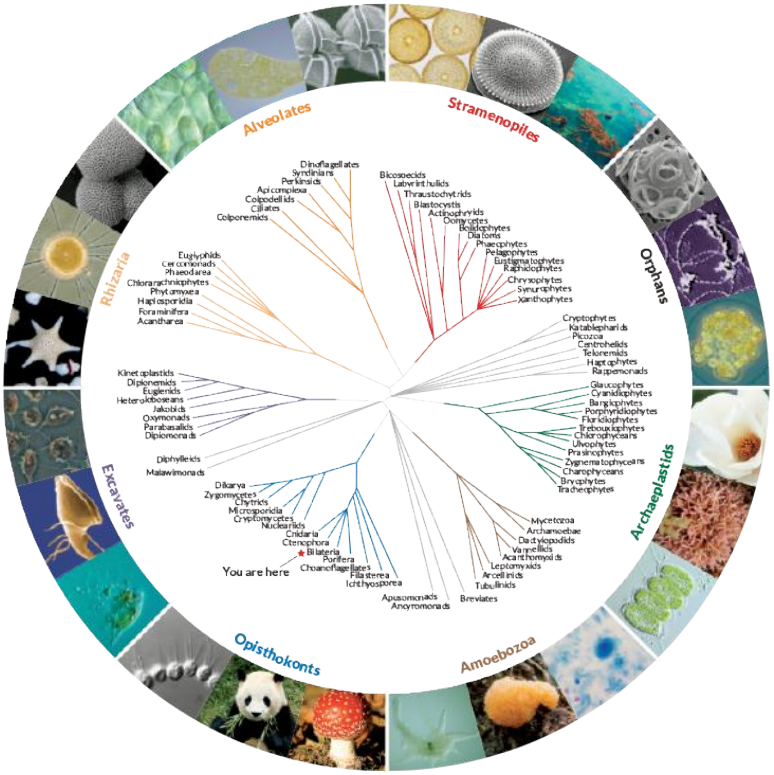
Bio Mar PT

Instituto Português do Mar e da Atmosfera

Guia técnico de curso de formação

Biologia Molecular e Bioinformática



Bárbara Frazão e Teixeira

João Paulo Machado

Créditos da imagem: A. Worden *et al*/*Science* 2015

Introdução à Biologia Molecular e à Bioinformática

1ª edição: 1 a 3 de Junho de 2016

2ª edição: 12 a 14 de Outubro de 2016

Curso BIOMAR PT 2016

Formadores

Bárbara Frazão

João Paulo Machado

Estrutura do curso

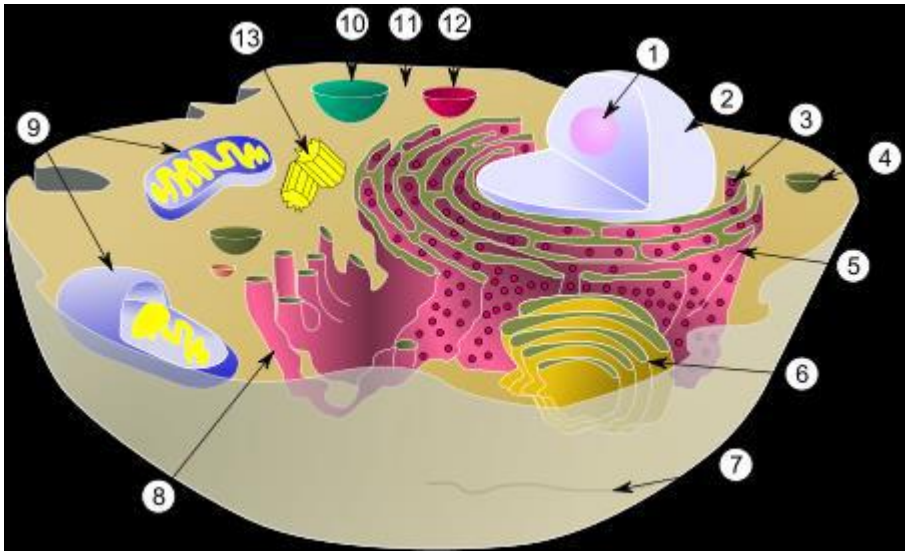
- 1ª aula (T) 4h - BM

  Conceitos básicos de Biologia molecular, preparação das amostras, Primer design, PCR, sequenciação
- 2ª aula (P) 3h –BM

  Extração DNA genómico, Electroforese em gel de agarose e Quantificação de DNA genómico
- 3ª aula (T) 4h- BI
  - Bases de dados, alinhamentos, modelos evolutivos, filogenias
- 4ª aula (P) 3h- BM
  - PCR e corrida em gel
- 5ª aula (TP) 3h – BI
  - Desenho de primers, BLAST, alinhamentos, determinação do modelo evolutivo
- 6ª aula (TP) 3h- BI
  - Construção de Filogenias

Conceitos básicos de Biologia molecular

- A **Biologia Molecular** é o estudo da Biologia a nível molecular, com ênfase na estrutura e função do material genético e seus produtos de expressão, as proteínas.



- Animal cell
  - cytoplasm
  - (1) nucleolus
  - (2) nucleus
  - (3) ribosome
  - (4) vesicle
  - (5) rough endoplasmic reticulum
  - (6) Golgi apparatus
  - (7) cytoskeleton
  - (8) smooth endoplasmic reticulum
  - (9) mitochondria
  - (10) vacuole
  - (11) cytosol
  - (12) lysosome
  - (13) centriole.

Conceitos básicos de Biologia molecular

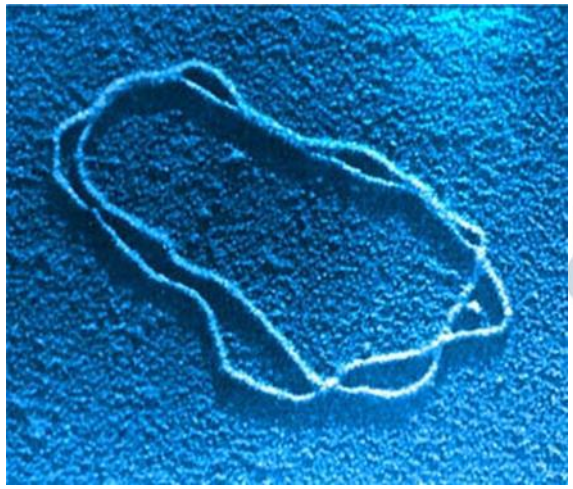
DNA

Descoberto por James Watson (norte-americano) e Francis Crick (britânico) em 1953

Prémio Nobel de Fisiologia ou Medicina em 1962

Ácido desoxirribonucleico

Composto orgânico cujas moléculas contêm as instruções genéticas que coordenam o desenvolvimento e funcionamento de todos os seres vivos e alguns vírus, e que transmitem as características hereditárias de cada ser vivo.



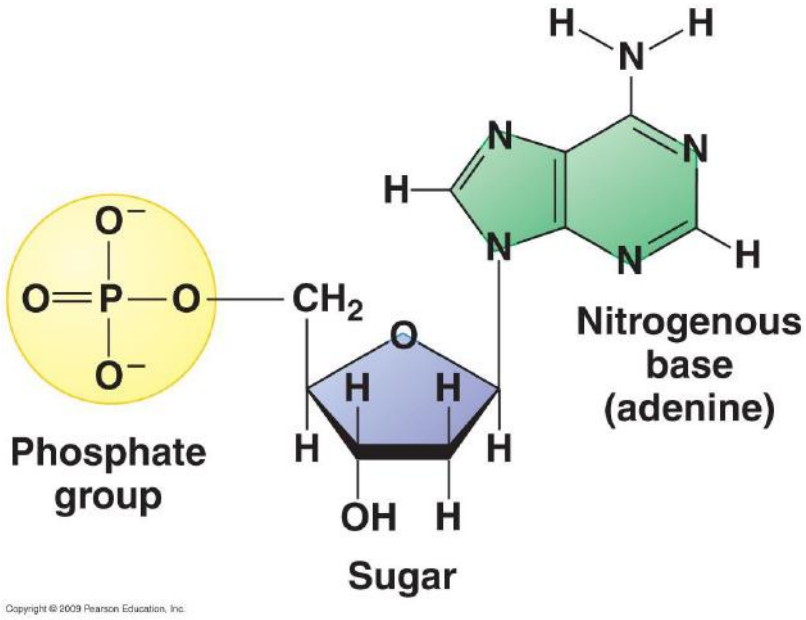
O seu principal papel é armazenar as informações necessárias para a construção das proteínas de RNAs.

Conceitos básicos de Biologia molecular

Ácidos Nucleicos

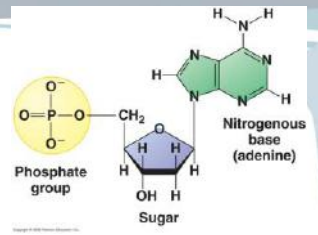
Polímero de unidades simples (monómeros)-

os nucleótidos

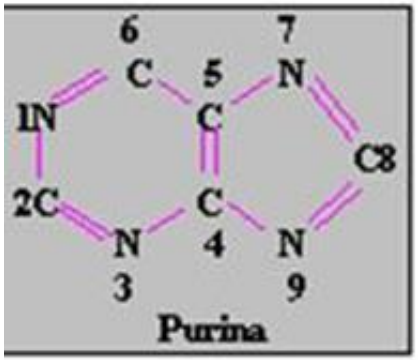


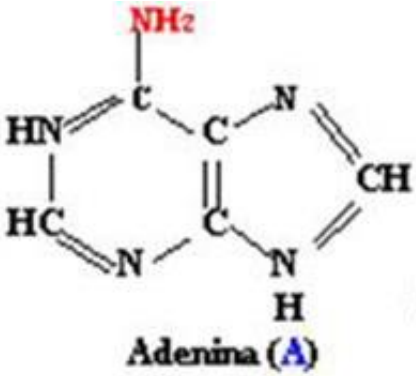
Conceitos básicos de Biologia molecular

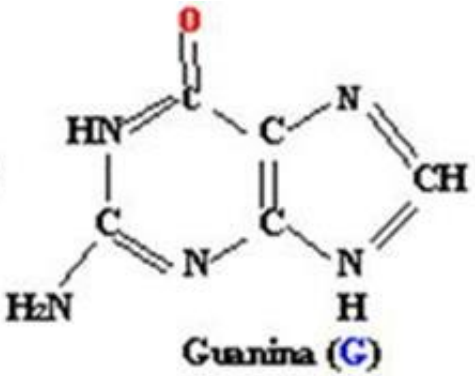
Ácidos Nucleicos

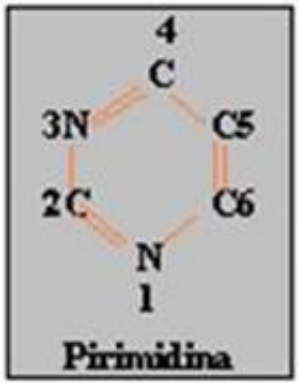


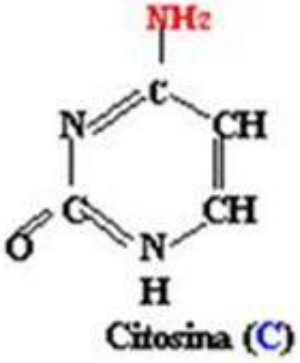
5 tipos de bases azotadas

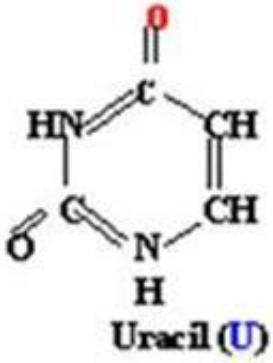


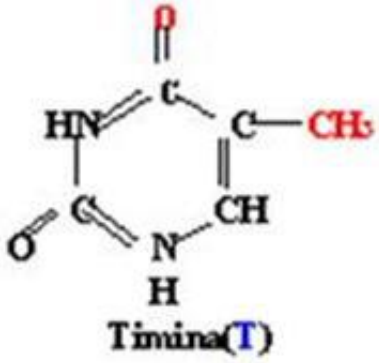






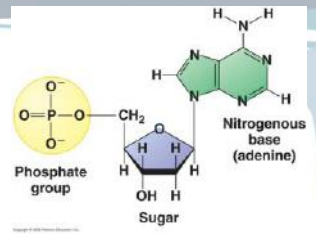




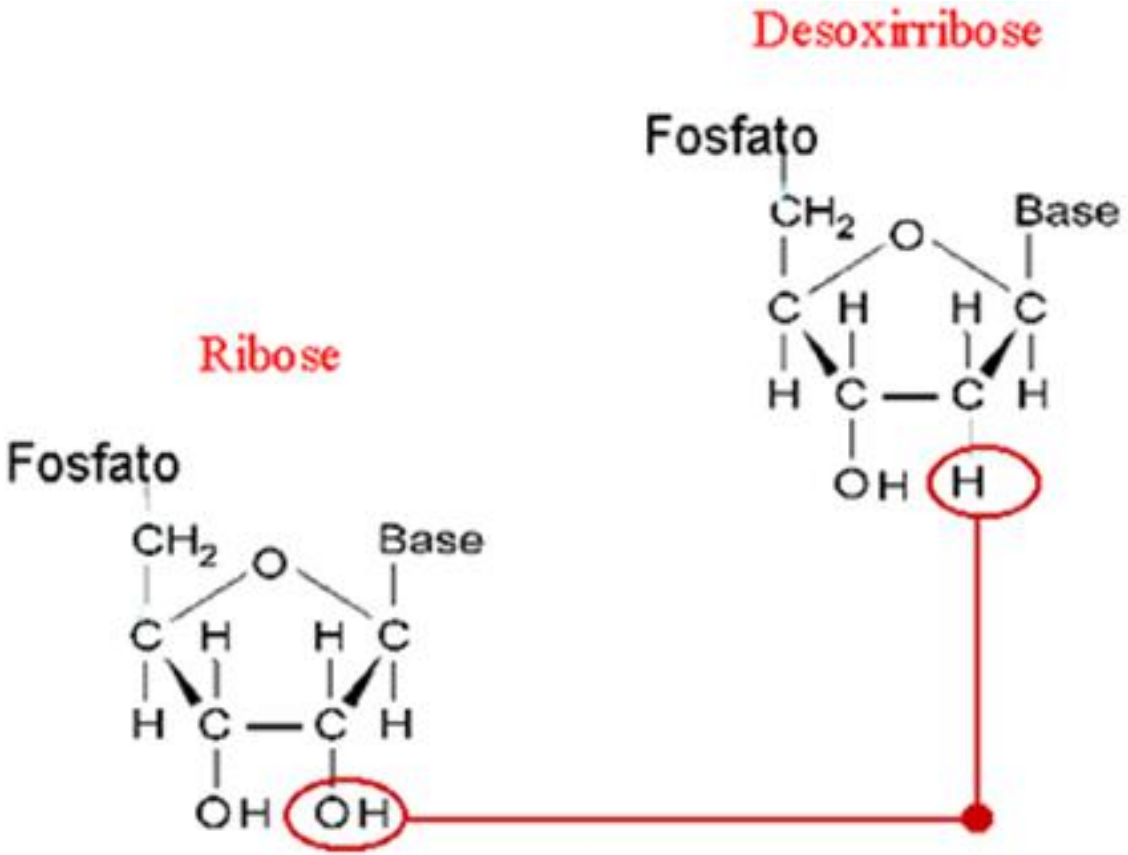


Conceitos básicos de Biologia molecular

Ácidos Nucleicos

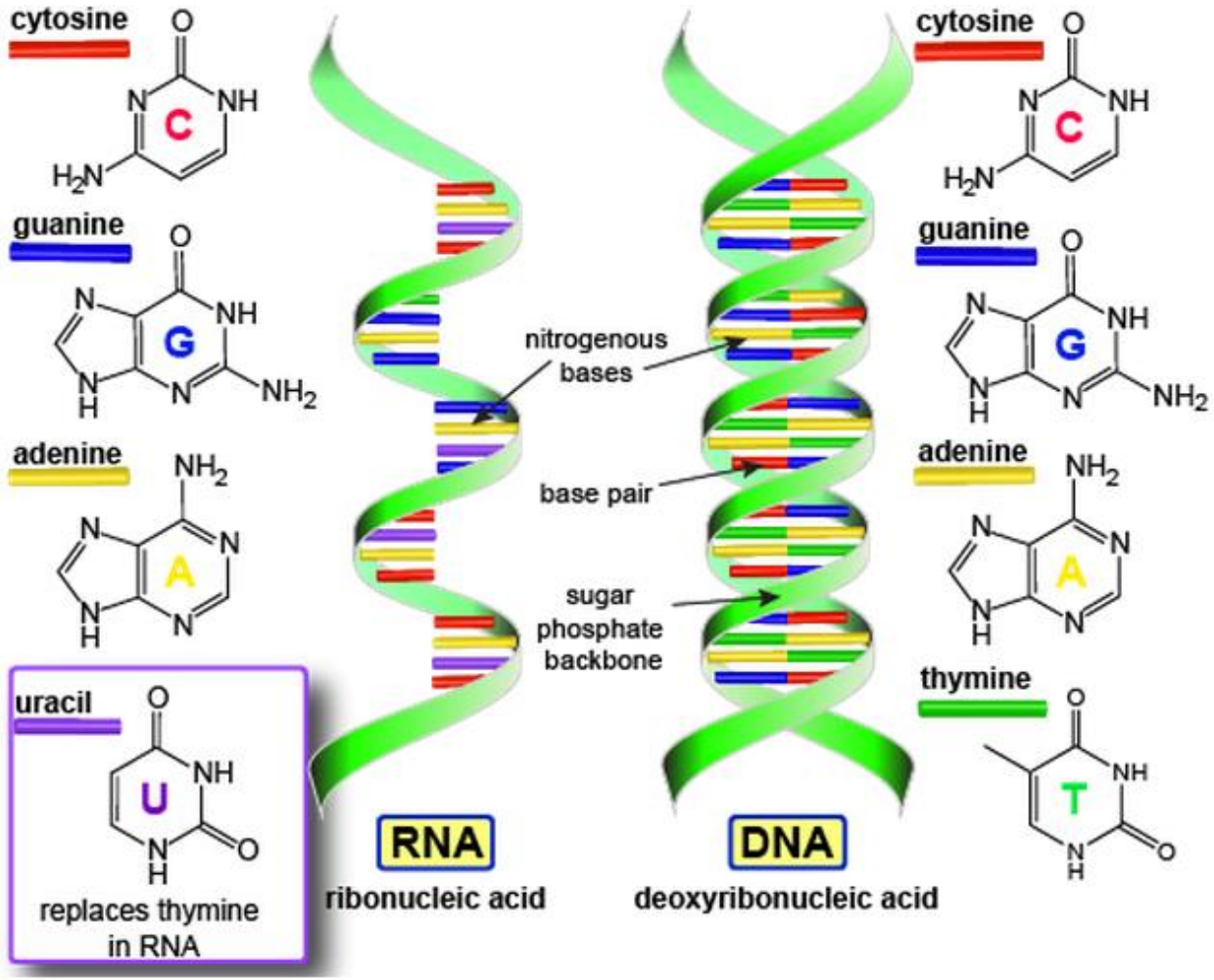


2 tipos de Pentoses



Conceitos básicos de Biologia molecular

Ácidos Nucleicos



Conceitos básicos de Biologia molecular

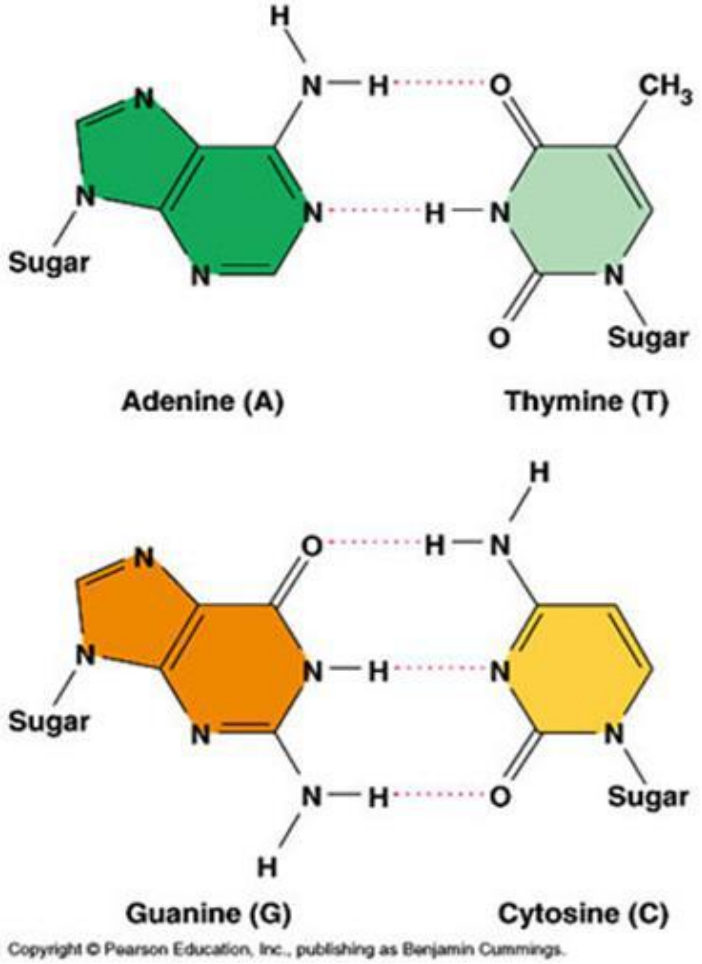
Ácidos Nucleicos

	DNA	RNA
Pentose	Desoxirribose	Ribose
Bases púricas	Adenina e Guanina	Adenina e Guanina
Estrutura	Citosina e Timina	Citosina e Uracila
Bases primídicas	Duas cadeias helicoidais	Uma cadeia
Enzima hidrolítica	Desoxirribonuclease (DNAase)	Ribonuclease (RNAase)
Origem	Replicação	Transcrição
Enzima sintética	DNA - polimerase	RNA - polimerase
Função	Informação genética	Síntese de proteínas

Conceitos básicos de Biologia molecular

DNA

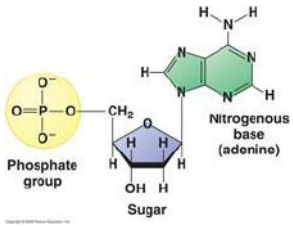
- Adenina complementar Timina
  - Ligação por 2 pontes de hidrogénio
- Citosina complementar com Guanina
  - Ligação por 3 pontes de hidrogénio

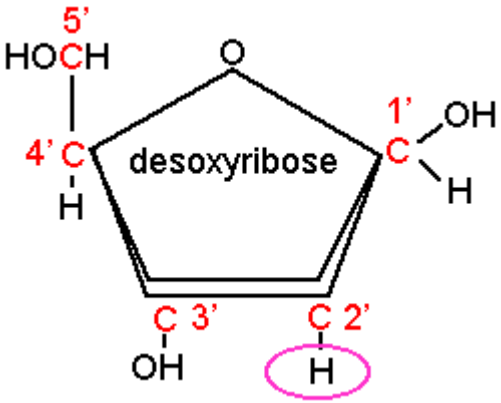


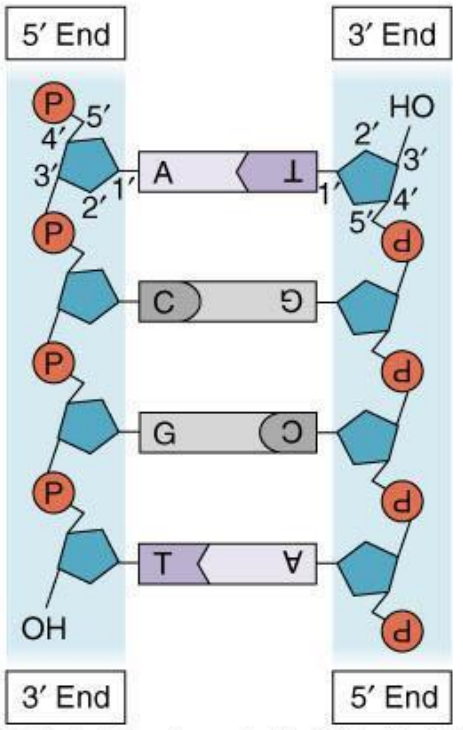
Conceitos básicos de Biologia molecular

DNA

- 2 cadeias helicoidais complementares e antiparalelas



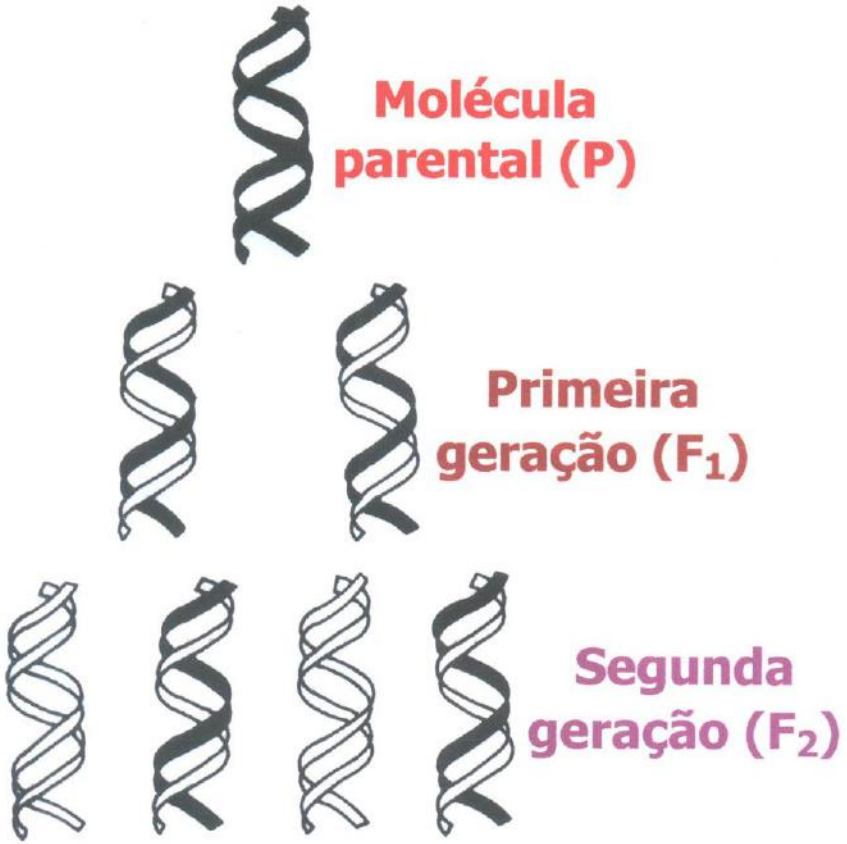




- Extremo 3’ – tem um grupo hidroxilo no 3º carbono
- Extremo 5´ - tem um grupo fosfato no 5º carbono
- Numeração dos carbonos- de 1´ a 5’ por convenção no sentido dos ponteiros do relógio, a partir do oxigénio
- Importância: síntese dos ácidos nucleicos dá-se no sentido 3´

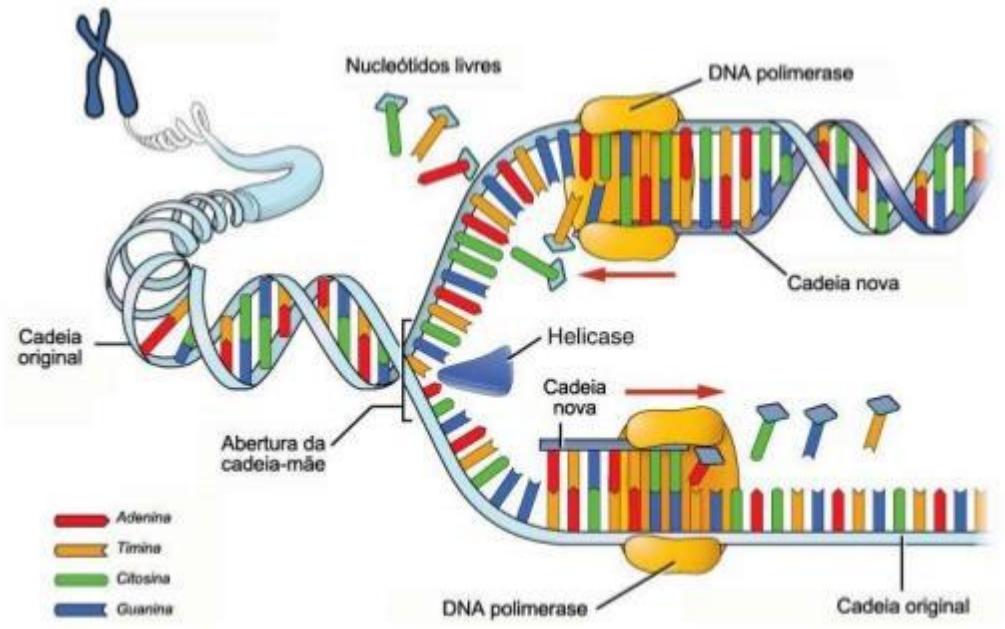
Conceitos básicos de Biologia molecular

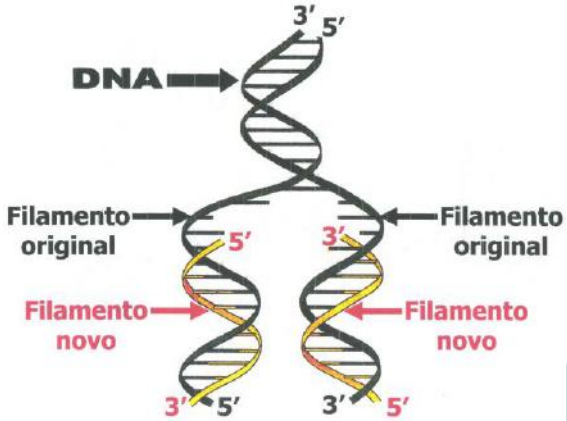
Replicação semi-conservativa do DNA



Conceitos básicos de Biologia molecular

Replicação semiconservativa do DNA





1. **Helicases**- separam as 2 cadeias da molécula
2. **DNA Polimerase III-** catalizam a adição de um nucleótido ao radical hidroxilo na extremidade 3´ da cadeia que se está formando. Desta forma as cadeias só podem crescer no sentido 5´ → 3´

Conceitos básicos de Biologia molecular

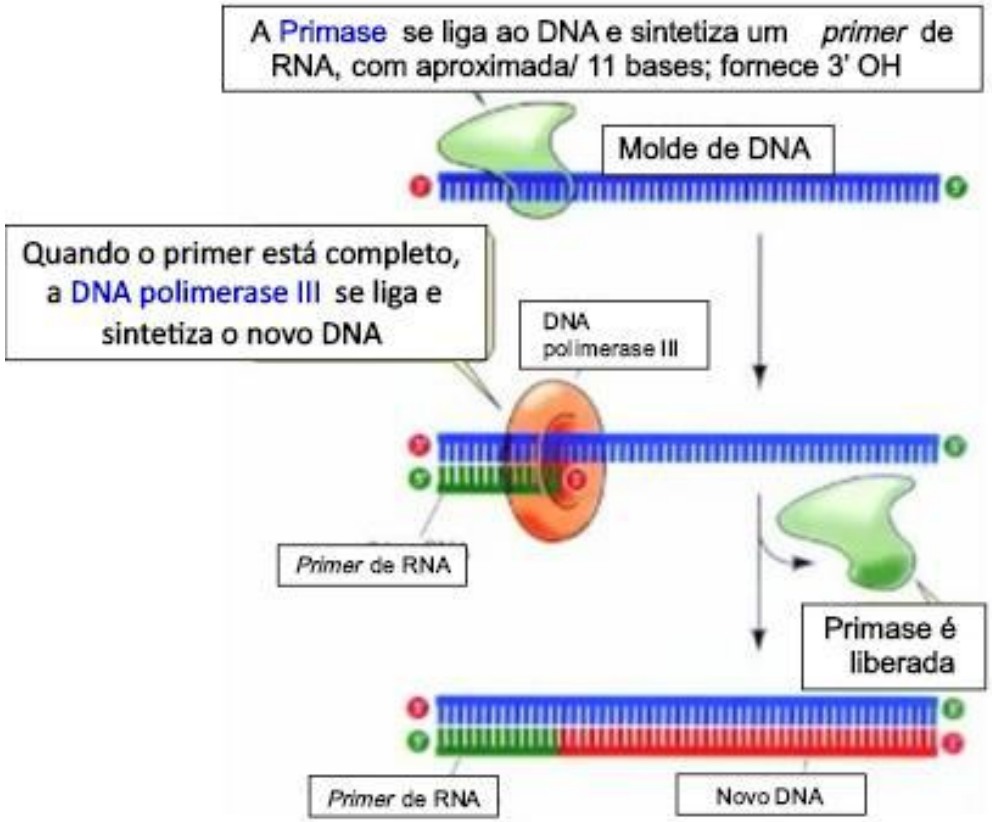
Replicação semiconservativa do DNA

Forquilha de replicação

- **Cadeia contínua-** sintetizada continuamente a partir de um iniciador na cadeia molde 3´ → 5´
- **Cadeia descontínua** – sintetizada descontinuamente a partir de múltiplos iniciadores

Conceitos básicos de Biologia molecular

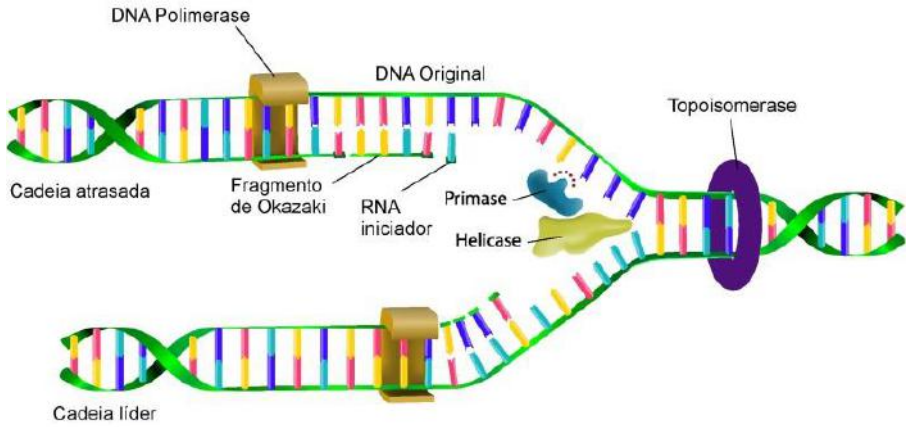
Replicação semiconservativa do DNA



3. **Primase**- Sintetiza pequenas moléculas de RNA utilizadas como iniciadores durante o processo de replicação

Conceitos básicos de Biologia molecular

Replicação semiconservativa do DNA

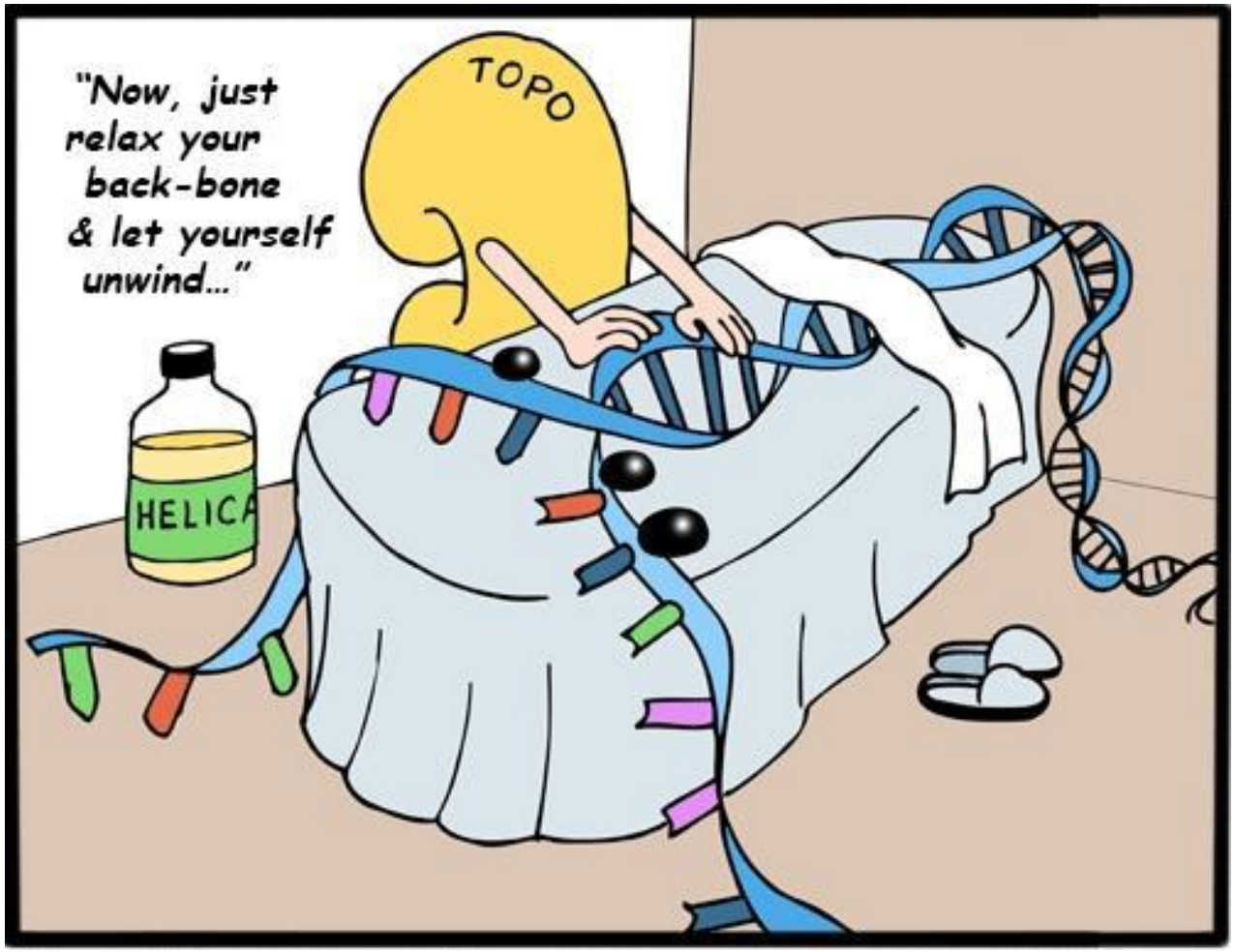


Topoisomerase

alivia a torção na parte da cadeia que não está a ser replicada

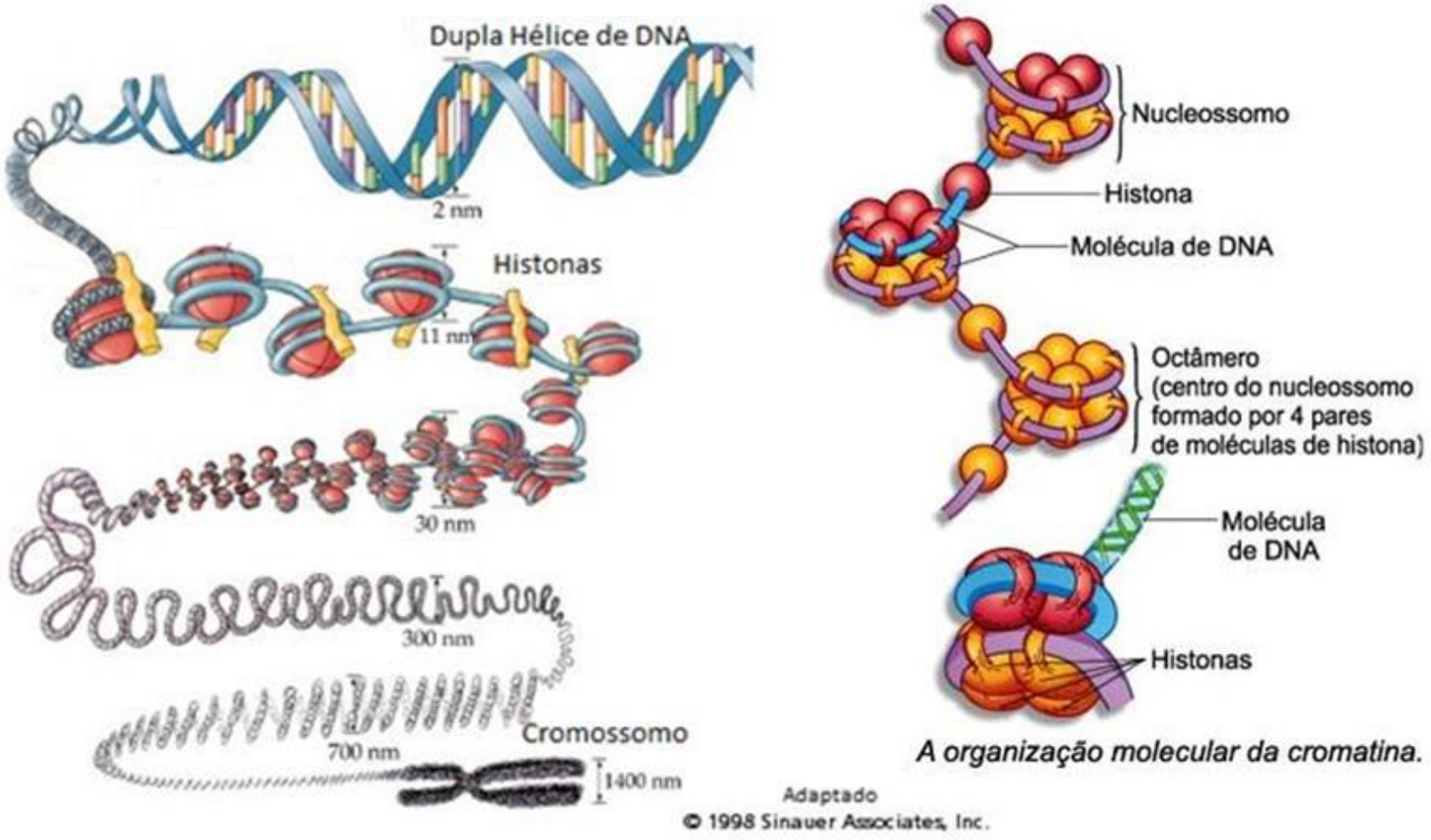
- Cada iniciador é alongado pela DNA polimerase resultando na formação de **Fragmentos de Okazaki**
- **DNA polimerase I** remove o primer do RNA do fragmento adjacente e preenche os gaps entre os fragmentos
- **DNA ligase** – liga os fragmentos de Okasaki

Conceitos básicos de Biologia molecular



Conceitos básicos de Biologia molecular

Organização do DNA

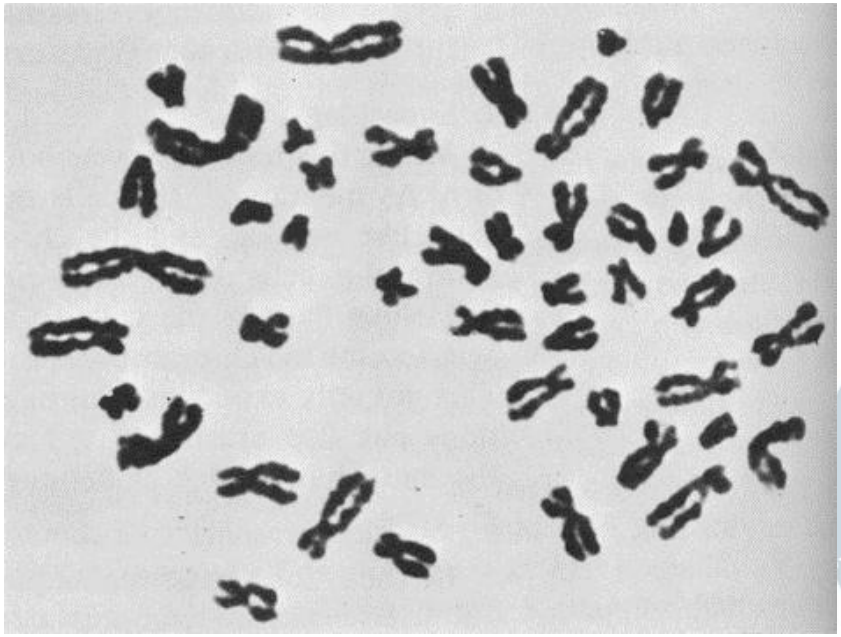


A organização molecular da cromatina.

Adaptado

Conceitos básicos de Biologia molecular

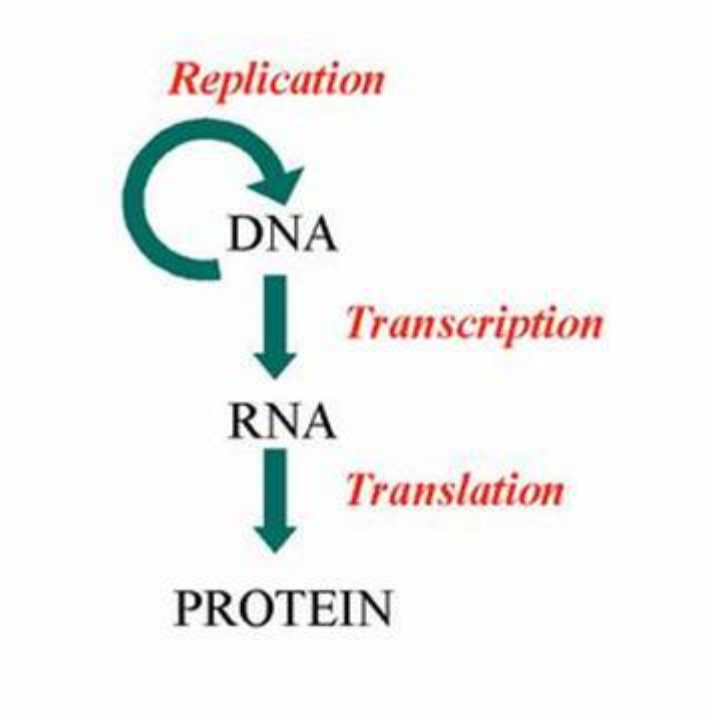
Cromossomas



Cariótipo
Conjunto de cromossomas de uma célula

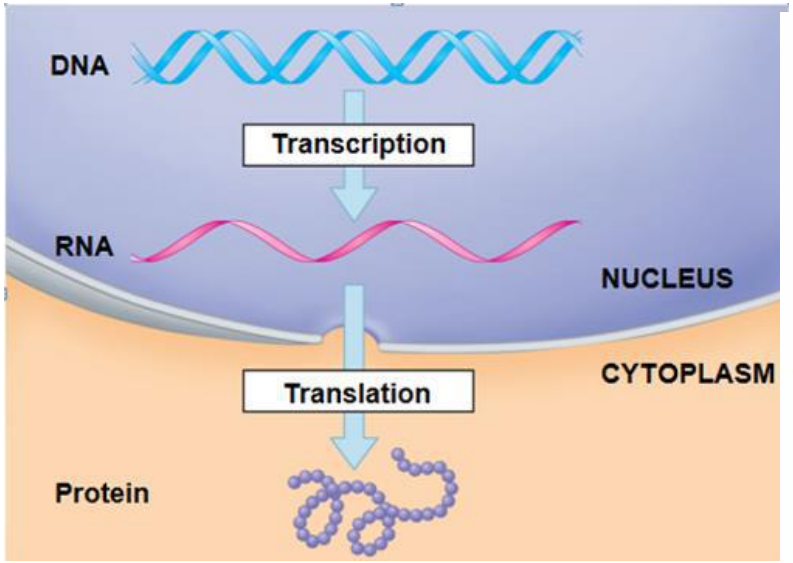
Conceitos básicos de Biologia molecular

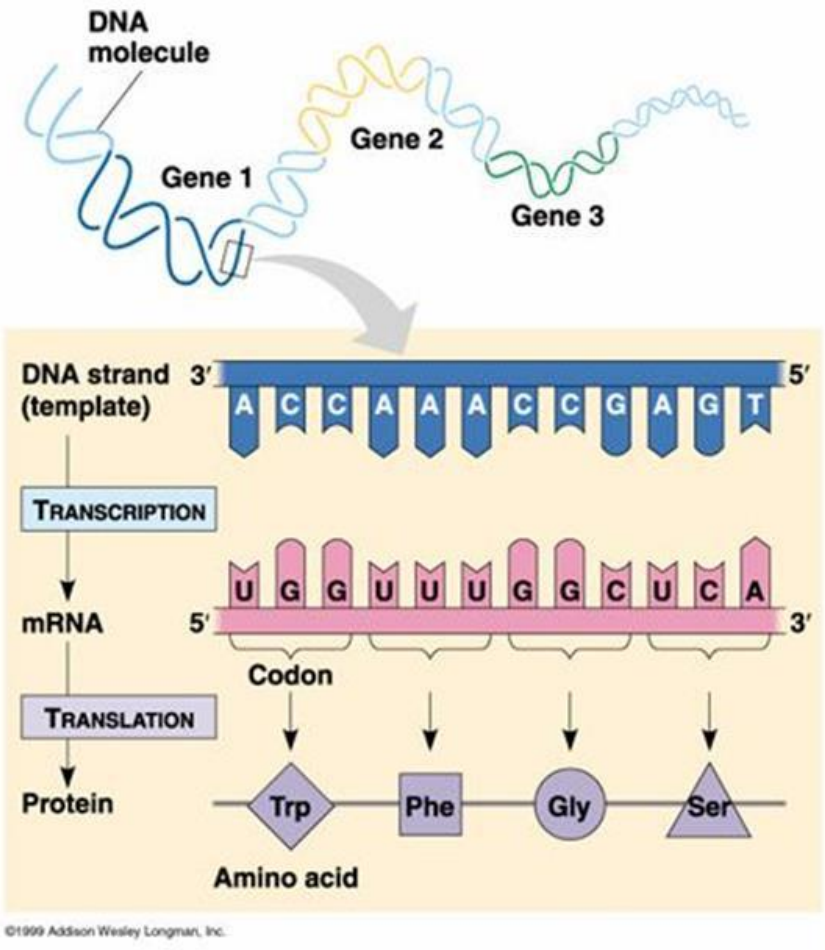
Transcrição e Tradução



Conceitos básicos de Biologia molecular

Transcrição e Tradução

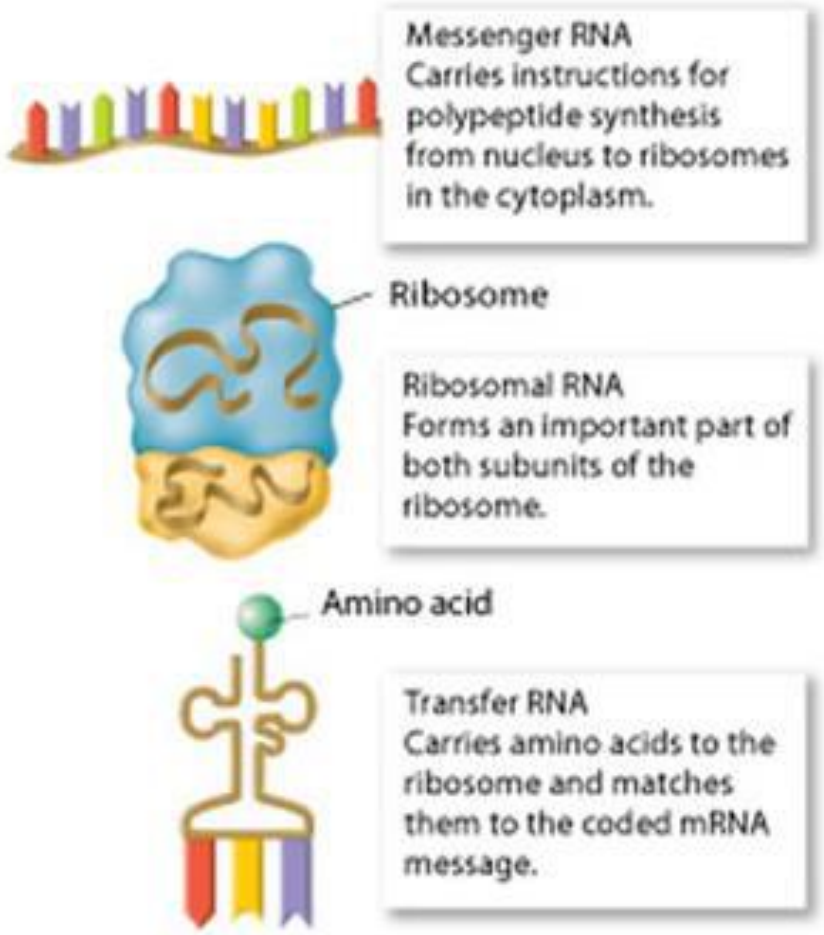




Conceitos básicos de Biologia molecular

Transcrição e Tradução

A **transcrição** é a passagem da informação genética, do núcleo para o citoplasma, sob a forma de **RNA mensageiro (mRNA),** cuja sequencia é complementar ao DNA de que foi transcrito



<u>Tipos de RNA</u>

-Mensageiro

-Ribossómico

-de Transferência

Conceitos básicos de Biologia molecular

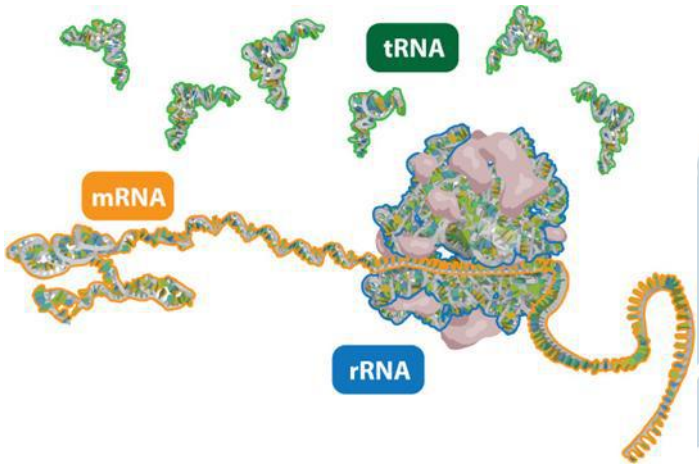
Código genético

Gene-região de DNA que codifica para determinada proteína ou RNA funcional
Genoma- todo o material genético de um organismo e inclui quer os genes quer as sequencias não-codificantes

Sequências não codificantes, e.g.

- RNA não codificantes
  - transferência RNA
  - ribosómico RNA
  - regulatório RNA

- Regulação da atividade das regiões codificantes



Conceitos básicos de Biologia molecular

Código genético

A sequência de bases ao longo da molécula de ADN constitui a informação genética. A leitura destas sequências é feita por intermédio do código genético, que especifica a sequência linear dos aminoácidos das proteínas.

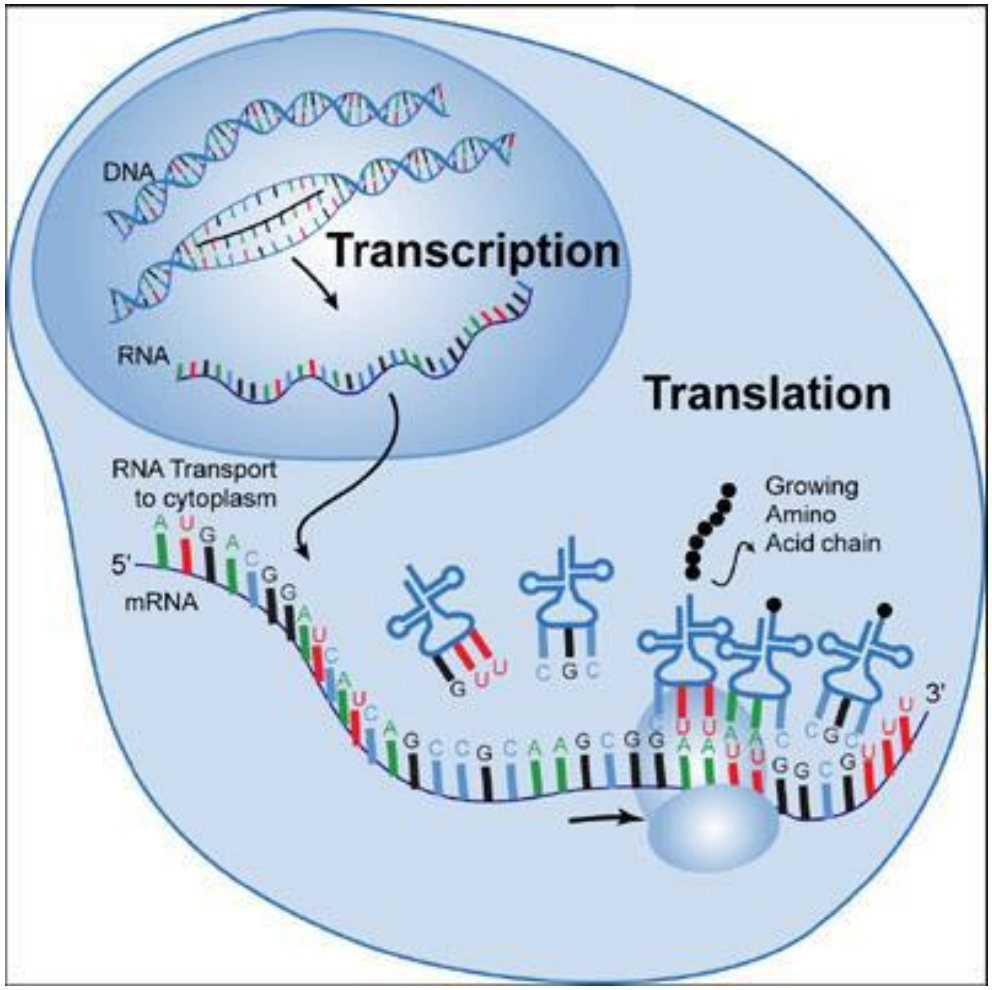
First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile **AUG Met**	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Codão- 3 nucleótidos seguidos do mRNA

Anticodão- as 3 bases do tRNA, que são complementares ao codão do mRNA

Conceitos básicos de Biologia molecular

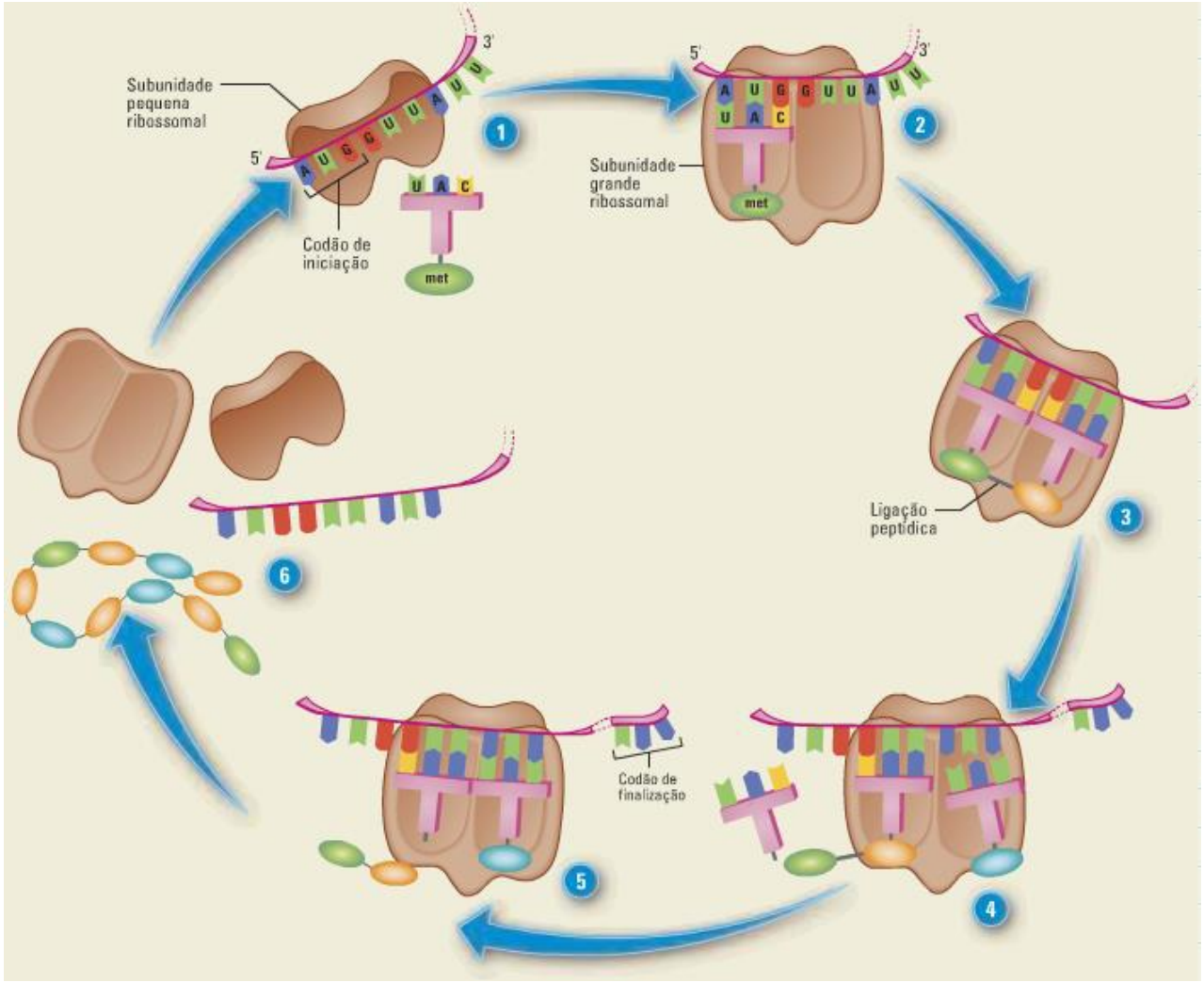
Tradução



Processo pelo qual o RNA maduro, serve de “template” para a síntese de uma nova proteína

Conceitos básicos de Biologia molecular

Tradução



Extração de DNA genómico

Método tradicional Fenólico

Ref: http://dx.doi.org/10.2216/i0031-8884-42-3-261.1

1. 50ml culture
2. Centrifugue and discard supernatant
3. Pellet ressuspended in 750ul filtered NSW
4. 3500g, 10min, 4ºC
5. Pellet stored in liquid nitrogen

1. Nitrogen pump – 10 cycles, 2400psi (to lyse)
2. 500ul lysis buffer at 65ºC, 30min

- 0.02M EDTA
- 2% CTAB
- 0.1M Tris
- 1.4M NaCl
- 9.2% 2-b-mercaptoetanol

Extração de DNA genómico

Método tradicional Fenólico

Lysis Buffer- permite quebrar as células para libertar o DNA, sem a sua degradação

Podem conter:

1. Sais-manter uma força iónica (concentração salina)
2. Detergentes- separar as proteínas da membrana
   - Triton X-100
   - CHAPS
   - SDS
3. RNase A – degrada RNA
4. Proteinase K- desnatura proteínas, mas geralmente tem um tempo definido de atuação

Extração de DNA genómico

Método tradicional Fenólico

3. 100ul phenol-chloroform-isoamyl alcohol (25:24:1)
4. 14000g 8min, 4ºC
5. Repeat step 3 com o sobrenadante
6. Chloroform-isoamyl alcohol (24:1)
7. 14000g 8min, 4ºC
8. 5ul glycogen ao sobrenadante
9. 15ul 3M NaOAc and isopropanol
10. Incubate -80ºC, 30min
11. 16000g, 15min
12. 1000ul, 70%ethanol
13. 16000g, 15min
14. Dried in a speed vacum
15. Ressuspend 50ul H2O

Extração de DNA genómico

Método tradicional : Fenólico

Phenol-chloroform-isoamyl alcohol

DNA extraction proporção 25:24:1

pH DNA extraction 6.7-8

(RNA – pH fenol 4.8)

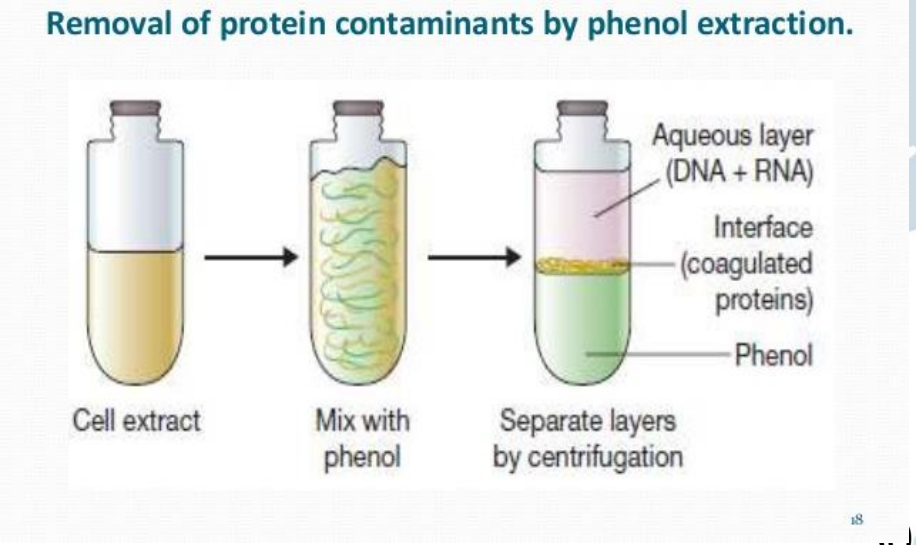
Clorofórmio- desnatura proteínas

Isoamyl alcohol- previne formação de espuma

Sais de Guanidina – reduzem os efeitos das nucleases

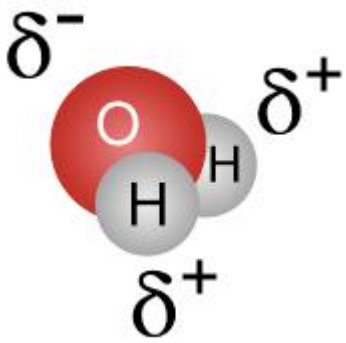
Glicogénio é insoluvel no etanol e forma um precipitado que agarra o ácido nucleico

Fenol ácido retem o RNA na fase aquosa, mas move o DNA para a fase fenólica

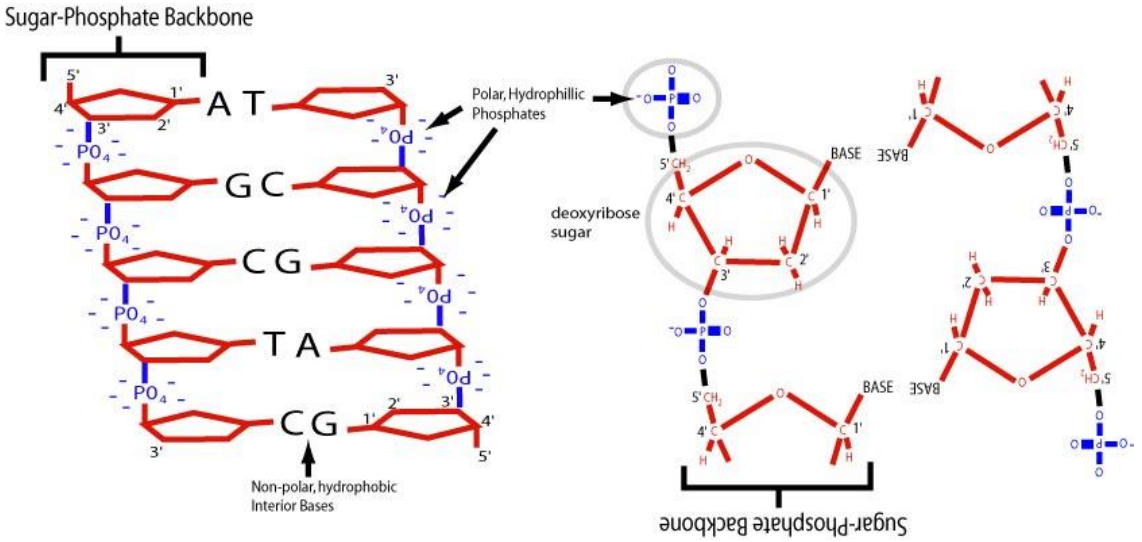


Extração de DNA genómico

Método tradicional : Fenólico



DNA rodeado por moléculas de água



Extração de DNA genómico

Método tradicional : Fenólico

Precipitação de DNA

1. Sal para neutralizer a carga do ácido nucleico. O DNA fica menos hidrofílico e precipita na solução (acetato de sódio, cloreto de sódio, cloreto de litio, acetate de amónio, etc)
2. Etanol 100% para precipitar o DNA (altera a estrutura do DNA de maneira em que as suas moléculas se agregam e precipitam)
3. Frio para arrefecer a amostra. Temperaturas baixas promovem a floculação dos ácidos nucleicos, para que formem um complex maior que facilite a formação de pellet na centrifugação (Over-night -4ºC ou -80ºC 1 hora)
4. Uma concentração elevada de ácido nucleico, suficiente para forçar recuperação do DNA (se não for elevada pode adicionar-se um “carrier” como o glicogénio para aumentar a recuperação
5. Centrifugação da amostra até formar um pellet

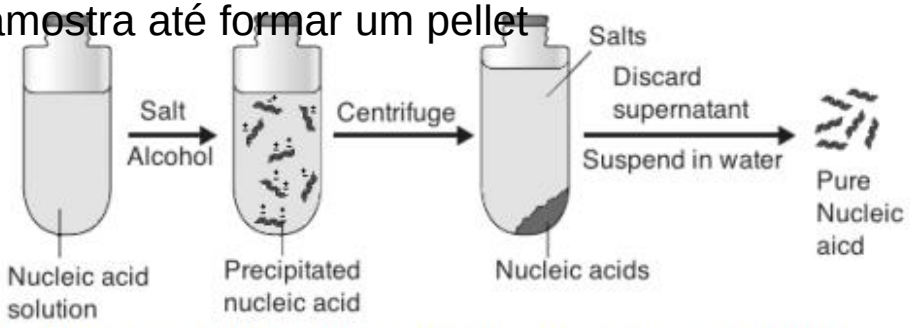


Figure 1. Schematic overview of an ethanol precipitation of nucleic acids.

Extração de DNA genómico

Método tradicional : Fenólico

Etanol	Isopropanol
Se couberem 2 volumes de etanol	Se couber apenas 1 volume de álcool (tubo pequeno)
Precipita fragmentos menores e maiores	Precipita apenas fragmentos maiores
Precipita a -20ºC	Precipita a RT
Precipita pouca concentração de amostra	Só precipita se em quantidade considerável de amostra
Precipitação demorada	Precipitação rápida

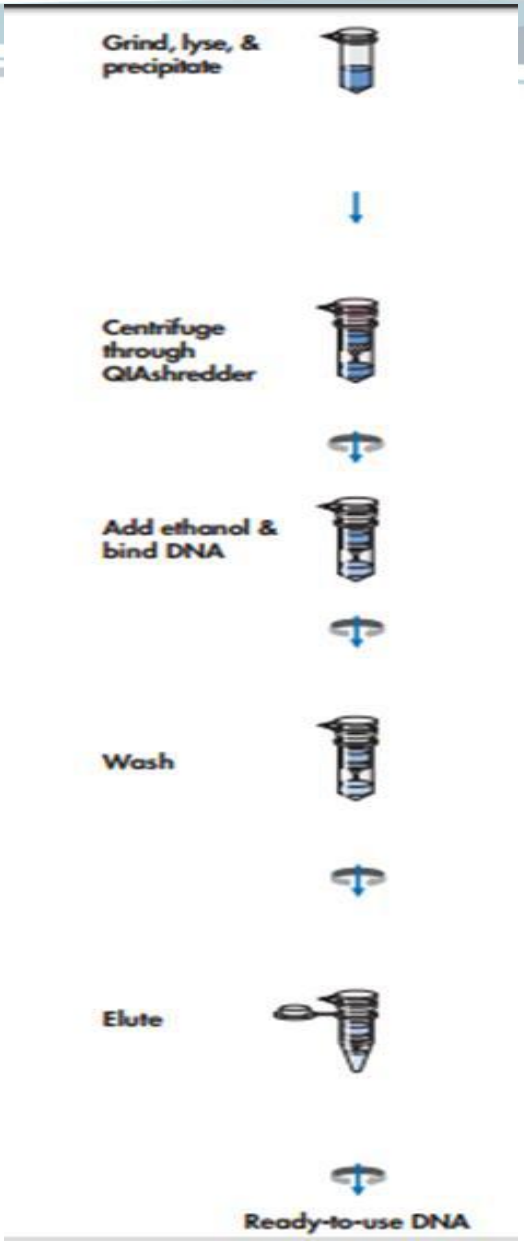
Lavagem do pellet com etanol a 70% ethanol. Percentagem na qual os sais são removidos e o DNA não é dissolvido

Secagem do pellet:

- Deve ser eficaz, para o etanol não estar presente na amostra
- Não deve ser demasiado, pois dificulta a dissolução na solução de eluição

Extração de DNA genómico

Kit da Qiagen- DNeasy plant



Extração de DNA genómico

Kit da Qiagen- DNeasy plant

1. Add 400 µl Buffer AP1 and 4 µl RNase A and vortex
2. Homogeneização da amostra no miniLys- 15s, 5000rpm

Extração de DNA genómico

Kit da Qiagen- DNeasy plant

- Mais volume amostra no mesmo volume de lysis buffer/Coluna, **não aumenta** eficiência de extração

8. **Incubate the mixture for 10 min at 65°C. Mix 2 or 3 times during incubation by inverting tube.**

   This step lyses the cells.

9. **Add 130 µl Buffer P3 to the lysate, mix, and incubate for 5 min on ice.**

   This step precipitates detergent, proteins, and polysaccharides.

10. **Recommended: Centrifuge the lysate for 5 min at 20,000 x *g* (14,000 rpm).**

    Some plant materials can generate very viscous lysates and large amounts of precipitates during this step. This can result in shearing of the DNA in the next step (see "Lysate filtration with QIAshredder", page 19). In this case, optimal results are obtained if the majority of these precipitates are removed by centrifugation for 5 min at 20,000 x *g* (14,000 rpm). After centrifugation, apply supernatant to QIAshredder Mini spin column and continue with step 11.

11. **Pipet the lysate into the QIAshredder Mini spin column (lilac) placed in a 2 ml collection tube, and centrifuge for 2 min at 20,000 x *g* (14,000 rpm).**

    It may be necessary to cut the end off the pipet tip to apply the lysate to the QIAshredder Mini spin column. The QIAshredder Mini spin column removes most precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be careful not to disturb this pellet in step 12.

12. **Transfer the flow-through fraction from step 11 into a new tube (not supplied) without disturbing the cell-debris pellet.**

    Typically 450 µl of lysate is recovered. For some plant species less lysate is recovered. In this case, determine the volume for the next step.

13. **Add 1.5 volumes of Buffer AW1 to the cleared lysate, and mix by pipetting.**

    For example, to 450 µl lysate, add 675 µl Buffer AW1. Reduce the amount of Buffer AW1 accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AW1, but this will not affect the DNeasy procedure.

    Note: Ensure that ethanol has been added to Buffer AW1. See "Things to do before starting", page 22.

    Note: It is important to pipet Buffer AW1 directly onto the cleared lysate and to mix immediately.

Extração de DNA genómico

Kit da Qiagen- DNeasy plant

14. **Pipet 650 µl of the mixture from step 13, including any precipitate that may have formed, into the DNeasy Mini spin column placed in a 2 ml collection tube (supplied). Centrifuge for 1 min at ≥6000 x *g* (corresponds to ≥8000 rpm for most microcentrifuges), and discard the flow-through.* Reuse the collection tube in step 15.**
15. **Repeat step 14 with remaining sample. Discard flow-through* and collection tube.**

* Flow-through fractions contain Buffer AW1, and are therefore not compatible with bleach. See page 6 for safety information.

16. **Place the DNeasy Mini spin column into a new 2 ml collection tube (supplied), add 500 µl Buffer AW2, and centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm). Discard the flow-through and reuse the collection tube in step 17.**

    Note: Ensure that ethanol is added to Buffer AW2. See "Things to do before starting", page 22.
17. **Add 500 µl Buffer AW2 to the DNeasy Mini spin column, and centrifuge for 2 min at 20,000 x *g* (14,000 rpm) to dry the membrane.**

    It is important to dry the membrane of the DNeasy Mini spin column since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during elution. Discard flow-through and collection tube.

    After washing with Buffer AW2, the DNeasy Mini spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW2, refer to "Darkly colored membrane or green/yellow eluate after washing with Buffer AW2" in the Troubleshooting Guide on page 43.

    Note: Following the centrifugation, remove the DNeasy Mini spin column from the collection tube carefully so the column does not come into contact with the flow-through, as this will result in carryover of ethanol.

Extração de DNA genómico

Kit da Qiagen- DNeasy plant

- Eluição em 30ul, optimiza quantidade de DNA
- Secagem total de etanol, Incubação RT (room temperature) e Elution Buffer 50-60ºC também optimiza quantidade de DNA

18. **Transfer the DNeasy Mini spin column to a 1.5 ml or 2 ml microcentrifuge tube (not supplied), and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate for 5 min at room temperature (15–25°C), and then centrifuge for 1 min at ≥6000 x *g* (≥8000 rpm) to elute.**

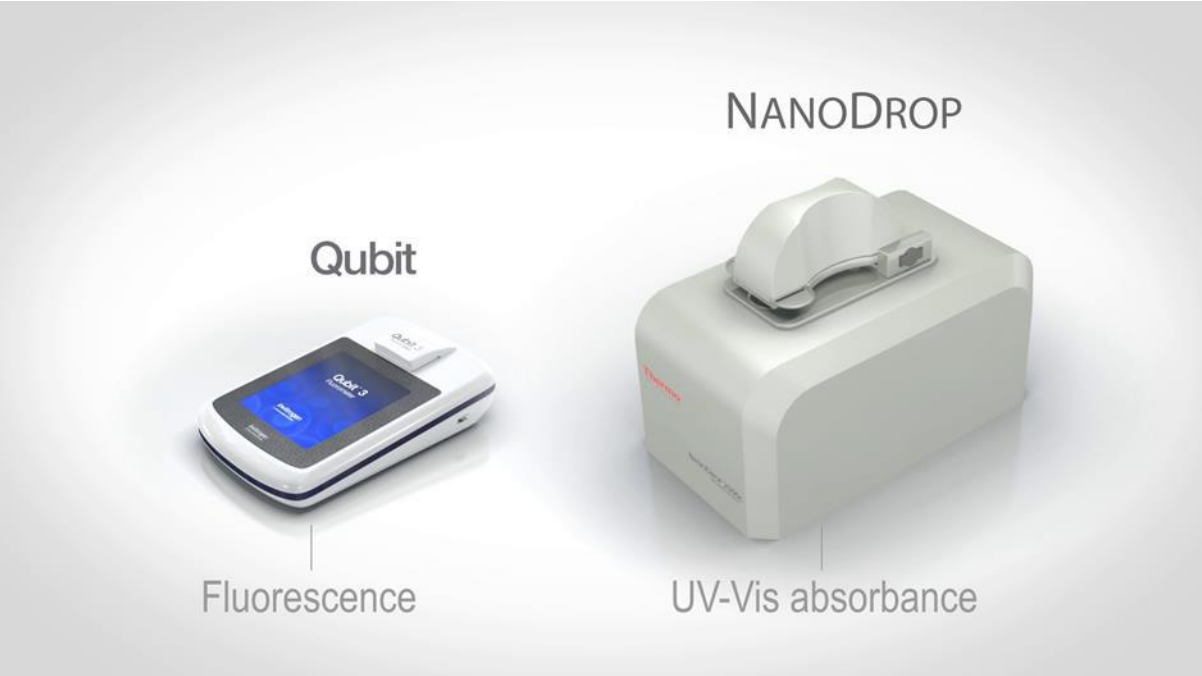
Elution with 50 µl (instead of 100 µl) increases the final DNA concentration in the eluate significantly, but also reduces overall DNA yield. If larger amounts of DNA (>20 µg) are loaded, eluting with 200 µl (instead of 100 µl) increases yield. See "Elution", page 19.

19. **Repeat step 18 once.**

A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates. See "Elution", page 19.

Note: More than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the DNeasy Mini spin column will come into contact with the eluate.

Quantificação do DNA



Quantificação do DNA

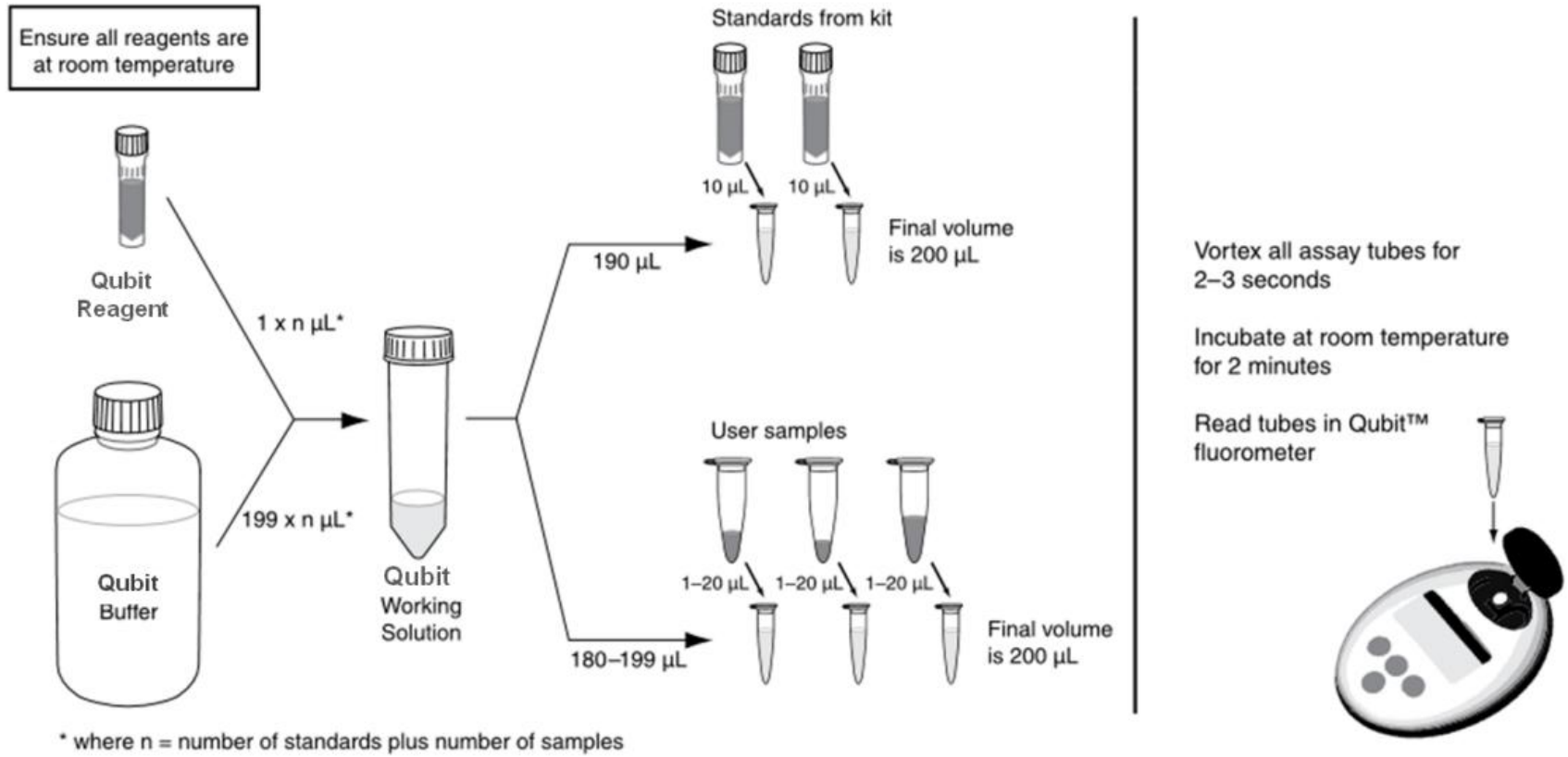
Table 3. Quantification method comparison.

	Qubit® Fluorometer	UV-absorbance microvolume spectrophotometer
Quantification method	Fluorescence-based dyes that bind specifically to DNA, RNA, or protein	UV absorbance measurements (measures absorbance at 260 nm and 260 nm/280 nm ratio)
Accuracy and precision at low concentrations (Figure 4)	Accurately quantifies DNA in samples with concentrations as low as 10 pg/µL	Not recommended for concentrations under 2 ng/µL; variation for sample concentrations <10 ng/µL is often high
Sensitivity and range (Figure 5)	The effective range covers a sample concentration range of 10 pg/µL to 1 µg/µL DNA	Covers a sample concentration range of 2 ng/µL to 15 µg/µL; uses 0.5–2 µL of sample
Can indicate contamination	No	Gives peaks revealing the presence of contaminants

Quantificação do DNA

Product	Cat. No.	No. of assays	Quantitation range	Applications
Qubit dsDNA BR Assay Kit	Q32850	100	2–1,000 ng	Quantitation of genomic and miniprep DNA samples
	Q32853	500		
Qubit dsDNA HS Assay Kit	Q32851	100	0.2–100 ng	Quantitation of PCR products, viral DNA, or samples for NGS
	Q32854	500		

Quantificação do DNA



Quantificação do DNA

- Pipetar 2ul de amostra, sem criar bolhas e sem tocar no equipamento
- Ler a absorvância do “Branco”- Solução na qual o DNA está eluído
- Ler a absorvância da nossa amostra
- Abs 260/280 deve estar entre 1.8 e 2. Valores >2 indicam contaminação com RNA. Valores inferiores a 1.8 indicam contaminação com Fenol ou proteínas, por exemplo.

Quantificação do DNA

Quantificação de DNA genómico
em gel de agarose

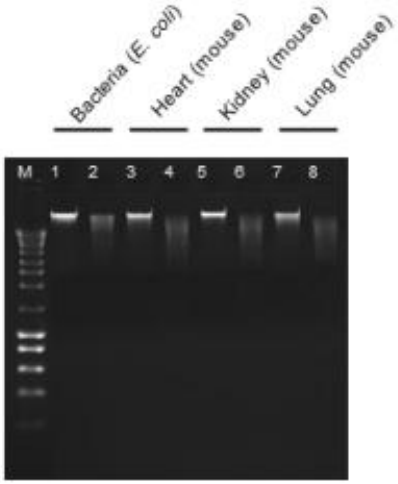
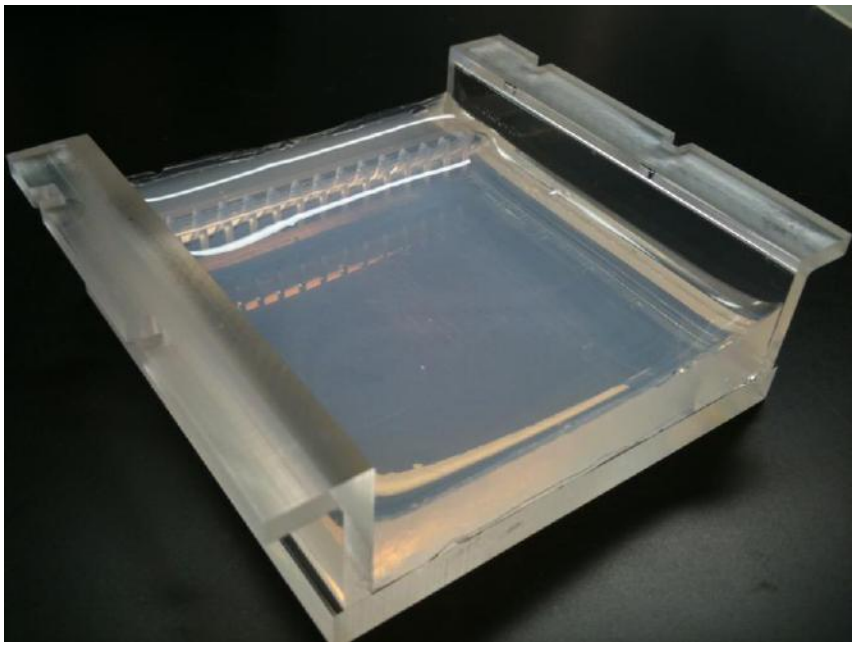
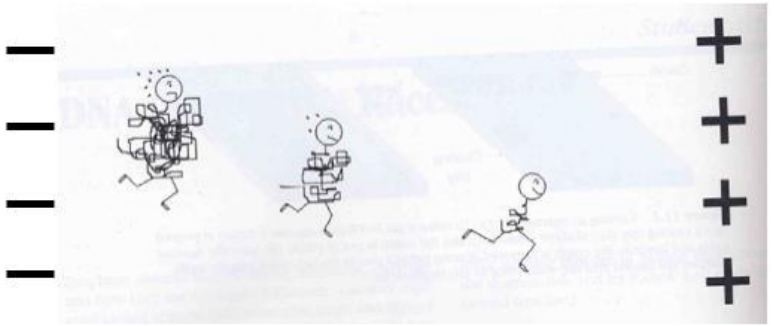


Fig. 1. Genomic DNA from various sources isolated with NZY Tissue gDNA Isolation kit. 0.25 µg of each isolated DNA was analysed on a 0.8% agarose gel. Lanes 2, 4, 6 and 8: gDNA digested with *Eco*R I. Lanes 1, 3, 5 and 7: undigested gDNA. M: NZYDNA Ladder III (MB0440); 1, 2: gDNA from bacteria (*E. coli*); 3, 4: gDNA from mouse heart; 5, 6: gDNA from mouse kidney; 7, 8: gDNA from mouse lung.

Electroforese em gel de agarose

A eletroforese em gel é uma técnica de separação de moléculas que envolve a migração de partículas num determinado gel durante a aplicação de uma diferença de potencial. As moléculas são separadas de acordo com o seu tamanho, pois as de menor massa irão migrar mais rapidamente que as de maior massa.





Electroforese em gel de agarose

- Separa e analisa DNA
- Quantifica e Isola uma determinada banda
- O DNA é visualizado pela adição
  - Brometo de etídio (altamente tóxico e mutagénico, mas dá mais visibilidade)
  - GelRed
  - GelGreen
  - SYBRSafe

  Liga-se ao DNA e fica fluorescente sob luz UV.

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
0.5%	1 kb to 30 kb
0.7%	800 bp to 12 kb
1.0%	500 bp to 10 kb
1.2%	400 bp to 7 kb
1.5%	200 bp to 3 kb
2.0%	50 bp to 2 kb

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

Electroforese em gel de agarose

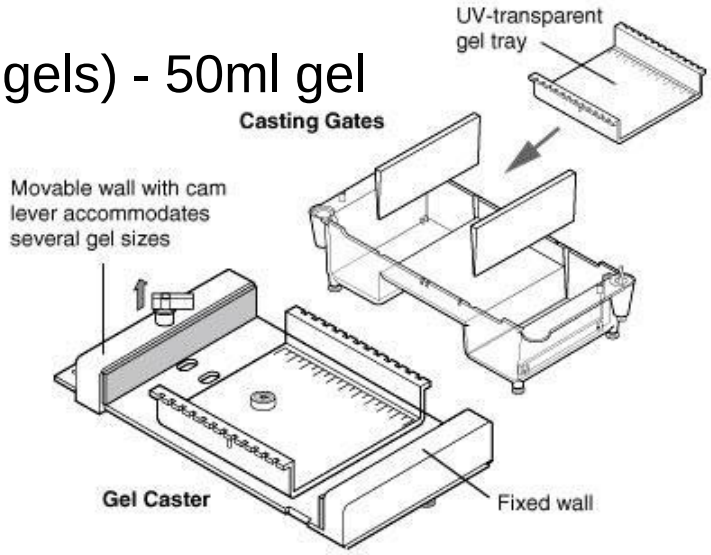
Polimerização dos géis

- Há géis que já se compram polimerizados
- Normalmente fazem-se em laboratório

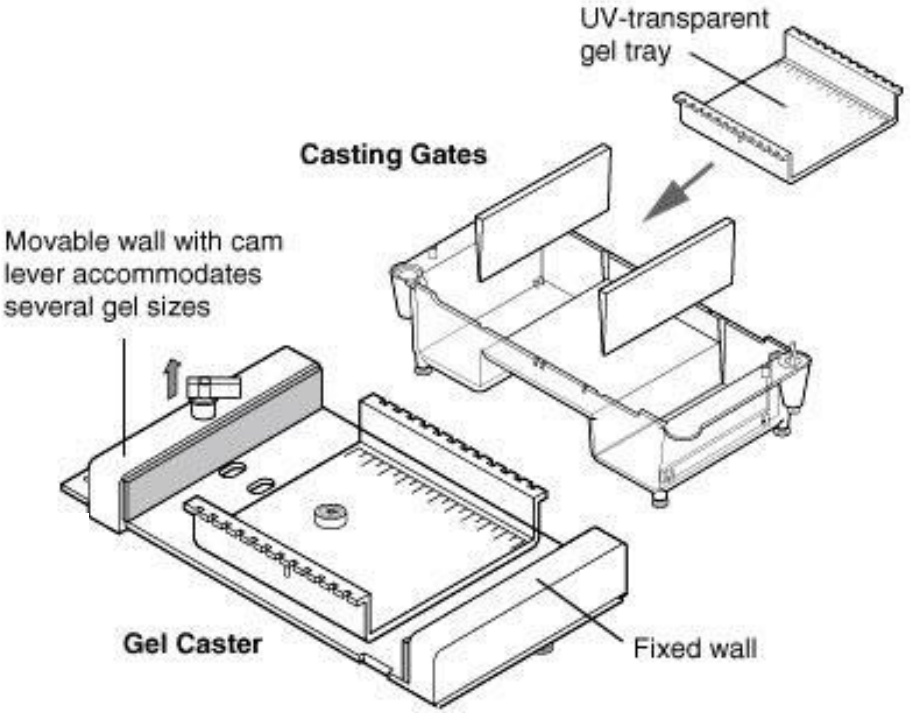
Qual o tanque a escolher

- Numero de amostras a carregar
- Velocidade de corrida

- Tinas pequenas 8x10 cm gels (minigels) - 50ml gel
- Tina grande - 100ml gel

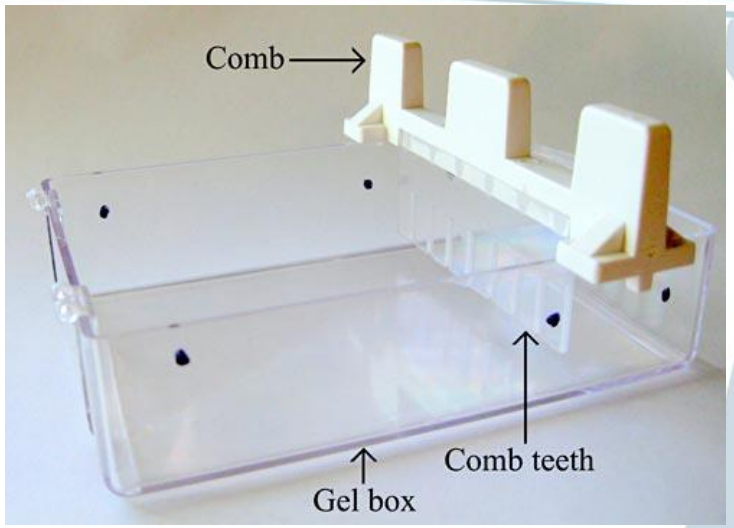


Electroforese em gel de agarose



Electroforese em gel de agarose

Qual pente a usar?



Depende volume que queremos carregar

Volume do poço - Depende largura poço e da espessura do dente do pente, e da espessura do gel

Tina pequena - pente com 8 poços (20 ou 40ul dependendo da espessura do dente) ou 15 poços (20ul)

Tina grande - pente com 15 ou 20 poços

Para além das amostras, não esquecer que temos de carregar, controlo positivo, controlo negativo, geralmente com um poço livre de intervalo e o marcador (1 ou 2 lanes)

Electroforese em gel de agarose

Que quantidade carregar?

- Normalmente, uma banda é visível com 20ng de DNA
- Se queremos apenas confirmar a banda, para mandar sequenciar - 2ul
- Se queremos visualizar a banda num 1º ensaio de PCR- 5ul
- Se queremos cortar a banda para mandar sequenciar - todo o produtos de PCR

  (20/25ul se a banda é boa ou 50ul se temos uma banda difusa “faint band”)

Electroforese em gel de agarose

Procedimento

1. Montar o dispositivo para solidificar o gel (pente inclusivé) e verificar que está nivelado

Como fazer o gel de agarose

2. Medir volume TBE (tina pequena-50ml de gel)
   - geis mais finos, dão maior resolução
3. Pesar e adicionar a agarose (1g – se 2%)
4. Dissolver no microondas
   - Cuidado para não queimar as mãos, ferver ou derramar-se
   - Ir agitando ocasionalmente
5. Deixar arrefecer um pouco na bancada
6. Adicionar GreenSafe Premium - 2ul/50ml gel
   - Brometo de etídeo (arrefecer a 60ºC (morno ao toque) para evitar vapores)
7. Agitar sem fazer bolhas

Electroforese em gel de agarose

Procedimento cont.

8. Verter o gel sem fazer bolhas. Eliminar as que possam surgir com uma ponta
9. Esperar 30min que solidifique
10. Mergulhar na tina com tampão de corrida TBE (a mesma que foi usada para fazer o gel), até que a solução cubra superficialmente os poços
    - Atenção, os poços devem estar junto do pólo negativo (PP-Preto-Poços)
11. Retirar com cuidado o pente
12. Se não quiser usar imediatamente, tapar o gel com papel de alumínio para evitar a degradação do Greensafe com a luz
13. Carregar o gel (1º amostras, 2º Controlo negativo com 1 poço de intervalo, 3ºmarcador (no 1º e último poço))
14. Colocar a correr (gel pequeno)
    - Voltagem pequeno :80V, 30min
    - Voltagem grande: 100V, 45min
        - Corridas maiores, separam melhor os fragmentos

Electroforese em gel de agarose

Soluções Tampão

- **TBE** (Tris/Borate/EDTA)
  - solução stock a 5X, ou outra concentração mais elevada, mas é menos estável pois há precipitação durante o armazenamento
  - solução ao uso 1X
- Tris-faz com que o DNA continue solúvel em água
- EDTA - quelante de iões divalentes, protege o DNA da degradação enzimática (iões são cofactores de muitas enzimas)
- Borato-inibidor de enzimas

- **TAE** (Tris/Acetate/EDTA)
  - Menor capacidade tampão (tem de se reutilizar menos vezes), mas DNA corre mais depressa. Necessita menos procedimentos para extrair DNA do gel
  - Porém há quem use os 2 tampões da mesma maneira

Electroforese em gel de agarose

Recipes for TAE and TBE Electrophoresis Buffers

Agarose gels are generally run two types of electrophoresis buffers. Nucleic acid agarose gel electrophoresis is usually conducted with either Tris-acetate-EDTA (TAE) buffer or Tris-borate-EDTA (TBE) buffer. While TAE buffer provides faster electrophoretic migration of linear DNA and better resolution of supercoiled DNA, TBE buffers have a stronger buffering capacity for longer or higher voltage electrophoresis runs.

Note: Because of higher voltages and resulting higher currents often used with Sub-Cell® Model 96 and 192 cells, we recommend that only TBE buffers be used for electrophoresis with these specific systems.

Tris-acetate-EDTA (TAE) buffer

TAE is often prepared in concentrated stock solutions of 10× or 50× in the laboratory. A 1× working solution is prepared prior to electrophoresis.

Composition of 1x TAE buffer

- 40 mM Tris (pH 7.6)
- 20 mM acetic acid
- 1 mM EDTA

Preparation of 50x TEA stock solution

To prepare 1 liter of 50× TAE dissolve following components in 600 ml of deionized water:

- 242 g Tris base (FW = 121)
- 57.1 ml glacial acetic aci
- 100 ml 0.5 M EDTA (pH 8.0)

Adjust the final volume to 1 liter with deionized water.

To prepare a 1× working solution from 50× stock buffer mix 50× stock buffer with DNAse free deionized water at 1:4 ratio.

Tris-borate-EDTA (TBE) buffer

TBE buffer can be made and stored in concentrated stocks of 5× or 10×.

Composition of 1x TBE buffer

- 89 mM Tris (pH 7.6)
- 89 mM boric acid
- 2 mM EDTA

Preparation of 10x TBE stock solution

To prepare 1 liter of 10× TBE dissolve following components in 600 ml deionized water:

- 108 g Tris base (FW = 121)
- 55 g boric acid (FW = 61.8)
- 40 ml 0.5 M EDTA (pH 8.0)

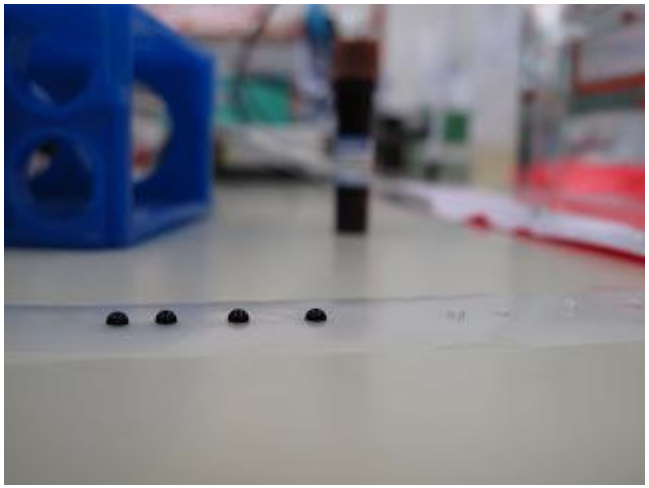
Adjust final volume to 1 liter with deionized water.

To prepare a 1x working solution from 10× stock buffer, mix 10× stock buffer with DNAse free deionized water at 1:9 ratio.

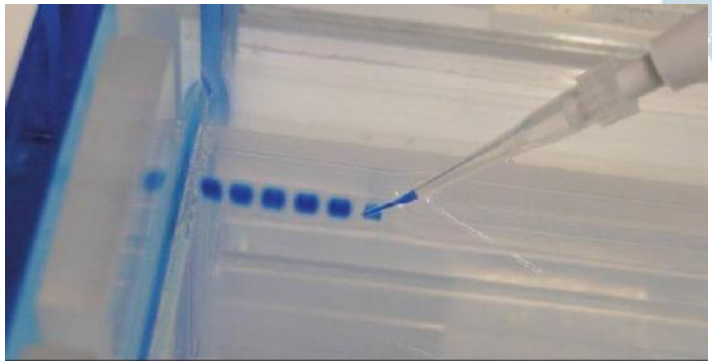
Electroforese em gel de agarose

Como carregar as amostras

1. Spin down da amostras a carregar
2. Cortar um pedaço de Parafilm®
3. Decidir o volume a carregar
4. Colocar cerca de 0,2% Loading Buffer no Parafilm
   - ex: 2ul por 10ul de amostra
   - nosso caso 1ul por 5ml produto PCR ou DNA genómico



4. Pipetar a amostra, despejar a amostra na gota de loading dye, agitar cima e baixo, sem bolhas e carregar no poço



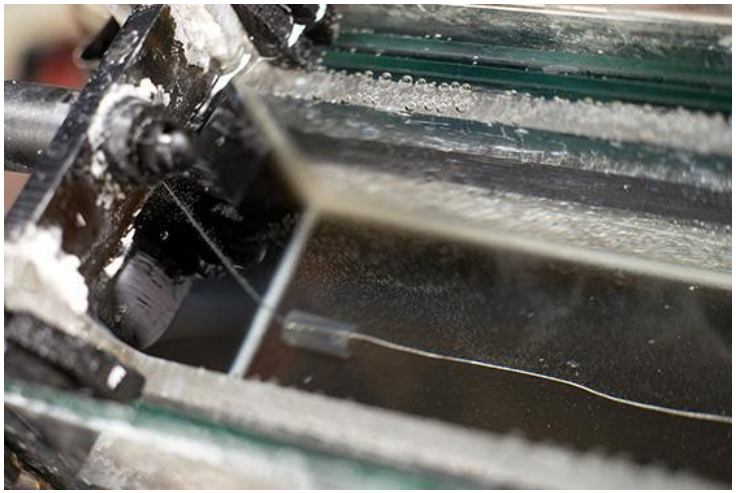
Nota: Algumas mix de PCR já incorporam o loading dye

Electroforese em gel de agarose

5. Carregar 2ul de Marcador/Ruller/Ladder
   - No inicio e fim das amostras
   - Se for possível, deixar os poços dos extremos pois costumam correr pior
   - Resolução do marcador escolhida relativamente ao peso do meu fragmento de interesse

- Carregar da esquerda para a direita, com os poços em cima (no gel).
  Por convenção, publicam-se os resultados assim.

6. Fechar a tampa, ligar aparelho, colocar a correr (80V, 30min)
7. Verificar se está a passar
   corrente eléctrica
   (ver bolhinhas à volta do fio)

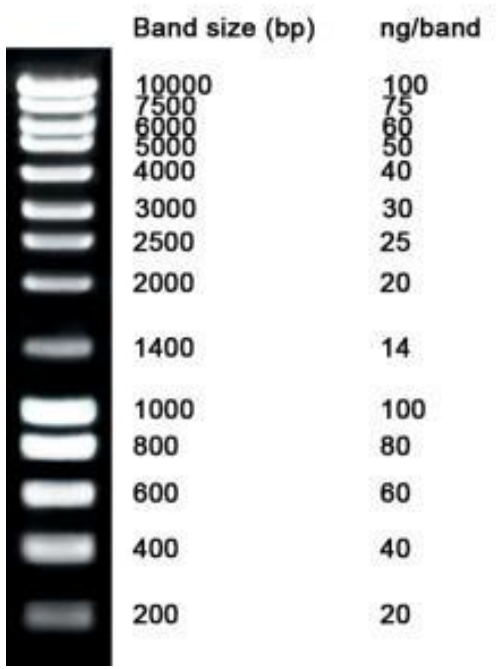


Electroforese em gel de agarose

Marcadores

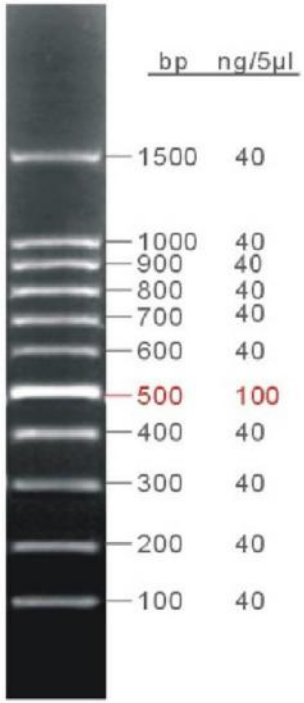
Alto peso molecular

NzyDNA ladder III



Baixo peso molecular

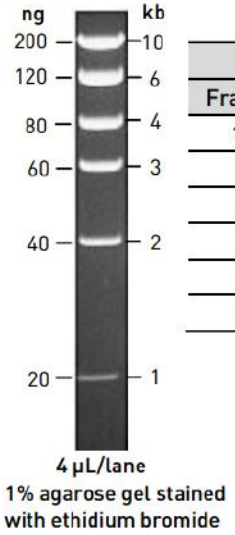
Grisp ladder 100pb



1.7% Agarose | 0.5x TBE | 50V - 1h
minigel - 5uL/lane

Marcador quantitativo

Mass Ladder Thermofisher



4 µL/lane
1% agarose gel stained
with ethidium bromide

Amount of DNA (ng) in each band			
Fragment size	2 µL	4 µL	8 µL
10,000 bp	100 ng	200 ng	400 ng
6000 bp	60 ng	120 ng	240 ng
4000 bp	40 ng	80 ng	160 ng
3000 bp	30 ng	60 ng	120 ng
2000 bp	20 ng	40 ng	80 ng
1000 bp	10 ng	20 ng	40 ng

Electroforese em gel de agarose

Loading buffers

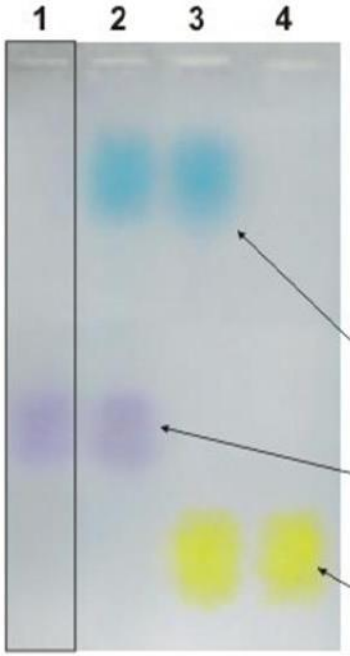
- Dá cor à amostra
  - bromophenol blue
  - xylene cyanol
  - Orange G
- Dá densidade à amostra
  - Glicerol
  - sucrose
- Permitem monitorizar a corrida
- Facilita o carregamento nos poços
- Carregados negativamente, logo migram no mesmo sentido que o DNA

Eletroforese em gel de agarose

Loading buffers

Typical recipe

- 25 mg bromophenol blue or xylene cyanol
- 4 g sucrose
- H2O to 10 mL
- The exact amount of dye is not important
- Store at 4ºC to avoid mould growing in the sucrose. 10 mL of loading buffer will last for years.
- Escolher o corante com base no fragmento que queremos ver, para não impedir a visualização da banda



6x DNA Loading Dye

Line 1: DNA L.Dye Buffer Blue
Line 2: DNA L.Dye Buffer Double Blue
Line 3: DNA L.Dye Buffer Orange, Blue
Line 4: DNA L.Dye Buffer Orange

In 1% agarose gel 1x TBE

Xylene Cyanol FF migrates along with~3500 bp fragments,

Bromophenol Blue migrates along with ~300 bp fragments and

Orange G migrates along with ~40 bp

Eletroforese em gel de agarose

Visualização das amostras

1. Após corrida do gel, desligar a Tina de Eletroforese
2. Colocar o gel no tabuleiro com papel absorvente, para retirar o excesso de solução tampão
3. Colocá-lo (com o suporte em acrílico) no transiluminador

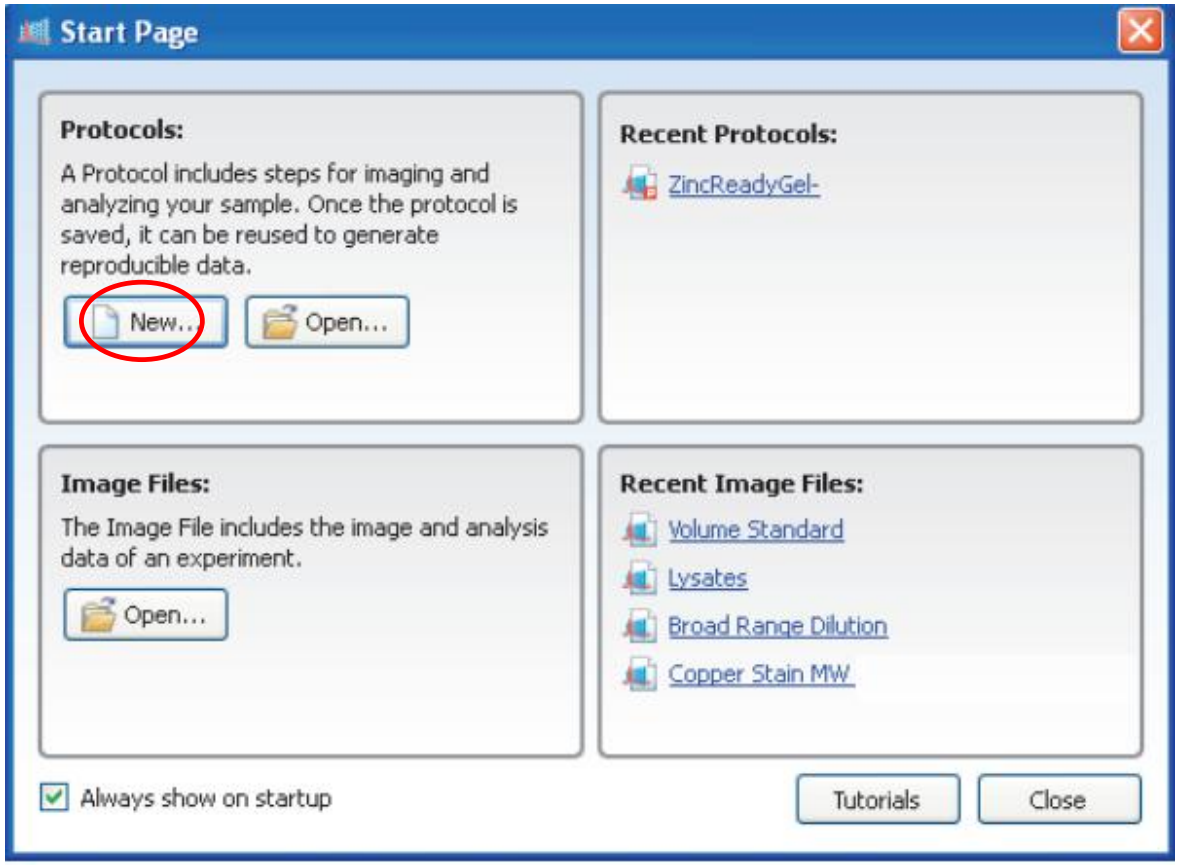
   -se o seu suporte não for apropriado para ver directamente o gel, retirá-lo do suporte
4. Ligar o transiluminador e o PC

Os UV são carcinogénicos-proteger pele e olhos.

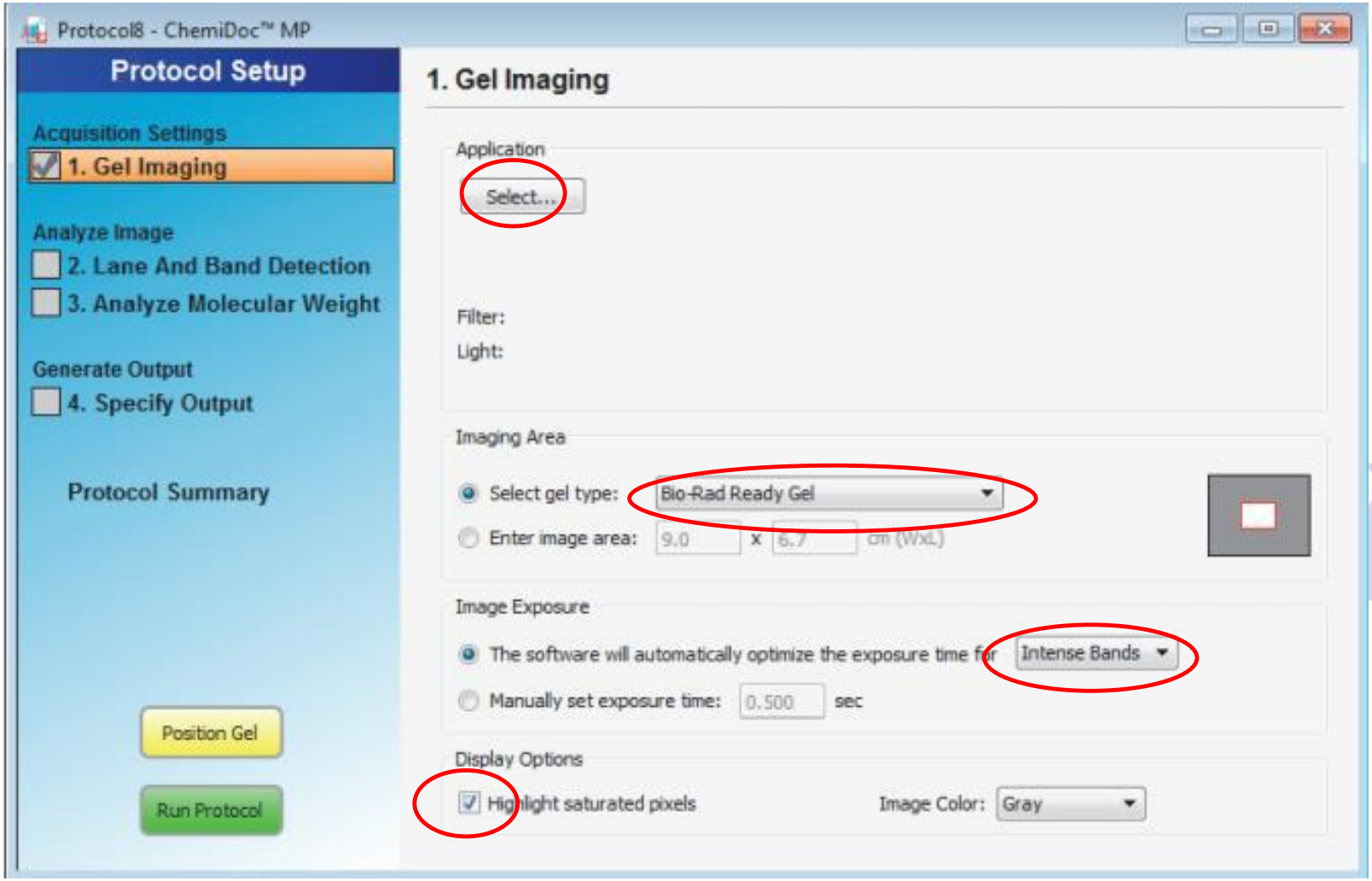
Eletroforese em gel de agarose

Visualização das amostras

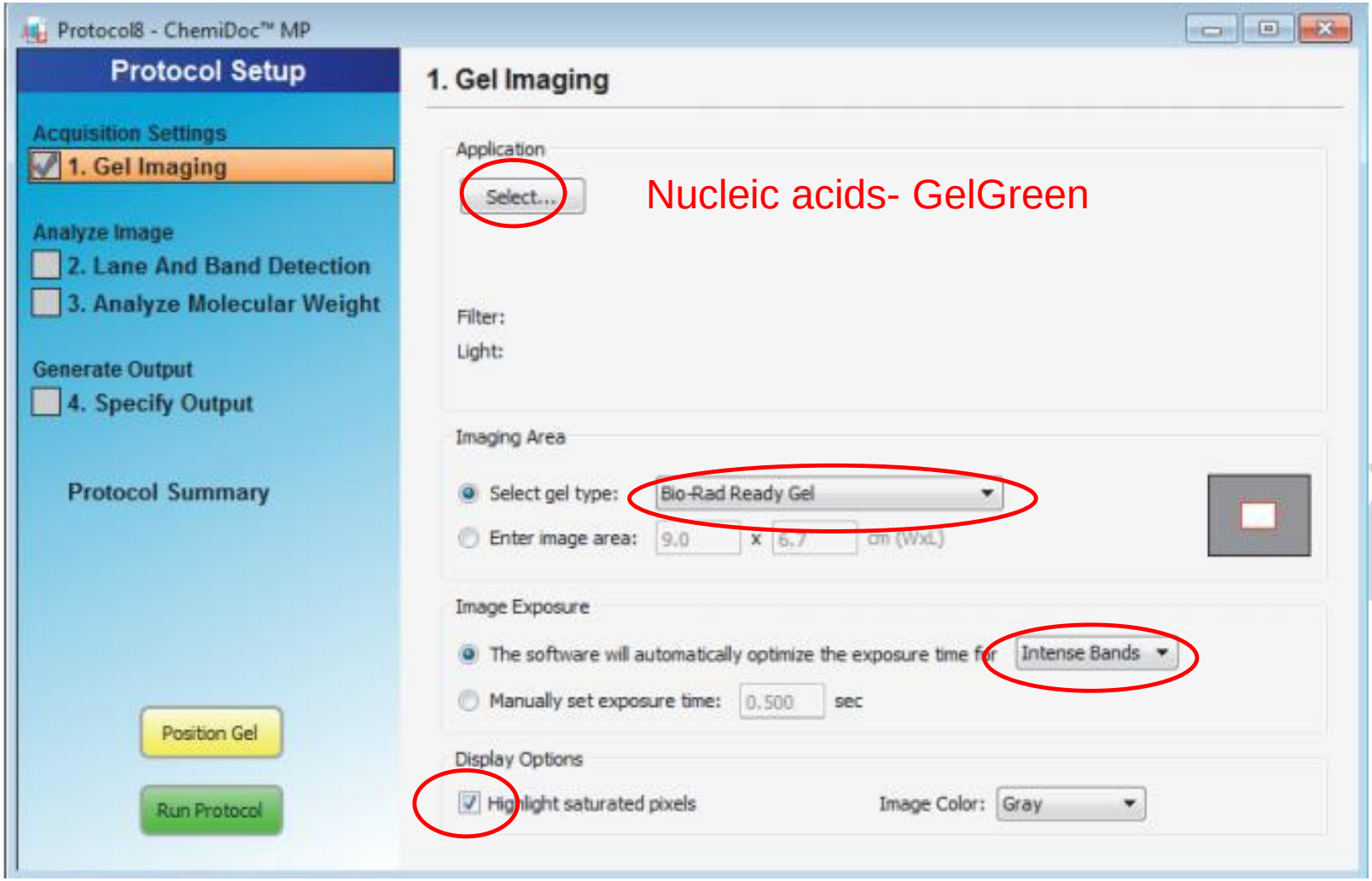
1. Abrir atalho gel-doc



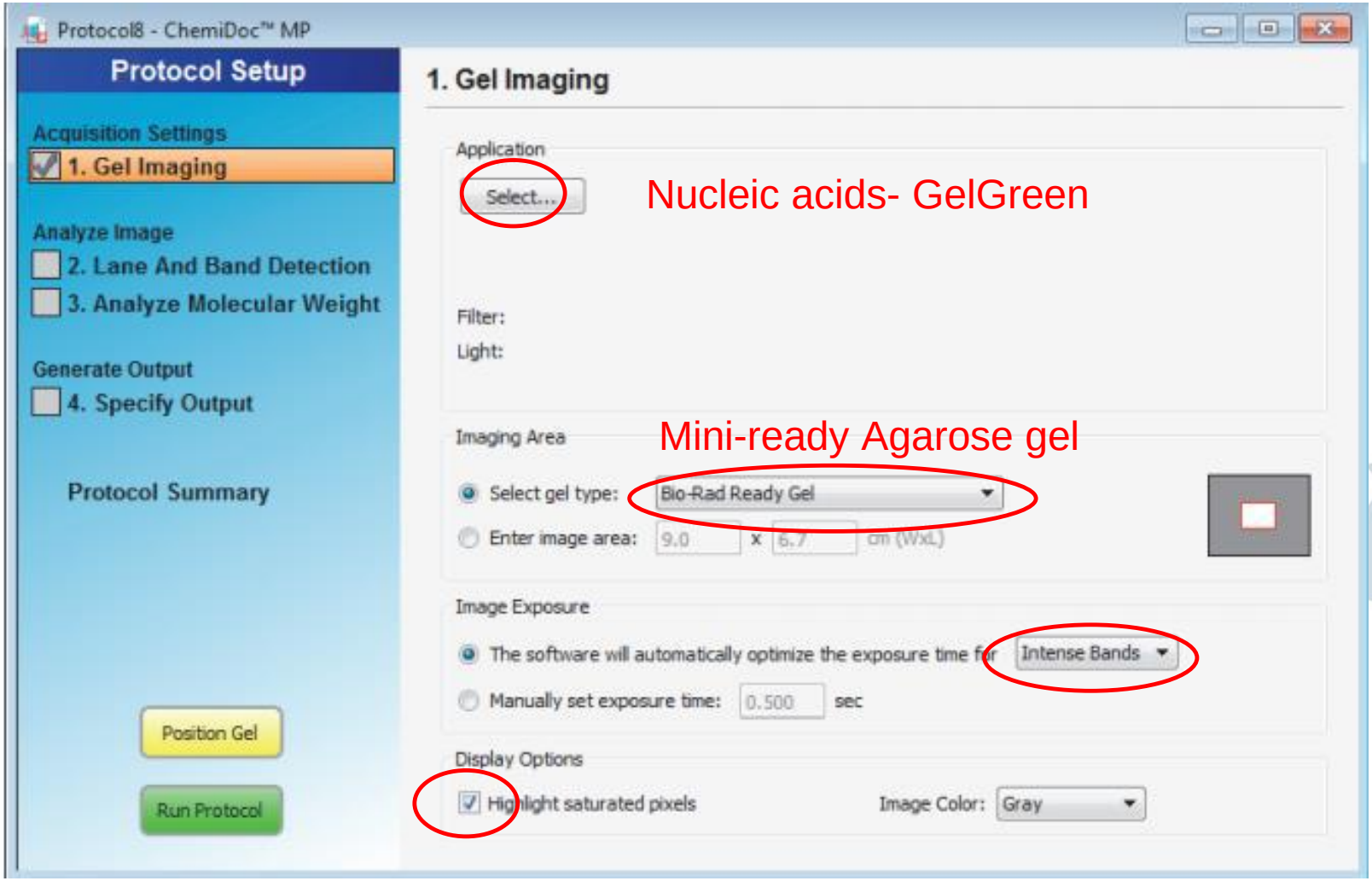
Eletroforese em gel de agarose



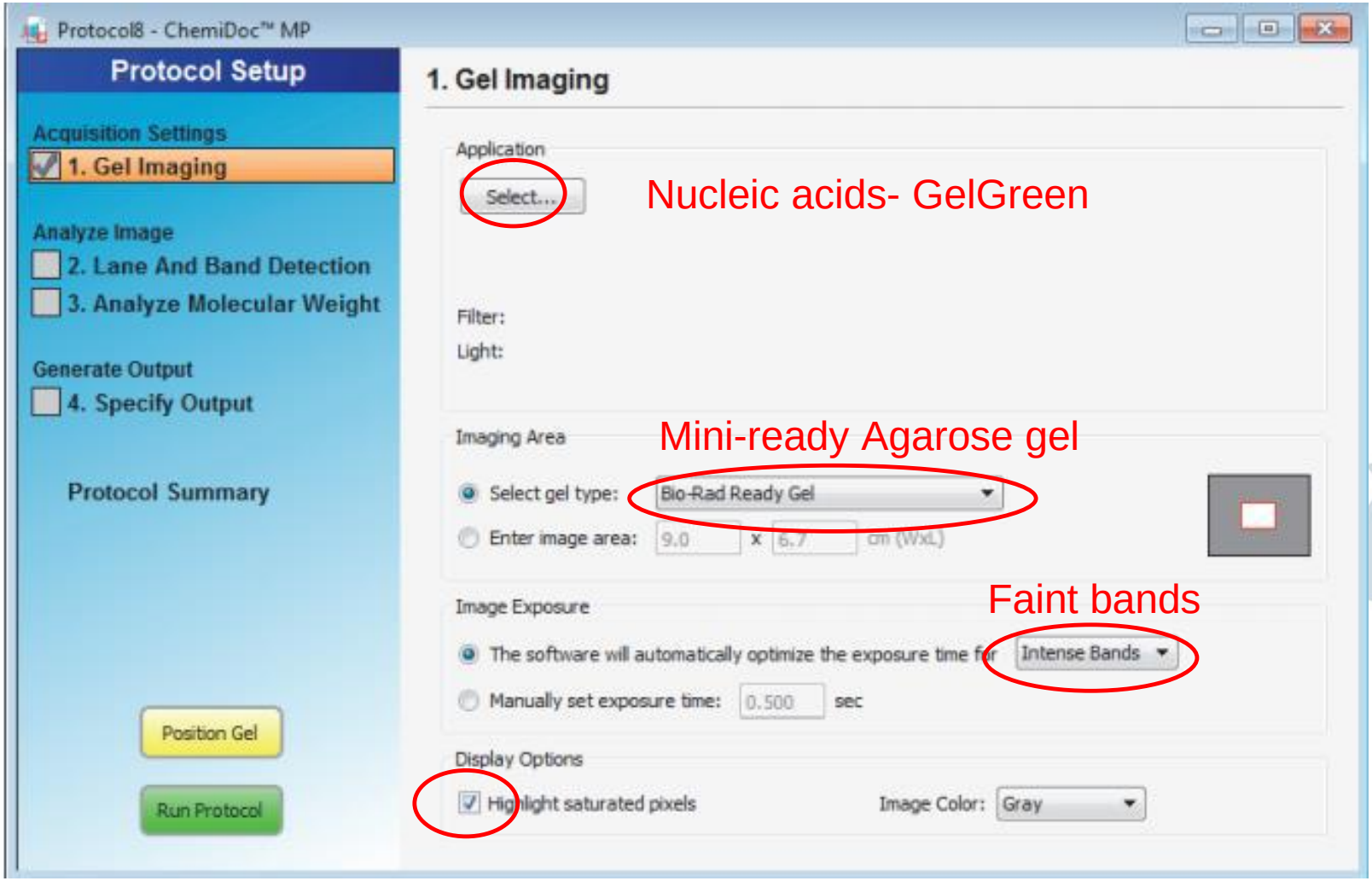
Eletroforese em gel de agarose



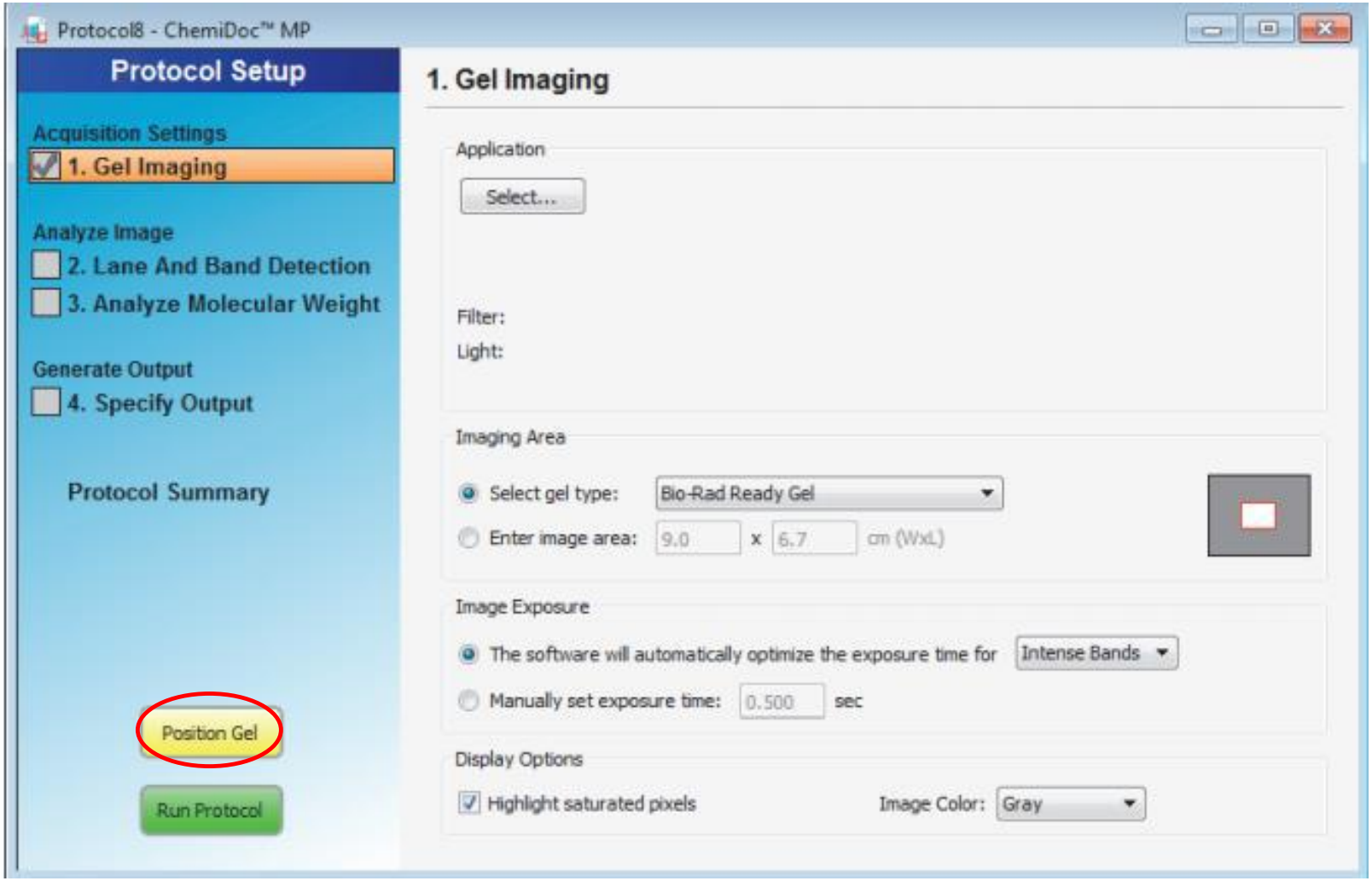
Eletroforese em gel de agarose



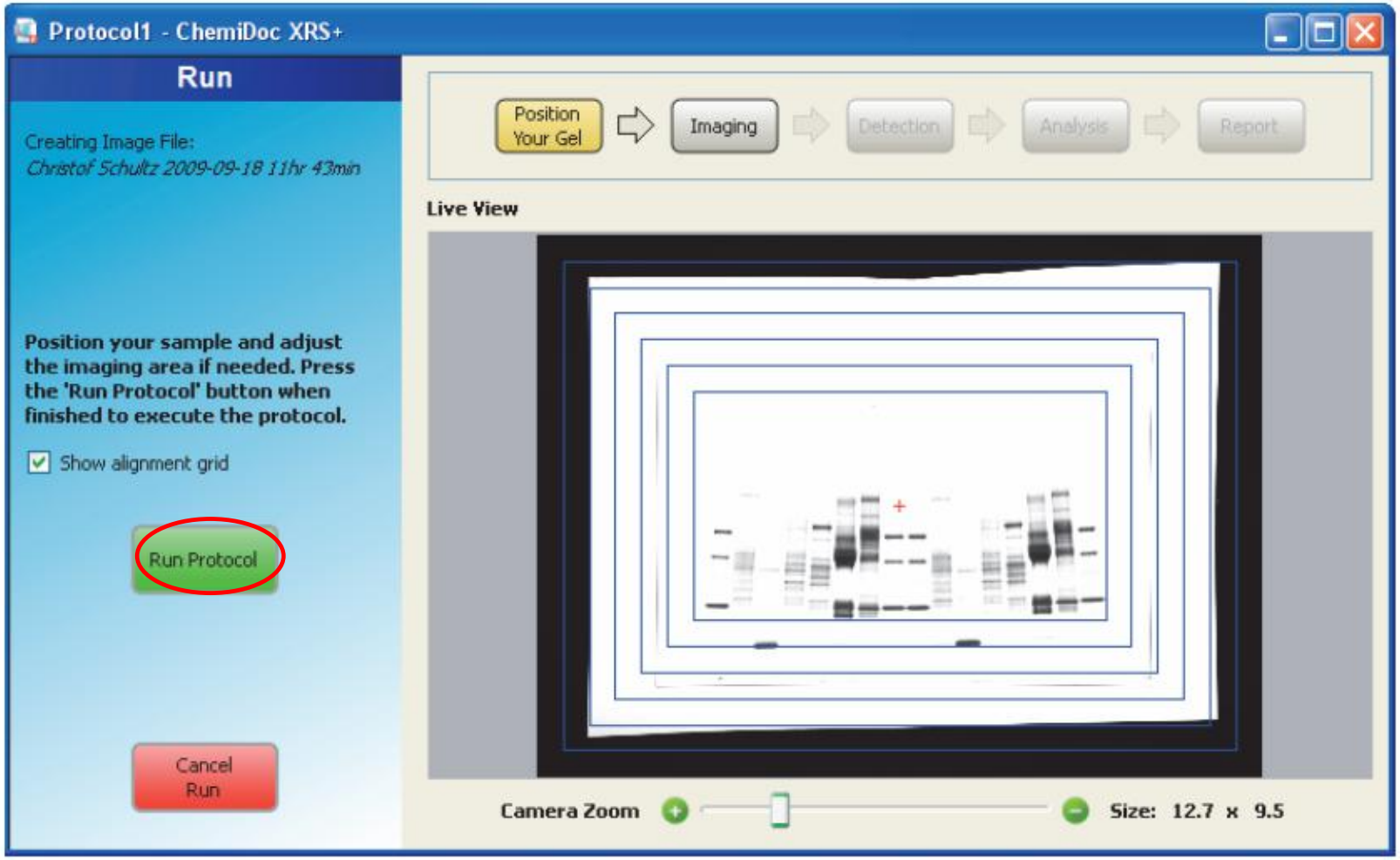
Eletroforese em gel de agarose



Eletroforese em gel de agarose



Eletroforese em gel de agarose



Quantificação do DNA

Quantificação de DNA genómico em gel de agarose

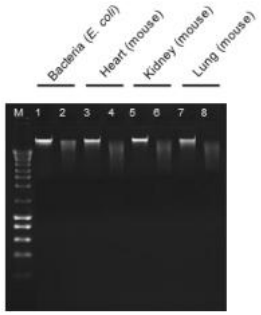
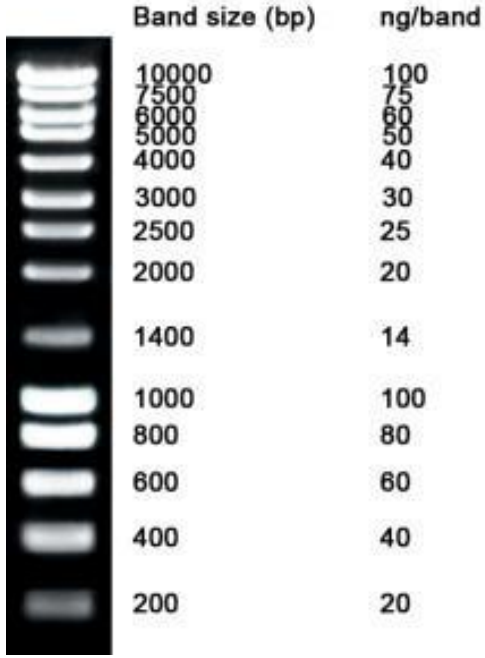


Fig. 1. Genomic DNA from various sources isolated with NZY Tissue gDNA Isolation kit. 0.25 µg of each isolated DNA was analysed on a 0.8% agarose gel. Lanes 2, 4, 6 and 8: gDNA digested with *Eco*R I. Lanes 1, 3, 5 and 7: undigested gDNA. M: NZYDNA Ladder III (MB0440); 1, 2: gDNA from bacteria (*E. coli*); 3, 4: gDNA from mouse heart; 5, 6: gDNA from mouse kidney; 7, 8: gDNA from mouse lung.



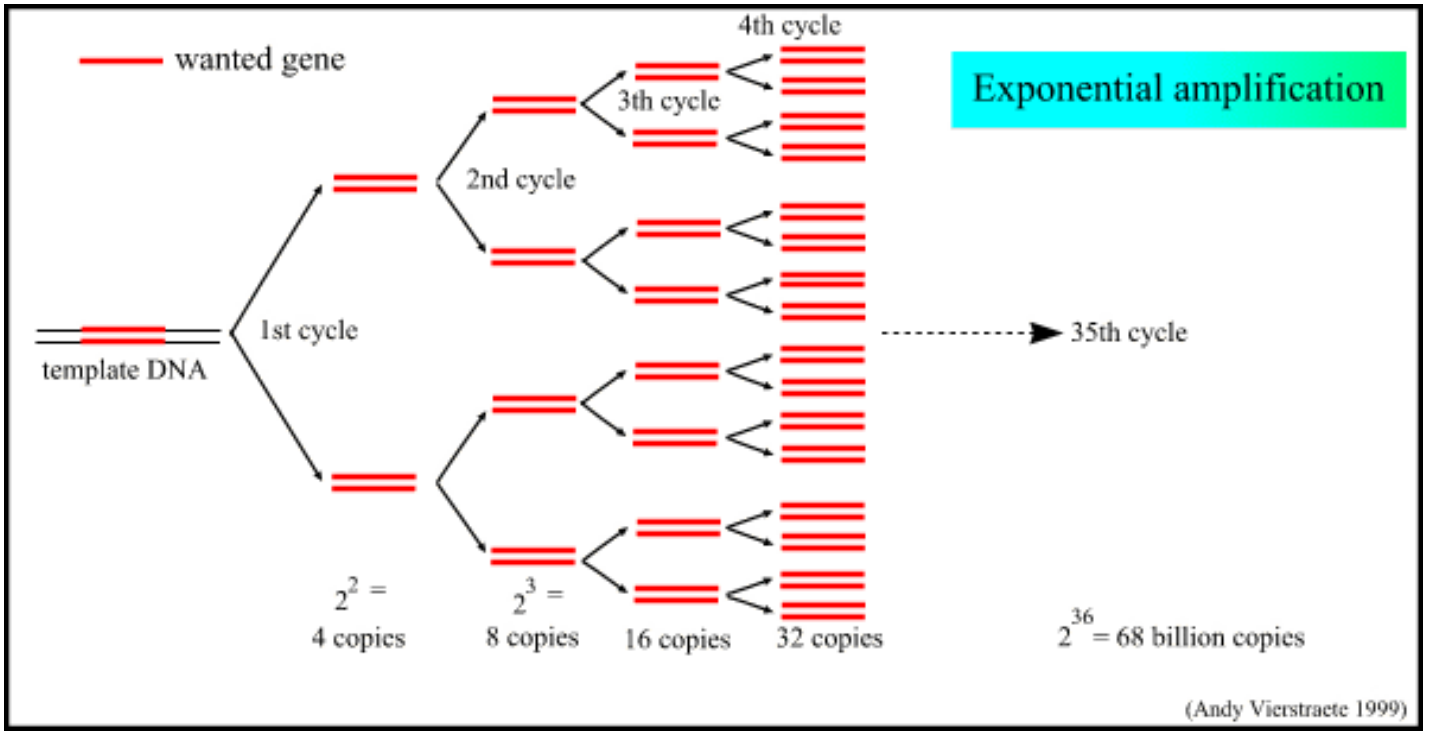
PCR-Polymerase Chain reaction

- Descrito por Kary Mullis, 1983,
  - Prémio Nobel da Química

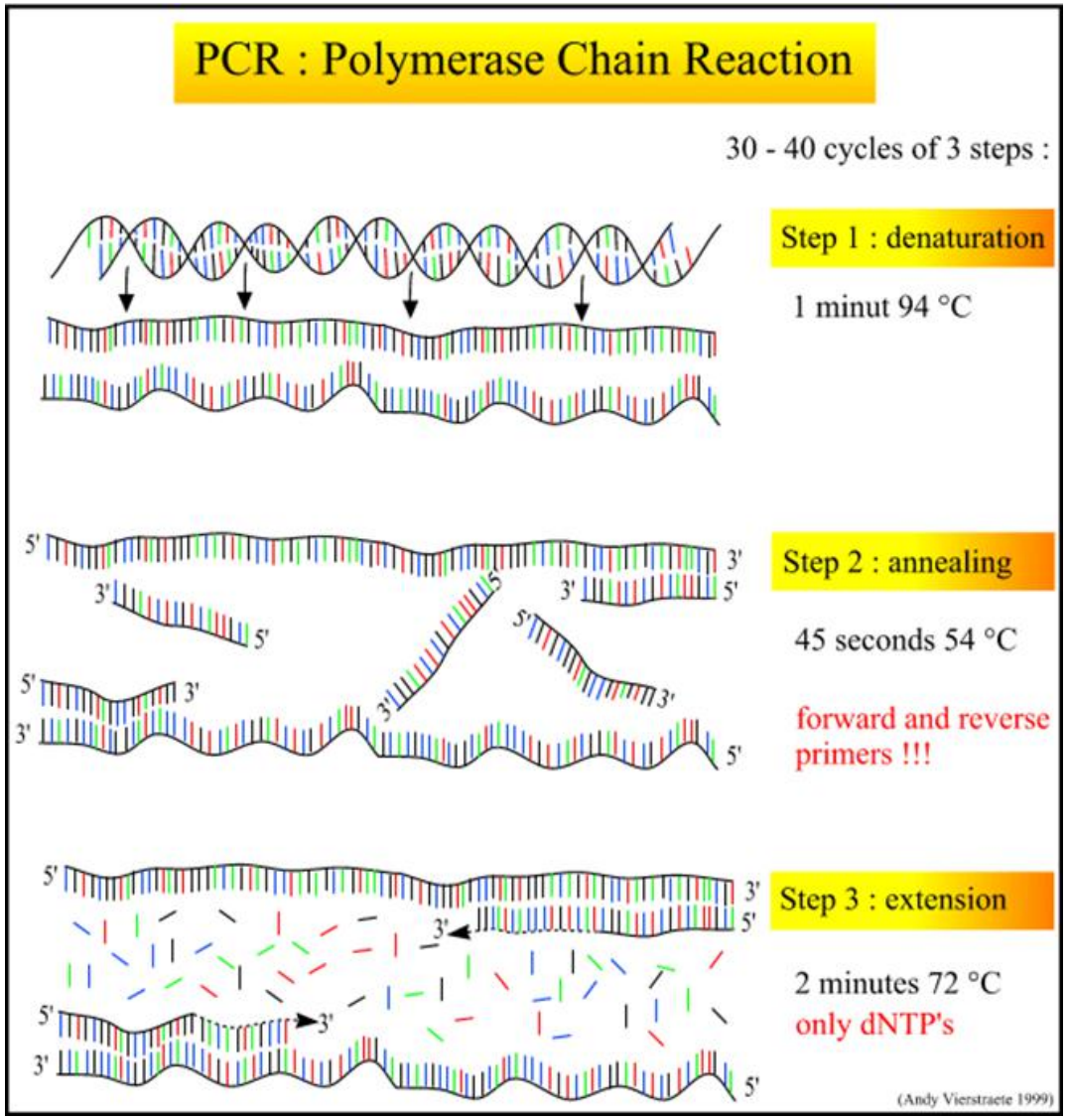
- É um método de amplificação (de criação de múltiplas cópias) de DNA

- Aplicações
  - Investigação médica e biológica
    - detecção de doenças hereditárias,
    - testes de paternidade,
    - exames para detecção de agentes patogénicos
    - etc

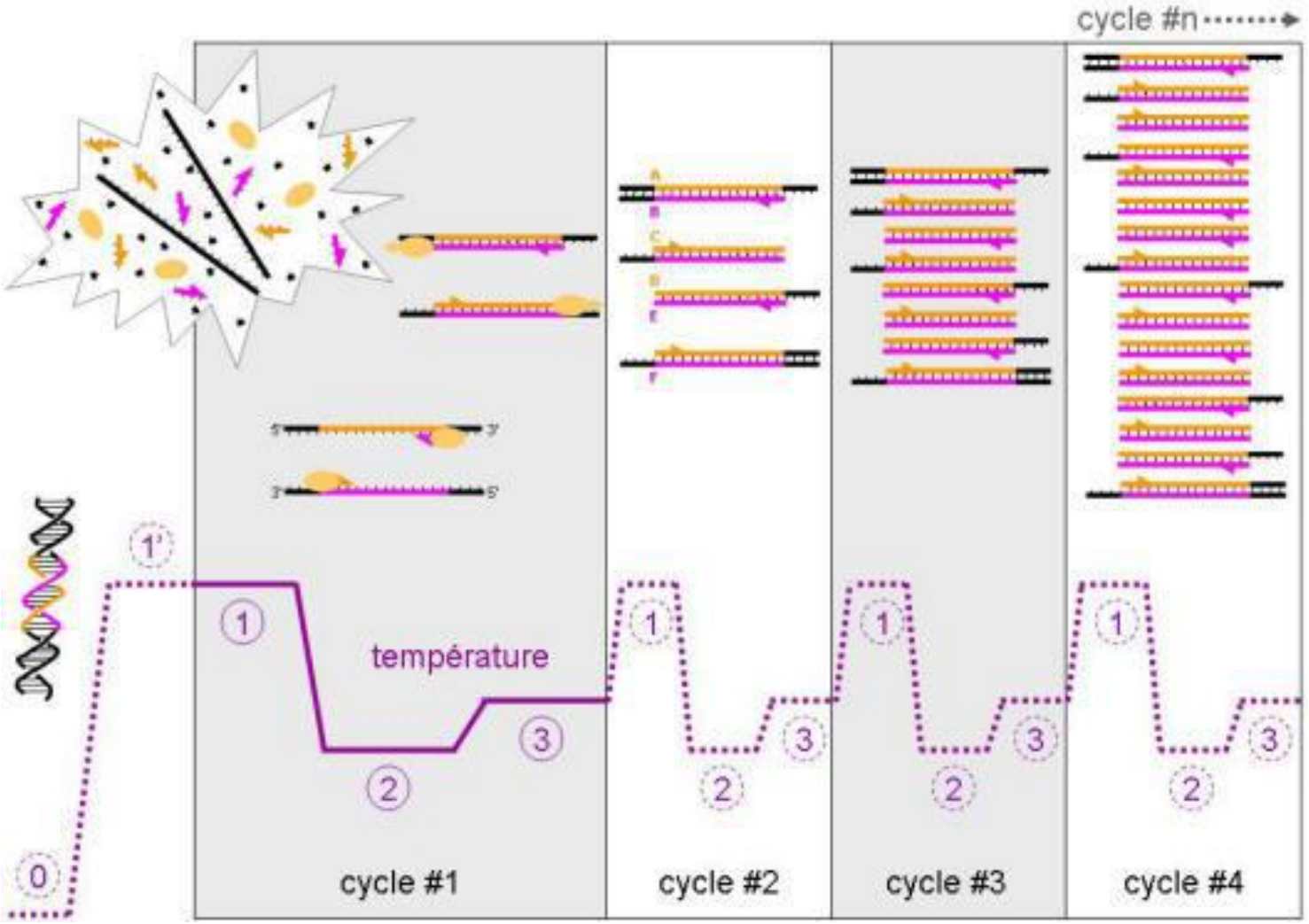
PCR-Polymerase Chain reaction



PCR-Polymerase Chain reaction



PCR-Polymerase Chain reaction



PCR-Polymerase Chain reaction

Componentes:

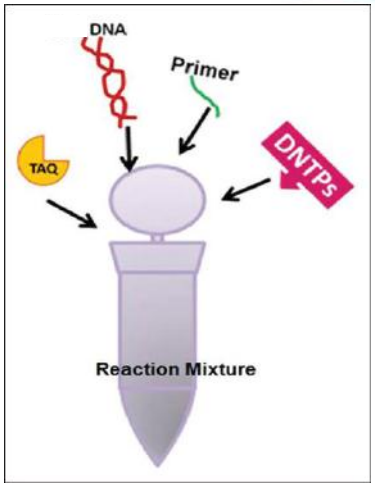
1. Cadeia molde “template” de DNA

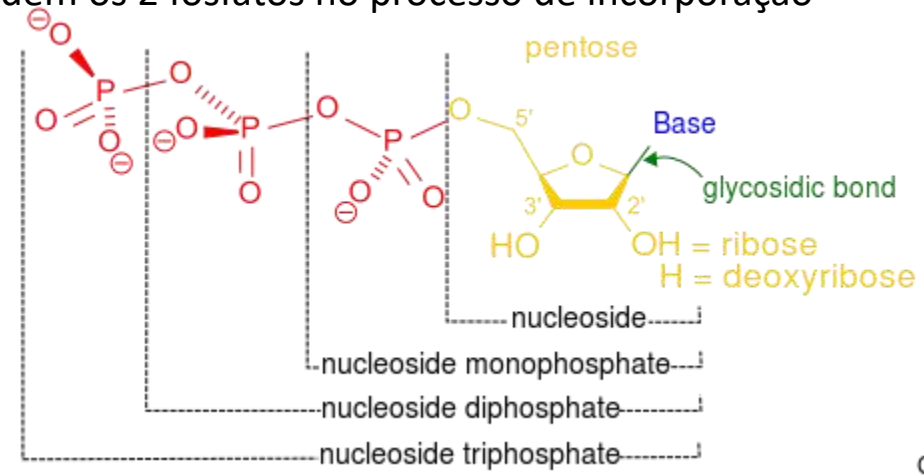
   10 a 100ng/ul

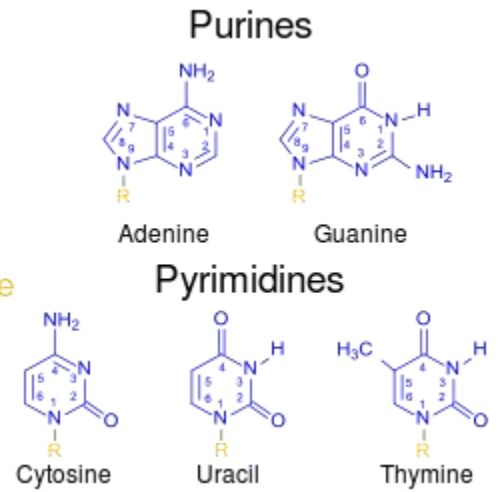
   Diluir se a concentração for elevada, pois elevadas concentrações inibem reação

2. dNTPs (desoxirribonucleotídeos trifosfatos), que são as bases nitrogenadas ligadas 3 três fosfatos (dATP, dGTP, dTTP, dCTP)

   Perdem os 2 fosfatos no processo de incorporação







PCR-Polymerase Chain reaction

dNTPs Working solution

Exercício:

- Temos dATP, dCTP, dGTP, dTTP – 100mM each
- Queremos 0,2mM each numa reação de PCR com Volume total 20ul
- Como fazemos nossa solução de trabalho?

PCR-Polymerase Chain reaction

dNTPs Working solution

Exercício:

- Temos dATP, dCTP, dGTP, dTTP – 100mM each
- Queremos 0,2mM each numa reação de PCR com Volume total 20ul
- Como fazemos nossa solução de trabalho?

Como fazer:

Ci x Vi = Cf x Vf

Ci (working solution) x 1ul = 0,2mM (no PCR) x 20ul (PCR)

Ci= 4mM

PCR-Polymerase Chain reaction

dNTPs Working solution

Exercício:

- Temos dATP, dCTP, dGTP, dTTP – 100mM each
- Queremos 0,2mM each numa reação de PCR com Volume total 20ul
- Como fazemos nossa solução de trabalho de 100ul?

Como fazer:

Ci x Vi = Cf x Vf

Ci (working solution) x 1ul = 0,2mM (no PCR) x 20ul (PCR)

Ci= 4mM

Ci x Vi = Cf x Vf

100mM x Vi (compra) = 4mM x 100ul (normalmente – volume working solution)

Vi= 4ul

PCR-Polymerase Chain reaction

dNTPs Working solution

Exercício:

- Temos dATP, dCTP, dGTP, dTTP – 100mM each
- Queremos 0,2mM each numa reação de PCR com Volume total 20ul
- Como fazemos nossa solução de trabalho?

Como fazer:

dATP (100mM)- 4ul

dCTP (100mM)- 4ul

dGTP (100mM)- 4ul

dTTP (100mM)- 4ul

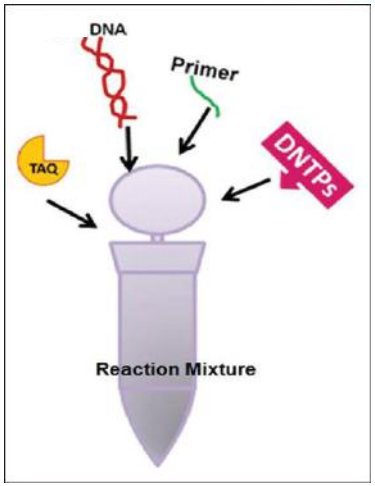
H2O – 84ul

Mix e spin

PCR-Polymerase Chain reaction

Componentes:

3. Água de uso molecular
4. Primers (também chamados de oligonucleotídeos ou iniciadores)
   - Pequena sequencia complementar ao DNA template, que flanqueia a minha região de interesse
   - Compram-se em solução ou liofilizados
     - Reconstituem-se em água de uso molecular
     - Manipulam-se numa câmara de PCR
     - Convém usar pontas com filtro

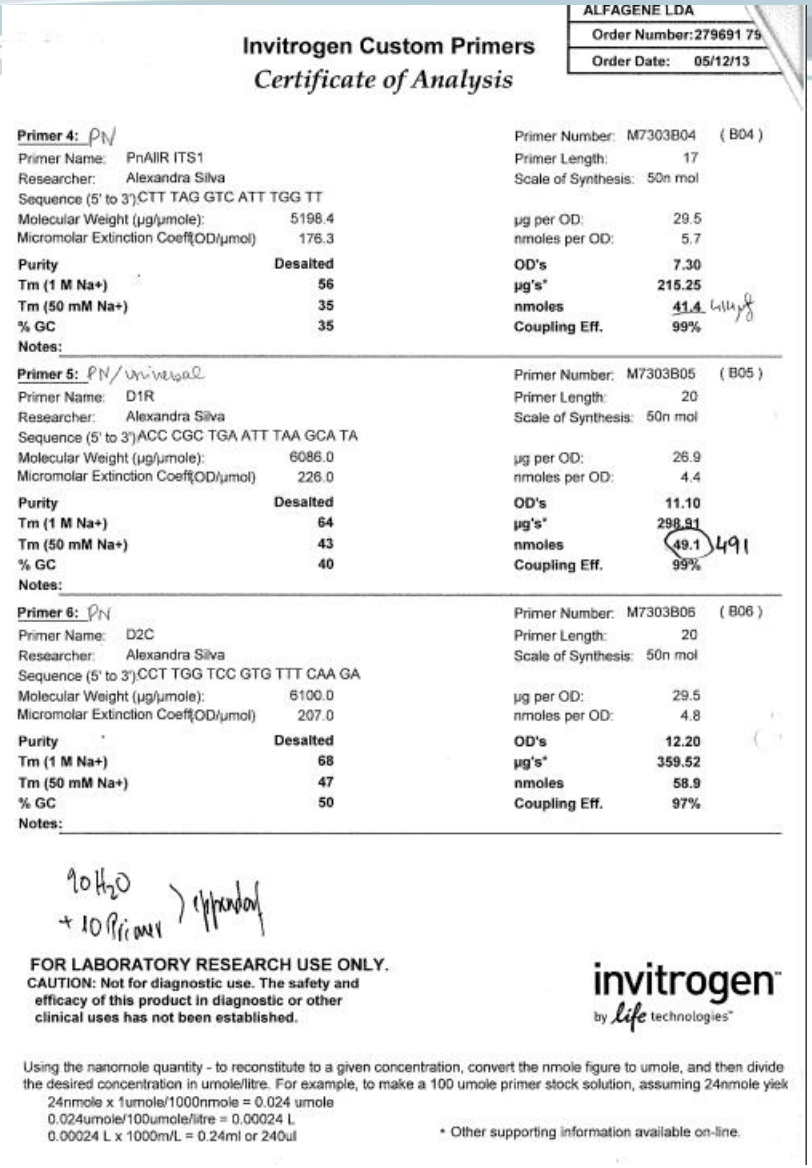


PCR-Polymerase Chain reaction

Como Reconstituir e

fazer working solution dos Primers?

D1R/D3Ca



ALFAGENE LDA
Order Number:279691 79
Order Date: 05/12/13

Invitrogen Custom Primers
Certificate of Analysis

Primer 4: PN
Primer Name: PnAlIR ITS1
Researcher: Alexandra Silva
Sequence (5' to 3'): CTT TAG GTC ATT TGG TT
Molecular Weight (µg/µmole): 5198.4
Micromolar Extinction Coeff(OD/µmol) 176.3
Purity: Desalted
Tm (1 M Na+): 56
Tm (50 mM Na+): 35
% GC: 35
Notes:
Primer Number: M7303B04 (B04)
Primer Length: 17
Scale of Synthesis: 50n mol
µg per OD: 29.5
nmoles per OD: 5.7
OD's: 7.30
µg's*: 215.25
nmoles: 41.4
Coupling Eff.: 99%

Primer 5: PN/universal
Primer Name: D1R
Researcher: Alexandra Silva
Sequence (5' to 3'): ACC CGC TGA ATT TAA GCA TA
Molecular Weight (µg/µmole): 6086.0
Micromolar Extinction Coeff(OD/µmol) 226.0
Purity: Desalted
Tm (1 M Na+): 64
Tm (50 mM Na+): 43
% GC: 40
Notes:
Primer Number: M7303B05 (B05)
Primer Length: 20
Scale of Synthesis: 50n mol
µg per OD: 26.9
nmoles per OD: 4.4
OD's: 11.10
µg's*: 298.91
nmoles: 49.1
Coupling Eff.: 99%

Primer 6: PN
Primer Name: D2C
Researcher: Alexandra Silva
Sequence (5' to 3'): CCT TGG TCC GTG TTT CAA GA
Molecular Weight (µg/µmole): 6100.0
Micromolar Extinction Coeff(OD/µmol) 207.0
Purity: Desalted
Tm (1 M Na+): 68
Tm (50 mM Na+): 47
% GC: 50
Notes:
Primer Number: M7303B06 (B06)
Primer Length: 20
Scale of Synthesis: 50n mol
µg per OD: 29.5
nmoles per OD: 4.8
OD's: 12.20
µg's*: 359.52
nmoles: 58.9
Coupling Eff.: 97%

90 H_2O + 10 Primer) Aliquot

FOR LABORATORY RESEARCH USE ONLY.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

invitrogen by life technologies

Using the nanomole quantity - to reconstitute to a given concentration, convert the nmole figure to umole, and then divide the desired concentration in umole/litre. For example, to make a 100 umole primer stock solution, assuming 24nmole yiek
24nmole x 1umole/1000nmole = 0.024 umole
0.024umole/100umole/litre = 0.00024 L
0.00024 L x 1000m/L = 0.24ml or 240ul

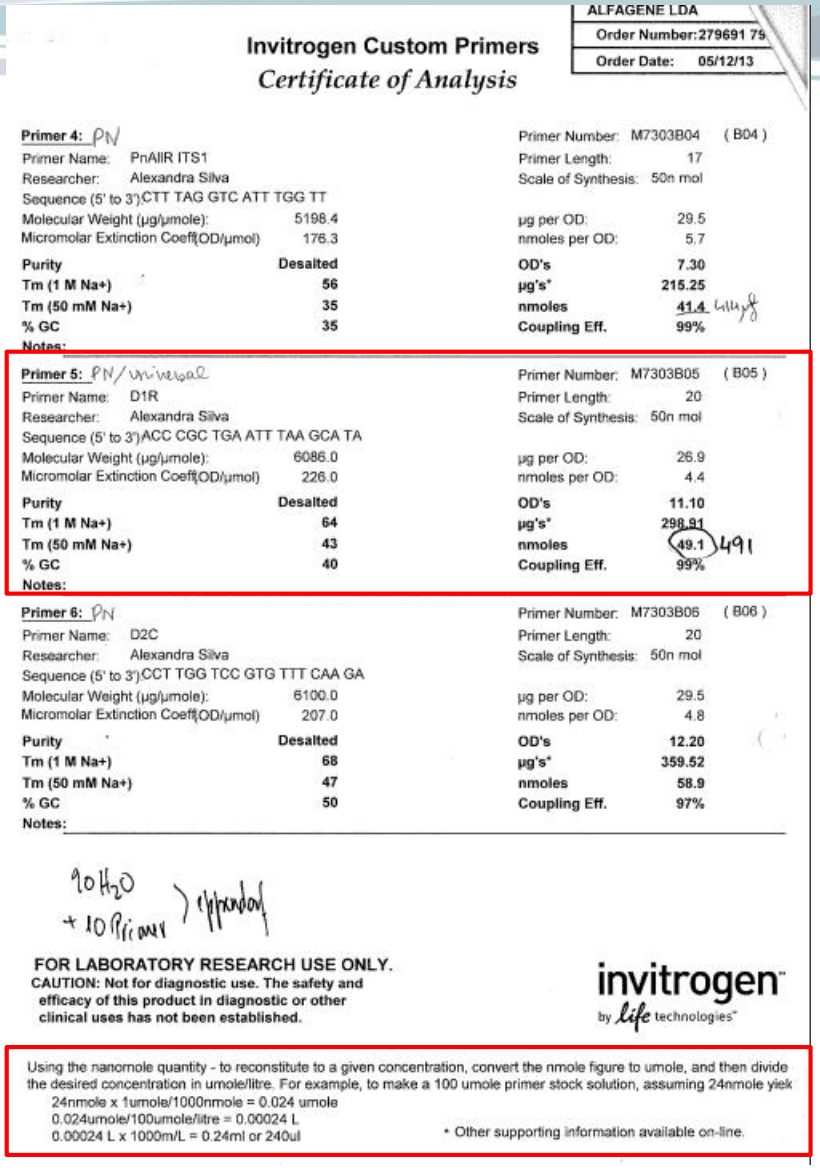
* Other supporting information available on-line.

PCR-Polymerase Chain reaction

Como Reconstituir e
fazer working solution dos Primers?
D1R/D3Ca

Solução stock-100uM
VH2O (uL)= nmoles x 10

Exercício?
Working solution 10uM, 100uL?



ALFAGENE LDA
Order Number:279691 79
Order Date: 05/12/13

Invitrogen Custom Primers
Certificate of Analysis

Primer 4: PN
Primer Name: PnAlR ITS1
Researcher: Alexandra Silva
Sequence (5' to 3') CTT TAG GTC ATT TGG TT
Primer Number: M7303B04 (B04)
Primer Length: 17
Scale of Synthesis: 50n mol

Molecular Weight (µg/µmole):	5198.4	µg per OD:	29.5
Micromolar Extinction Coeff(OD/µmol)	176.3	nmoles per OD:	5.7
Purity	Desalted	OD's	7.30
Tm (1 M Na+)	56	µg's*	215.25
Tm (50 mM Na+)	35	nmoles	41.4
% GC	35	Coupling Eff.	99%

Notes:

Primer 5: PN/universal
Primer Name: D1R
Researcher: Alexandra Silva
Sequence (5' to 3') ACC CGC TGA ATT TAA GCA TA
Primer Number: M7303B05 (B05)
Primer Length: 20
Scale of Synthesis: 50n mol

Molecular Weight (µg/µmole):	6086.0	µg per OD:	26.9
Micromolar Extinction Coeff(OD/µmol)	226.0	nmoles per OD:	4.4
Purity	Desalted	OD's	11.10
Tm (1 M Na+)	64	µg's*	298.91
Tm (50 mM Na+)	43	nmoles	49.1
% GC	40	Coupling Eff.	99%

Notes:

Primer 6: PN
Primer Name: D2C
Researcher: Alexandra Silva
Sequence (5' to 3') CCT TGG TCC GTG TTT CAA GA
Primer Number: M7303B06 (B06)
Primer Length: 20
Scale of Synthesis: 50n mol

Molecular Weight (µg/µmole):	6100.0	µg per OD:	29.5
Micromolar Extinction Coeff(OD/µmol)	207.0	nmoles per OD:	4.8
Purity	Desalted	OD's	12.20
Tm (1 M Na+)	68	µg's*	359.52
Tm (50 mM Na+)	47	nmoles	58.9
% GC	50	Coupling Eff.	97%

Notes:

90 H_2O
+ 10 Primer

FOR LABORATORY RESEARCH USE ONLY.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

invitrogen by life technologies

Using the nanomole quantity - to reconstitute to a given concentration, convert the nmole figure to umole, and then divide the desired concentration in umole/litre. For example, to make a 100 umole primer stock solution, assuming 24nmole yiek
24nmole x 1umole/1000nmole = 0.024 umole
0.024umole/100umole/litre = 0.00024 L
0.00024 L x 1000ml/L = 0.24ml or 240ul

* Other supporting information available on-line.

PCR-Polymerase Chain reaction

Como Reconstituir e

fazer working solution dos Primers?

D1R/D3Ca

Solução stock-100uM

VH2O (uL)= nmoles x 10

Exercício?

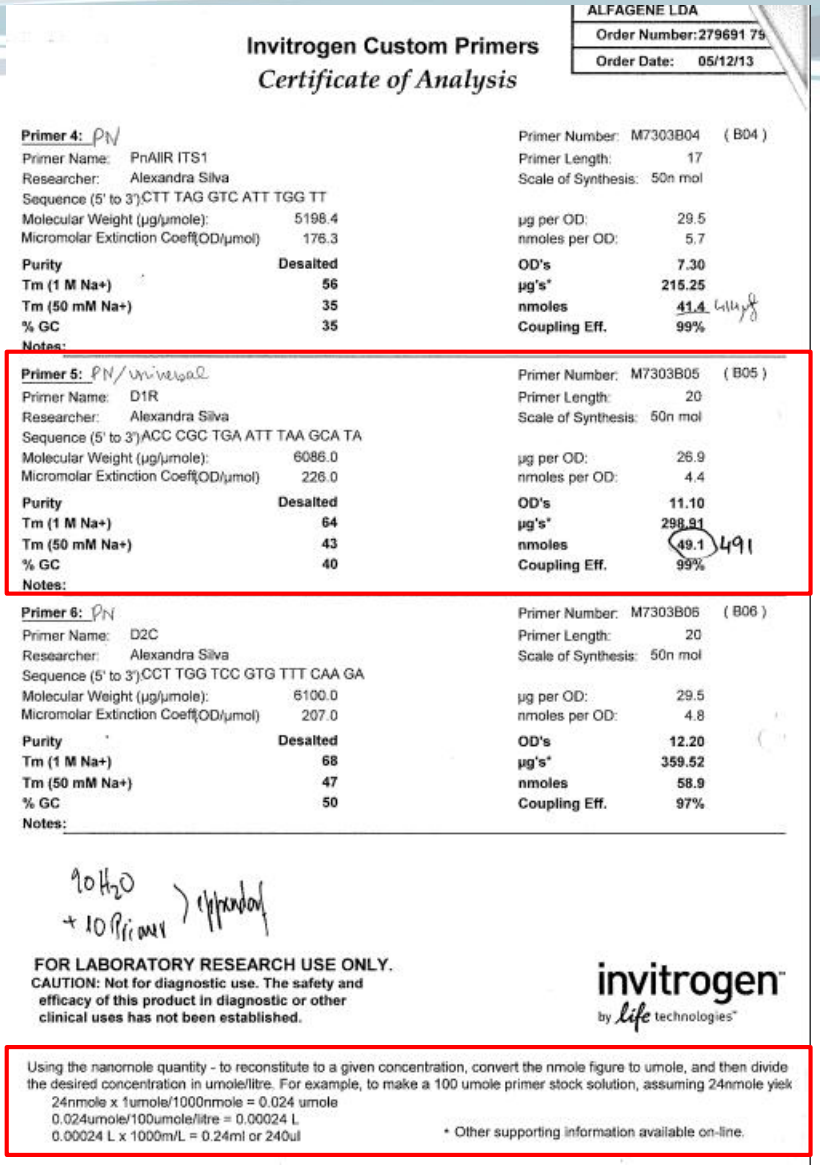
Working solution 10uM, 100uL?

Ci x Vi = Cf x Vf

100uM x Vi = 10uM x 100ul

Vi= 10 ul

Working solution 10uM= 10ul stock + 90 ul H2O



ALFAGENE LDA
Order Number:279691 79
Order Date: 05/12/13

Invitrogen Custom Primers
Certificate of Analysis

Primer 4: PN
Primer Name: PnAlR ITS1
Researcher: Alexandra Silva
Sequence (5' to 3'): CTT TAG GTC ATT TGG TT
Molecular Weight (µg/µmole): 5198.4
Micromolar Extinction Coeff(OD/µmol) 176.3
Purity: Desalted
Tm (1 M Na+): 56
Tm (50 mM Na+): 35
% GC: 35
Notes:
Primer Number: M7303B04 (B04)
Primer Length: 17
Scale of Synthesis: 50n mol
µg per OD: 29.5
nmoles per OD: 5.7
OD's: 7.30
µg's*: 215.25
nmoles: 41.4
Coupling Eff.: 99%

Primer 5: PN/universal
Primer Name: D1R
Researcher: Alexandra Silva
Sequence (5' to 3'): ACC CGC TGA ATT TAA GCA TA
Molecular Weight (µg/µmole): 6086.0
Micromolar Extinction Coeff(OD/µmol) 226.0
Purity: Desalted
Tm (1 M Na+): 64
Tm (50 mM Na+): 43
% GC: 40
Notes:
Primer Number: M7303B05 (B05)
Primer Length: 20
Scale of Synthesis: 50n mol
µg per OD: 26.9
nmoles per OD: 4.4
OD's: 11.10
µg's*: 298.91
nmoles: 49.1
Coupling Eff.: 99%

Primer 6: PN
Primer Name: D2C
Researcher: Alexandra Silva
Sequence (5' to 3'): CCT TGG TCC GTG TTT CAA GA
Molecular Weight (µg/µmole): 6100.0
Micromolar Extinction Coeff(OD/µmol) 207.0
Purity: Desalted
Tm (1 M Na+): 68
Tm (50 mM Na+): 47
% GC: 50
Notes:
Primer Number: M7303B06 (B06)
Primer Length: 20
Scale of Synthesis: 50n mol
µg per OD: 29.5
nmoles per OD: 4.8
OD's: 12.20
µg's*: 359.52
nmoles: 58.9
Coupling Eff.: 97%

90 H_2O + 10 Primer (appendix)

FOR LABORATORY RESEARCH USE ONLY.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

invitrogen by life technologies

Using the nanomole quantity - to reconstitute to a given concentration, convert the nmole figure to umole, and then divide the desired concentration in umole/litre. For example, to make a 100 umole primer stock solution, assuming 24nmole yiek
24nmole x 1umole/1000nmole = 0.024 umole
0.024umole/100umole/litre = 0.00024 L
0.00024 L x 1000ml/L = 0.24ml or 240ul

* Other supporting information available on-line.

PCR-Polymerase Chain reaction

Primers D1R/D3Ca

Primer 5: PN/Universal

Primer Name:	D1R
Researcher:	Alexandra Silva
Sequence (5' to 3')	ACC CGC TGA ATT TAA GCA TA
Molecular Weight (µg/µmole):	6086.0
Micromolar Extinction Coeff (OD/µmol)	226.0
Purity	**Desalted**
Tm (1 M Na+)	**64**
Tm (50 mM Na+)	**43**
% GC	**40**
Notes:	

Primer Number:	M7303B05 (B05)
Primer Length:	20
Scale of Synthesis:	50n mol
µg per OD:	26.9
nmoles per OD:	4.4
OD's	**11.10**
µg's*	**298.91**
nmoles	**49.1** 491
Coupling Eff.	**99%**

Primer 7: PN/Universal

Primer Name:	D3Ca
Researcher:	Alexandra Silva
Sequence (5' to 3')	ACG AAC GAT TTG CAC GTC AG
Molecular Weight (µg/µmole):	6127.0
Micromolar Extinction Coeff (OD/µmol)	225.0
Purity	**Desalted**
Tm (1 M Na+)	**68**
Tm (50 mM Na+)	**47**
% GC	**50**
Notes:	

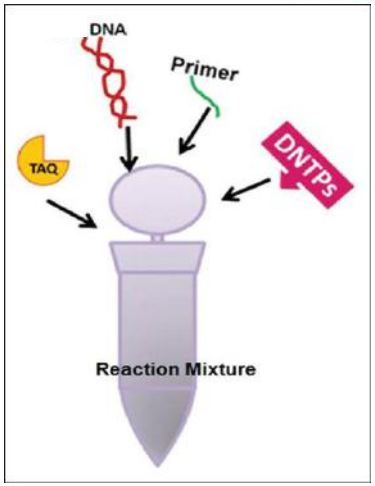
Primer Number:	M7303B07 (B07)
Primer Length:	20
Scale of Synthesis:	50n mol
µg per OD:	27.2
nmoles per OD:	4.4
OD's	**14.50**
µg's*	**394.85**
nmoles	**64.4** 644
Coupling Eff.	**99%**

PCR-Polymerase Chain reaction

Componentes:

3. Enzima Taq-polimerase
   - DNA polimerase
   - Origem na bactéria ***Thermus aquaticus*** que tolera elevadas temperaturas
4. Solução tampão
   - MgCl2 (remove os fosfatos da solução)

     Cofactor da Taq
     - Elevadas conc. levam aumento da inespecificidade
     - Baixas concentrações levam diminuição de produto
     - 1.5-2.0 mM



5. Termociclador, equipamento que faz ciclos de temperatura pré-estabelecidos com tempos exatos específicos para cada reação (fragmento a ser amplificado).

PCR-Polymerase Chain reaction

Eppendorf plus

Biorad

TC-plus

PCR-Polymerase Chain reaction

Reação de PCR
Ex. GoTaq, Promega

In a sterile, nuclease-free microcentrifuge tube, combine the following on ice:

Component	Final Volume	Final Concentration
5X Green or Colorless GoTaq® Reaction Buffer[1]	10µl	1X (1.5mM $MgCl_2$)[2]
PCR Nucleotide Mix, 10mM each	1µl	0.2mM each dNTP
upstream primer	Xµl	0.1–1.0µM
downstream primer	Yµl	0.1–1.0µM
GoTaq® DNA Polymerase (5u/µl)	0.25µl	1.25u
template DNA	Zµl	<0.5µg/50µl
Nuclease-Free Water to	50µl	

[1]Completely thaw and thoroughly vortex the buffer prior to use.
[2]More $MgCl_2$ can be added to the reaction using 25mM $MgCl_2$ Solution (Cat.# A3511).

PCR-Polymerase Chain reaction

Reação de PCR
Ex. GoTaq, Promega

Table 1. Recommended Thermal Cycling Conditions for GoTaq® DNA Polymerase-Mediated PCR Amplification. These guidelines are optimal for the Perkin Elmer thermal cycler model 480 or comparable thermal cyclers.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	25–35 cycles
Annealing	42–65°C*	0.5–1 minute	
Extension	72°C	1min/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*Annealing temperature should be optimized for each primer set based on the primer T_m.

PCR-Polymerase Chain reaction

Reação de PCR
GoTaq, Promega

Componentes	Concentrações finais	Nossa concentração stock	Reação base (1 amostra) ul	Nossa reação (1+ Cpos+ Cneg)=3 +1 (erros pipetagem)
Flexi buffer GoTaq	1x	5x	4	
dNTPs	0,2mM	5mM each	1	
MgCl2	1-4mM	25mM	1,5 (1,8mM)	
D1R (Fw)	0,1-1uM	10mM	1 (0.5uM)	
D3Ca (Rv)	0,1-1uM	10mM	1 (0.5uM)	
Taq	1-1.5U	5U/uL	0.1	
DNA template	10pg-1ug	10ng-100ng	1	
H2O	Variável		10.4	
Volume total	-		20ul	

PCR-Polymerase Chain reaction

Reação de PCR
GoTaq, Promega

Componentes	Concentrações finais	Nossa concentração stock	Reação base (1 amostra) ul	Nossa reação (1+ Cpos+ Cneg)=3 +1 (erros pipetagem)
Flexi buffer GoTaq	1x	5x	4	12
dNTPs	0,2mM	5mM each	1	3
MgCl2	1-4mM	25mM	1,5 (1,8mM)	4,5
D1R (Fw)	0,1-1uM	10mM	1 (0.5uM)	3
D3Ca (Rv)	0,1-1uM	10mM	1 (0.5uM)	3
Taq	1-1.5U	5U/uL	0.1	0.3
DNA template	10pg-1ug	10ng-100ng	1	
H2O	Variável		10.4	31.2
Volume total	-		20ul	

PCR-Polymerase Chain reaction

Programa do termociclador para D1R/D3Ca

Step	Temperature ºC	Time	Nº cycles
Desnaturação inicial	95	3 min	1
Desnaturação	95	30 seg	
Emparelhamento	62	35 seg	40
Extensão	72	1 min	
Extensão final	72	10 min	1
Pausa	4	∞	

PCR-Polymerase Chain reaction

Reação de PCR

1. Limpar superfícies de trabalho com Lixivia, esperar 10 min e passar por água estéril (lixivia é corrosiva)
   Para descontaminar DNA prévio
2. Colocar pipetas de PCR, Eppendorfs, tubos PCR, caneta de acetato na câmara de UV
3. Ligar câmara de UVs 15min
   - Pode realizar-se a mistura fora de uma câmara de PCR
   - UVs destroem o DNA por formar ligações covalentes entre bases
4. Programar o termociclador
5. Gelo num tabuleiro
6. Ordenar todos os componentes que vamos usar (à excepção da taq)
7. Descongelar completamente, mix e spin de todos os componentes (componentes que precipitam no frio)
8. Ir para a Câmara de PCR
9. Marcar tubos de PCR

PCR-Polymerase Chain reaction

Reação de PCR

10. Fazer a mix a começar pelos maiores volumes
11. Adicionar a Taq
12. Mix com o dedo
13. Distribuir por todos os tubos 19ul
14. O que restar será no Cneg. Fechar tubo
15. Colocar o DNA nos tubos
16. Dirigir à sala pós-PCR
17. Colocar no termociclador (não retirar as escoras)
18. Run

PCR-Polymerase Chain reaction

PCR Master mix
-Buffer + MgCl2 + Taq
-faltam primers, DNA, H2O
- Há com e sem Loading Dye

Vantagens MIX
-Menos erros pipetagem
-Processo mais rápido

Desvantagem
- Não se pode optimizar condições de Magnésio ou dNTPs
- Se contaminação, há maior desperdicio

PCR-Polymerase Chain reaction

Direct PCR Master mix

Phire tissue Master Mix – “Extração de DNA” + PCR directo

6.1 Solid samples

Animal tissues

1. **Direct protocol:** Take a sample of 0.35-0.5 mm in diameter from tissue with a sterile scalpel (or small peace, e.g. one *Drosophila* leg) or by a tissue puncher. Place the sample directly into the PCR reaction (50 µL of volume). It is recommended to place the sample into the liquid rather than into an empty tube. Make sure that you see the sample in the solution.
2. **Dilution & storage protocol:** Before beginning, warm a heat block to 98 °C. Place the tissue sample into 20 µL of Dilution Buffer. Add 0.5 µL of DNARelease Additive. Mix by vortexing the tube briefly, and spin down the solution. If a larger sample is used, adjust the volume of the Dilution Buffer and DNARelease Additive accordingly. Make sure the sample is covered with the solution. Incubate the reaction for 2–5 minutes at room temperature and then place the tube into the pre-heated (98 °C) block for 2 minutes. Spin down the remaining tissue and store the supernatant at –20 °C if not used immediately. Usually 1 µL of supernatant is sufficient for a 20 µL PCR reaction. In some cases the supernatant may have to be diluted 1:10 or 1:100, or the PCR reaction performed in a 50 µL volume.

PCR-Polymerase Chain reaction

Direct PCR Master mix

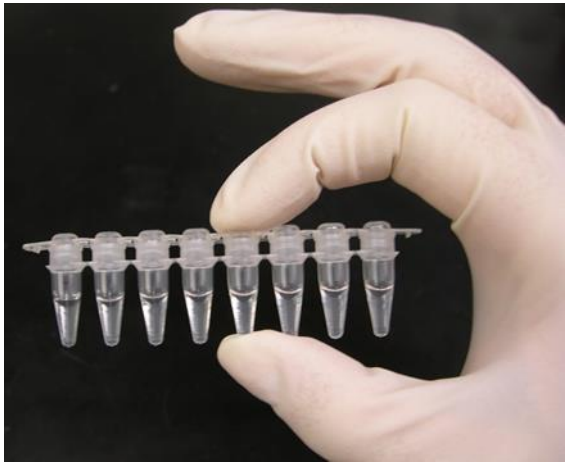
Phire tissue Master Mix – “Extração de DNA” + PCR directo

Table 1. Pipetting instructions

Component	20 µL rxn	50 µL rxn*	Final conc.
H_2O	add to 20 µL	add to 50 µL	-
2X Phire Tissue Direct PCR Master Mix	10 µL	25 µL	1X
Primer A	X µL	X µL	0.5 µM
Primer B	X µL	X µL	0.5 µM
Sample (see Section 6) Direct protocol:	-	Amount depends on the sample**	-
Dilution & Storage protocol:	0.5-1 µL	2.5 µL	

*50 µL reaction volume is recommended for the direct protocol.

**0.5 mm punch or a small sample of tissue (see www.thermoscientific.com/directpcr)

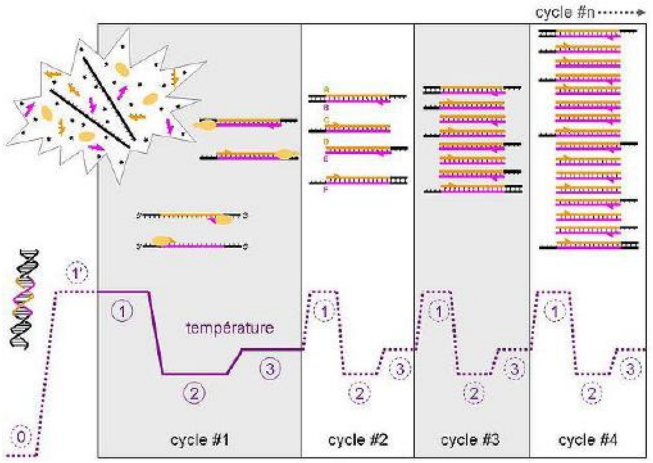


PCR-Polymerase Chain reaction

Programa de PCR

1. Desnaturação inicial

   Fundamental, pois compromete todos os passos seguintes
   1-3 min a 95ºC - GC content é 50% ou menos
   até 10min - GC-rich templates



PCR-Polymerase Chain reaction

Programa de PCR

2. Melting/Desnaturação

   quebra das pontes de hidrogénio

   separação da dupla cadeia de DNA

   0.5-2 min entre 94 a 96ºC

3. Annealing/Emparelhamento

   Primers emparelham com cadeia molde

   Depende da quantidade de citosina (C) e guanina (G)

   5ºC abaixo temperatura melting primer-template

   0.5-2min entre 50 a 60 ºC

   Se produtos inespecíficos-aumentar com incrementos de 1-2ºC

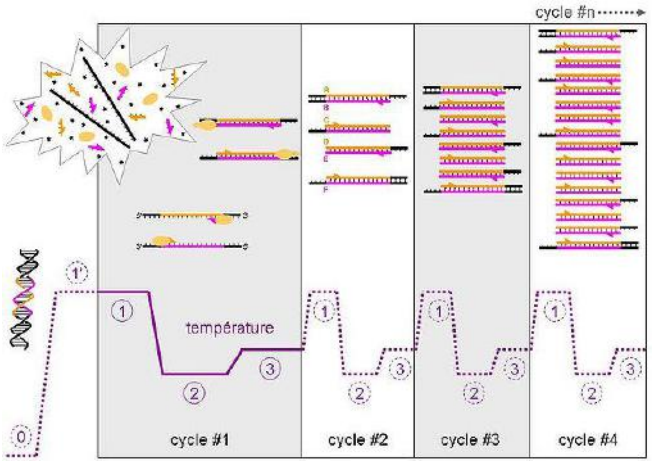
4. Extension/Extensão

   A enzima sintetiza a nova molécula

   70-75ºC

   1min até 2Kb

   Quando fragmentos maiores – adiciona 1min/1000bp



PCR-Polymerase Chain reaction

Programa de PCR

5. Ciclos

   Depende da quantidade de DNA template e do nº de cópias que pretendo no final

   25 a 40 ciclos

   taxa de replicação é exponencial

6. Extensão final

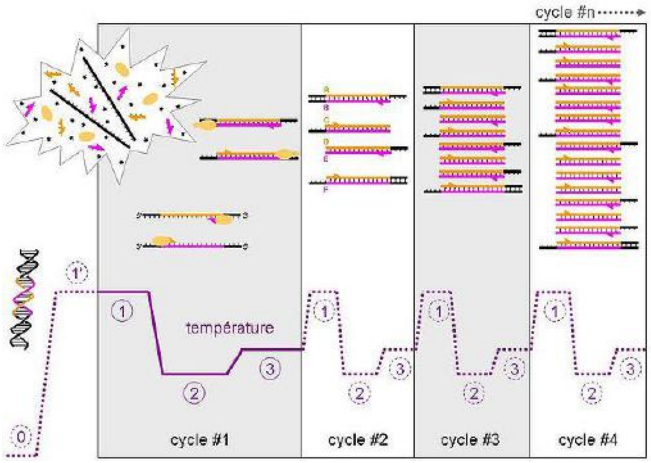
   72ºC – 5-15min

   terminar o final das cadeias

   Taq adiciona poly-A tails ao extremo 3´ (importante para a clonagem)

7. Final hold

   4ºC - ∞



PCR-Polymerase Chain reaction

PCR additives

•**DMSO**: Reduce secondary structure that could inhibit the progress of the polymerase. Especially useful for GC rich templates. Use at a final concentration of 5-10%.

•**Glycerol**: Reduces secondary structure. Use 5-10%

•**Betaine monohydrate**: Reduces secondary structure. Use 1 to 3M

•**BSA**: Very useful for templates that may be contaminated with humic acids (e.g. environmental samples contaminated with soil) and is also reported to prevent reaction components from sticking to the tube wall. Use up to 0.8 mg/ml

•**Tween-20**: Can neutralize SDS left over from template DNA preparation that would inhibit the reaction. Use 0.25 to 1% final concentration

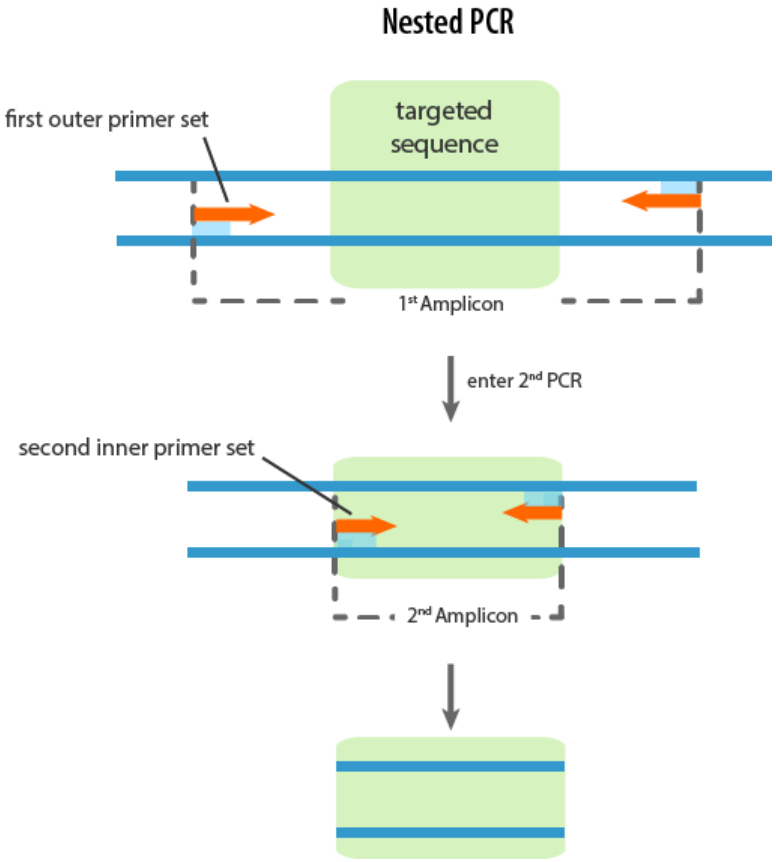
•**Formamide**: Increases the stringency of primer annealing, resulting in less non-specific priming and increased amplification efficiency. Use 1-10%

•**Tetramethyl ammonium chloride**: Similar to formamide. Use 10-100mM

•**7-deaza-2'-deoxyguanosine**: A dGTP analogue that is especially useful for extremely GC rich templates. Success is reported with up to 83% GC. Use a 1:3 ratio of dGTP:7-deaza-2'-deoxyguanosine

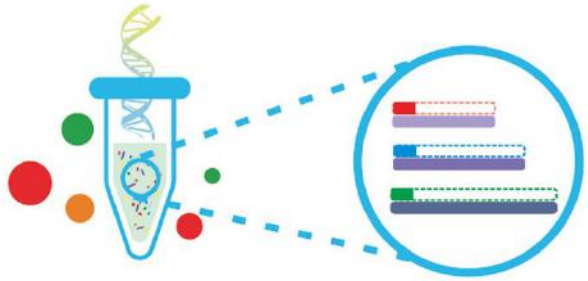
Nested PCR

- Aumentar a especificidade do PCR
- 2 pares de primers para o mesmo locus
  - 1 par mais externo (1ª amplificação)
  - Outro par mais interno (restantes amplificações)
- Adicionar-se em 2 reações de PCR consecutivas



Multiplex PCR

- Vários alvos numa única reação PCR
- Mesma mistura de PCR
  - 1 template
  - Vários pares de primers
- Normalmente os produtos amplificados têm vários tamanhos
- Temperaturas de annealing e desenho de primers têm de ser optimizados para todos trabalharem correctamente numa mesma reação

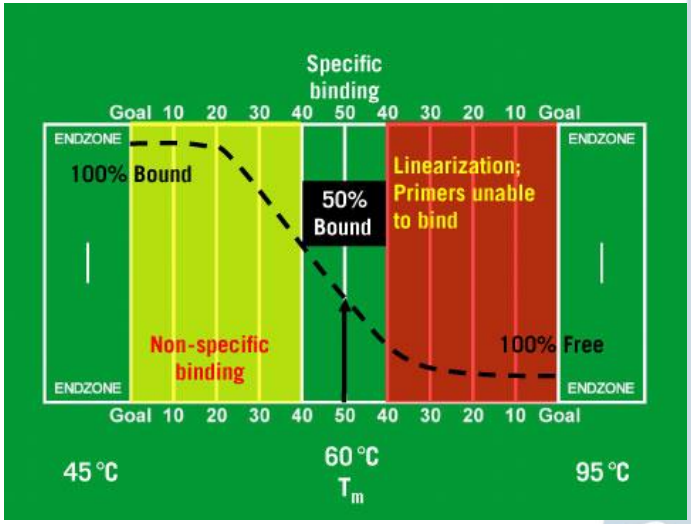


Touchdown PCR

- Reduz amplificações não-especificas, pelo decréscimo da temperature de annealing
- Início- 3-5ºC acima T_m
  - Elevada especificidade
- PCR continua – temp. em cada ciclo decresce 0.2ºC até chegar 3-5ºC abaixo da T_m.

- Temperaturas elevadas – elevada especificidade
- Temperaturas baixas – amplificações mais eficientes, do produto formando nos 1ºs ciclos

- Logo a 1ª sequência amplificada é a única entre as regiões de maior especificidade do primer e será a mais abundante no final do PCR



Hot-start PCR

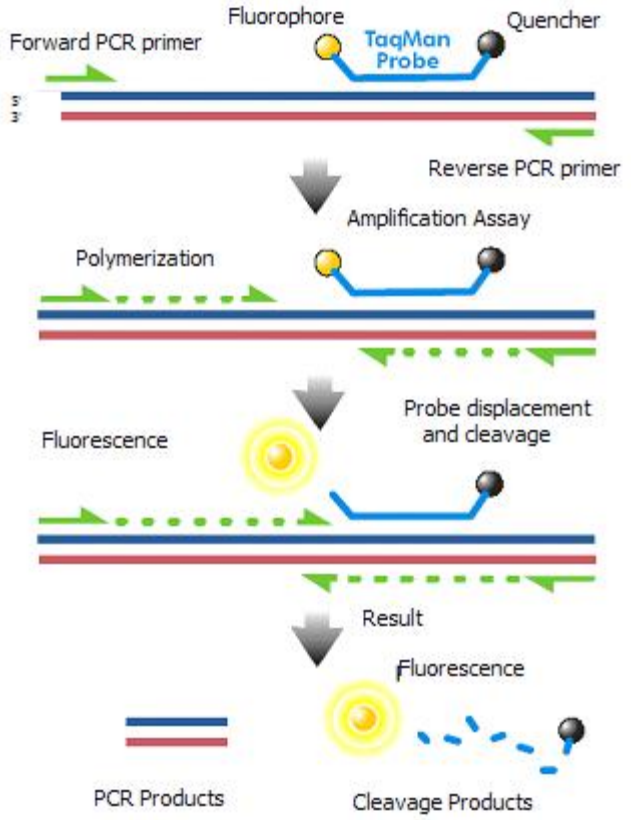
- A reação de PCR é ativada quando a temperatura atinge 94ºC
- Aumenta a especificidade do PCR, pois a DNA polimerase contém um anticorpo, que se desnatura e ativa a enzima ao atingir a temperatura de 94ºC.
- DNA polimerases que não possuem este inibidor podem amplificar produtos inespecíficos à temperatura ambiente.

https://youtu.be/ID6KY1QBR5s

https://youtu.be/mvvP90Cpdfc

Real-time PCR (qPCR)

- PCR que quantifica o DNA obtido por fluorescência
- Resultados mais rápidos e mais precisos
- Detecção SyberGreen
  - Corante não especifico que se intercala na molécula de DNA de cadeia dupla
- Detecção sondas Taqman (ex.)
  - Sequencias específicas complementares ao meu alvo, tipo “primers” marcadas com uma molécula fluorescente que permite deteção depois da hibridação



PCR-Polymerase Chain reaction

PCR convencional	Real-time PCR
Menor precisão	Maior
Menor sensibilidade	Maior
Menor resolução	Maior
Não automatizado	Automatizado
Resultados não numéricos	Numéricos
Possibilidade de produtos inespecíficos	Não influenciado por amplificação não específica
Amplificação monitorizada no final	Amplificação monitorizada em tempo real
Gel de agarose	Não há processamentos pós-pcr
Menor rapidez na corrida	Maior
Maior quantidade DNA	Requer 1000X menos DNA
Coleta de dados na fase final	Coleta de dados na fase exponencial

Preparação de amostras

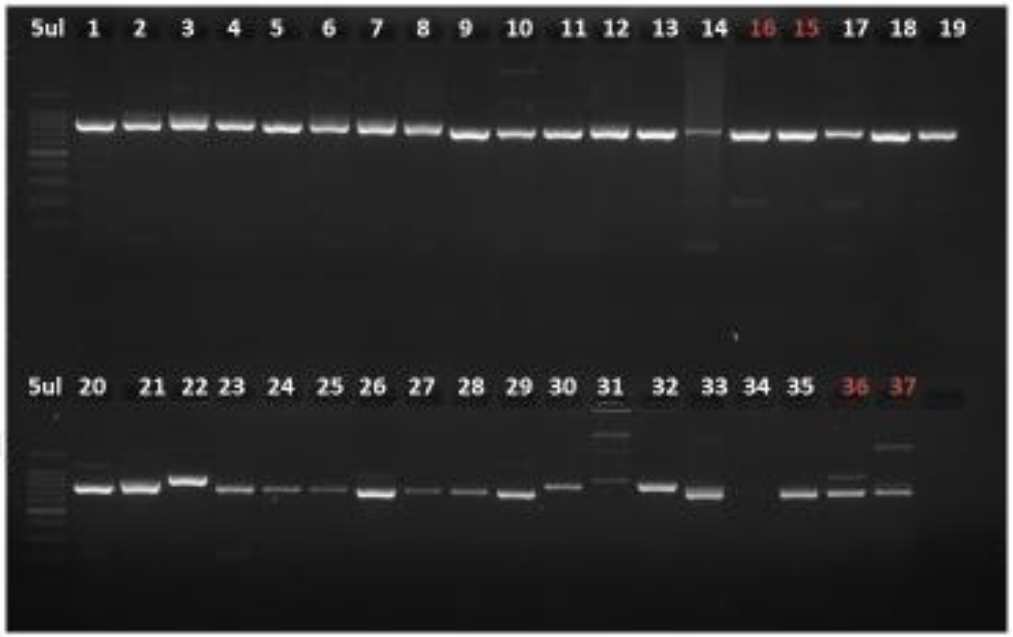
Resultados

2016Mat03
Primers:
D1R / D3Ca

ver amostras caderno ou slide seguinte

95ºC- 3min
94ºC-30"
62ºC – 35" – 40x
72ºC – 1min
72ºC – 10min
4ºC - ∞

H2O-7
Phire-10
D1r-1
D3Ca-1
DNA-1



2% Agarose- 100ml de gel
10W-40min
5 ul pcr
4 greensafe

Preparação de amostras

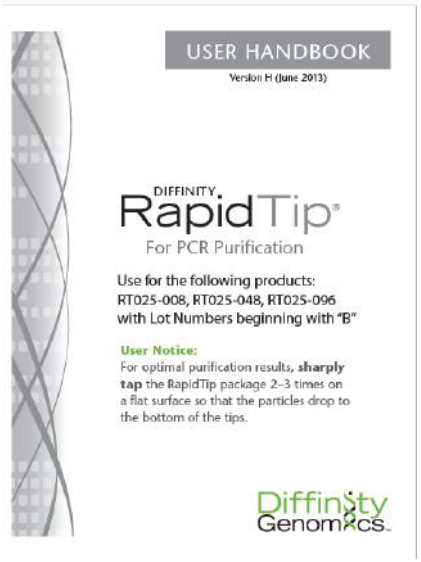
Purificação de produtos de PCR

Eliminam primers, dNTPs, enzimas, produtos PCR pequenos e inespecíficos e sais de fragmentos de PCR >100 bp, que podem afectar as aplicações seguintes

- Silica based columnns (mais comum)
  - Sal caotrópico (quebra pontes de H) desnatura o DNA que se liga à resina de sílica da coluna, permitindo que se separe dos restantes components da amostra
  - Depois da lavagem, o DNA é eluído com uma solução baixa em sal, que permite que se reorganize e perca afinidade com a membrane
  - Eluir em H2O (não afecta procedimentos subsequentes, menos estável). Eluir em Tris-EDTA (mais estável, mas sais podem afectar procedimentos subsequentes)

Preparação de amostras

Purificação de produtos de PCR



RapidTip PCR Purification Protocol

Prepare Samples:

Diffinity RapidTip is optimized for a 25 µl PCR reaction size.

RT025-008, 048, 096
Purifies 20-30 µl sample size.
For PCR volumes >30 µl, aliquot 25 µl into a tube.
For PCR volumes <20 µl, please dilute to 25 µl.

Dilution works best with highly concentrated DNA samples (>50 ng/µl) as your sample concentration will be reduced.

Prepare Tips:

Diffinity RapidTip for PCR Purification contains proprietary particles that purify a PCR reaction; these particles can adhere to the pipette tip walls during shipping. For optimal results, sharply tap the box 2–3 times on a flat surface so that particles are at the bottom of the tips (near the retainer).

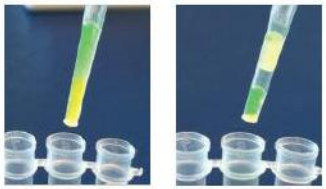
Please note that it is normal to see fine dust-like particles on the side of the tip. After tapping the box, you should expect to see about 1-2mm of white particles above the retainer at the small end of the pipette tip.

Purify Samples:

1. For 25 ± 5 µl samples, program pipettor to aspirate 30 µl.
2. Place Diffinity RapidTip on pipettor — single or multichannel.
   Please note that you can use a multi-channel pipettor to mix more than 1 sample at a time for even higher productivity.

Page 12 www.diffinitygenomics.com

Pipetting Examples



A. Correct mixing: Sample and particles mixing well.

B. Incorrect mixing: Particles separated by air bubble.

Preparação de amostras

Purificação de produtos de PCR

RapidTip PCR Purification Protocol

Prepare Samples:

Diffinity RapidTip is optimized for a 25 µl PCR reaction size.

RT025-008, 048, 096
Purifies 20-30 µl sample size.
For PCR volumes >30 µl, aliquot 25 µl into a tube.
For PCR volumes <20 µl, please dilute to 25 µl.

Dilution works best with highly concentrated DNA samples (>50 ng/µl) as your sample concentration will be reduced.

Prepare Tips:

Diffinity RapidTip for PCR Purification contains proprietary particles that purify a PCR reaction; these particles can adhere to the pipette tip walls during shipping. For optimal results, sharply tap the box 2–3 times on a flat surface so that particles are at the bottom of the tips (near the retainer).

Please note that it is normal to see fine dust-like particles on the side of the tip. After tapping the box, you should expect to see about 1-2mm of white particles above the retainer at the small end of the pipette tip.

Purify Samples:

1. For 25 ± 5 µl samples, program pipettor to aspirate 30 µl.
2. Place Diffinity RapidTip on pipettor — single or multichannel.
   Please note that you can use a multi-channel pipettor to mix more than 1 sample at a time for even higher productivity.
3. Place pipette tip into PCR sample solution.
4. Pre-wet the particles on first aspirate (see page 10).

   Aspirate about half the sample and then pause for 5 seconds to ensure complete wetting of the particles before mixing. Aspirate the rest of the sample and then dispense.
5. Set timer and mix for 60 seconds (approximately 15 aspirate/dispense cycles).

   Please note that pipetting will be slower than normal — wait for liquid to completely fill the tip to begin the next mix. It is not necessary to drive liquid completely out of the tip on every dispense. The particles should mix completely with the solution and make it appear cloudy while inside the tip.
6. On the final dispense, you can use your pipettor's blowout mode for maximum liquid recovery.

Your purified PCR Amplicon is now ready for downstream Sanger amplification.

Preparação de amostras

Purificação de produtos de PCR

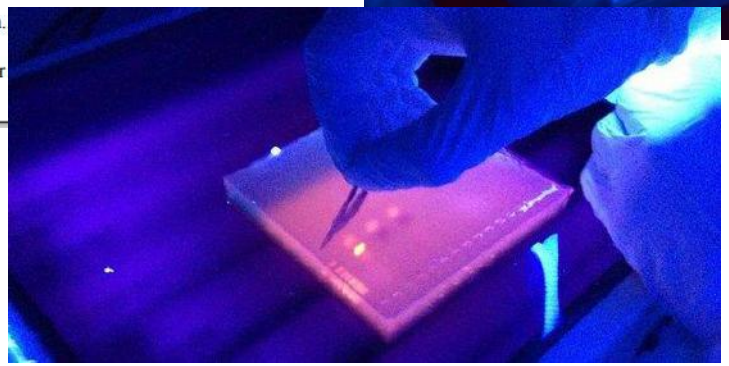
5.2 Cutting Gels

1. Open the drawer until it is fully extended and the Trans UV LED stops blinking.
2. Place the clear acrylic UV protection screen in the horizontal slot in the inside of the drawer facing the transilluminator.

!! CAUTION!! !! VORSICHT !!

WARNING: EXPOSURE TO UV RADIATION IS HAZARDOUS TO HEALTH. PLEASE WEAR PROPER UV PROTECTIVE CLOTHING AND FACE AND EYE SHIELDS WHEN CUTTING GELS. THE UV SHIELD PROVIDED WITH THE SYSTEM IS NOT ADEQUATE PROTECTION AGAINST UV GENERATED BY THIS SYSTEM.

3. Press the **Trans UV** button to turn on the UV illumination.
4. Cut the gel.
5. Press the **Trans UV** button and the UV transilluminator protection shield before closing the drawer.



Preparação de amostras

Purificação de produtos de PCR

grisp Research Solutions

Cut&Spin Gel Extraction Columns

#GS131.0250: 250 columns, #GS131.0500: 500 columns

Cut&Spin Gel Extraction Columns are ready-to-use spin columns for the extraction of DNA from agarose gels.

Protocol

Just cut out the desired DNA-band from the agarose gel, place it on the top of the column media, and centrifuge at 5.000-6.000g at room temperature for only 5-10 minutes.

Supplied Material

Spin columns are supplied in bags of 10, inserted in a 1,5ml receiver tube and closed with an attached receiver tube lid.

Preparação de amostras

Send to sequence

- Macrogen
- Stabvida
- GATC (Nzytech)
- …

Mandar sequenciar à **GATC**

(intermediário Nzytech (diogo.comprido@nzytech.com))

LightRun (LR)

1. Preparação das amostras
   - **a.** Purificar.
   - **b.** Tubo 1 – 10ul exactamente (tubo de 1,5ml)
     5ul DNA (20-80ng/ul) + 5ul Primer (5uM)
     ou 9ul DNA + 1ul Primer (desde que a conc. DNA seja entre 100 a 400ng e o primer 25pmol)
   - **c.** Se quiser sequenciar o reverse tenho de criar mais um tubo com PCR product e primer reverse
2. Labelling das amostras
   - **a.** Colar um barcode branco ao tubo (Light Run for Tubes)
   - **b.** Se quiser o Reverse, colo outro barcode
   - **c.** Colar o outro autocolante no caderno para meu registo
3. Preparar envelope (?)
   - **a.** Imprimir ou guardar assim que clica no label
   - **b.** Colar num envelope/saco
4. Chamar UPS – °707 232323
   - i. Recolha etiqueta pré-paga – UPS saver
   - ii. Até 1kg
   - iii. Alemanha
   - iv. Morada

     IPMA - Fitoplâncton Lab.
     Rua Alfredo Magalhães Ramalho, 6
     1495-006 Lisboa

Preparação de amostras

Send to sequence

Macrogen
Stabvida
GATC (Nzytech)

Mandar sequenciar à GATC

(intermediário Nzytech (diogo.comprido@nzytech.com))

SupremeRun (SR)

1. Preparação das amostras
   - a. Não preciso de purificar. Está incluido no preço
   - b. DNA sample-20ul exactamente, com 10-50ng/ul, no tubo 1 (tubo de 1,5ml)
   - c. Primer FW- 20ul exactamente, com 10uM (o que usamos), no tubo 2
   - d. Primer Rv- 20ul exactamente, com 10uM (o que usamos), no tubo 3
     - i. Estes 20ul dão para 6 rnx

2. Labelling das amostras
   - a. Colar um barcode verde ao tubo 1 (GATC DNA)
   - b. Colar outro barcode amarelo ao tubo 2 (GATC Primer)
   - c. Colar outro barcode amarelo ao tubo 3 (GATC Primer)

3. Ir à myGATC account
   - a. Sanger sequencing
   - b. Supreme Run order
   - c. Tubes
   - d. PCR fragments
   - e. Can be purified by GATC
   - f. Select DNA Barcode
   - g. Nomear a minha amostra
   - h. Select Primer Barcode
   - i. Não preciso de colocar sequência
   - j. Clicar + Reuse DNA

4. Preparar envelope
   - a. Imprimir ou guardar assim que clica no label
   - b. Colar num envelope/saco

5. Chamar UPS – 707 232323
   - i. Recolha etiqueta pré-paga – UPS saver
   - ii. Até 1kg
   - iii. Alemanha
   - iv. Morada

IPMA - Fitoplâncton Lab
Rua Alfredo Magalhães Ramalho, 6
1495-006 Lisboa

Preparação de amostras

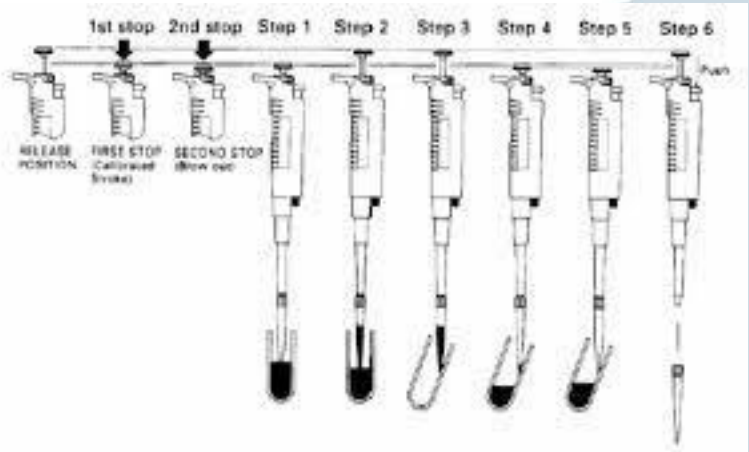
Micropipetas

Como pipetar

1. Aspirar lentamente e na vertical
2. Descartar lentamente e com ângulo de 40º, com a ponta encostada ao recipiente, até à 1ª posição
3. Carregar até à 2ª posição, arrastando a ponta pelo recipente

Manutenção e Limpeza das Micropipetas

- Manter a pipeta sempre em posição vertical e no volume máximo (a pipeta deitada de um dia para o outro é suficiente para a descalibrar. A mola em tensão pode provocar também uma descalibração)
- Para encaixar as pontas, não bater. Rodar nas monocanais e encaixar frente e trás nas pluricanais.
- Limpar externamente a mp com alcool a 70º, sempre que necessário.
- Limpeza interna - 5 cargas/descargas **sem** ponta, com alcool a 70º, e deixar a secar no fim de semana ou 30min na estufa até 60ºC, se houver urgência
- Lixívia e autoclavagem para situações mais complicadas



INTRODUÇÃO À BIOLOGIA MOLECULAR E BIOINFORMÁTICA:

Bioinformatics

Lisboa, 1-3 Junho

João Machado

Bárbara Frazão

Bioinformatic

- What is it? Bioinformatics is the creation , development and operation of databases and other computational tools to collect, organize and interpret data

- Data Sources ? They are usually derived from biological data experiences that provide quantitative and qualitative data

Use of databases in bioinformatics as repositories and sources of information

Data Warehouses

- From 1982 databases began to be created for storing information and sequences of nucleotides

Examples

- European Molecular Biology Laboratory: http://www.embl.org/ (Europe)
- National Institutes of Health: http://www.ncbi.nlm.nih.gov (North America)
- DNA Databank (DDBJ): http://www.ddbj.nig.ac.jp/ (Japan)

Data Warehouses

- From 1986 databases of amino acids (proteins)

Exemplos

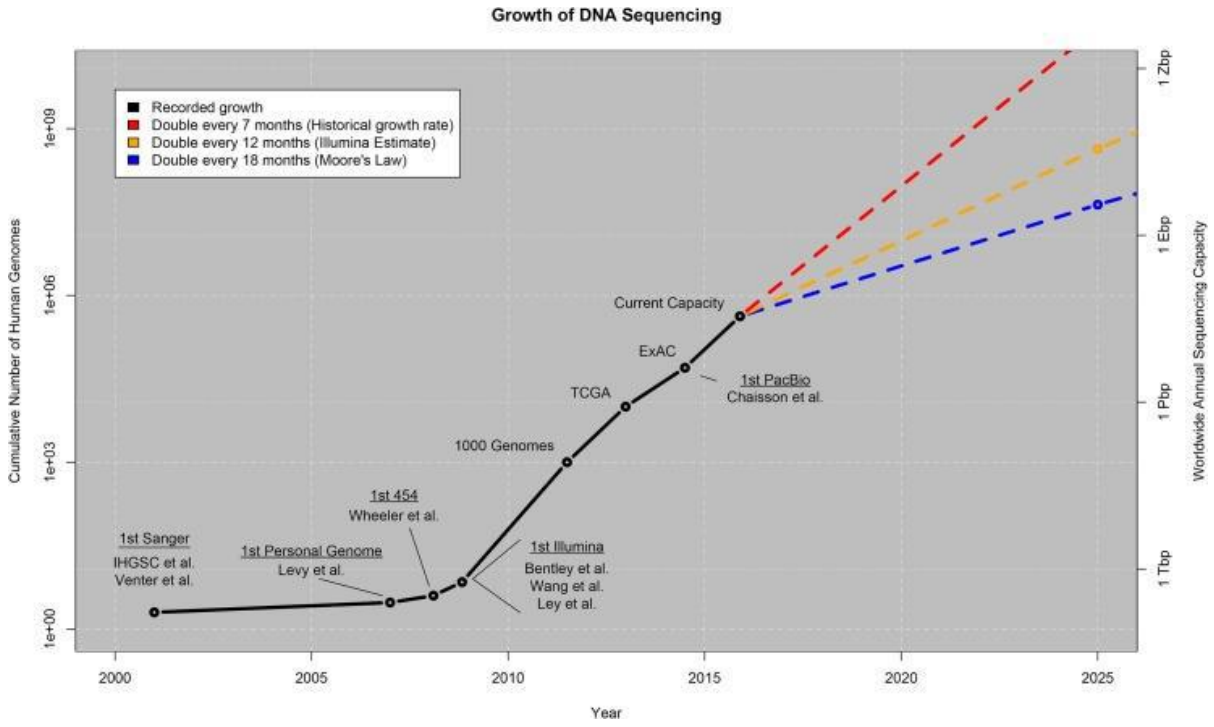
- Swissprot/TrEMBL
- PIR

- In 2002 joined in UniProtKb (http://www.uniprot.org/)

- **UniProtKB/Swiss-Prot** which is manually annotated and is reviewed and
- **UniProtKB/TrEMBL** which is automatically annotated and is not reviewed.

Data growth

- "100-gigabase" in August 2005. 200 billion bp in September 2007. The amount of data doubles every 18 months.



PLoS Biol. 2015 Jul; 13(7): e1002195.

Conventions

GenBank	gb|accession.version
EMBL	emb|accession.version
DDBJ	dbj|accession.version
NCBI	RefSeq ref|accession.version
PDB	pdb|entry|chain
Patents	pat|country|number
NBRF PIR	pir||entry
SWISS-PROT	sp|accession|entry
Protein Research Foundation	prf|name
GenInfo Backbone	Id bbs|number
General database identifier	gnl|database|identifier
Local Sequence identifier	lcl|identifier

Conventions

RefSeq categories

Experimentally determined and curated		Genome annotation (computational predictions from DNA)	
NC	Complete genomic molecules		
NG	Incomplete genomic region		
NM	mRNA	XM	Model mRNA
NR	RNA (non-coding)		
NP	Protein	XP	Model protein

Conventions

IUB/IUPAC nucleotide and ambiguity codes

A	adenosine	M	A or C (amino)	V	A, C, or G
C	cytidine	K	G or T (keto)	H	A, C, or T
G	guanine	R	A or G (purine)	D	A, G, or T
T	thymidine	Y	C or T (pyrimidine)	B	C, G, or T
U	uridine	S	A or T (strong)	–	Gap of indeterminate length
		W	C or G (weak)	N	A, C, G, or T (any or unknown)

R	A,G
Y	C,T
M	A,C
K	G,T
S	C,G
W	A,T
H	A,C,T
B	C,G,T
V	A,C,G
D	A,G,T
N	A,C,G,T

Conventions

- Gene Names (http://www.genenames.org/)

File Formats

- Fasta files

```
>seq1
----------------------KSKERYKDENGGNYFQLREDWWDANRE
>seq2
---------------YEGLTTANGXKEYYQDKNGGNFFKLREDWWTANRE
>seq3
-----------------------SQRHYKD-DGGNYFQLREDWWTANRH
>seq4
--------------------NVAALKTRYEK-DGQNFYQLREDWWTANYF
```

File Formats

- Phylip interleaved

- The first line of the input file contains the number of species and the number of characters separated by blanks. The information for each species follows, starting with a ten-character species name (which can include punctuation marks and blanks), and continuing with the characters for that species. Phylip format files can be interleaved, as in the example below, or sequential.

```
4 123
seq1 ---------- ---------- ---KSKERYK DENGGNYFQL REDWWDANRE
seq2 ---------- -----YEGLT TANGXKEYYQ DKNGGNFFKL REDWWTANRE
seq3 ---------- ---------- ----SQRHYK D-DGGNYFQL REDWWTANRH
seq4 ---------- ---------- NVAALKTRYE K-DGQNFYQL REDWWTANRA

 TVWKAITCNA --GGGKYFRN TCDG--GQNP TETQNNCRCIG---------
 TVWKAITCGA P-GDASYFHA TCDSGDGRGG AQAPHKCRCD G---------
 TVWEAITCSA DKGNA-YFRR TCNSADGKSQ SQARNQCRC- --KDENGKN-
 TIWEAITCSA DKGNA-YFRA TCNSADGKSQ SQARNQCRC- --KDENGXN-
```

File Formats

- Phylip sequencial

```
4 123
seq1 ---------- ---------- ---KSKERYK DENGGNYFQL REDWWDANRE
TVWKAITCNA --GGGKYFRN TCDG--GQNP TETQNNCRCI G---------
seq2 ---------- -----YEGLT TANGXKEYYQ DKNGGNFFKL REDWWTANRE
TVWKAITCGA P-GDASYFHA TCDSGDGRGG AQAPHKCRCD G---------
seq3 ---------- ---------- ----SQRHYK D-DGGNYFQL REDWWTANRH
TVWEAITCSA DKGNA-YFRR TCNSADGKSQ SQARNQCRC- --KDENGKN-
seq4 ---------- ---------- NVAALKTRYE K-DGQNFYQL REDWWTANRA
TIWEAITCSA DKGNA-YFRA TCNSADGKSQ SQARNQCRC- --KDENGXN-
```

File Formats

- Nexus

```
#NEXUS
BEGIN DATA;
     DIMENSIONS NTAX=10 NCHAR=22;
     FORMAT MISSING=? DATATYPE=DNA GAP=- EQUATE="0=A 1=C";
     OPTIONS GAPMODE=MISSING;
MATRIX

                        [0000000000000000000000]
                        [0000000000000000000000]
                        [0000000001111111111222]
                        [1234567890123456789012]

TaxonA                   AAAAAAAAAAAAAAAA000000
TaxonB                   AA-----AAA--AAAA1--100
TaxonC                   AAA--AAAAAA--AAA010010
TaxonD                   AAAGAA-AAAAGAA-A001001
TaxonE                   ACGTACGTACGTACGT000000
TaxonF                   AAAAAAAAAAAAAAAA000000
TaxonG                   AA-----AAA--AAAA1--100
TaxonH                   AAA--AAAAAA--AAA010010
TaxonI                   AAAGAA-AAAAGAA-A001001
TaxonJ                   ACGTACGTACGTACGT000000
;
END;

[ Indel Character      Sequence Region ]
[ ---------------      --------------- ]
[                                      ]
[ 17              3-7          ]
[ 18              4-5          ]
[ 19              7-7          ]
[ 20              11-12          ]
[ 21              12-13          ]
[ 22              15-15          ]
```

File Formats

GenBank

```
LOCUS       AF023787                618 bp    DNA     linear   PLN 02-MAY-1998
DEFINITION  Bryum stenotrichum small ribosomal protein 4 (rps4) gene,
            chloroplast gene encoding chloroplast protein, partial cds.
ACCESSION   AF023787
VERSION     AF023787.1  GI:3098167
KEYWORDS    .
SOURCE      chloroplast Bryum stenotrichum
  ORGANISM  Bryum stenotrichum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Bryophyta;
            Moss Superclass V; Bryopsida; Bryidae; Bryanae; Bryales; Bryaceae;
            Bryum.
REFERENCE   1  (bases 1 to 618)
  AUTHORS   Cox,C.J. and Hedderson,T.A.J.
  TITLE     Phylogenetic relationships among the ciliate arthrodontous mosses:
            evidence from chloroplast and nuclear DNA sequences
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 618)
  AUTHORS   Cox,C.J. and Hedderson,T.A.J.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-SEP-1997) Dept. of Botany, School of Plant Sciences,
            University of Reading, Whiteknights, Reading, Berkshire RG6 6AS,
            United Kingdom
FEATURES             Location/Qualifiers
     source          1..618
                     /organism="Bryum stenotrichum"
                     /organelle="plastid:chloroplast"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:66994"
     gene            <1..573
                     /gene="rps4"
     CDS             <1..573
                     /gene="rps4"
                     /codon_start=1
                     /product="small ribosomal protein 4"
                     /protein_id="AAC15532.1"
                     /db_xref="GI:3098168"
                     /translation="RRLGSLPGLTNKTPQLKTNSINQSISNKKISQYRIRLEEKQKLR
                     FHYGITERQLLNYVRIARKAKGSTGEVLLQLLEMRLDNVIFRLGMAPTIPGARQLVNH
                     RHILVNDRIVNIPSYRCKPEDSITIKDRQKSQAIISKNLNLYQKYKTPNHLTYNFLKK
                     KGLVNQILDRESIGLKINELLVVEYYSRQA"
ORIGIN
        1 cgccgtttag gatctttacc aggactaact aataaaacac cccagttaaa aactaattcg
       61 atcaatcaat caatatctaa taaaaaaatt tctcaatatc gcattcgttt ggaagaaaaa
      121 caaaaattac gttttcatta tggaataaca gagcgacaat tacttaatta tgtacgtatt
      181 gctagaaaag ctaaagggtc aacaggtgaa gtcttattac aattacttga aatgcgctta
      241 gataacgtta tttttcgatt aggtatggct cctacaattc ctggagcaag gcaactagta
      301 aatcatagac atattttagt taatgatcgt atagtaaata taccaagtta tcggtgtaaa
      361 cctgaggatt ctattactat aaaagatcga caaaaatctc aggctataat tagtaaaaat
      421 ttaaatttgt atcaaaaata taaaacacca aatcatttaa cttataattt tttaaaaaaa
      481 aaaggattgg ttaatcaaat actagatcgt gaatccattg gtttaaaaat aaatgaatta
      541 ttagttgtag aatattattc tcgccaagct taattaacaa ctaagagtat ttgtaattat
      601 atacataata aaaatttg
//
```

File Convertions

- Software (e.g. Seaview, Mega, Mesquite, Bioedit, etc)
  - Requires instalation

- Scripts (generally in Python or Perl)
  - Several are freely distributed in github (https://github.com/)

- Web-based tool (easy)

Hands-On 1

- https://goo.gl/VFwldq

1) Descarregar as sequencias na pasta hands_on_1

2) Usar o site: http://www.ebi.ac.uk/Tools/sfc/emboss_seqret/

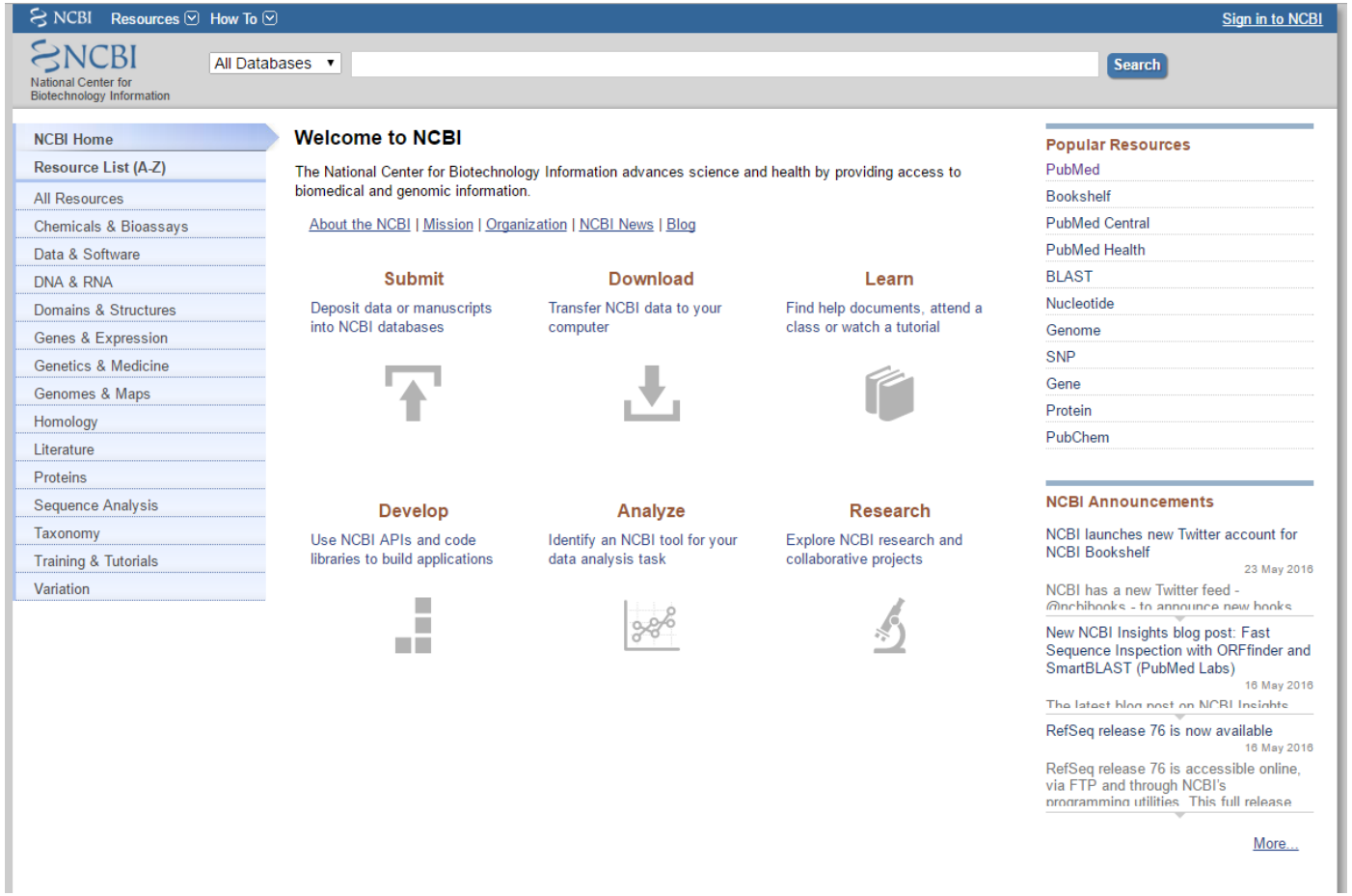
3) Analisar o formato original das sequencias

4) Converter as sequencias para Fasta

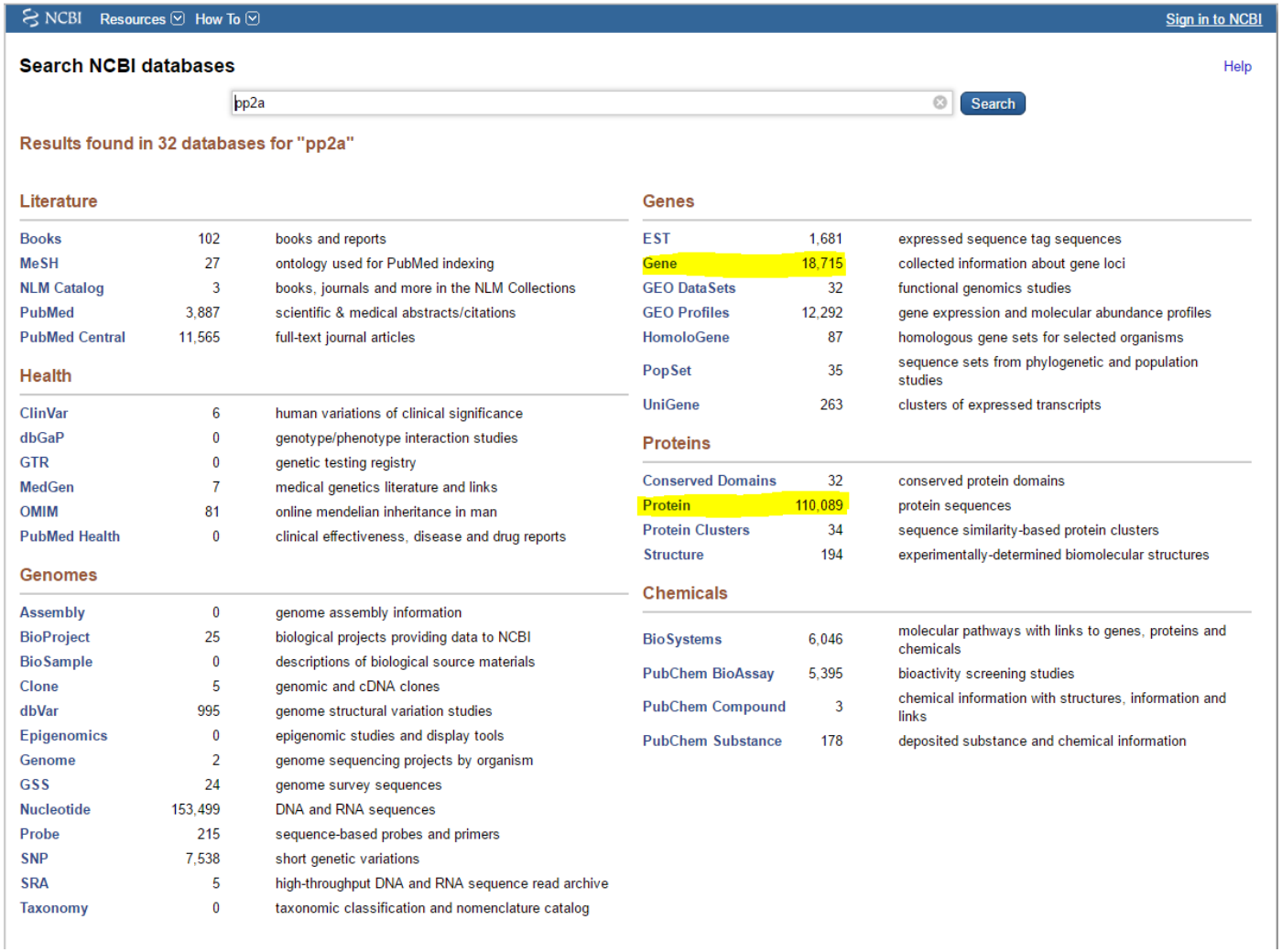
5) Adicionar num ficheiro separado usando editor de texto

Search Methods

Search NCBI


NCBI Resources How To
Sign in to NCBI
NCBI
National Center for Biotechnology Information
All Databases
Search
NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation
Welcome to NCBI
The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.
About the NCBI | Mission | Organization | NCBI News | Blog
Submit
Deposit data or manuscripts into NCBI databases
Download
Transfer NCBI data to your computer
Learn
Find help documents, attend a class or watch a tutorial
Develop
Use NCBI APIs and code libraries to build applications
Analyze
Identify an NCBI tool for your data analysis task
Research
Explore NCBI research and collaborative projects
Popular Resources
PubMed
Bookshelf
PubMed Central
PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem
NCBI Announcements
NCBI launches new Twitter account for NCBI Bookshelf
23 May 2016
NCBI has a new Twitter feed - @ncbibooks - to announce new books
New NCBI Insights blog post: Fast Sequence Inspection with ORFfinder and SmartBLAST (PubMed Labs)
16 May 2016
The latest blog post on NCBI Insights
RefSeq release 76 is now available
16 May 2016
RefSeq release 76 is accessible online, via FTP and through NCBI's programming utilities. This full release
More...

Search NCBI



NCBI Resources How To Sign in to NCBI

Search NCBI databases Help

pp2a Search

Results found in 32 databases for "pp2a"

Literature

Books	102	books and reports
MeSH	27	ontology used for PubMed indexing
NLM Catalog	3	books, journals and more in the NLM Collections
PubMed	3,887	scientific & medical abstracts/citations
PubMed Central	11,565	full-text journal articles

Health

ClinVar	6	human variations of clinical significance
dbGaP	0	genotype/phenotype interaction studies
GTR	0	genetic testing registry
MedGen	7	medical genetics literature and links
OMIM	81	online mendelian inheritance in man
PubMed Health	0	clinical effectiveness, disease and drug reports

Genomes

Assembly	0	genome assembly information
BioProject	25	biological projects providing data to NCBI
BioSample	0	descriptions of biological source materials
Clone	5	genomic and cDNA clones
dbVar	995	genome structural variation studies
Epigenomics	0	epigenomic studies and display tools
Genome	2	genome sequencing projects by organism
GSS	24	genome survey sequences
Nucleotide	153,499	DNA and RNA sequences
Probe	215	sequence-based probes and primers
SNP	7,538	short genetic variations
SRA	5	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	1,681	expressed sequence tag sequences
Gene	18,715	collected information about gene loci
GEO DataSets	32	functional genomics studies
GEO Profiles	12,292	gene expression and molecular abundance profiles
HomoloGene	87	homologous gene sets for selected organisms
PopSet	35	sequence sets from phylogenetic and population studies
UniGene	263	clusters of expressed transcripts

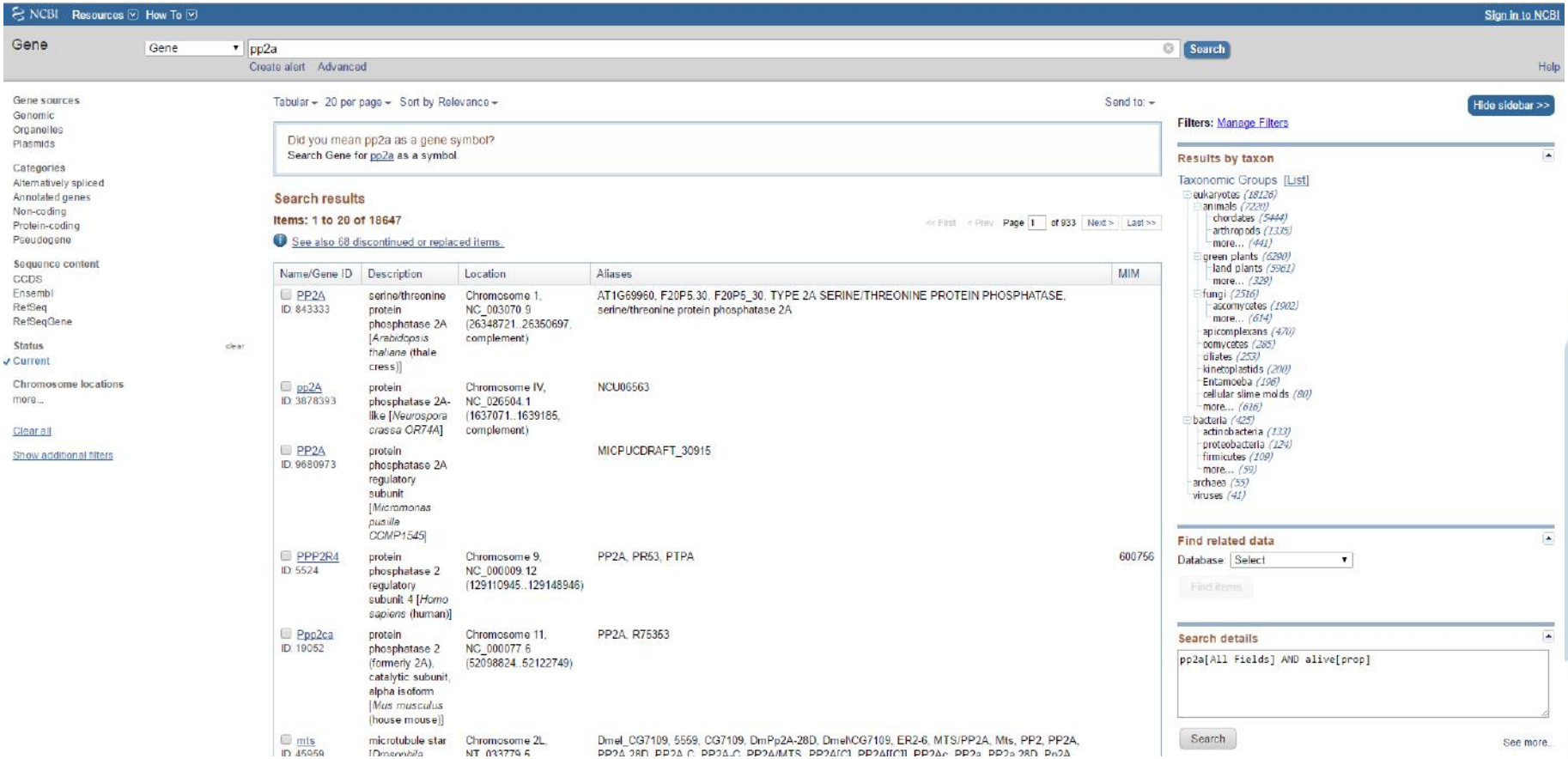
Proteins

Conserved Domains	32	conserved protein domains
Protein	110,089	protein sequences
Protein Clusters	34	sequence similarity-based protein clusters
Structure	194	experimentally-determined biomolecular structures

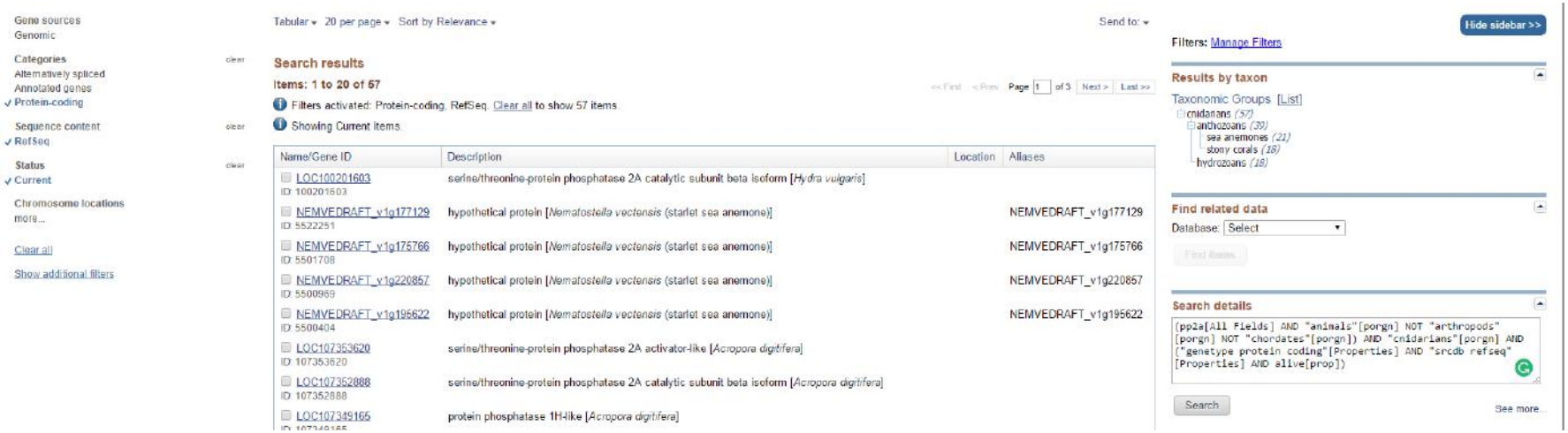
Chemicals

BioSystems	6,046	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	5,395	bioactivity screening studies
PubChem Compound	3	chemical information with structures, information and links
PubChem Substance	178	deposited substance and chemical information

Search NCBI


NCBI Resources How To
Sign in to NCBI
Gene
Gene
pp2a
Search
Create alert Advanced
Help
Gene sources
Genomic
Organelles
Plasmids
Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene
Sequence content
CCDS
Ensembl
RefSeq
RefSeqGene
Status
Current
Chromosome locations
more...
Clear all
Show additional filters
Tabular 20 per page Sort by Relevance
Send to:
Hide sidebar >>
Filters: Manage Filters
Did you mean pp2a as a gene symbol?
Search Gene for pp2a as a symbol.
Search results
Items: 1 to 20 of 18647
Page 1 of 933 Next > Last >>
See also 68 discontinued or replaced items.
Name/Gene ID
Description
Location
Aliases
MIM
PP2A
ID: 843333
serine/threonine protein phosphatase 2A [Arabidopsis thaliana (thale cress)]
Chromosome 1, NC_003070.9 (26348721..26350697, complement)
AT1G69960, F20P5.30, F20P5_30, TYPE 2A SERINE/THREONINE PROTEIN PHOSPHATASE, serine/threonine protein phosphatase 2A
pp2A
ID: 3878393
protein phosphatase 2A-like [Neurospora crassa OR74A]
Chromosome IV, NC_026504.1 (1637071..1639185, complement)
NCU06563
PP2A
ID: 9680973
protein phosphatase 2A regulatory subunit [Micromonas pusilla CCMP1545]
MICPUCDRAFT_30915
PPP2R4
ID: 5524
protein phosphatase 2 regulatory subunit 4 [Homo sapiens (human)]
Chromosome 9, NC_000009.12 (129110945..129148946)
PP2A, PR53, PTPA
600756
Ppp2ca
ID: 19052
protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform [Mus musculus (house mouse)]
Chromosome 11, NC_000077.6 (52098824..52122749)
PP2A, R75353
mts
microtubule star
Chromosome 2L, NT_033779.5
Dmel_CG7109, 5559, CG7109, DmPp2A-28D, DmelCG7109, ER2-6, MTS/PP2A, Mts, PP2, PP2A,
Results by taxon
Taxonomic Groups [List]
eukaryotes (18126)
animals (7220)
chordates (5444)
arthropods (1335)
more... (441)
green plants (6290)
land plants (5961)
more... (329)
fungi (2516)
ascomycetes (1902)
more... (614)
apicomplexans (470)
oomycetes (285)
ciliates (253)
kinetoplastids (200)
Entamoeba (196)
cellular slime molds (80)
more... (616)
bacteria (425)
actinobacteria (133)
proteobacteria (124)
firmicutes (109)
more... (59)
archaea (53)
viruses (41)
Find related data
Database: Select
Find items
Search details
pp2a[All Fields] AND alive[prop]
Search
See more...

Search NCBI


Gene sources
Genomic
Categories
Alternatively spliced
Annotated genes
Protein-coding
Sequence content
RefSeq
Status
Current
Chromosome locations
more...
Clear all
Show additional filters
Tabular
20 per page
Sort by Relevance
Send to:
Search results
Items: 1 to 20 of 57
Page 1 of 3
Next >
Last >>
Filters activated: Protein-coding, RefSeq. Clear all to show 57 items.
Showing Current items.
Name/Gene ID
Description
Location
Aliases
LOC100201603
ID: 100201603
serine/threonine-protein phosphatase 2A catalytic subunit beta isoform [Hydra vulgaris]
NEMVEDRAFT_v1g177129
ID: 5522251
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g177129
NEMVEDRAFT_v1g175766
ID: 5501708
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g175766
NEMVEDRAFT_v1g220857
ID: 5500969
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g220857
NEMVEDRAFT_v1g195622
ID: 5500404
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g195622
LOC107353620
ID: 107353620
serine/threonine-protein phosphatase 2A activator-like [Acropora digitifera]
LOC107352888
ID: 107352888
serine/threonine-protein phosphatase 2A catalytic subunit beta isoform [Acropora digitifera]
LOC107349165
protein phosphatase 1H-like [Acropora digitifera]
Hide sidebar >>
Filters: Manage Filters
Results by taxon
Taxonomic Groups [List]
cnidarians (57)
anthozoans (39)
sea anemones (21)
stony corals (18)
hydrozoans (18)
Find related data
Database: Select
Search details
(pp2a[All Fields] AND "animals"[porgn] NOT "arthropods"[porgn] NOT "chordates"[porgn]) AND "cnidarians"[porgn] AND ("genetype protein coding"[Properties] AND "srcdb refseq"[Properties] AND alive[prop])
Search
See more...

Search NCBI

LOC107353620 serine/threonine-protein phosphatase 2A activator-like [*Acropora digitifera*]

Gene ID: 107353620, updated on 16-Apr-2016

Summary

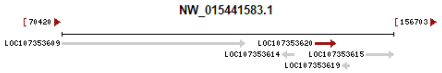
Gene symbol	LOC107353620
Gene description	serine/threonine-protein phosphatase 2A activator-like
Gene type	protein coding
RefSeq status	MODEL
Organism	Acropora digitifera
Lineage	Eukaryota; Metazoa; Cnidaria; Anthozoa; Hexacorallia; Scleractinia; Astrocoeniina; Acroporidae; Acropora

Genomic context

Location: chromosome: Un — See LOC107353620 in Genome Data Viewer

Exon count: 6

Annotation release	Status	Assembly	Chr	Location
100	current	Adig_1.1 (GCF_000222465.1)	Unplaced Scaffold	NW_015441583.1 (136387..141496)



Genomic regions, transcripts, and products

Genomic Sequence: NW_015441583.1 Unplaced Scaffold Reference Adig_1.1 Primary Assembly

Go to reference sequence details

Go to nucleotide: Graphics FASTA GenBank

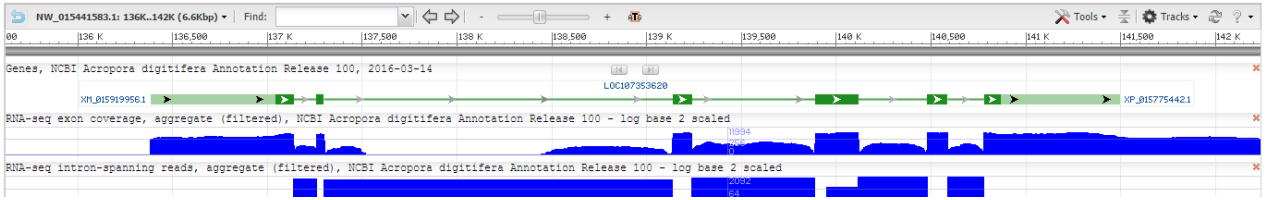


Table of contents

Summary
Genomic context
Genomic regions, transcripts, and products
Bibliography
General protein information
NCBI Reference Sequences (RefSeq)
Related sequences

Genome Browsers

Genome Data Viewer

Related information

BioProjects
Conserved Domains
Gene neighbors
Nucleotide
Protein
RefSeq Proteins
RefSeq RNAs
Taxonomy

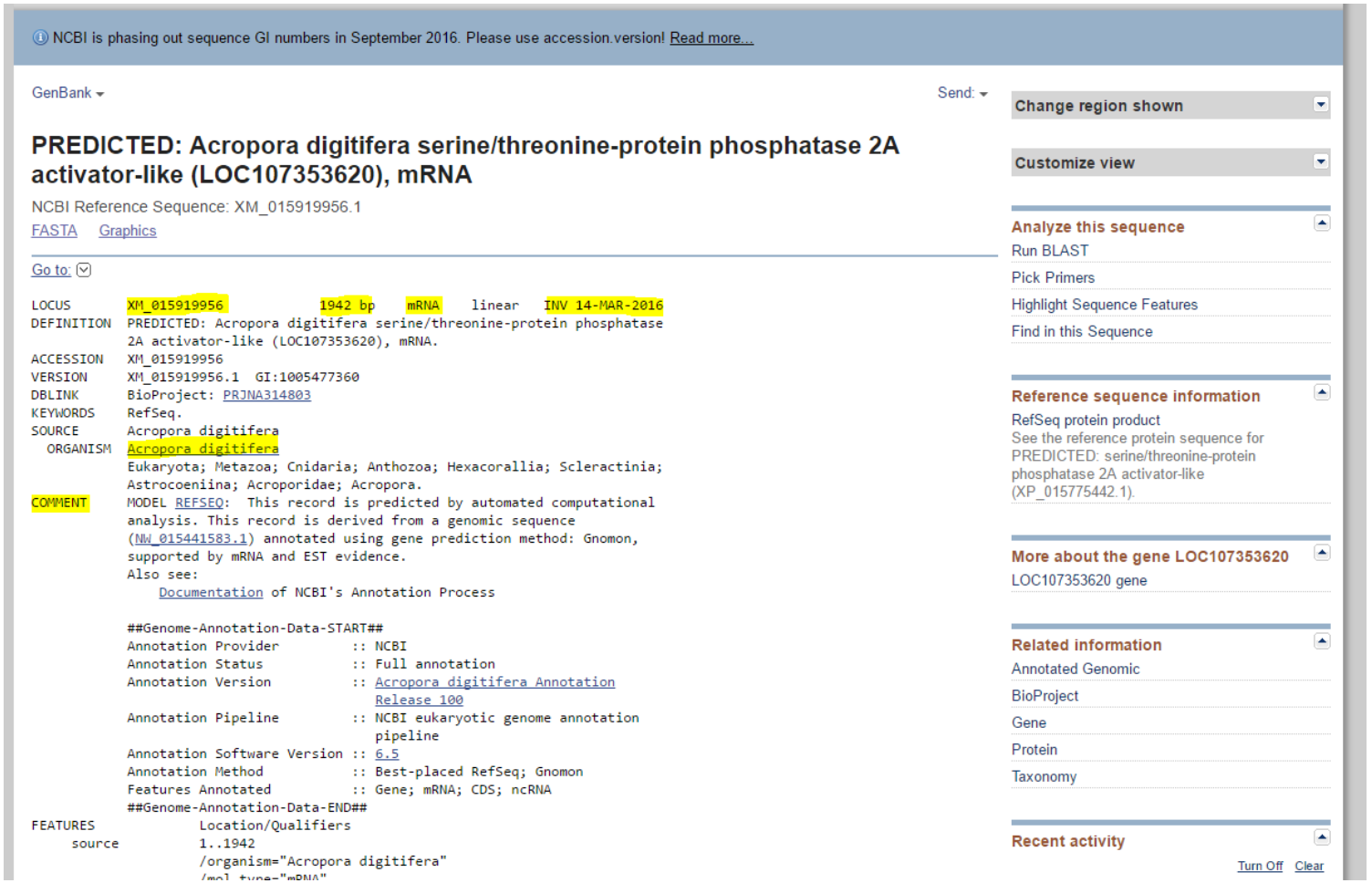
General information

About Gene
FAQ
FTP site
Help
My NCBI help
NCBI Handbook
Statistics

Related sites

BLAST

Search NCBI



NCBI is phasing out sequence GI numbers in September 2016. Please use accession.version! Read more...

GenBank ▾ Send: ▾

PREDICTED: Acropora digitifera serine/threonine-protein phosphatase 2A activator-like (LOC107353620), mRNA

NCBI Reference Sequence: XM_015919956.1

FASTA Graphics

Go to:

```
LOCUS       XM_015919956           1942 bp    mRNA    linear   INV 14-MAR-2016
DEFINITION  PREDICTED: Acropora digitifera serine/threonine-protein phosphatase
            2A activator-like (LOC107353620), mRNA.
ACCESSION   XM_015919956
VERSION     XM_015919956.1  GI:1005477360
DBLINK      BioProject: PRJNA314803
KEYWORDS    RefSeq.
SOURCE      Acropora digitifera
  ORGANISM  Acropora digitifera
            Eukaryota; Metazoa; Cnidaria; Anthozoa; Hexacorallia; Scleractinia;
            Astrocoeniina; Acroporidae; Acropora.
COMMENT     MODEL REFSEQ:  This record is predicted by automated computational
            analysis. This record is derived from a genomic sequence
            (NW_015441583.1) annotated using gene prediction method: Gnomon,
            supported by mRNA and EST evidence.
            Also see:
                Documentation of NCBI's Annotation Process

            ##Genome-Annotation-Data-START##
            Annotation Provider         :: NCBI
            Annotation Status           :: Full annotation
            Annotation Version          :: Acropora digitifera Annotation
                                           Release 100
            Annotation Pipeline         :: NCBI eukaryotic genome annotation
                                           pipeline
            Annotation Software Version :: 6.5
            Annotation Method           :: Best-placed RefSeq; Gnomon
            Features Annotated          :: Gene; mRNA; CDS; ncRNA
            ##Genome-Annotation-Data-END##
FEATURES             Location/Qualifiers
     source          1..1942
                     /organism="Acropora digitifera"
                     /mol_type="mRNA"
```

Change region shown

Customize view

Analyze this sequence
- Run BLAST
- Pick Primers
- Highlight Sequence Features
- Find in this Sequence

Reference sequence information

RefSeq protein product
See the reference protein sequence for PREDICTED: serine/threonine-protein phosphatase 2A activator-like (XP_015775442.1).

More about the gene LOC107353620

LOC107353620 gene

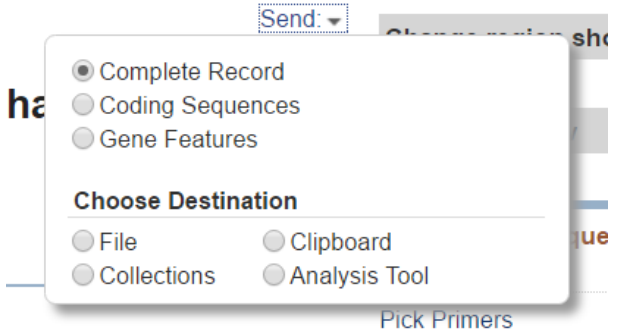
Related information
- Annotated Genomic
- BioProject
- Gene
- Protein
- Taxonomy

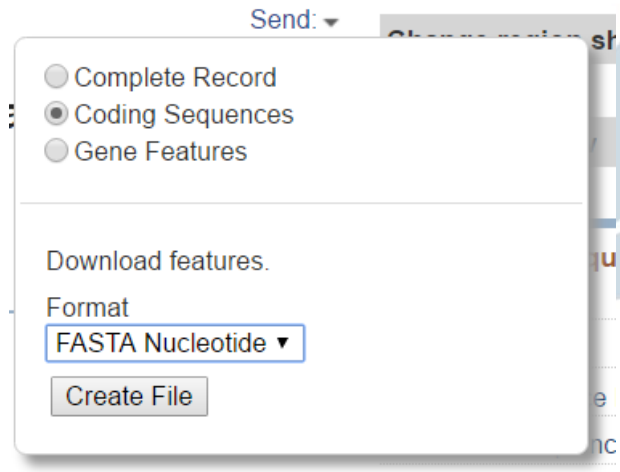
Recent activity

Turn Off Clear

Search NCBI

```
FEATURES             Location/Qualifiers
     source          1..1942
                     /organism="Acropora digitifera"
                     /mol_type="mRNA"
                     /db_xref="taxon:70779"
                     /chromosome="Unknown"
                     /country="Japan:Okinawa, Kunigami, Oku"
     gene            1..1942
                     /gene="LOC107353620"
                     /note="Derived by automated computational analysis using
                     gene prediction method: Gnomon. Supporting evidence
                     includes similarity to: 6 mRNAs, 3 ESTs, 25 Proteins, and
                     100% coverage of the annotated genomic feature by RNAseq
                     alignments, including 25 samples with support for all
                     annotated introns"
                     /db_xref="GeneID:107353620"
     CDS             659..1315
                     /gene="LOC107353620"
                     /codon_start=1
                     /product="serine/threonine-protein phosphatase 2A
                     activator-like"
                     /protein_id="XP_015775442.1"
                     /db_xref="GI:1005477361"
                     /db_xref="GeneID:107353620"
                     /translation="MPLQQSVHSLVQPLLPDKFLGAAIELTAYLKDAFGNKTRIDYGT
                     GHEASFAAFLCCLFKLRVLDQSDCAAIVFKVFQRYLELMRRLQLTYRMEPAGSQGVWG
                     LDDFQFLPFIWGSAQLIGHTSLEPQHFTCEKNVEEHHNKYMFLGCIRFINQMKRGPFA
                     EHSNTLWGISSVKTWEKVNSGLMKMYKAEVLSKFPVIQHFVFGTLMSIKEGETFKKPL
                     "
ORIGIN
        1 tcttgagtac tgtagtcttt cccagcattg actgacctag gctggtgaac aatgtttttt
       61 ttttttttt ttttttcat ttgtttctaa gggtaggaga aactactatg aaaggctggg
      121 aaagtcaatt ttatttcata cattaacttg tcactatata agtatgcttg tcactataaa
      181 ctgaaaaatt aaatgtgatt cttttaattc cattttcttt tgtttttttac attttgcaaa
      241 gtcctcaaaa agggtgatat agcaaacact tttctcattc acagctttcg cacatccttg
      301 gttattgtga cttggccaaa agataggaaa atttttcctg ctccagggag aaatataata
      361 ttgcttgatt ctcagaatgg cctgcttata atctgagaaa ttgatcattt ctggtaatta
      421 aggtattgcc ttaatttatt tgtaatggca attggatttt gcttgatgca gcttattaag
      481 aaagccatga attagaagag tccacatcat ttggttgcag ttaagtaatt tccatagttt
```



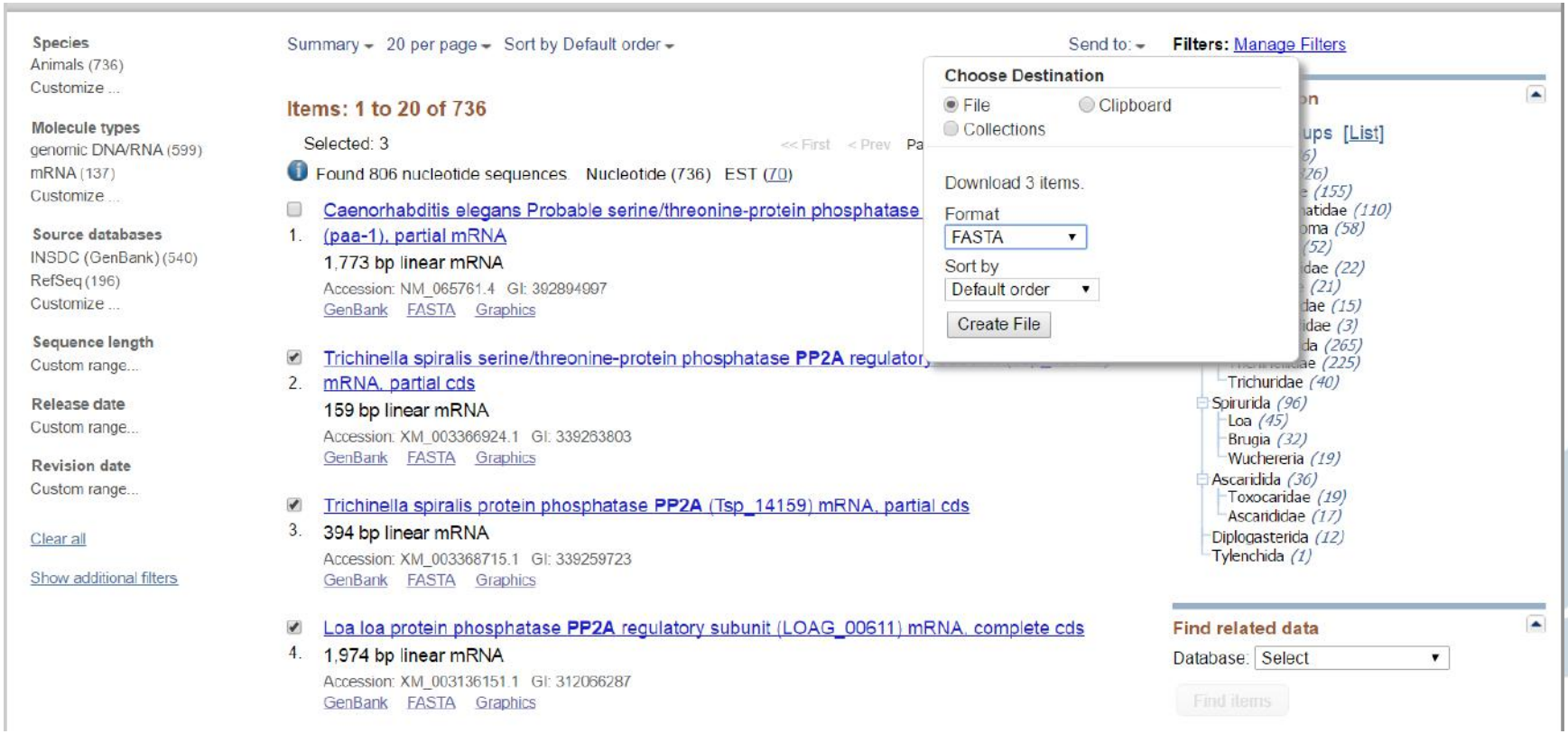


Search NCBI

sequence.txt

```
>lcl|XM_015919956.1_cds_XP_015775442.1_1 [gene=LOC107353620] [protein=serine/threonine-protein phosphatase 2A activator-like] [protein_id=XP_015775442.1] [location=659..1315]
ATGCCTCTTCAACAGAGTGTTCACTCGTTAGTTCAACCTCTTCTACCAGACAAGTTCCTTGGGGCTGCCA
TTGAATTAACTGCATATTTAAAAGATGCCTTTGGAAATAAAACAAGAATAGACTATGGAACAGGTCATGA
AGCTTCCTTTGCTGCATTTCTTTGTTGTTTATTCAAGCTCAGAGTATTGGACCAAAGTGACTGTGCTGCT
ATTGTGTTCAAGGTTTTTCAGAGGTATTTAGAACTGATGAGACGATTGCAGCTCACTTACAGAATGGAAC
CAGCTGGCAGTCAGGGTGTGTGGGGGCTGGATGATTTTCAGTTTCTCCCTTTCATTTGGGGAAGTGCTCA
GCTGATAGGCCATACAAGTCTAGAGCCACAGCACTTCACTTGTGAAAAAAACGTAGAGGAGCATCATAAC
AAGTACATGTTCCTGGGCTGCATCCGTTTTATAAACCAAATGAAAAGAGGACCCTTTGCAGAACATTCCA
ACACTTTGTGGGGAATAAGCTCTGTTAAAACATGGGAAAAAGTAAACTCTGGTTTGATGAAAATGTATAA
AGCTGAGGTTCTATCCAAGTTCCCAGTCATTCAGCATTTTGTTTTTGGTACATTAATGTCTATAAAAGAA
GGAGAAACGTTTAAAAAACCCCTGTAA
```

Search NCBI

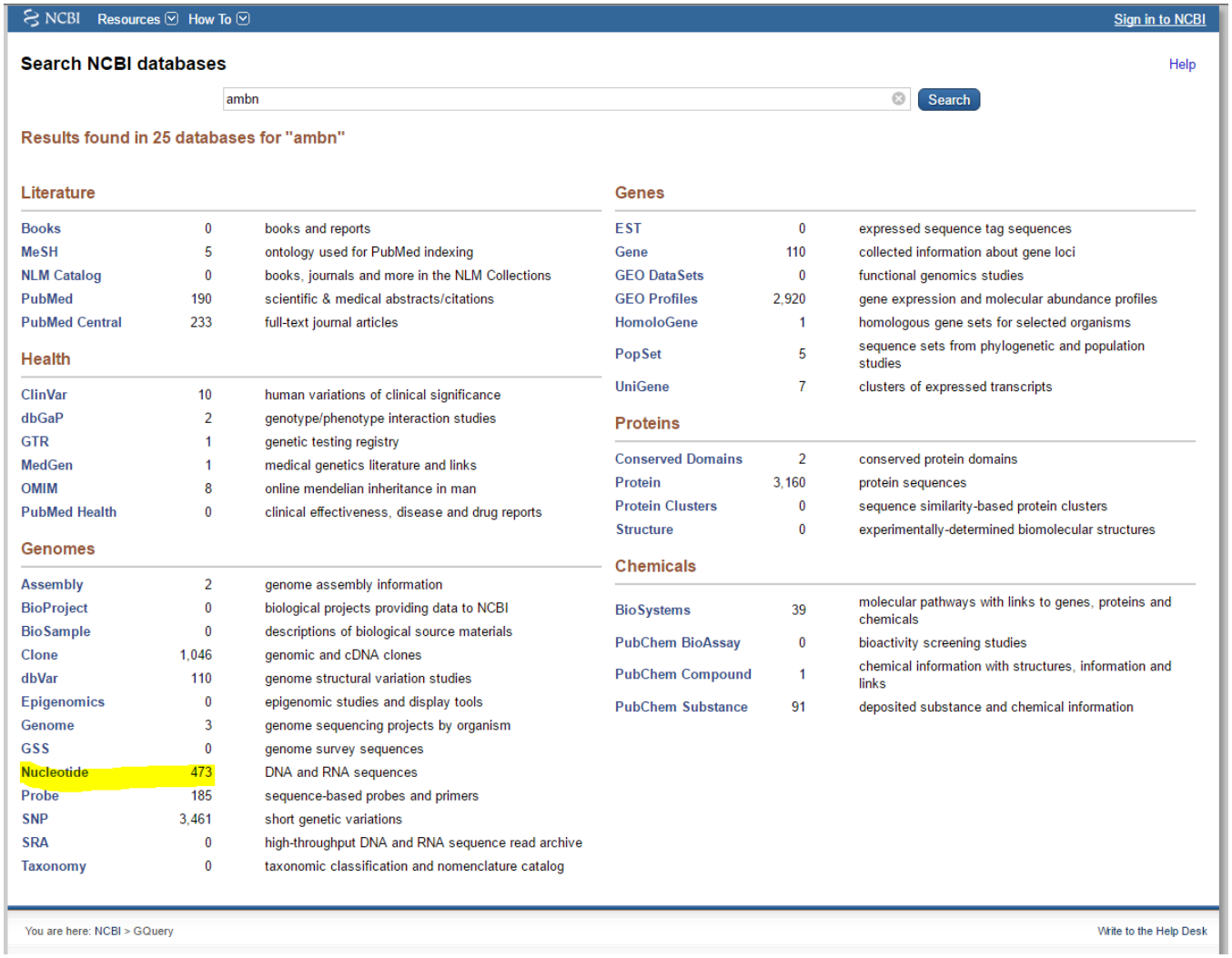

Species
Animals (736)
Customize ...
Molecule types
genomic DNA/RNA (599)
mRNA (137)
Customize ...
Source databases
INSDC (GenBank) (540)
RefSeq (196)
Customize ...
Sequence length
Custom range...
Release date
Custom range...
Revision date
Custom range...
Clear all
Show additional filters
Summary 20 per page Sort by Default order
Send to: Filters: Manage Filters
Items: 1 to 20 of 736
Selected: 3
Found 806 nucleotide sequences Nucleotide (736) EST (70)
1. Caenorhabditis elegans Probable serine/threonine-protein phosphatase (paa-1), partial mRNA
1,773 bp linear mRNA
Accession: NM_065761.4 GI: 392894997
GenBank FASTA Graphics
2. Trichinella spiralis serine/threonine-protein phosphatase PP2A regulator mRNA, partial cds
159 bp linear mRNA
Accession: XM_003366924.1 GI: 339263803
GenBank FASTA Graphics
3. Trichinella spiralis protein phosphatase PP2A (Tsp_14159) mRNA, partial cds
394 bp linear mRNA
Accession: XM_003368715.1 GI: 339259723
GenBank FASTA Graphics
4. Loa loa protein phosphatase PP2A regulatory subunit (LOAG_00611) mRNA, complete cds
1,974 bp linear mRNA
Accession: XM_003136151.1 GI: 312066287
GenBank FASTA Graphics
Choose Destination
File Clipboard
Collections
Download 3 items.
Format
FASTA
Sort by
Default order
Create File
Trichuridae (40)
Spirurida (96)
Loa (45)
Brugia (32)
Wuchereria (19)
Ascaridida (36)
Toxocaridae (19)
Ascarididae (17)
Diplogasterida (12)
Tylenchida (1)
Find related data
Database: Select
Find items

Search NCBI


Courier New
11
3 total sequences
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
gi|339263803|ref|XM_003366924.
gi|339259723|ref|XM_003368715.
gi|312066287|ref|XM_003136151.


Courier New
11
3 total sequences
Mode: Select / Slide
Selection: null
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
gi|339263803|ref|XM_003366924.
gi|339259723|ref|XM_003368715.
gi|312066287|ref|XM_003136151.

Nucleotide Search



NCBI Resources How To Sign in to NCBI

Search NCBI databases Help

ambn Search

Results found in 25 databases for "ambn"

Literature

Books	0	books and reports
MeSH	5	ontology used for PubMed indexing
NLM Catalog	0	books, journals and more in the NLM Collections
PubMed	190	scientific & medical abstracts/citations
PubMed Central	233	full-text journal articles

Health

ClinVar	10	human variations of clinical significance
dbGaP	2	genotype/phenotype interaction studies
GTR	1	genetic testing registry
MedGen	1	medical genetics literature and links
OMIM	8	online mendelian inheritance in man
PubMed Health	0	clinical effectiveness, disease and drug reports

Genomes

Assembly	2	genome assembly information
BioProject	0	biological projects providing data to NCBI
BioSample	0	descriptions of biological source materials
Clone	1,046	genomic and cDNA clones
dbVar	110	genome structural variation studies
Epigenomics	0	epigenomic studies and display tools
Genome	3	genome sequencing projects by organism
GSS	0	genome survey sequences
Nucleotide	473	DNA and RNA sequences
Probe	185	sequence-based probes and primers
SNP	3,461	short genetic variations
SRA	0	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	0	expressed sequence tag sequences
Gene	110	collected information about gene loci
GEO DataSets	0	functional genomics studies
GEO Profiles	2,920	gene expression and molecular abundance profiles
HomoloGene	1	homologous gene sets for selected organisms
PopSet	5	sequence sets from phylogenetic and population studies
UniGene	7	clusters of expressed transcripts

Proteins

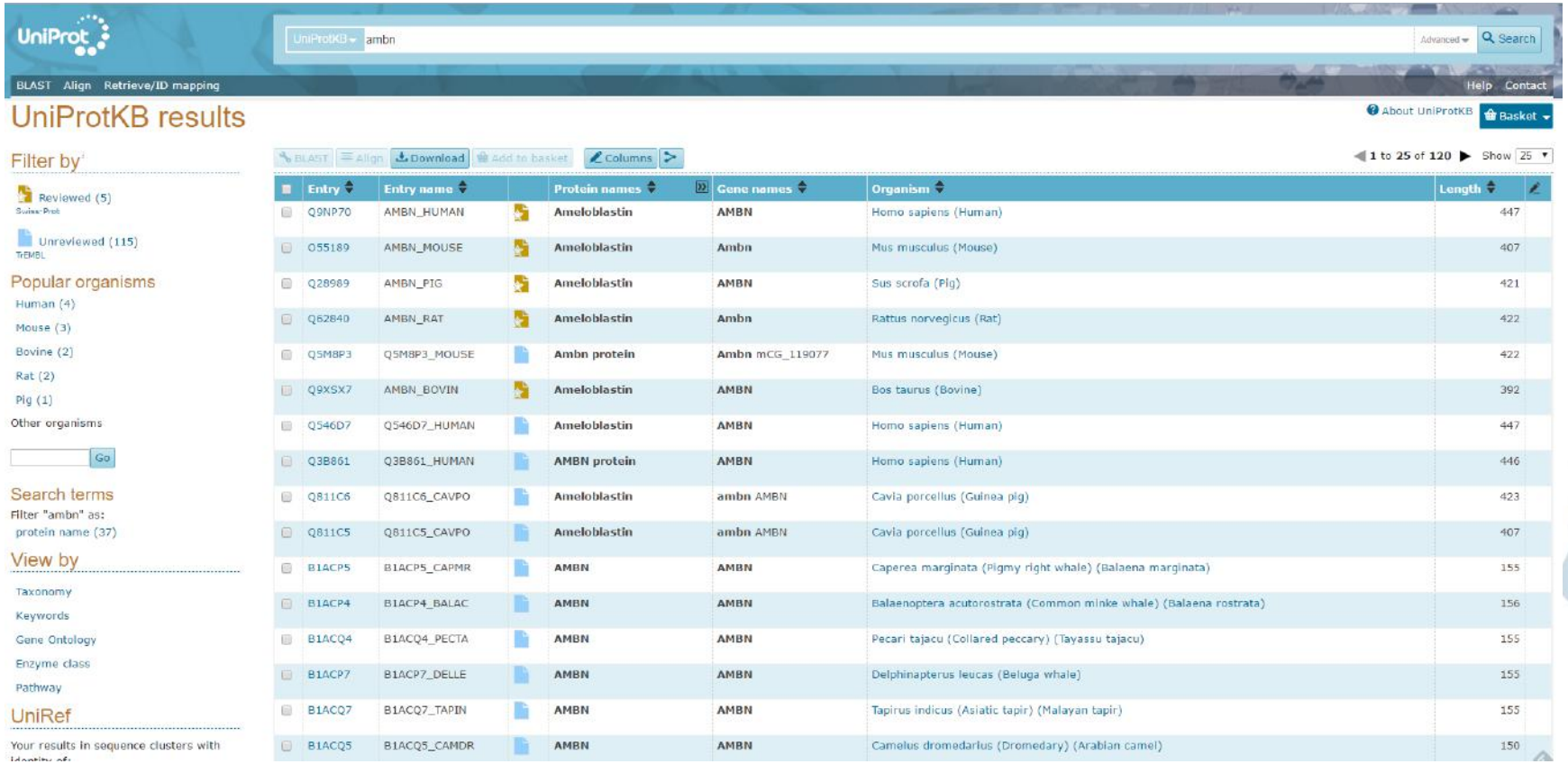
Conserved Domains	2	conserved protein domains
Protein	3,160	protein sequences
Protein Clusters	0	sequence similarity-based protein clusters
Structure	0	experimentally-determined biomolecular structures

Chemicals

BioSystems	39	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	0	bioactivity screening studies
PubChem Compound	1	chemical information with structures, information and links
PubChem Substance	91	deposited substance and chemical information

You are here: NCBI > GQuery Write to the Help Desk

UniProt



Entry	Entry name	Protein names	Gene names	Organism	Length
Q9NP70	AMBN_HUMAN	Ameloblastin	AMBN	Homo sapiens (Human)	447
O55189	AMBN_MOUSE	Ameloblastin	Ambn	Mus musculus (Mouse)	407
Q28989	AMBN_PIG	Ameloblastin	AMBN	Sus scrofa (Pig)	421
Q62840	AMBN_RAT	Ameloblastin	Ambn	Rattus norvegicus (Rat)	422
Q5M8P3	Q5M8P3_MOUSE	Ambn protein	Ambn mCG_119077	Mus musculus (Mouse)	422
Q9XSX7	AMBN_BOVIN	Ameloblastin	AMBN	Bos taurus (Bovine)	392
Q546D7	Q546D7_HUMAN	Ameloblastin	AMBN	Homo sapiens (Human)	447
Q3B861	Q3B861_HUMAN	AMBN protein	AMBN	Homo sapiens (Human)	446
Q811C6	Q811C6_CAVPO	Ameloblastin	ambn AMBN	Cavia porcellus (Guinea pig)	423
Q811C5	Q811C5_CAVPO	Ameloblastin	ambn AMBN	Cavia porcellus (Guinea pig)	407
B1ACP5	B1ACP5_CAPMR	AMBN	AMBN	Caperea marginata (Pigmy right whale) (Balaena marginata)	155
B1ACP4	B1ACP4_BALAC	AMBN	AMBN	Balaenoptera acutorostrata (Common minke whale) (Balaena rostrata)	156
B1ACQ4	B1ACQ4_PECTA	AMBN	AMBN	Pecari tajacu (Collared peccary) (Tayassu tajacu)	155
B1ACP7	B1ACP7_DELLE	AMBN	AMBN	Delphinapterus leucas (Beluga whale)	155
B1ACQ7	B1ACQ7_TAPIN	AMBN	AMBN	Tapirus indicus (Asiatic tapir) (Malayan tapir)	155
B1ACQ5	B1ACQ5_CAMDR	AMBN	AMBN	Camelus dromedarius (Dromedary) (Arabian camel)	150

UniProt

PTM / Processing

Molecule processing

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Signal peptide	1 – 26	26	Sequence analysis			Add BLAST
Chain	27 – 447	421	Ameloblastin		PRO_0000001192	Add BLAST

Amino acid modifications

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Modified residue	37 – 37	1	Hydroxyproline By similarity			
Modified residue	43 – 43	1	Phosphoserine By similarity			
Glycosylation	112 – 112	1	O-linked (GalNAc...) By similarity			

Keywords - PTM

Glycoprotein, Hydroxylation, Phosphoprotein

Proteomic databases

PaxDb	Q9NP70.
PRIDE	Q9NP70.

PTM databases

PhosphoSite	Q9NP70.

Expression

Tissue specificity

Ameloblast-specific. Located at the Tomes processes of secretory ameloblasts and in the sheath space between rod-interrod enamel.

Gene expression databases

UniProt

Interaction

GO - Molecular function

- growth factor activity Source: BHF-UCL

Protein-protein interaction databases

BioGrid	106756. 2 interactions.
STRING	9606.ENSP00000313809.

Structure

3D structure databases

ProteinModelPortal	Q9NP70.
ModBase	Search...
MobiDB	Search...

Family & Domains

Domains and Repeats

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Repeat	189 – 201	13	1			Add BLAST
Repeat	202 – 214	13	2			Add BLAST

Sequence similarities

Belongs to the ameloblastin family. Curated

Keywords - Domain

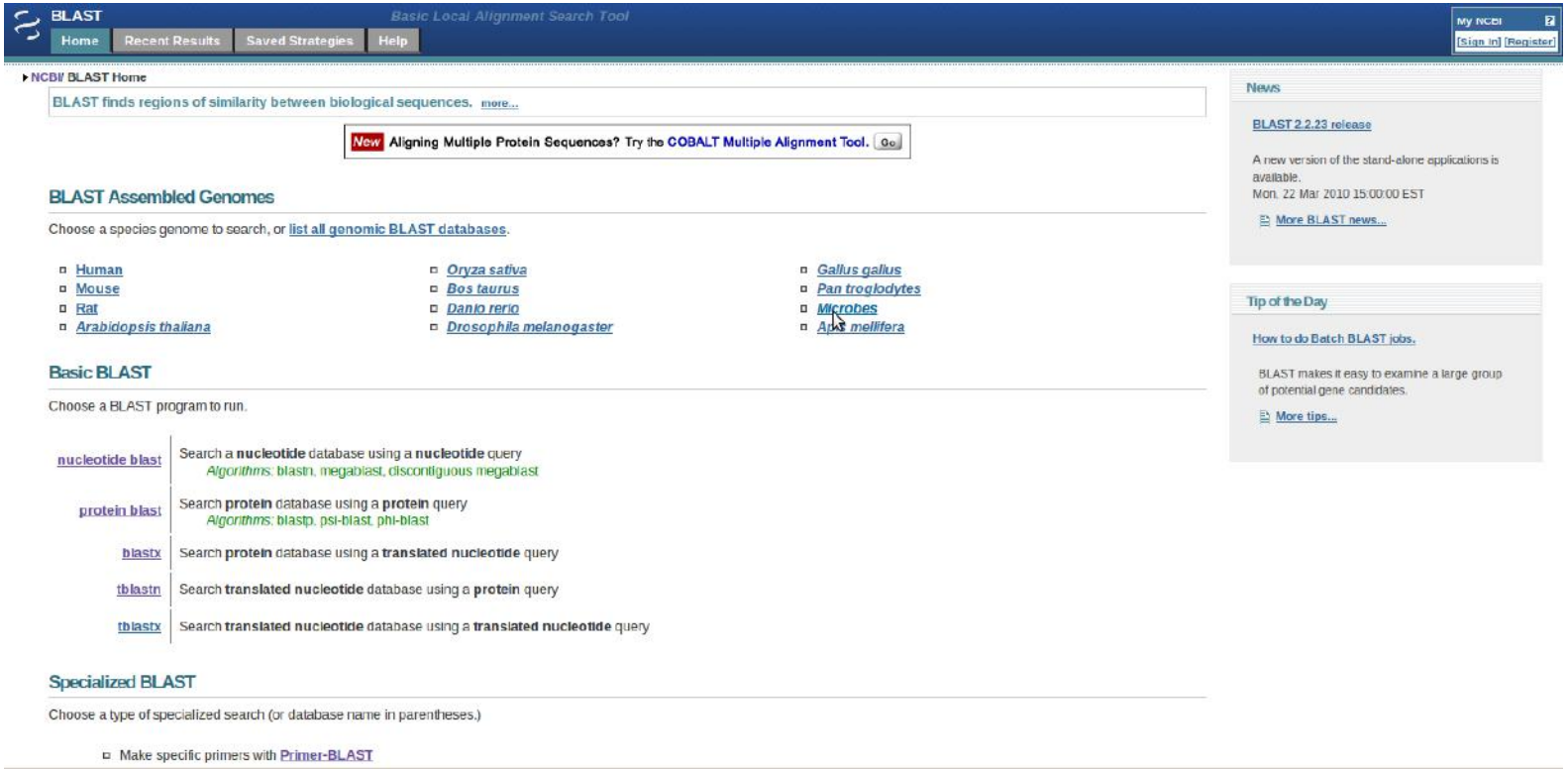
Repeat, Signal

Hands-On 2

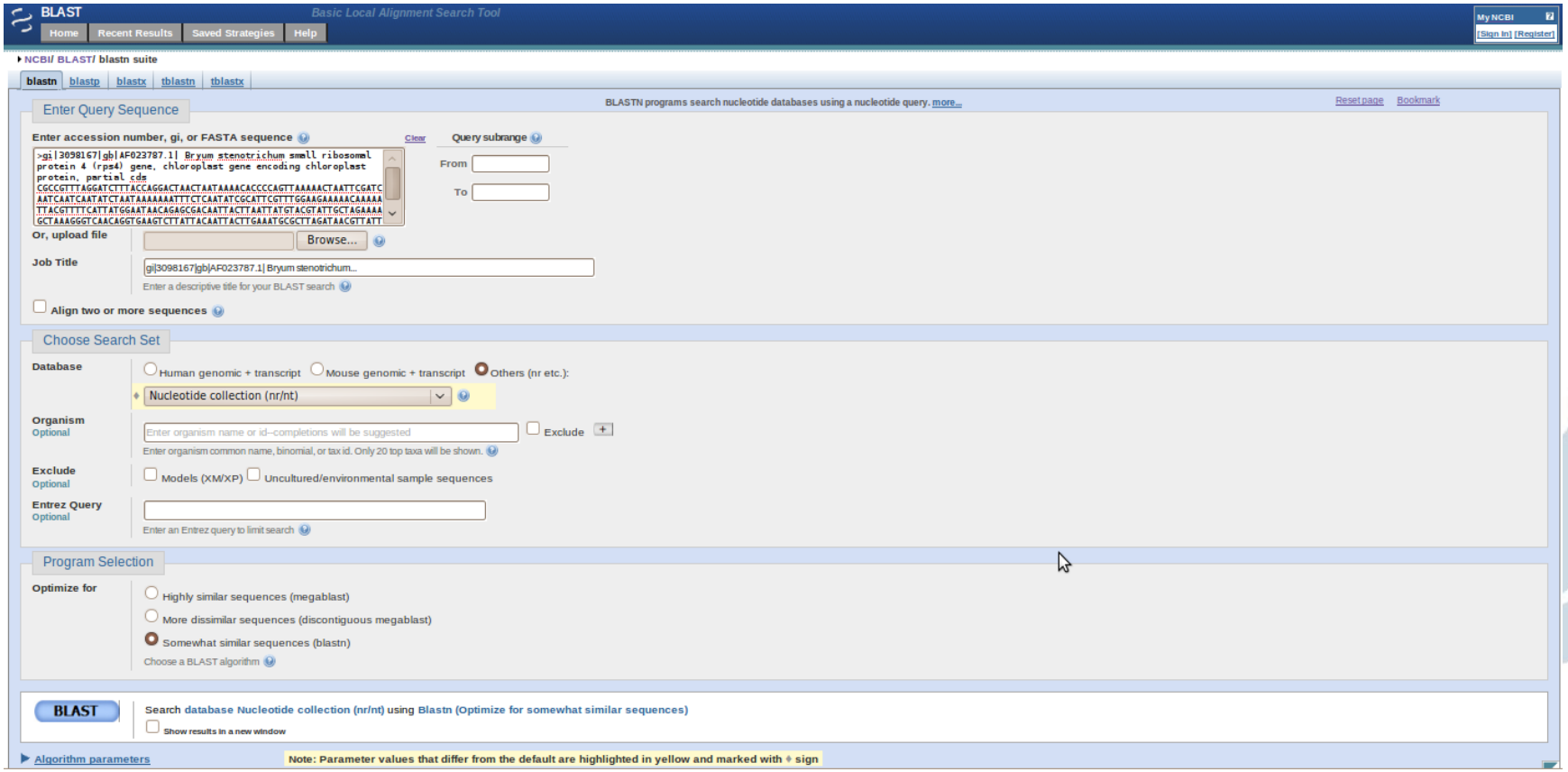
1. Escolher dois genes:
   - Acetylcholinesterase (ACHE)
   - Ameloblastin (AMBN)
   - Enamelin (ENAM)
   - Hemopexin (HPX)

2. Ir à página do NCBI e descarregar sequencias de 10 espécies

NCBI – BLAST (Basic Local Alignment Serach Tool)



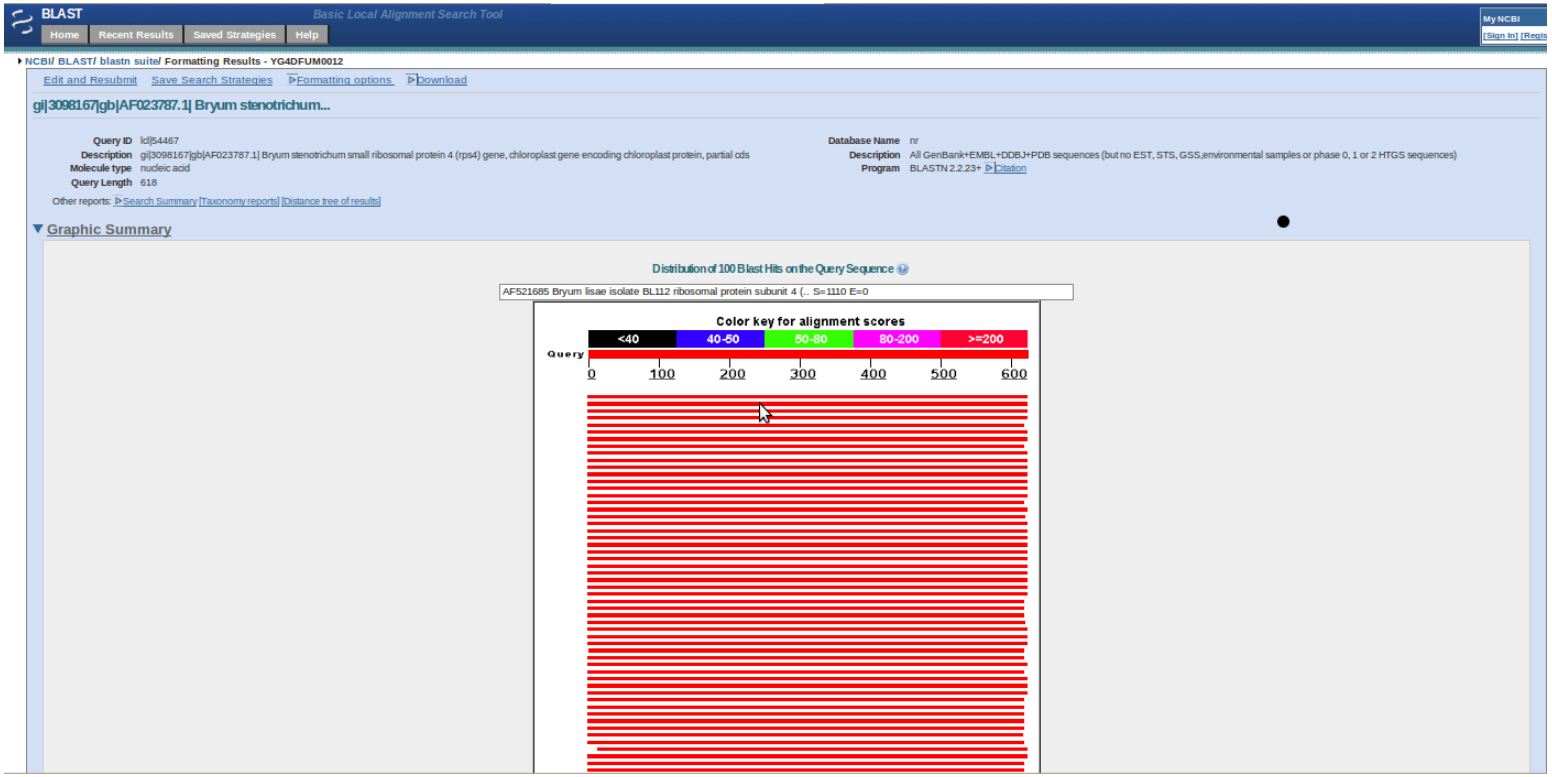
Blast Search



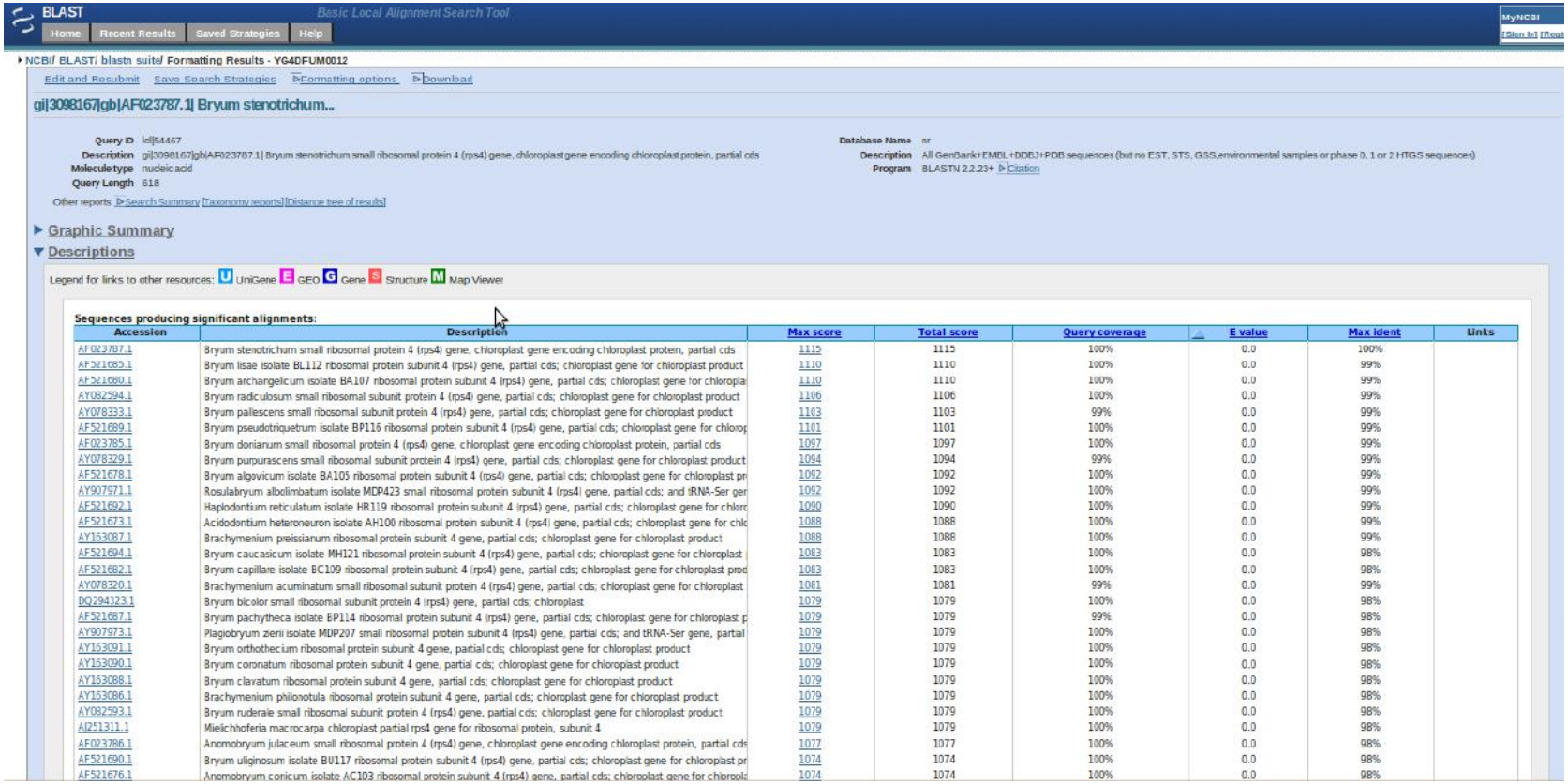
Blast methods

- blastn
- blastp
- blastx
- tblastn
- tblastx

NCBI – nucleotide BLAST result



NCBI – nucleotide BLAST result



E-values: 10^{-4} ***often considered*** good enough for an assumption of homology

```
Query  961   GGAGGTGCACAAGGCTCCCCTATGCCGGAGGCCAACCCAGACAATCTAGAAAACCCAGCT  1020
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1062  GGAGGTGCACAAGGCTCCCCTATGCCGGAGGCCAACCCAGACAATCTAGAAAACCCAGCT  1121

Query  1021  TTCCTTACAGAGCTAGAACCTGCTCCCCACGCAGGGCTCCTTGCTCTCCCTAAGGATGAC  1080
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1122  TTCCTTACAGAGCTAGAACCTGCTCCCCACGCAGGGCTCCTTGCTCTCCCTAAGGATGAC  1181

Query  1081  ATTCCCGGCCTGCCAAGGAGCCCTTCAGGGAAGATGAAGGGACTCCCCAGCGTCACCCCA  1140
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1182  ATTCCCGGCCTGCCAAGGAGCCCTTCAGGGAAGATGAAGGGACTCCCCAGCGTCACCCCA  1241

Query  1141  GCAGCTGCTGACCCACTGATGACCCCTGAATTAGCTGATGTTTATAGGACCTACGATGCT  1200
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1242  GCAGCTGCTGACCCACTGATGACCCCTGAATTAGCTGATGTTTATAGGACCTACGATGCT  1301

Query  1201  GACATGACCACATCCGTGGATTTCCAGGAAGAAGCAACCATGGATACCACGATGGCCCCA  1260
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1302  GACATGACCACATCCGTGGATTTCCAGGAAGAAGCAACCATGGATACCACGATGGCCCCA  1361

Query  1261  AACTCTCTGCAAACATCCATGCCAGGAAACAAAGCCCAGGAGCCCGAGATGATGCATGAC  1320
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1362  AACTCTCTGCAAACATCCATGCCAGGAAACAAAGCCCAGGAGCCCGAGATGATGCATGAC  1421

Query  1321  GCATGGCATTTCCAAGAGCCCTG  1343
              |||||||||||||||||||||||
Sbjct  1422  GCATGGCATTTCCAAGAGCCCTG  1444
```

Range 2: 633 to 671 GenBank Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
53.6 bits(58)	0.011	35/39(90%)	0/39(0%)	Plus/Plus

```
Query  610  CTCCCAGGATTGGATTTTGCTGATCCACAAGGTTCAACA  648
            |||||||| | ||||||||||||||||||| || ||
Sbjct  633  CTCCCAGGAGTAGATTTTGCTGATCCACAAGGTCCATCA  671
```

Range 3: 711 to 749 GenBank Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
53.6 bits(58)	0.011	35/39(90%)	0/39(0%)	Plus/Plus

```
Query  532  CTCCCAGGAGTAGATTTTGCTGATCCACAAGGTCCATCA  570
            |||||||| | ||||||||||||||||||| || ||
Sbjct  711  CTCCCAGGATTGGATTTTGCTGATCCACAAGGTTCAACA  749
```

Warnings

- Predictions methods can fail and sometimes accurancy is not available
- Prediction is always made of known issues
- Databases can contain incorrect data
- Avoid overvaloration of results

Hands-On 3

1) Descarregar sequencias da pasta hands_on_3

2) Fazer o “blast” para as sequencias

3) Identificar gene e espécies

4) Descarregar 3 top hits

Methods for multiple sequence alignments

Multiple Sequence Alignments (MSA)

- Web-based
- Local (software)
- Scripts

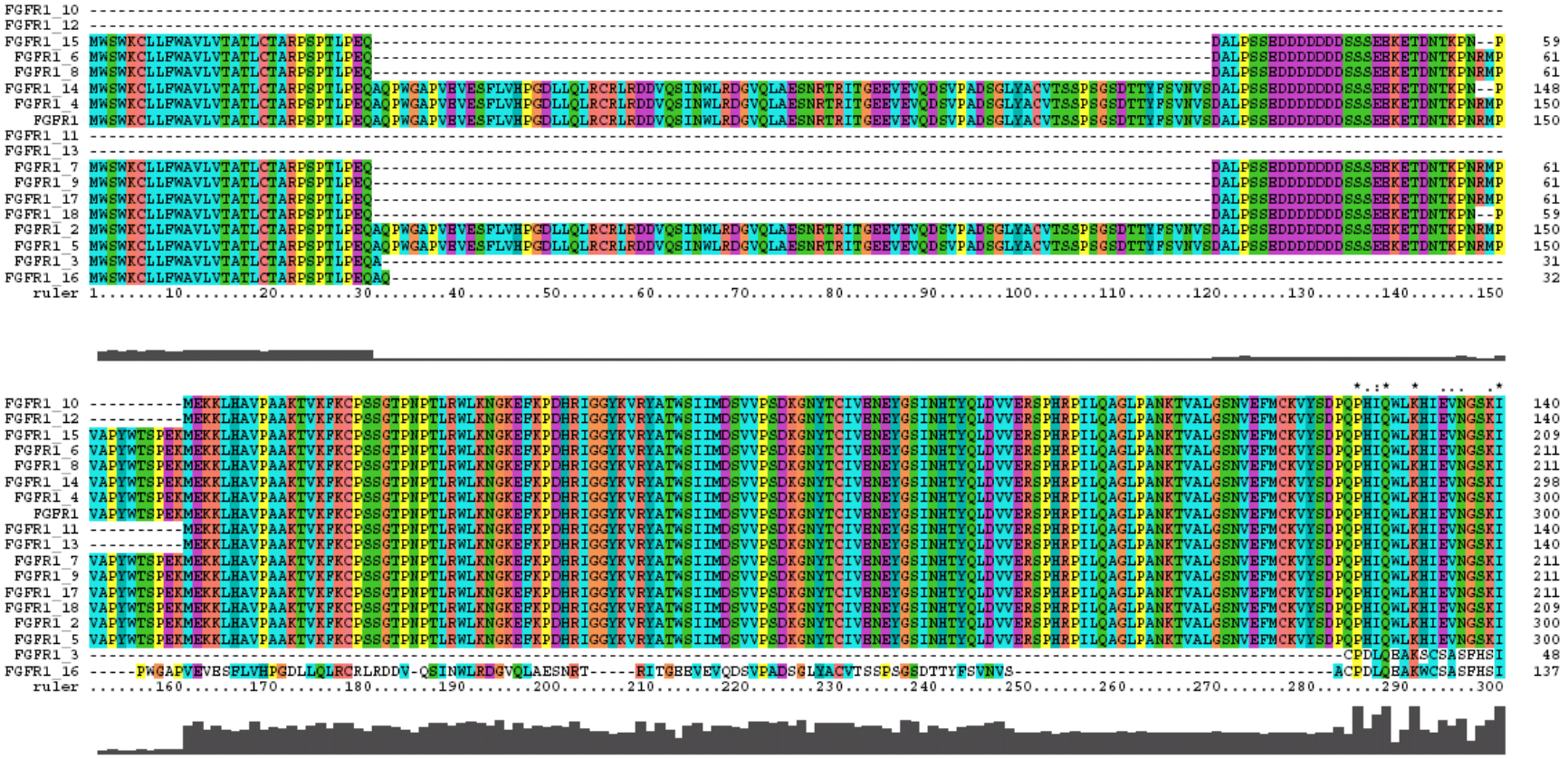
MSA

- Model
- Can be biased
- Quality can be checked (e.g. http://guidance.tau.ac.il/ver2/)
- Parameters:
  - Gap penalties
  - Mismatch
  - Iterations
  - Guiding tree

MSA Algorithms

- ClustalW
- Muscle
- T-Coffee
- MAFT
- ClustalOmega
- Prank

MSA


FGFR1_10
FGFR1_12
FGFR1_15
FGFR1_6
FGFR1_8
FGFR1_14
FGFR1_4
FGFR1
FGFR1_11
FGFR1_13
FGFR1_7
FGFR1_9
FGFR1_17
FGFR1_18
FGFR1_2
FGFR1_5
FGFR1_3
FGFR1_16
ruler
1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150
160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300

Hands-On 4

1) Requisitos: Seaview e/ou Mega6

2) Descarregar sequencias da pasta Hands_on_4

3) Importar ficheiro para o Mega

4) Alinhar usado o Muscle/ClustalW

5) Traduzir para aminoácidos

6) Repetir o passo 3

7) Repetir passos 3 a 6 usando o Seaview

Q & A

INTRODUÇÃO À BIOLOGIA MOLECULAR E BIOINFORMÁTICA:

Bioinformatics 2

Lisboa, 1-3 Junho

João Machado

Bárbara Frazão

Phylogenetic trees

The tree of Life

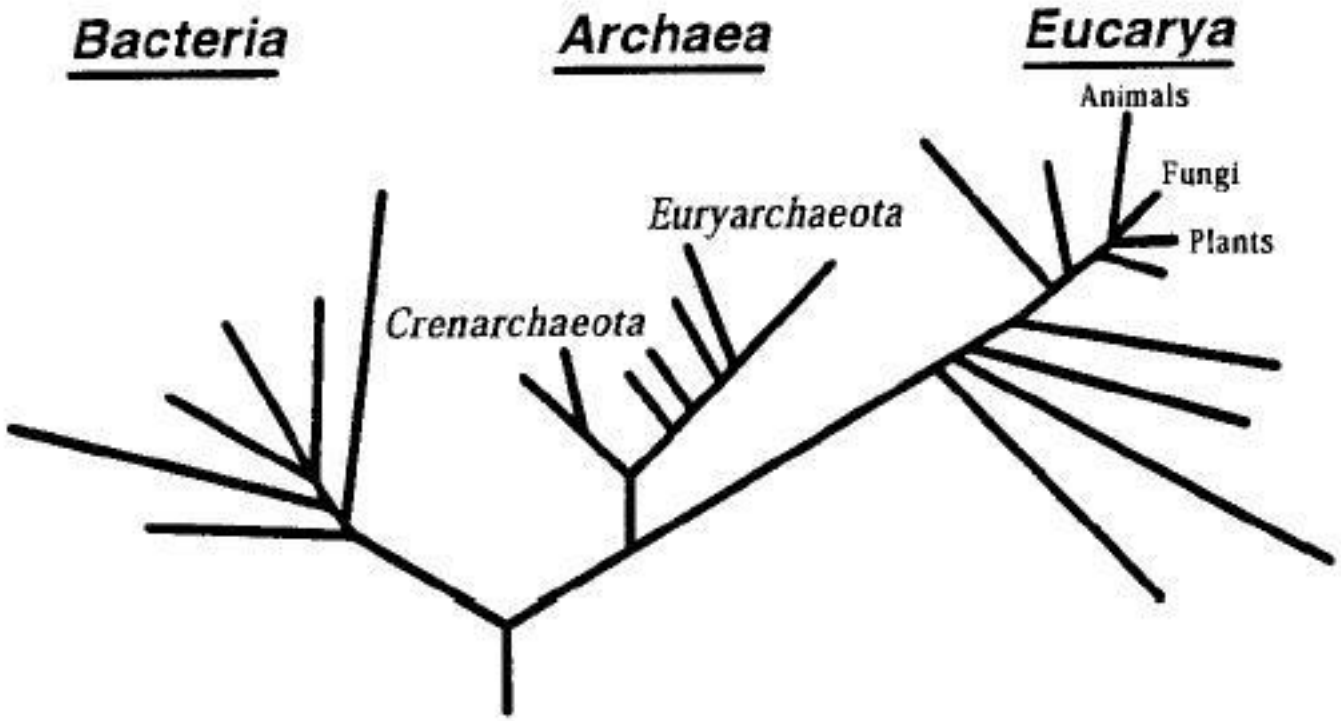
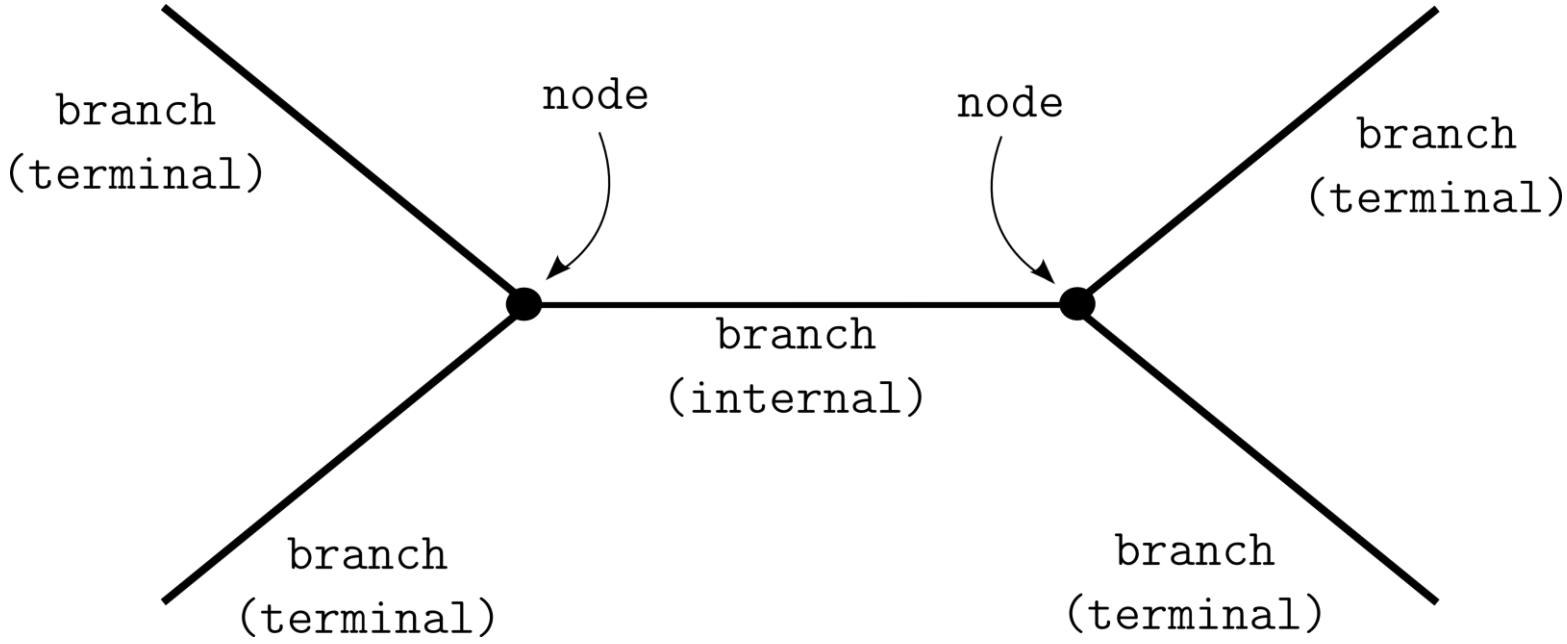


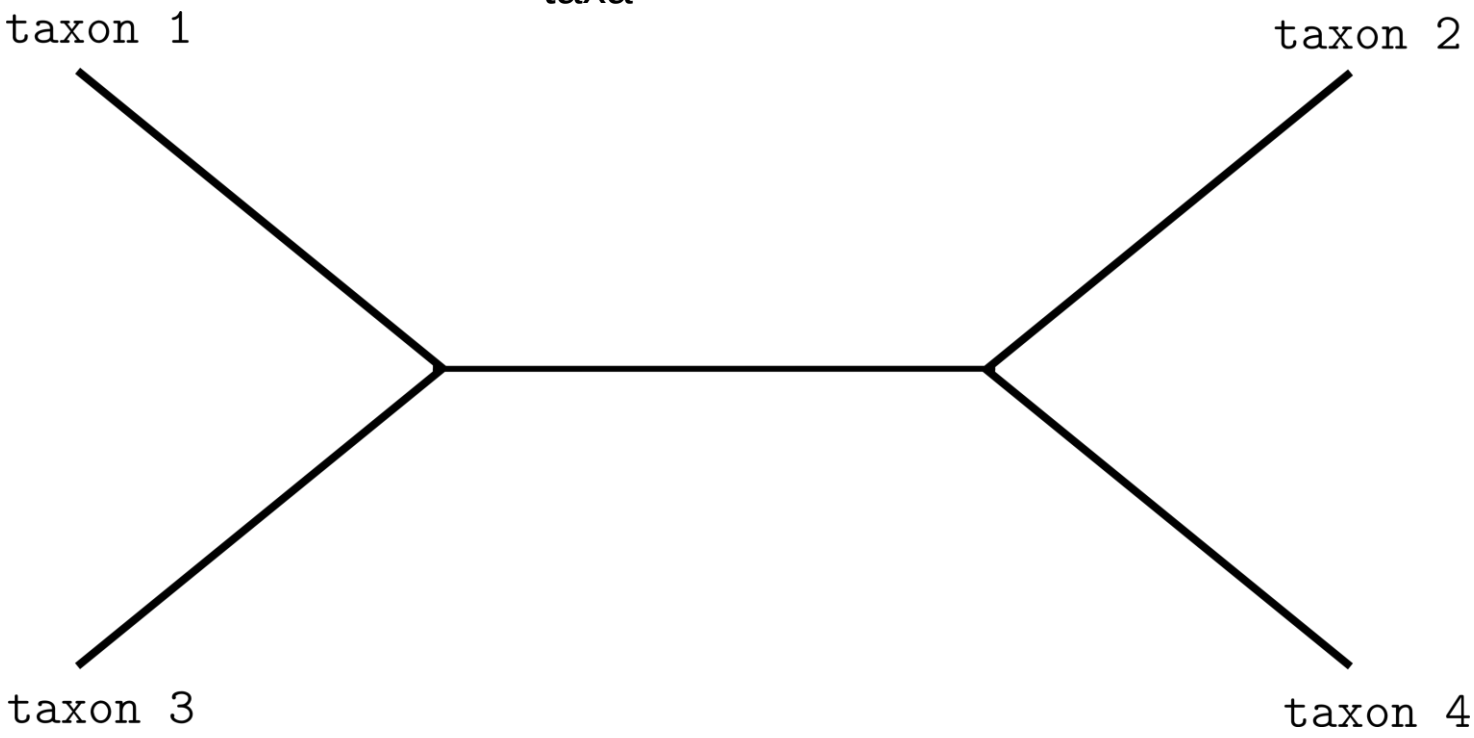
Fig. 1. The basal universal phylogenetic tree inferred from comparative analyses of rRNA sequences (4, 5). The root has been determined by using the paralogous gene couple EF-Tu/EFG (6).

Reading Trees



Reading Trees

Terminals are taxa



Rooted / Unrooted Tree

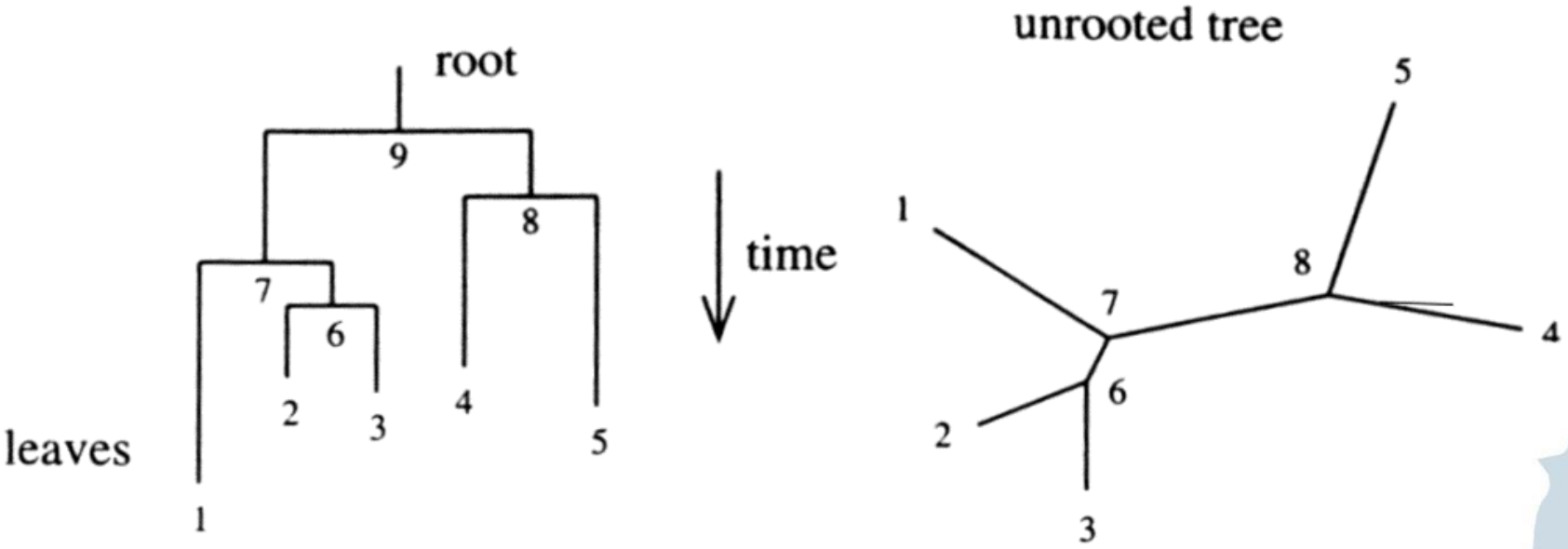
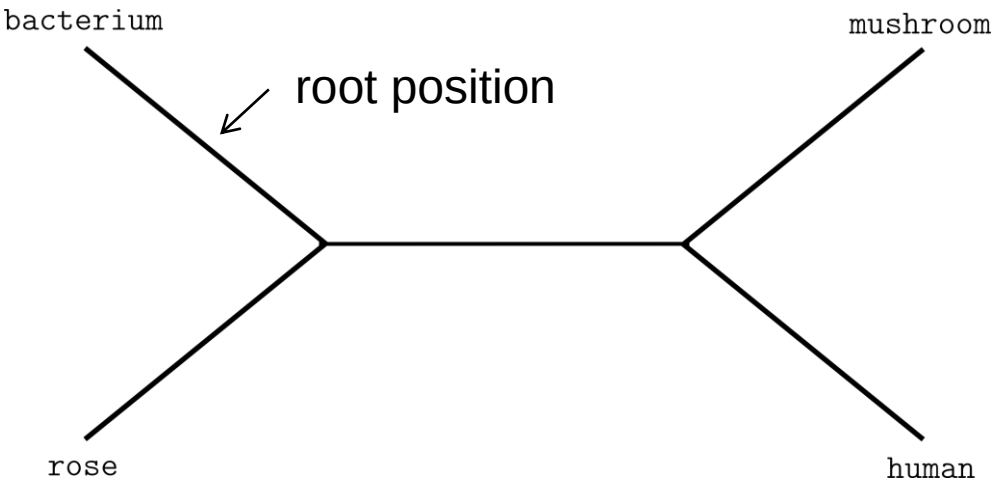


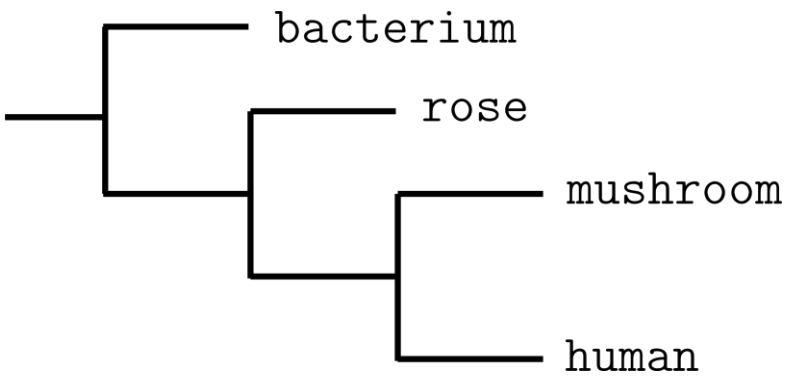
Figure 7.2 *An example of a binary tree, showing the root and leaves, and the direction of evolutionary time (the most recent time being at the bottom of the figure). The corresponding unrooted tree is also shown; the direction of time here is undetermined.*

Rooted / Unrooted Tree

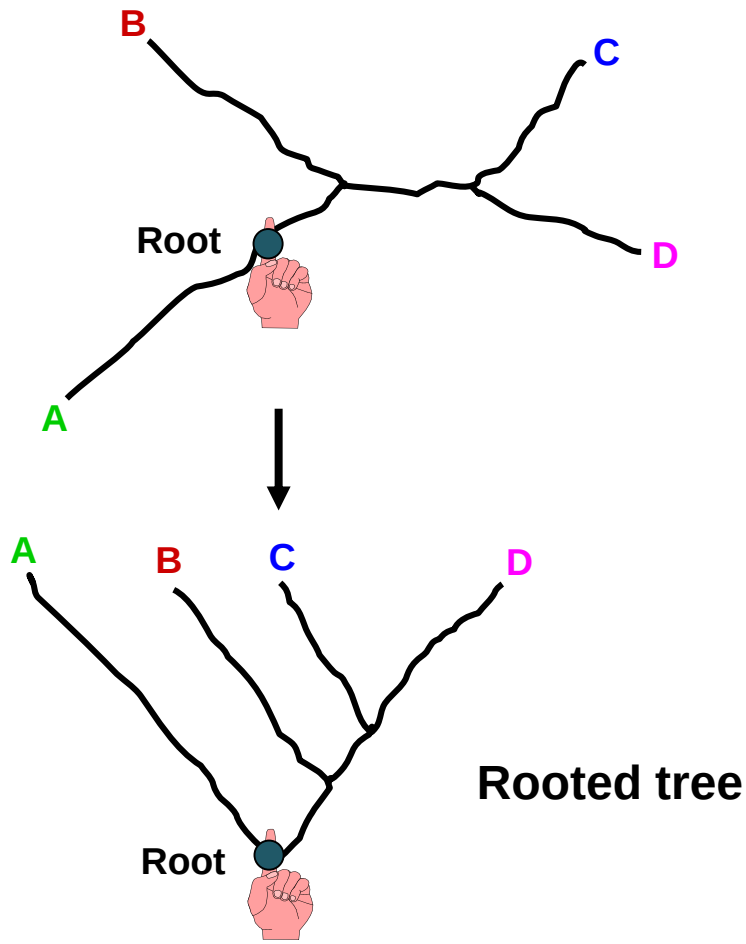
unrooted tree



rooted phylogenetic tree



Rooted / Unrooted Tree



Note that in this rooted tree, taxon A is no more closely related to taxon B than it is to C or D.

Counting Trees

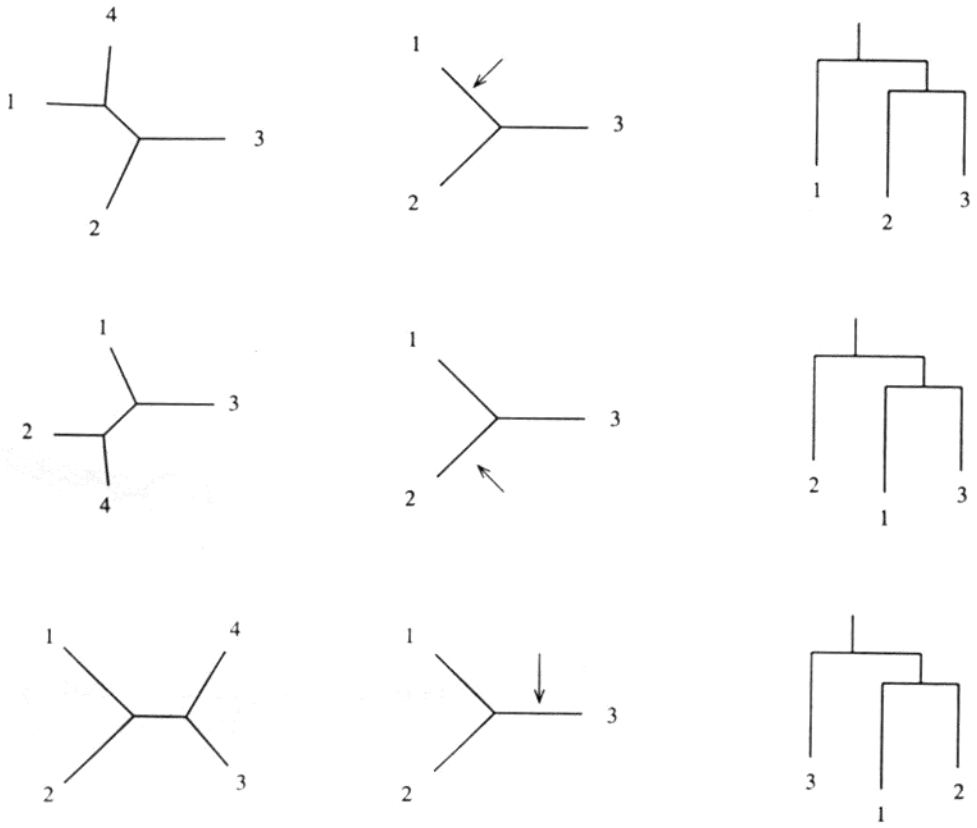
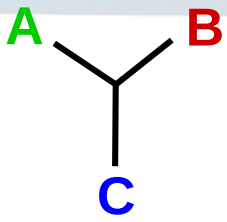
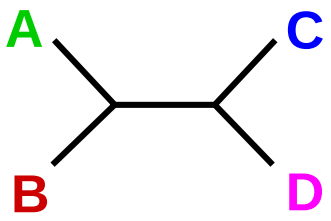
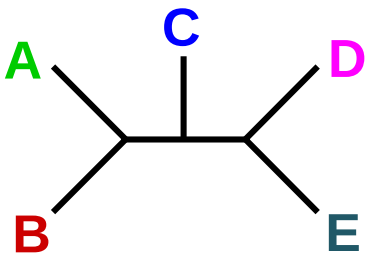


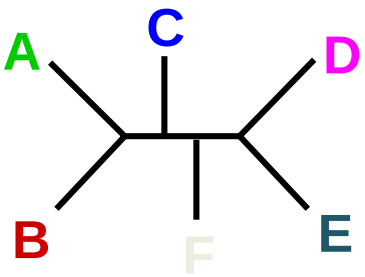
Figure 7.3 *The rooted trees (right-hand column) derived from the unrooted tree for three sequences by picking different edges as positions for the root (arrows).*

Counting Trees







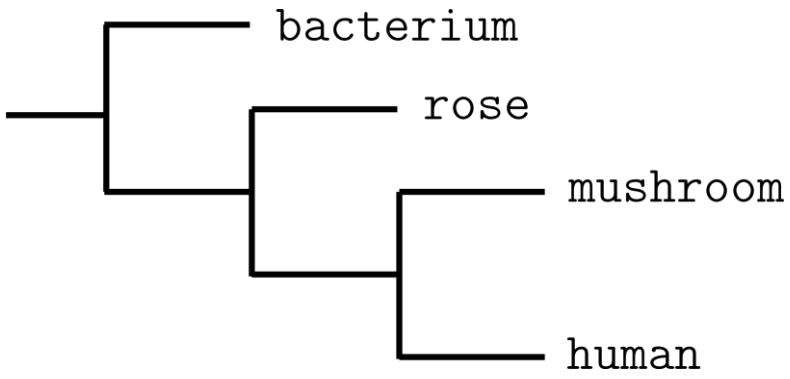


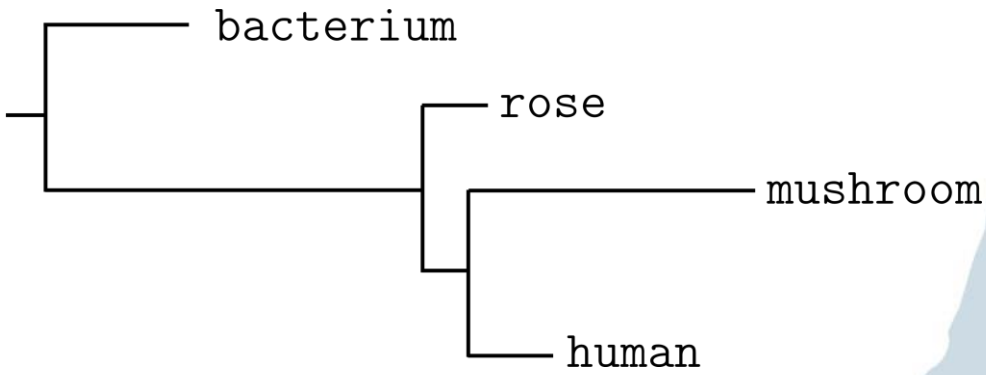
# Taxa (N)	# Unrooted trees
3	1
4	3
5	15
6	105
7	945
8	10,935
9	135,135
10	2,027,025
.	.
.	.
.	.
.	.
30	≈3.58 x 10^{36}

(2N - 5)!! = # unrooted trees for N taxa
(2N- 3)!! = # rooted trees for N taxa

172

Cladogram vs Phylogram



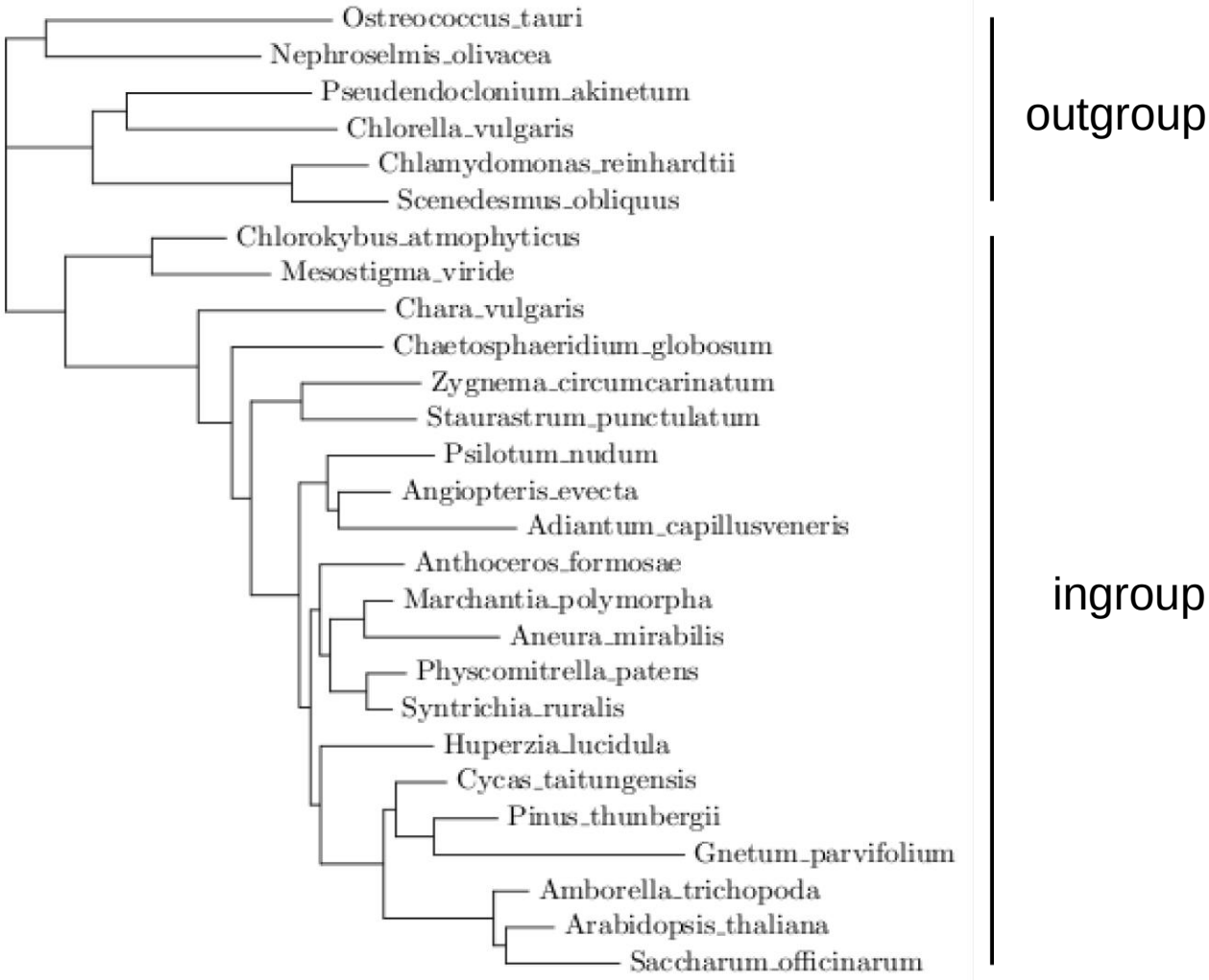


cladogram – arbitrary length branches

phylogram – branch length proportional

to some measure of genetic distance

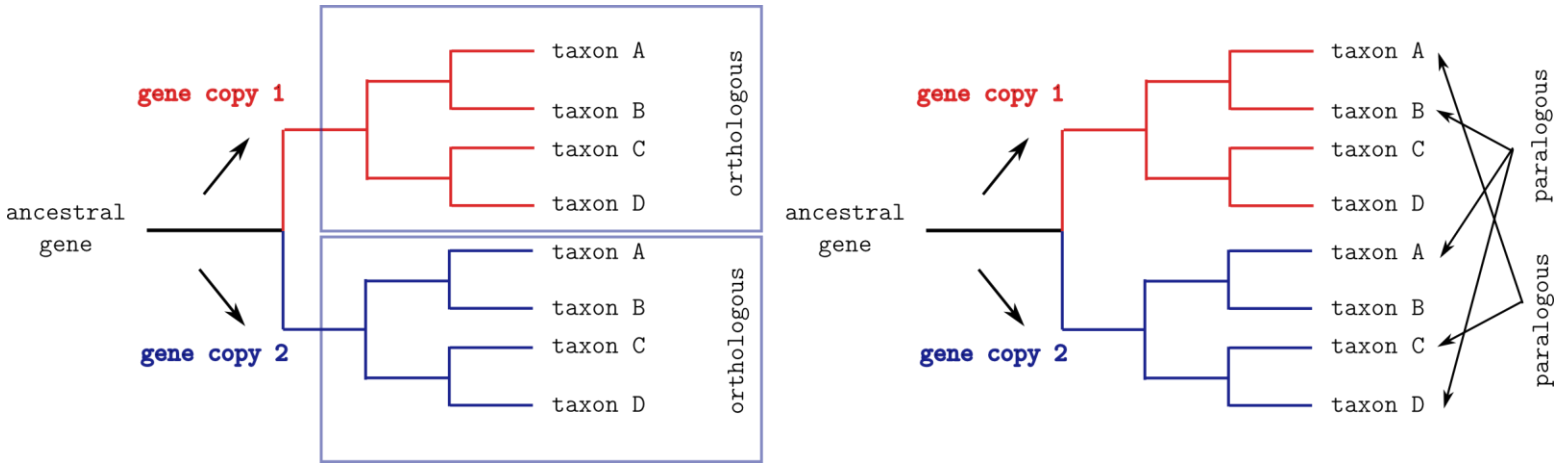
Outgroups


Ostreococcus_tauri
Nephroselmis_olivacea
Pseudendoclonium_akinetum
Chlorella_vulgaris
Chlamydomonas_reinhardtii
Scenedesmus_obliquus
Chlorokybus_atmophyticus
Mesostigma_viride
Chara_vulgaris
Chaetosphaeridium_globosum
Zygnema_circumcarinatum
Staurastrum_punctulatum
Psilotum_nudum
Angiopteris_evecta
Adiantum_capillusveneris
Anthoceros_formosae
Marchantia_polymorpha
Aneura_mirabilis
Physcomitrella_patens
Syntrichia_ruralis
Huperzia_lucidula
Cycas_taitungensis
Pinus_thunbergii
Gnetum_parvifolium
Amborella_trichopoda
Arabidopsis_thaliana
Saccharum_officinarum
outgroup
ingroup

Orthologs and Paralogs

Orthology – homologous gene sequences

Paralogy – gene sequences separated by a gene duplication event



Rooting

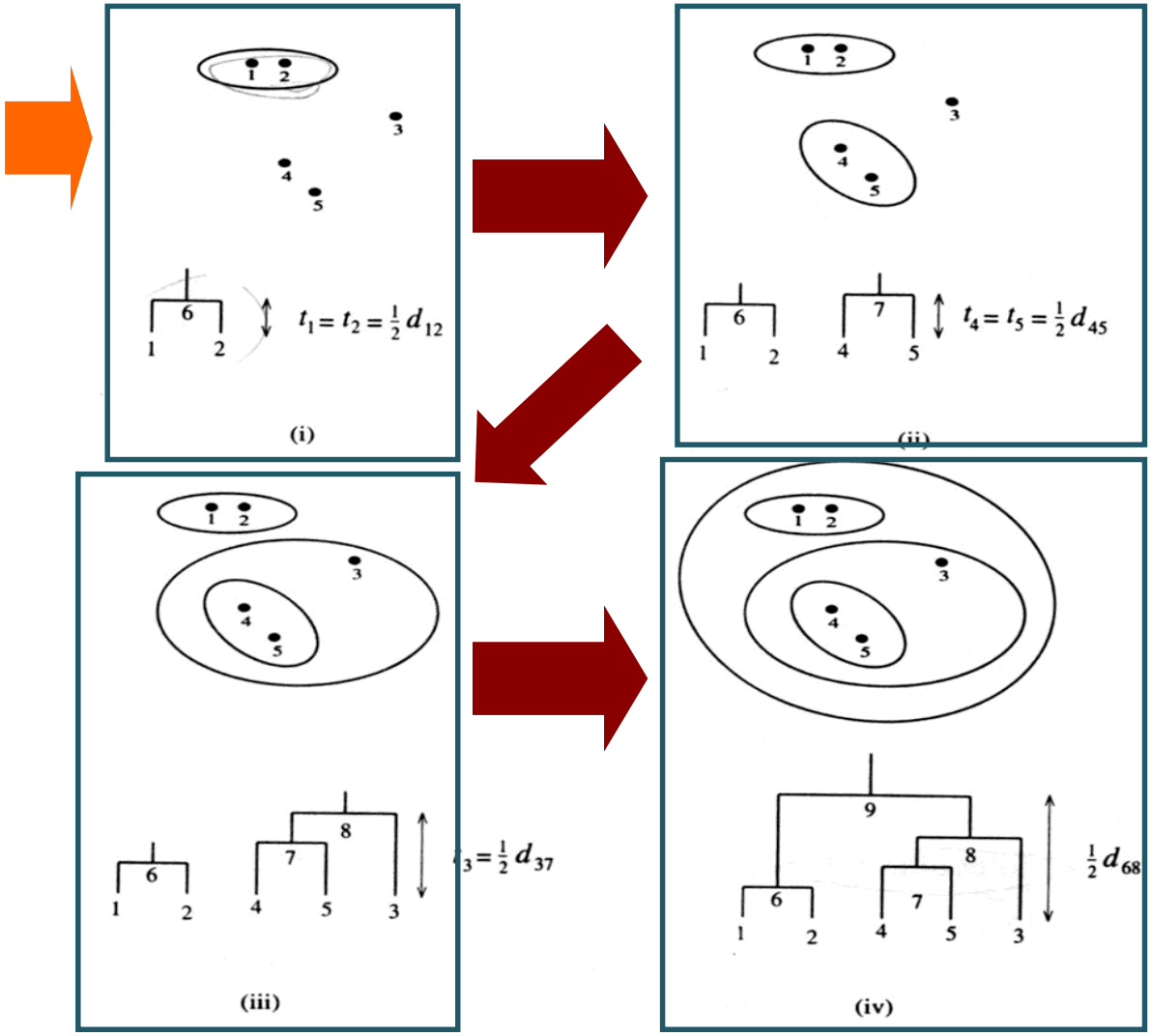
- A phylogenetic analysis most often results in an formally unrooted network
- For phylogenetic analyses can be included an “outgroup” which will be used to root the tree
- The taxa of interested in are called the “ingroup”
- The assumption is that the ingroup taxa are more closely related to each other than any is to the outgroup
- If this assumption is wrong, then the interpretations of the phylogenetic tree will be wrong!

Methods

- Distance-based methods:
- Neighbor-joining
- UPGMA

- Character-based methods:
- Maximum parsimony
- Model-based methods:
  - Maximum likelihood
  - Bayesian inference

UPGMA


1 2
3
4
5
6
1 2
$t_1 = t_2 = \frac{1}{2} d_{12}$
(i)
1 2
3
4 5
6
1 2
7
4 5
$t_4 = t_5 = \frac{1}{2} d_{45}$
(ii)
1 2
3
4 5
6
1 2
7
4 5
8
3
$t_3 = \frac{1}{2} d_{37}$
(iii)
1 2
3
4 5
9
6
1 2
8
7
4 5
3
$\frac{1}{2} d_{68}$
(iv)

UPGMA

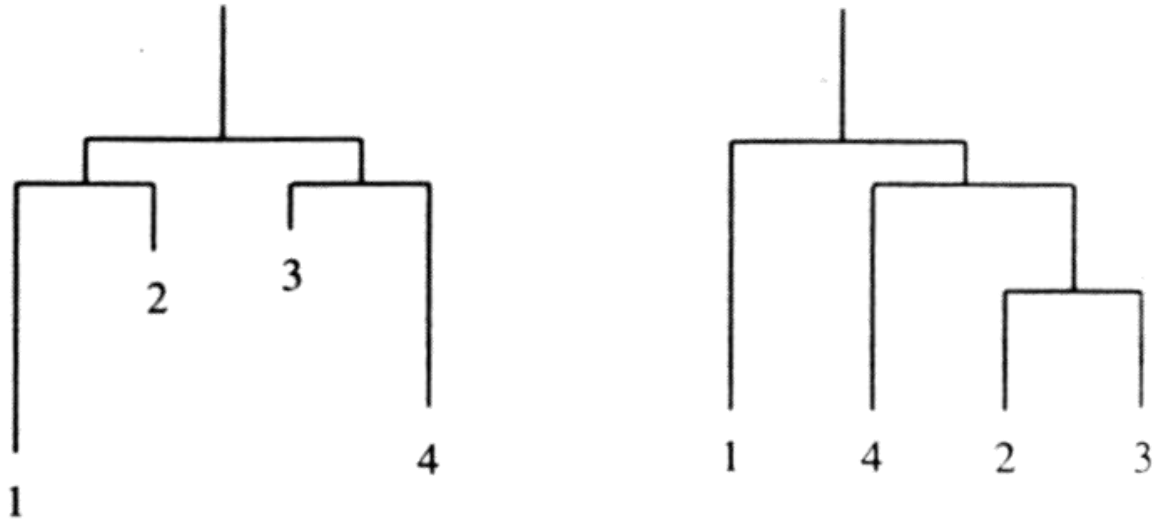
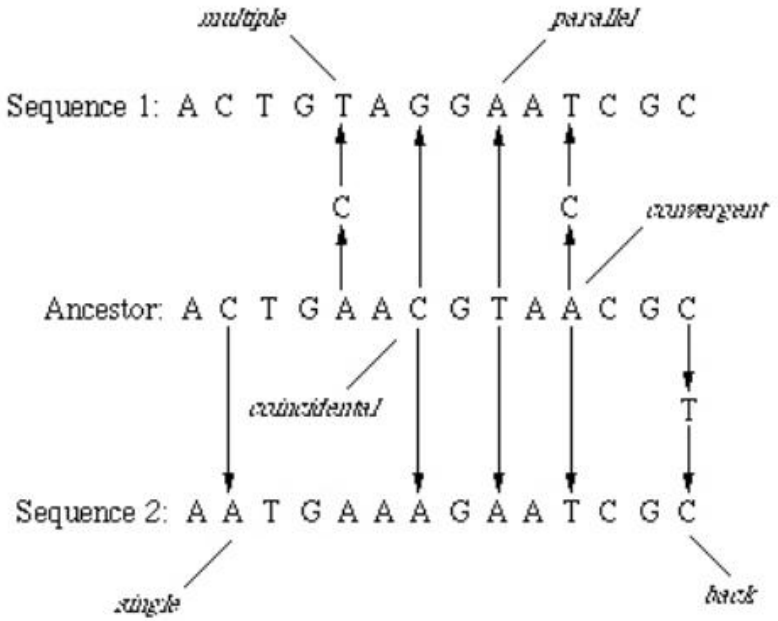


Figure 7.5 *A tree (left) that is reconstructed incorrectly by UPGMA (right).*

Neighbor Joining

- Very popular method
- Does not make molecular clock assumption : modified distance matrix constructed to adjust for differences in evolution rate of each taxon
- Produces unrooted tree
- Assumes additivity: distance between pairs of leaves = sum of lengths of edges connecting them

Neighbor Joining



Use models of substitution to correct these values

File Formats

- Newick (.nwk)

((species1:BranchLength,species2)Bootstrap,species 3);

Distance methods

- Advantages:
  - easy to perform
  - quick calculation
  - fit for sequences having high similarity scores
- Disadvantages:
  - the sequences are not considered as such (loss of information)
  - all sites are generally equally treated (do not take into account differences of substitution rates)
  - not applicable to distantly divergent sequences.

Hands On 5

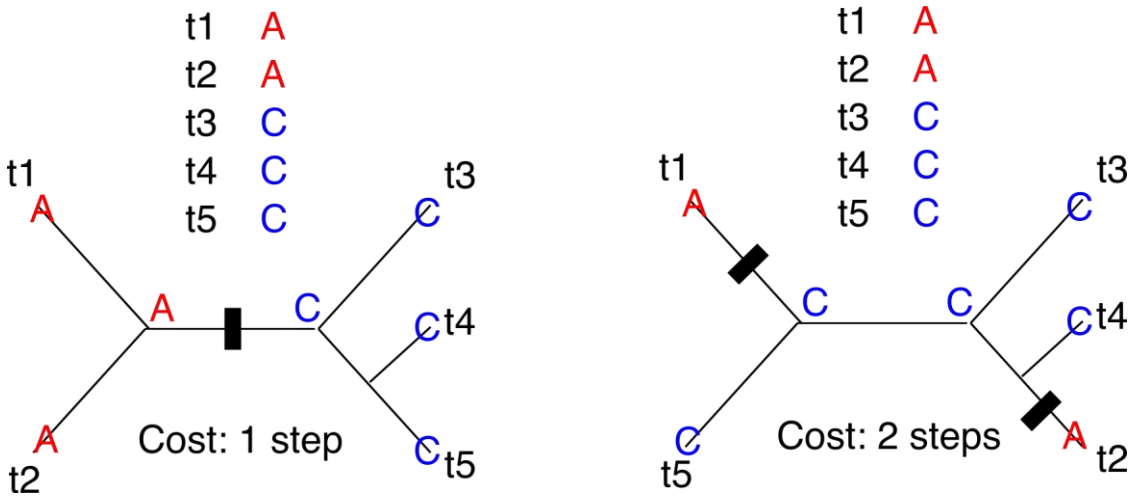
1. Requisitos Seaview e Mega
2. Descarregar sequencias na pasta hands_on_5
3. Alinhar usando Muscle em Aminoácidos
4. Fazer uma NJ de nucleótidos usando os parâmetros por omissão, 1000 bootstrap
5. Repetir o passo fazendo uma árvore de aminoácidos

Characters based tree

Maximum parsimony

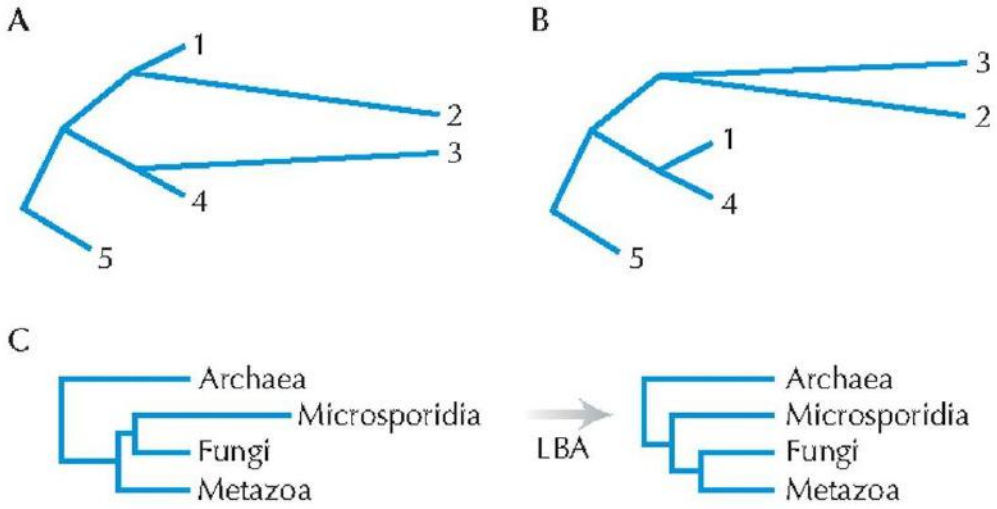
- Object is to minimise the number of changes necessary for the evolution of all characters on a tree.
- Character changes are *typically* treated as equally-weighted ie. the "cost" of changing from one state to another is the same between all states, but various weighting schemes can be applied
- Can be used with both morphological and molecular data, morphological characters may be ordered and polarised
- The tree with the fewest changes/steps is the MP tree. Might find many most- parsimonious solutions, which are often presented as a 50% majority-rule tree

Maximum parsimony



- Fitch's algorithm can be used to determine the most-parsimonious character reconstruction on any tree, the total score (or length) of a tree is the number of steps (changes) required by the most-parsimonious reconstructions of all characters, and the tree (or trees) with the lowest total score is the MP tree (or MP trees)

Long Branch Attraction



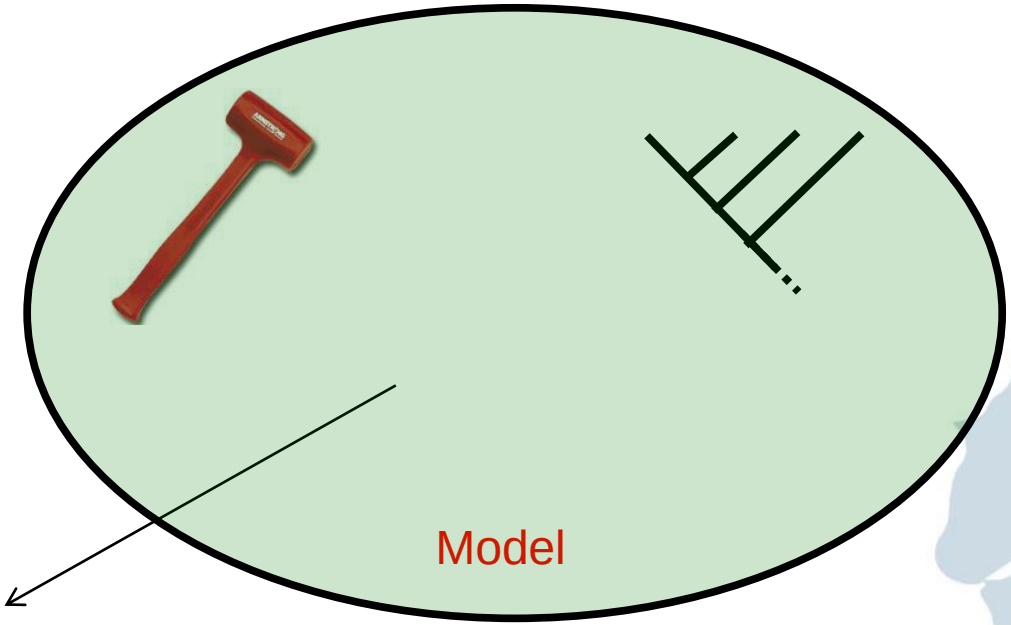
Maximum likelihood and Bayesian inference

- Both use explicit models of character change that are evaluated on a tree using the likelihood function $\propto \mathcal{P}rob(\mathcal{D} \mid \mathcal{H})$

- They differ in their use of statistical paradigms

Model as mechanism of change and tree

Data

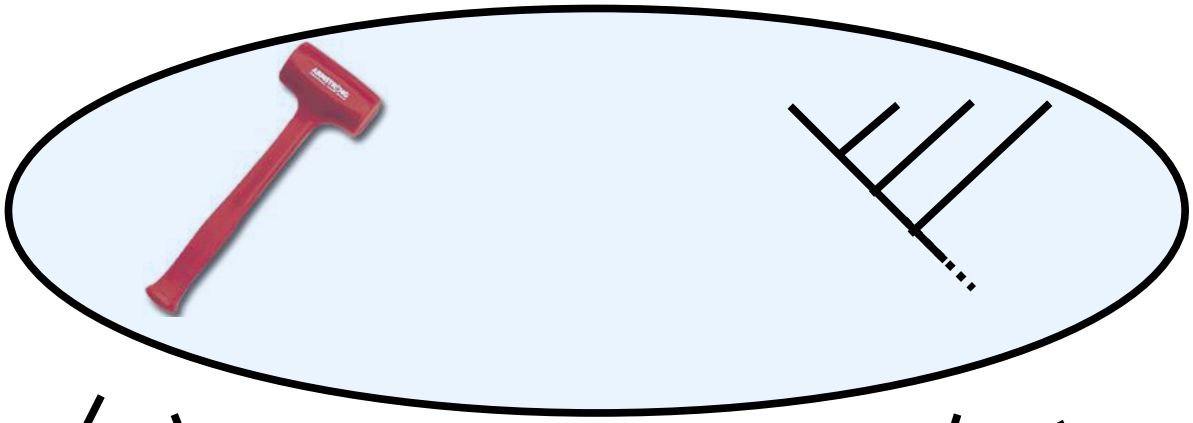


likelihood $\propto Prob(\mathcal{D} \mid \mathcal{H})$

The likelihood is proportional to the probability of *data* given the hypothesis

(a model of character change plus tree topology)

Model as mechanism of change and tree



model

tree

Composition

Substitution rates

Topology

Branch lengths

Instantaneous rate matrix

e.g. general time-reversible model

Models

- 1) the base composition:
- π_a, π_c, π_g, & π_t
  - The base composition frequency parameters remain constant over time (i.e. they are at equilibrium)
  - They express the rate at which changes ***to*** each base occur
  - Hence, the rate of change to a rare base would be low, whereas the change to a common base would be high
    - could be equal: 0.25, 0.25, 0.25, 0.25
    - or not: 0.3, 0.4, 0.2, 0.1
    - perhaps values are estimated from the data

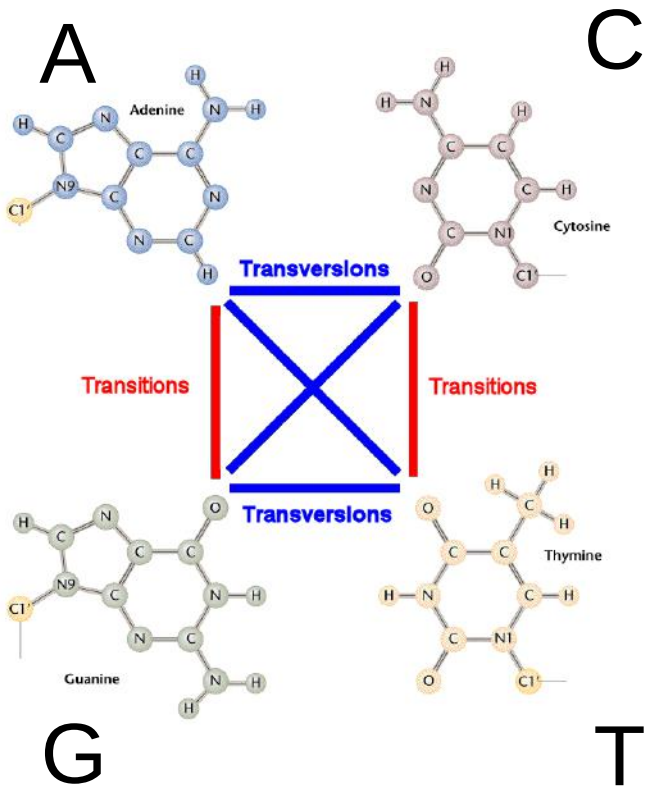
Models

2) Substitution rates:

$$\mathcal{R} = \begin{array}{c} \begin{matrix} \text{A} & \text{C} & \text{G} & \text{T} \end{matrix} \\ \begin{pmatrix} - & r_{A\to C} & r_{A\to G} & r_{A\to T} \\ r_{C\to A} & - & r_{C\to G} & r_{C\to T} \\ r_{G\to A} & r_{G\to C} & - & r_{G\to T} \\ r_{T\to A} & r_{T\to C} & r_{T\to G} & - \end{pmatrix} \end{array} \begin{matrix} \\ \text{A} \\ \text{C} \\ \text{G} \\ \text{T} \end{matrix}$$

Transitions and transversions

purines



pyrimidines

Model GTR

$$\mathcal{Q} = \begin{pmatrix} - & \mu r_{A\to C}\pi_C & \mu r_{A\to G}\pi_G & \mu r_{A\to T}\pi_T \\ \mu r_{C\to A}\pi_A & - & \mu r_{C\to G}\pi_G & \mu r_{C\to T}\pi_T \\ \mu r_{G\to A}\pi_A & \mu r_{G\to C}\pi_C & - & \mu r_{G\to T}\pi_T \\ \mu r_{T\to A}\pi_A & \mu r_{T\to C}\pi_C & \mu r_{T\to G}\pi_G & - \end{pmatrix}$$

$$\mathcal{GTR} = \begin{array}{c} \\ A \\ C \\ G \\ T \end{array} \begin{array}{c} \begin{array}{cccc} A & C & G & T \end{array} \\ \begin{pmatrix} - & \mu r_i\pi_C & \mu r_j\pi_G & \mu r_k\pi_T \\ \mu r_i\pi_A & - & \mu r_l\pi_G & \mu r_m\pi_T \\ \mu r_j\pi_A & \mu r_l\pi_C & - & \mu r_n\pi_T \\ \mu r_k\pi_A & \mu r_m\pi_C & \mu r_n\pi_G & - \end{pmatrix} \end{array}$$

GTR derived

Model derived from the *GTR* model – few have been implemented in phylogenetics

- GTR – unequal base frequencies and 6 substitution types
- SYM – equal base frequencies and 6 substitution types
- HKY85 – unequal base frequencies and 2 substitution types (transitions and transversion)
- F81 – unequal base frequencies and single substitution type
- JC – equal base frequencies and single substitution type

Model selection

2) The Akaike Information Criterion: $AIC_i = -2logL_i + 2p_i$

where: i is the hypothesis (model + tree), and

p is the number of free parameters

- Does not requite models to be nested
- Calculate AIC for each model
- Choose model with lowest AIC score
- To be preferred over the LRT

Model selection

1) The Likelihood Ratio Test (LRT):

where: L_0 is a restricted (simpler) version of L_1

e.g.:

Null model = HKY+G
Alternative model = GTR+G
2(lnL1-lnL0) = 23.2451
P-value = 0.000113

-lnL0 = 7918.9556
-lnL1 = 7907.3330
df = 4

Tools

- Modeltest conducts the LRT/AIC (and others) among a set of nested models
  – 14 substitution matrices with and without a *pinvar* and *gdasrv*
- Uses a crude distance tree to calculate the likelihoods of the models
- MrModeltest calculates similar for 24 models (those used by MrBayes)
- For amino acid models use ProtTest or ModelGenerator

Models parameters

- It is commonly recognised that not all sites evolve at the same rate – some may constrained by selection. This can be incorporated into the model:

1. *Site-specific rate categories* - defined *a priori*, e.g. first, second, third codon positions of a protein-coding gene.

2. *Proportion of invariant sites* (pinvar) - assumes some proportion of sites is incapable of changing and all other sites vary at the same rate.

3. *Gamma distributed among site rate variation* (gdasrv) - uses a number of discrete categories of rates that partitions a gamma distribution - the shape of the distribution is described by the parameter α

Amino acid substituion models

- Poisson model – (equiv. to JC) equal substitution rates and frequencies
- Proportional model – as Poisson but with unequal (empirically observed) frequencies

Empirically observed transition matrices:

- Dayhoff - derived from Dayhoff, et al.'s (1978) empirical substitution matrix
- JTT - Jones, Taylor, Thornton
- WAG - Whelan and Goldman

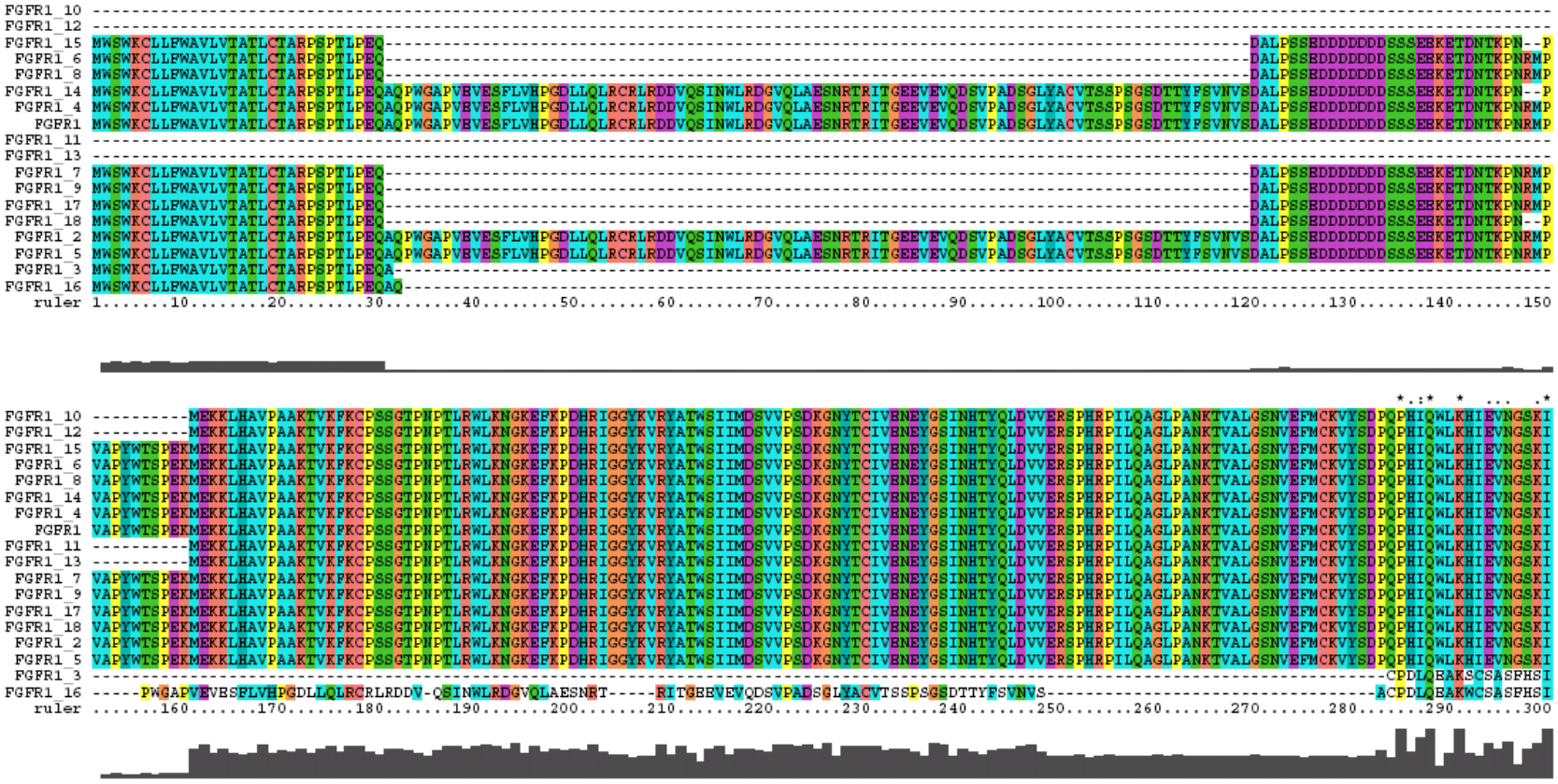
Bootstrap

- The bootstrap is a statistical method that is designed to test the reliability of the result by using pseudo-replicates drawn from the original data

- Draw characters/sites from the original data, with replacement, from the original data set to make a new one the same size. Repeat the phylogenetic analysis on this bootstrap replicate and repeat the process many times (100-1000).

- Interpretation of the bootstrap is difficult. It is known to be biased, but for a particular support values it not known where it is biased up or down. Its usually reckoned that 70% is statistically significant.

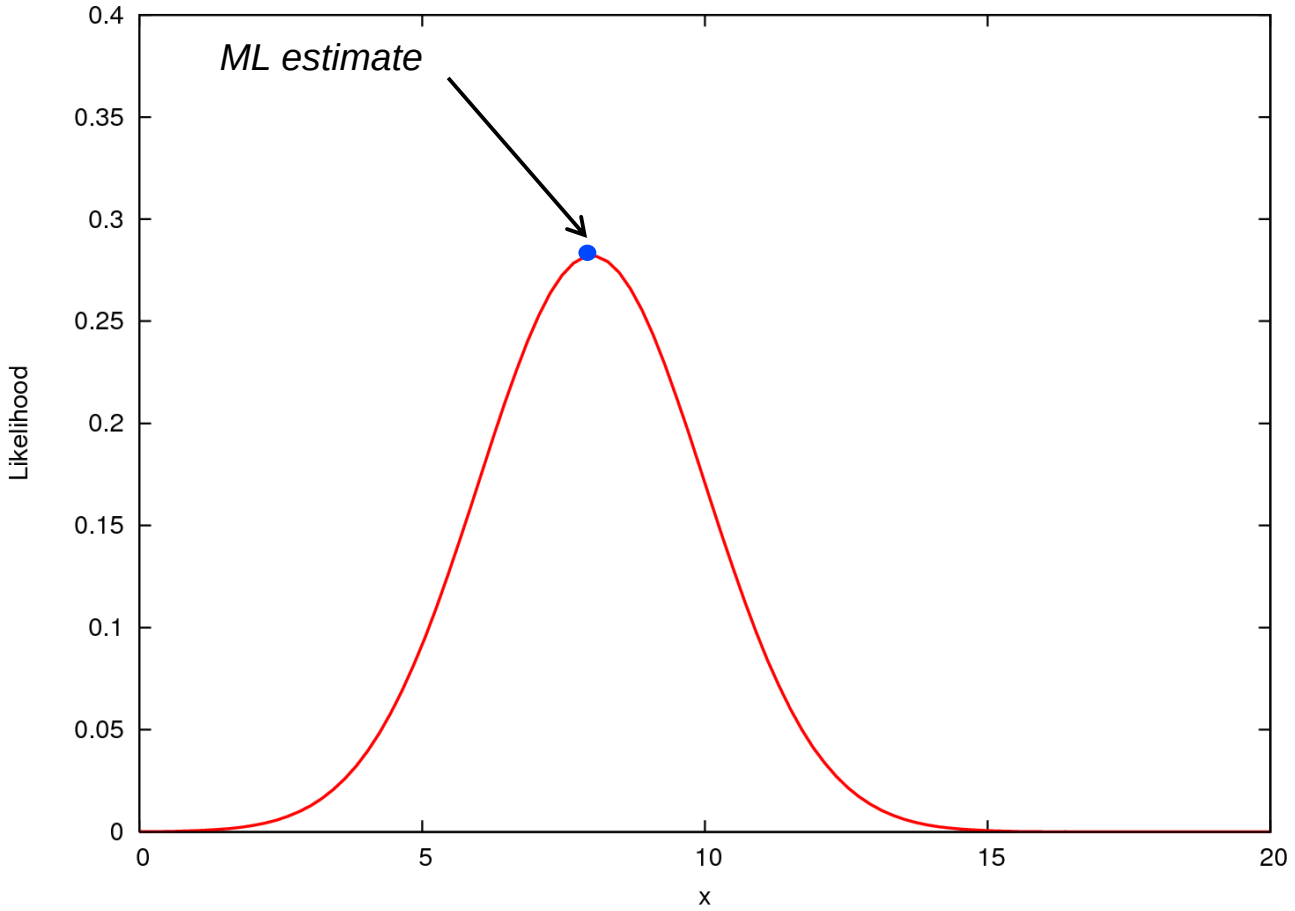
Bootstrap



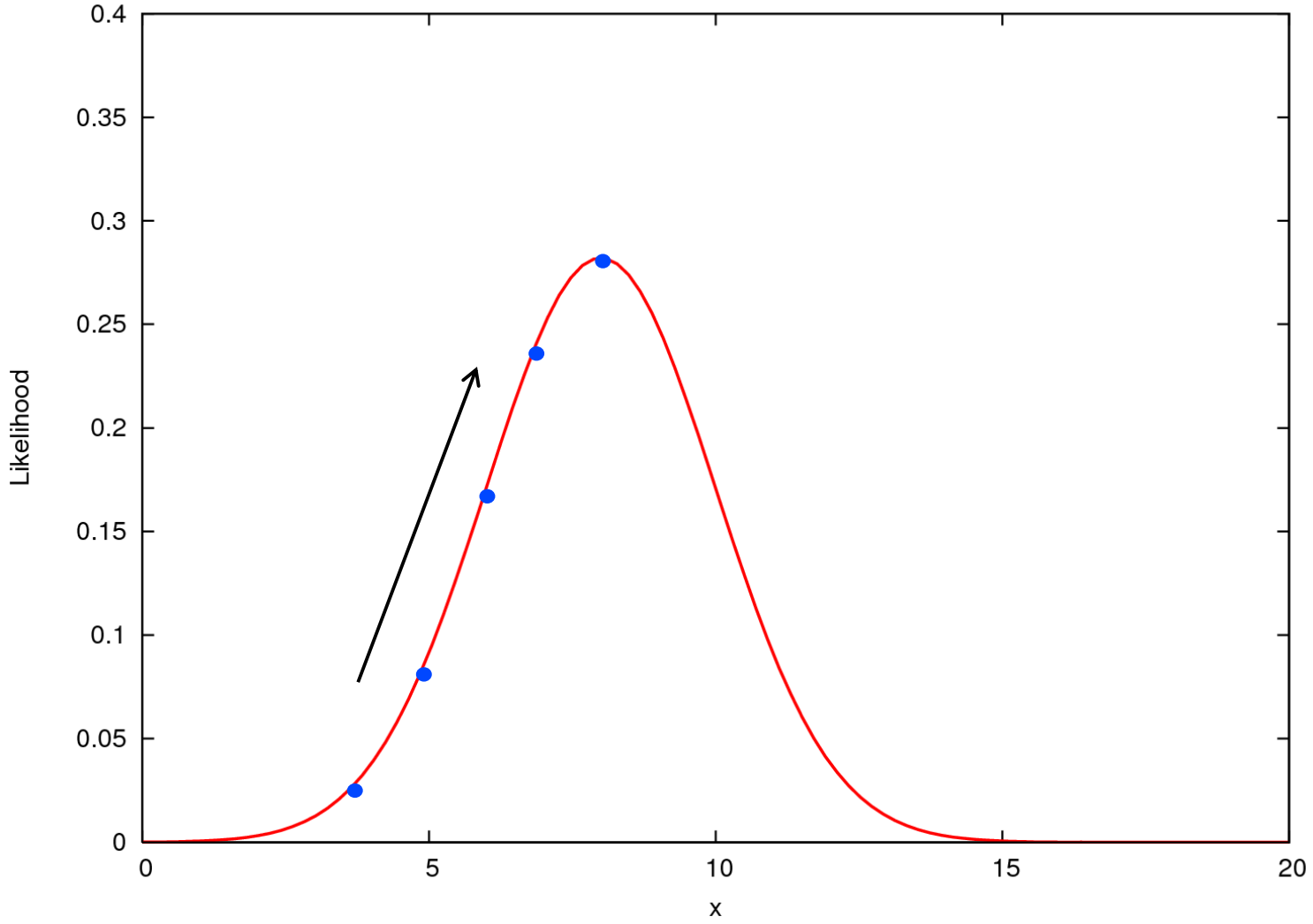
Hands On 6

1. Requisitos Seaview+jModelTest e/ou Mega
2. Alinhar usando MUSCLE
3. Determinar modelo evolucionário
4. Fazer uma ML usando 100 de bootstrap e o modelo determinado no passo 3

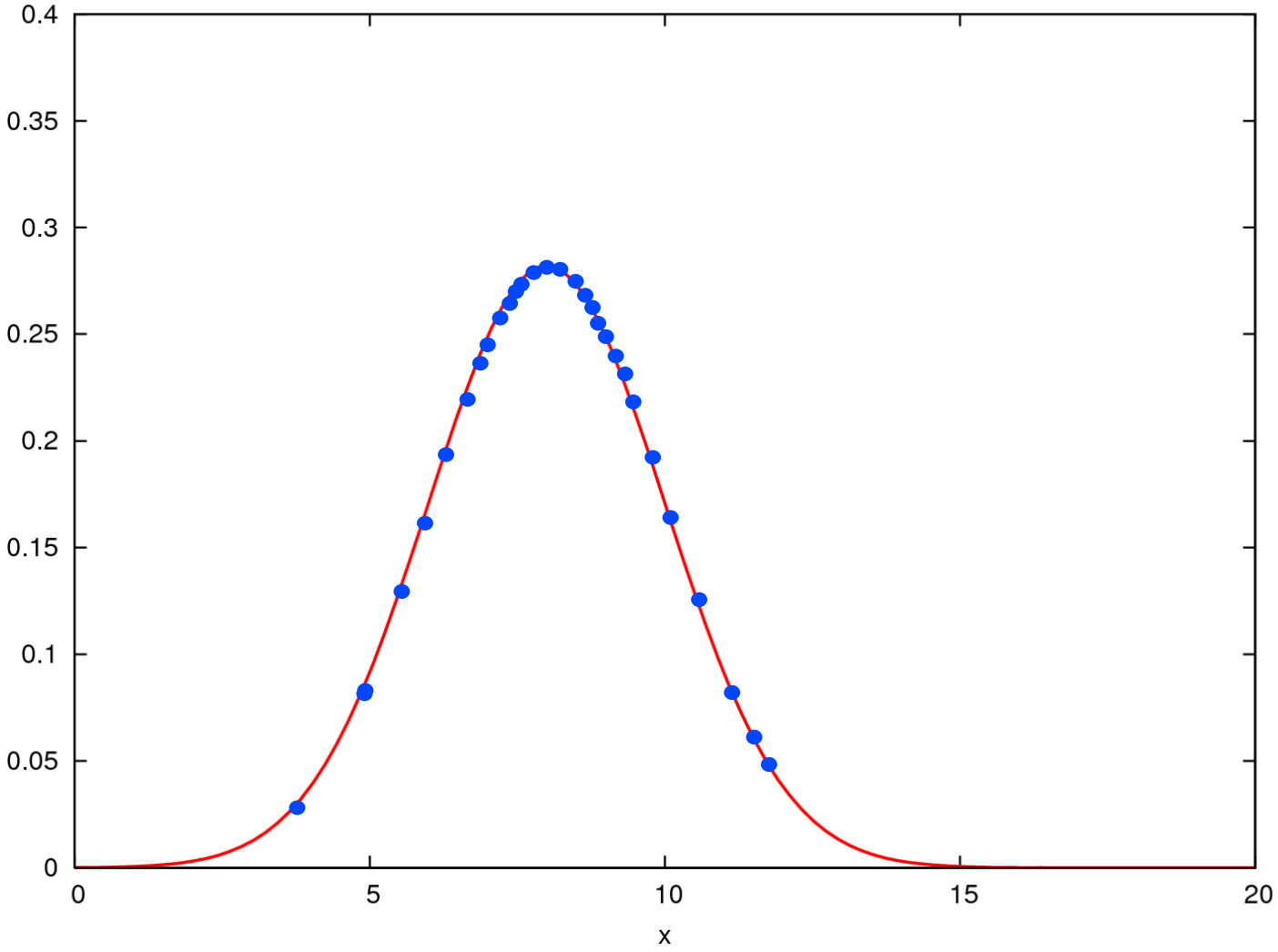
ML and PP density



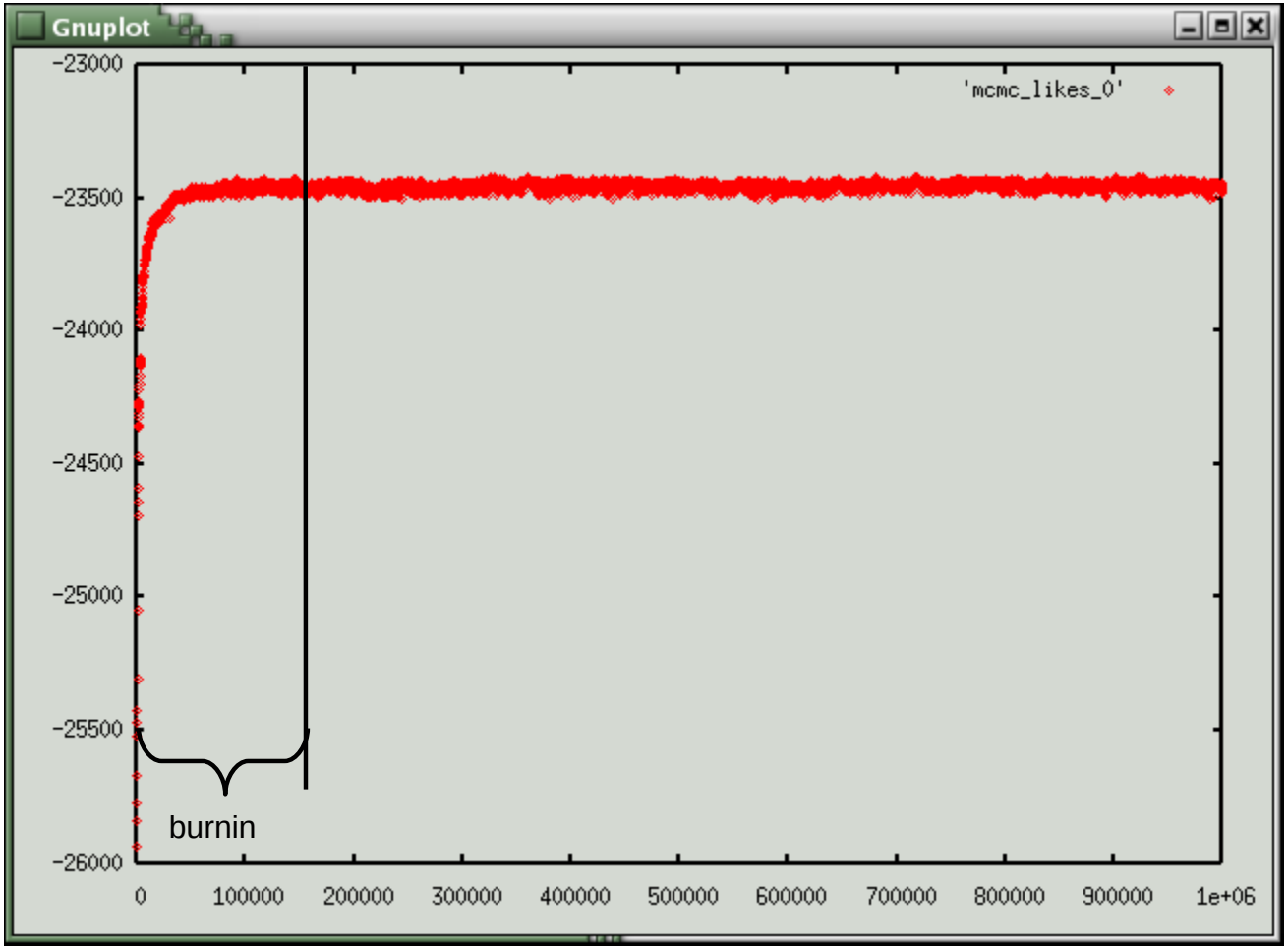
ML and PP density



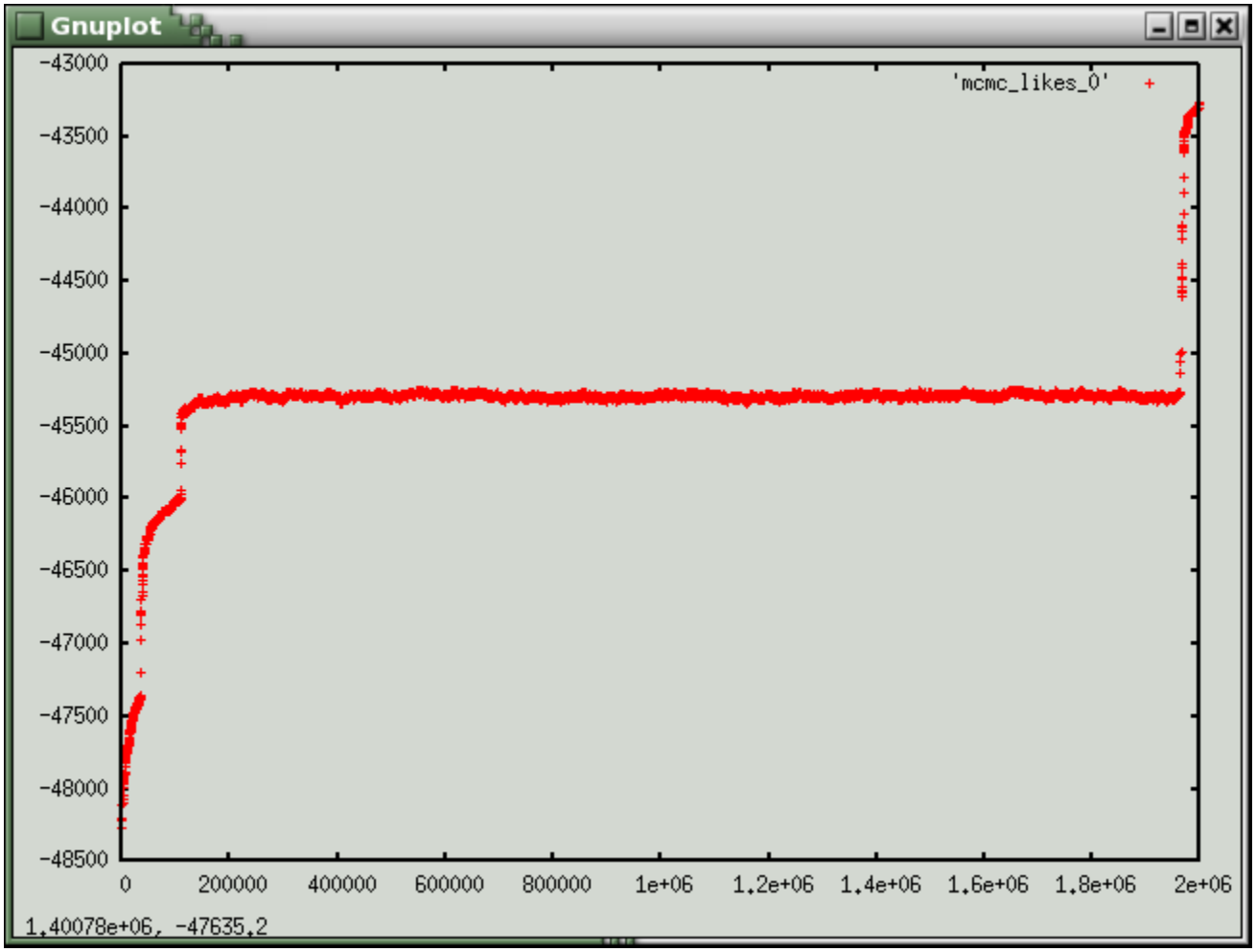
ML and PP density



Likelihood plot



Likelihood plot



Mcmc output

Composition *Substitution Rate* *gdasrv*

gens	A	C	G	T	A-C	A-G	A-T	C-G	C-T	G-T	alpha
100	0.22515466	0.24584661	0.30733981	0.22165891	0.11970749	0.23917488	0.08580185	0.14260800	0.32394051	0.08876728	0.874116
200	0.22581011	0.23935950	0.28160072	0.25322967	0.11361513	0.22751384	0.10471647	0.16653072	0.26859341	0.11903044	1.930917
300	0.23172551	0.27706732	0.27019486	0.22101230	0.12093299	0.20611349	0.08865832	0.15770240	0.28720945	0.13938334	4.380087
400	0.22999880	0.26063025	0.28697633	0.22239461	0.09333566	0.24749557	0.12001019	0.14071384	0.29772513	0.10071961	10.893358
500	0.24616074	0.25080719	0.27680877	0.22622330	0.10333398	0.20508574	0.10527309	0.16562894	0.31464847	0.10602977	14.875529
600	0.21219225	0.28281963	0.28884274	0.21614538	0.11325671	0.25977835	0.12678584	0.11935713	0.28575591	0.09506605	8.521777

etc...

```
tree t_100 = [&U] (((1:0.263151, 2:0.0564195):0.206267, 3:0.223034):0.73243, 4:0.107335, 5:0.0742962);
tree t_200 = [&U] (((5:0.115119, 4:0.0936513):1.14124, 2:0.0686334):0.206054, 3:0.338046, 1:0.223061);
tree t_300 = [&U] ((2:0.0799976, (4:0.0892171, 5:0.115119):1.10016):0.174534, 3:0.461212, 1:0.236187);
tree t_400 = [&U] ((2:0.0800969, (5:0.126667, 4:0.09186):1.39515):0.21926, 3:0.445261, 1:0.335234);
tree t_500 = [&U] (((4:0.100586, 5:0.149979):1.92335, 2:0.0800969):0.268639, 1:0.335234, 3:0.556183);
tree t_600 = [&U] (((3:0.671303, 1:0.413087):0.347891, 2:0.178729):2.18893, 5:0.0845881, 4:0.143907);
```

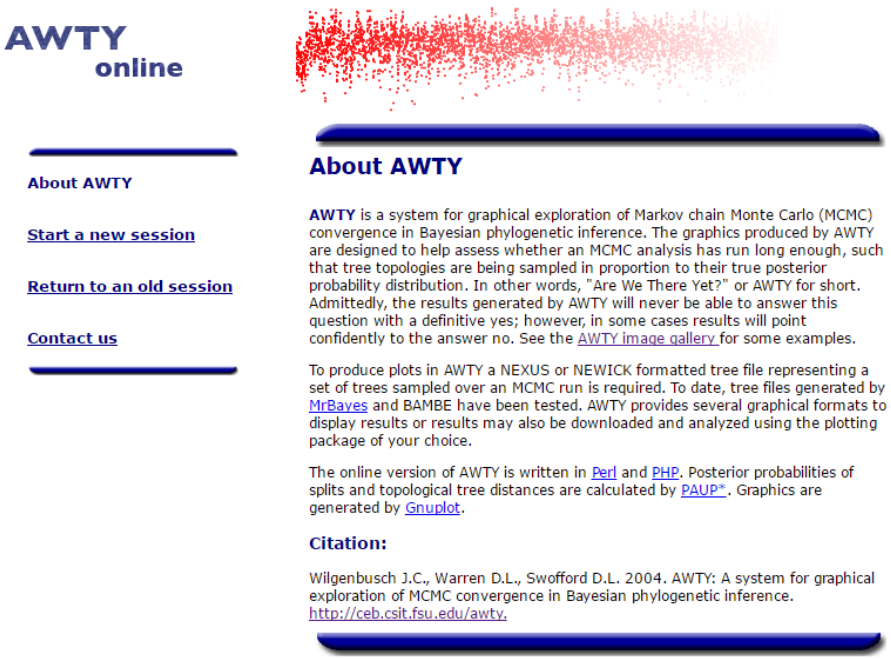
etc...

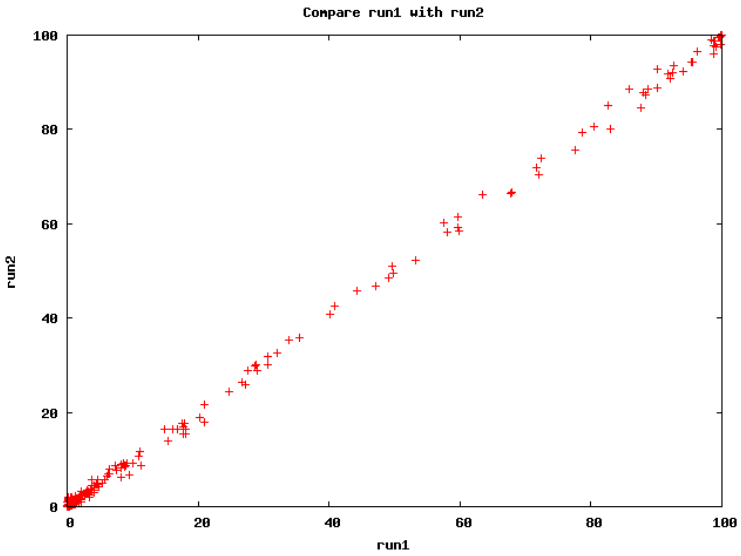
- *Note that at each generation the parameter values are known (i.e. they are the current values of the chain) hence the likelihood is easy and quick to calculate, this makes BA a relatively quick method when compared to ML*

Likelihood plot

- Are we there yet?

AWTY is a system for the graphical exploration of MCMC convergence, written by Jim Wilgenbusch, Dan Warren, and David Swofford.

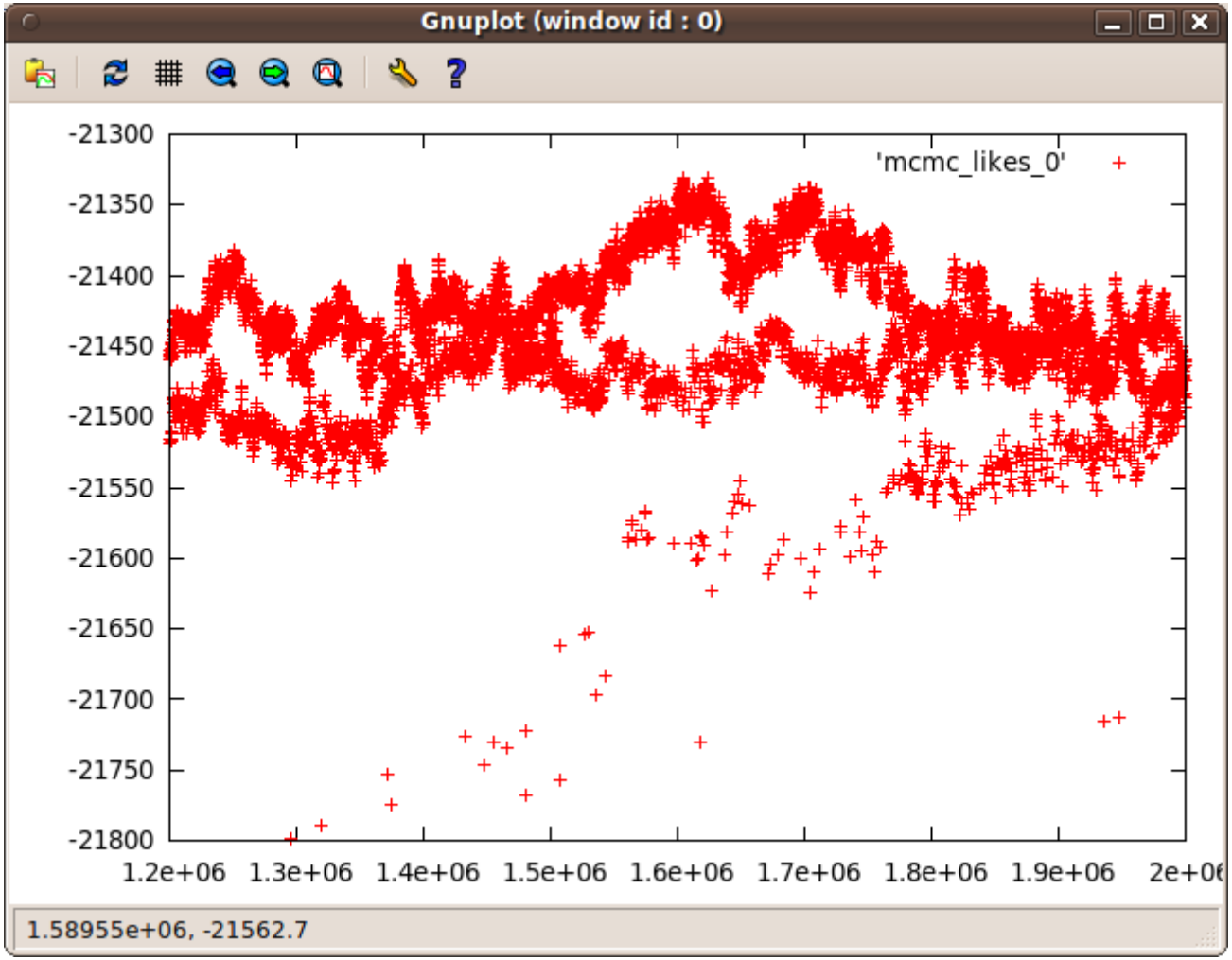




Convergence

- Run the analysis more that once and check that the separate runs give similar results
- Monitor the average standard deviation of split support between two separate runs (MrBayes does this by default)

Likelihood plot



Alternatives

- http://www.thines-lab.senckenberg.de/simba/
- http://phylemon.bioinfo.cipf.es/
- http://phylogeny.lirmm.fr/phylo_cgi/index.cgi

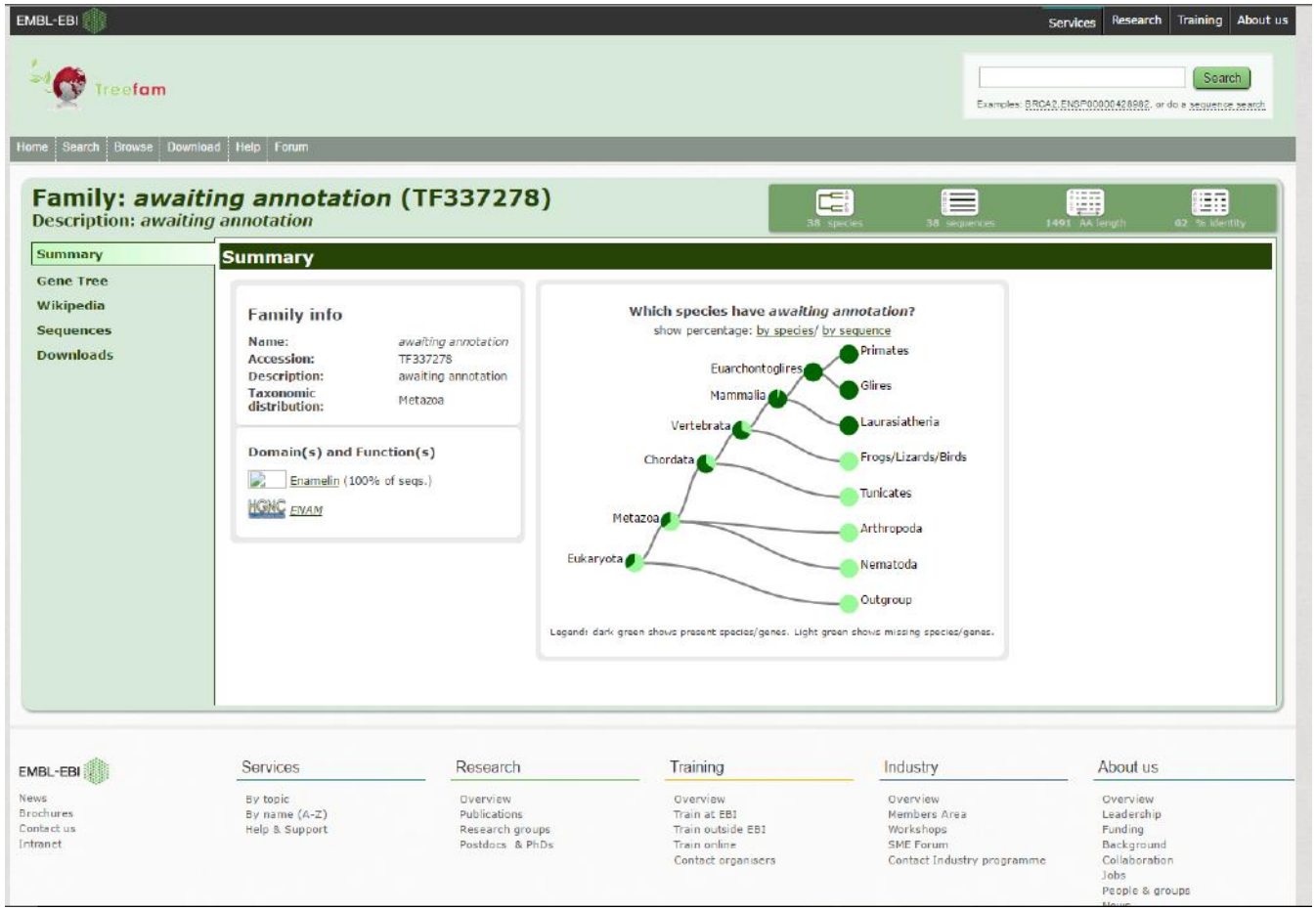
Hands On 7

1. Requisitos Mr. Bayes e TreeGraph
2. Abrir executável do Mr.Bayes (32 ou 64 bits)
3. “Execute nomedoficheiro.nex”
4. “help lset”
5. “Lset nst=6 rates=invgamma” (modelo GTR+I+G)
6. “help mcmc”
7. “mcmc ngen=500000”
8. “sump” (confirmar parâmetros)
9. “sumt conformat=simple”
10. Executar TreeGraph e abrir ficheiro .con gerado no Mr. Bayes

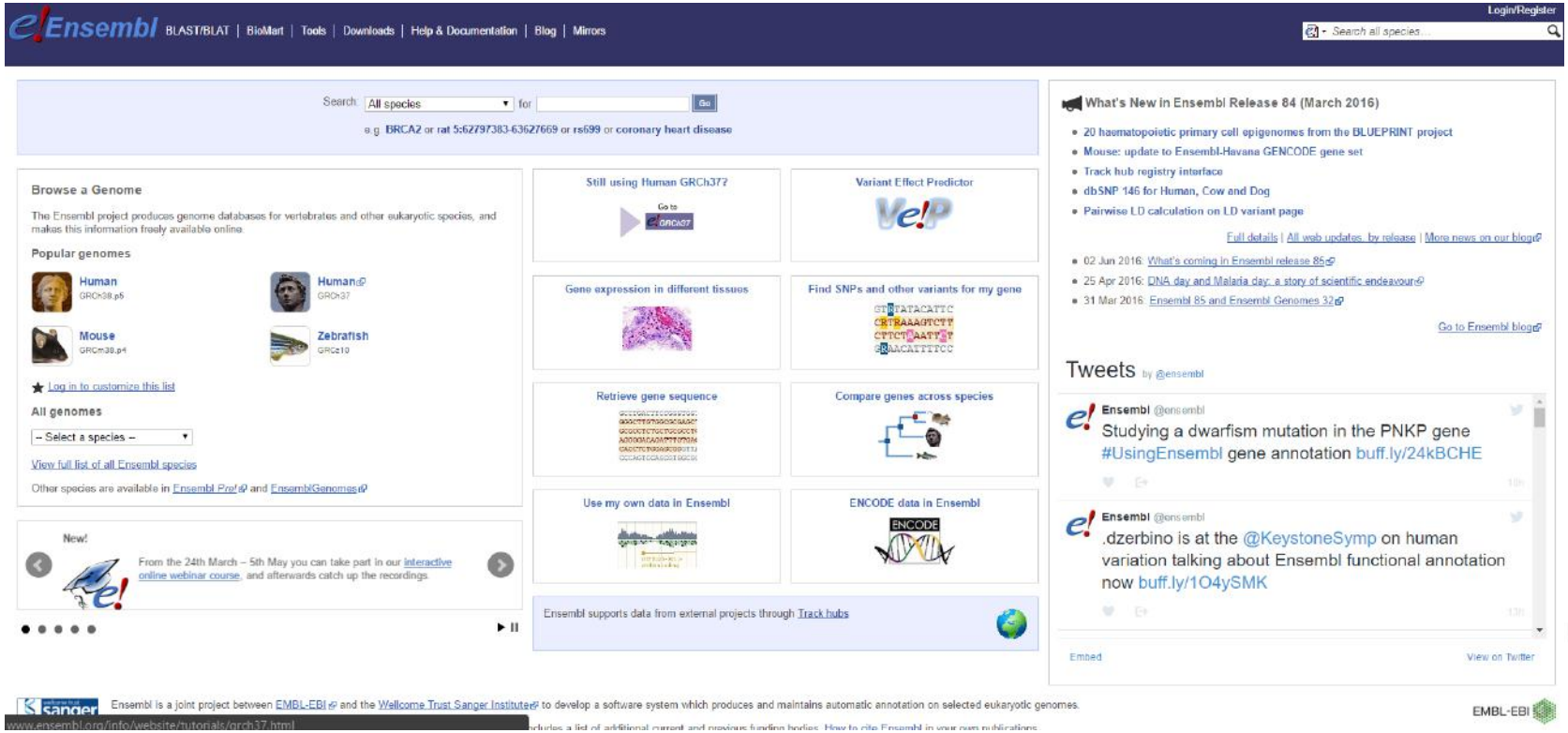
Hands On 8

1. Requisitos Tree-Puzzle
2. Abrir puzzle-windows-mingw.exe
3. k + enter -> K Tree search procedure? Evaluate user defined trees
4. m + enter até ao modelo GTR
5. w + enter até -> w Model of rate heterogeneity? Mixed (1 invariable + 4 Gamma rates)
6. y + enter
7. my_trees.txt (ficheiros com as árvores)
8. Abrir ficheiro .puzzle
9. No final do ficheiro aparece a comparação entre as 3 árvores “COMPARISON OF USER TREES (NO CLOCK)”

Data Warehouses Again



Data Warehouses Again



Primer design

Good primer design is essential for successful reactions

1. **Primer Length:**
   - 18-22 bp.
   - Grande o suficiente para ter especificidade
   - Curta o suficiente para se ligar facilmente ao template

Primer design

2. **GC Content**
   - O número de G's e C's no primer como % no número total de bases, deve ser entre 40-60%
3. **GC Clamp**
   - A presença de G ou C nas últimas 5 bases do extremo 3'
   - Ajuda a promover a ligação específica, devido à ligação ser mais forte entre G e C
   - Mais de 3 G's or C's devem ser evitados nas últimas 5 bases do extremo 3‘

Primer design

4. **Primer Melting Temperature (T_m)**
   - Por definição: temperature à qual metade da cadeia se dissocia e fica “single stranded” e indica a estabilidade do duplex
   - Primers com Tm entre 52-58 ^oC dão bons resultados
   - Primers com Tm > 65^oC têm tendência para “secondary annealing”
   - O conteúdo GC indica a T_m

5. **Primer Pair Tm Mismatch Calculation**
   - O par de primers deve ter uma Tm aproximada, para maximizer o produto de PCR.
   - A diferença não deve exceder 5ºC, ou pode levar à não amplificação

Primer design

6. Primer Annealing Temperature

- A temperature de melting é determinante para a temperatura de annealing
- Ta elevada produz pouca hidridação entre Primer-Template, o que conduz a baixo produto de PCR
- Ta baixas levam a produtos inespecíficos, desencadeados por ligações erradas entre bases
- 3 a 5ºC abaixo da Tm. Se for muito inespecífico, vamos subindo.

Primer design

7. Primer Secondary Structures

- Entre primers ou dentro do mesmo primer
- Levam a pouco ou nenhum produto de PCR
- Afectam annealing e consequentemente a amplificação
- Reduzem disponibilidade dos primers no mix

Primer design

6.1. Hairpins

- Dentro do primer
- Optimally a 3' end hairpin with a ΔG of -2 kcal/mol and an internal hairpin with a ΔG of -3 kcal/mol is tolerated generally.

ΔG

- Traduz a estabilidade do harpin
- A energia necessária para quebrar a estrutura secundária
- Valores negativos elevados: harpins estáveis, logo indesejáveis

Primer design

6.2. Self Dimer

- Entre 2 primers iguais (mesmo sentido)
- Produz menos produto de PCR, uma vez que há muitos mais primers disponíveis na amostra que de gene alvo
- Optimally a 3' end self dimer with a ΔG of -5 kcal/mol and an internal self dimer with a ΔG of -6 kcal/mol is tolerated generally.

6.3. Cross Dimer

- Entre o Fw e Rv
- Optimally a 3' end cross dimer with a ΔG of -5 kcal/mol and an internal cross dimer with a ΔG of -6 kcal/mol is tolerated generally.

7. 3' End Stability

- É o máximo ΔG das últimas 5 bases do extremo 3' end
- Um extremo 3' instável (ΔG menos negativo) resulta em “false priming”

Primer design

8. **Repeats**
   - É um di-nucleótido que ocorre muitas vezes consecutivamente, ATATATAT
   - Evitar pois leva a ligações erradas
   - O número máximo é de 4 di-nucleotidos
9. **Runs**
   - Evitar muitas bases iguais seguidas, AGCGGGGGATGGGG
   - Levam a ligações erradas
   - O nº máximo aceitável é de 4bp

Primer design

10. Evitar Template Secondary Structure

- Caso contrário a cadeia template impede a ligação dos primers

11. Avoid Cross Homology

- Primers apenas devem ligar-se a zonas únicas, caso contrário tornam-se inespecíficos
- Testar numa base de dados

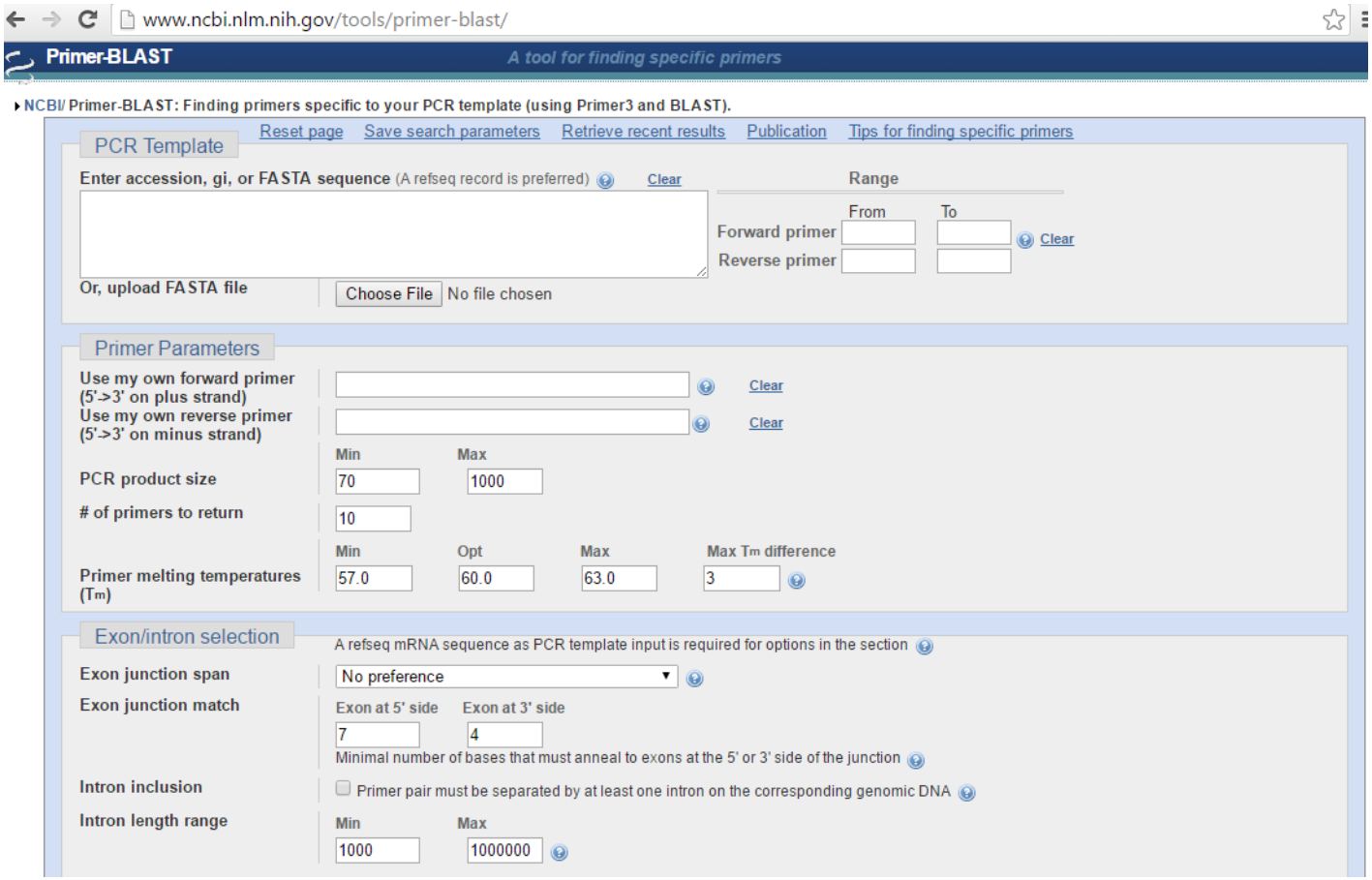
12. Amplicon Length

- qPCR- ronda 100bp
- PCR – até 1000bp
- Determinado pela posição Fw e RV

Primer design

Primer Design using Software

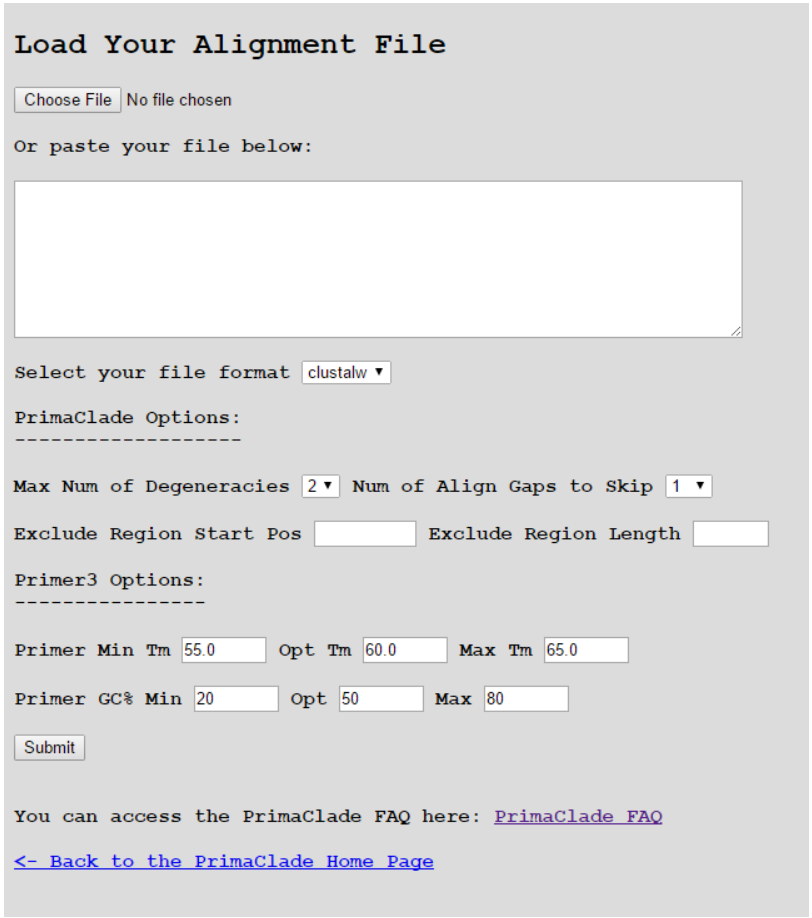
- 1 Sequência



Primer design

Primer Design using Software

- Várias sequências (alinhamento)



Primaclade

Primer design

Primer Design using Software

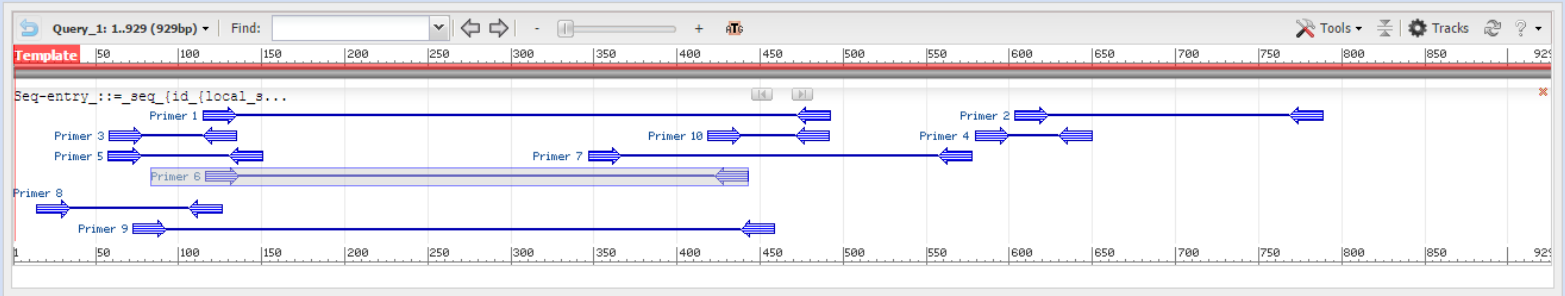
- PrimerBlast

Primer-BLAST *Primer-Blast results*

CBI/ Primer-BLAST : results: Job id=anGaZ-ltQEI7d395HldNBwV6fxYWZWIT more...

Input PCR template	gi|365767550|gb|JQ247713.1| Heterocapsa niei isolate IFR10-193 large subunit ribosomal RNA gene, partial sequenceTACCCGCTGAATATAAGCATATAAGTAAGCGGAGGATAAGAAACTAAATAGGATTCCCTCAGTAATGGCG
Range	1 - 929
Specificity of primers	Primer pairs are specific to input template as no other targets were found in selected database: Refseq mRNA (Organism limited to Homo sapiens)
Other reports	▷ Search Summary

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GAGGGTGAGAGTCCCGTTTG	Plus	20	115	134	60.04	60.00	3.00	0.00
Reverse primer	CTACCATGTCCCTGGCGTTT	Minus	20	492	473	60.04	55.00	4.00	0.00
Product length	378								

Primer pair 2

Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity

Primer design

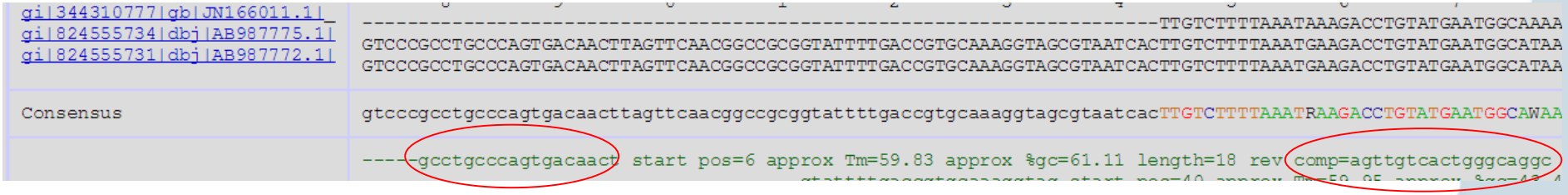
Primer Design using Software

- Primaclade

Primer design

Primer Design using Software

- Primaclade

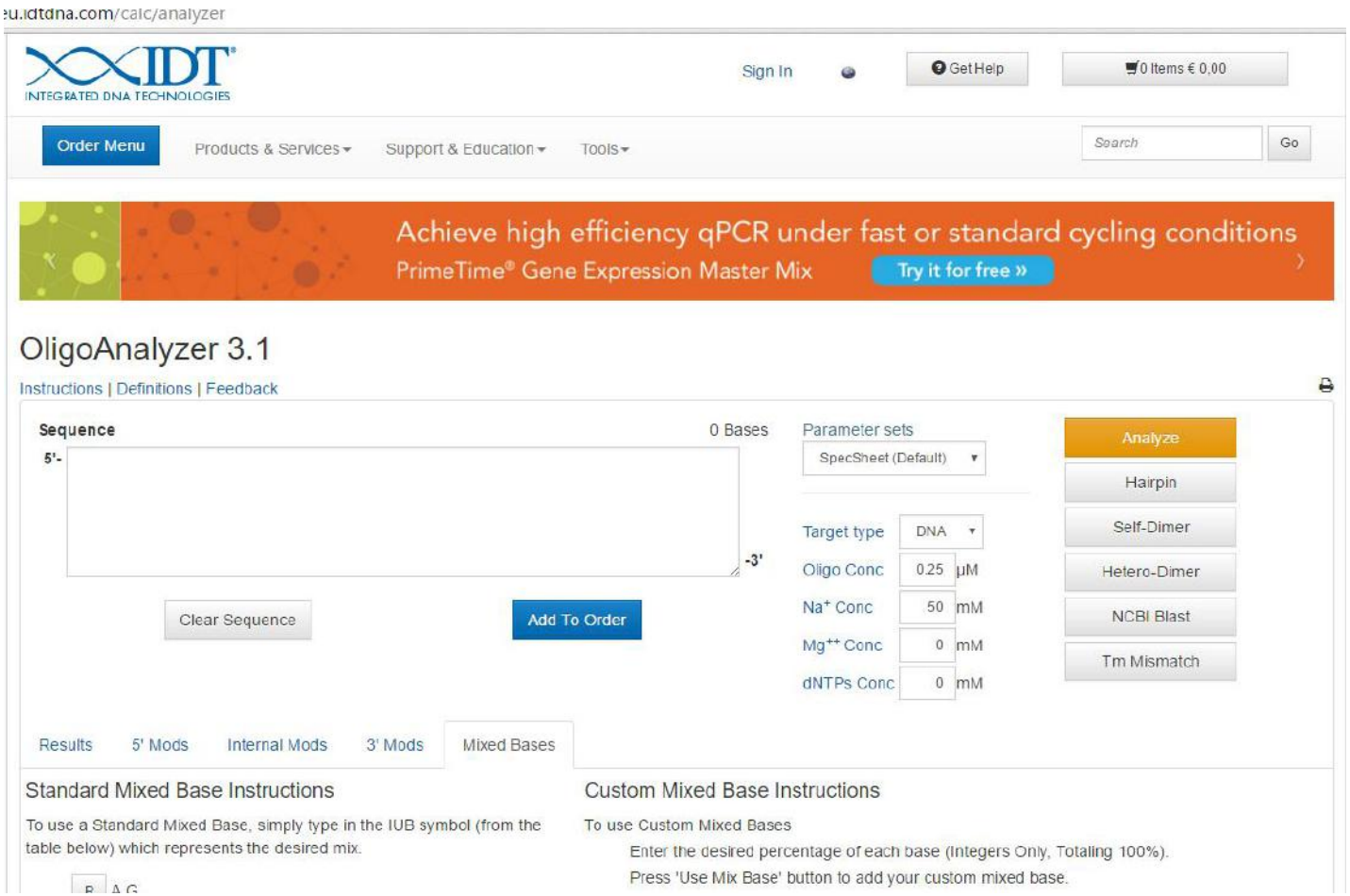


Fw

Rev

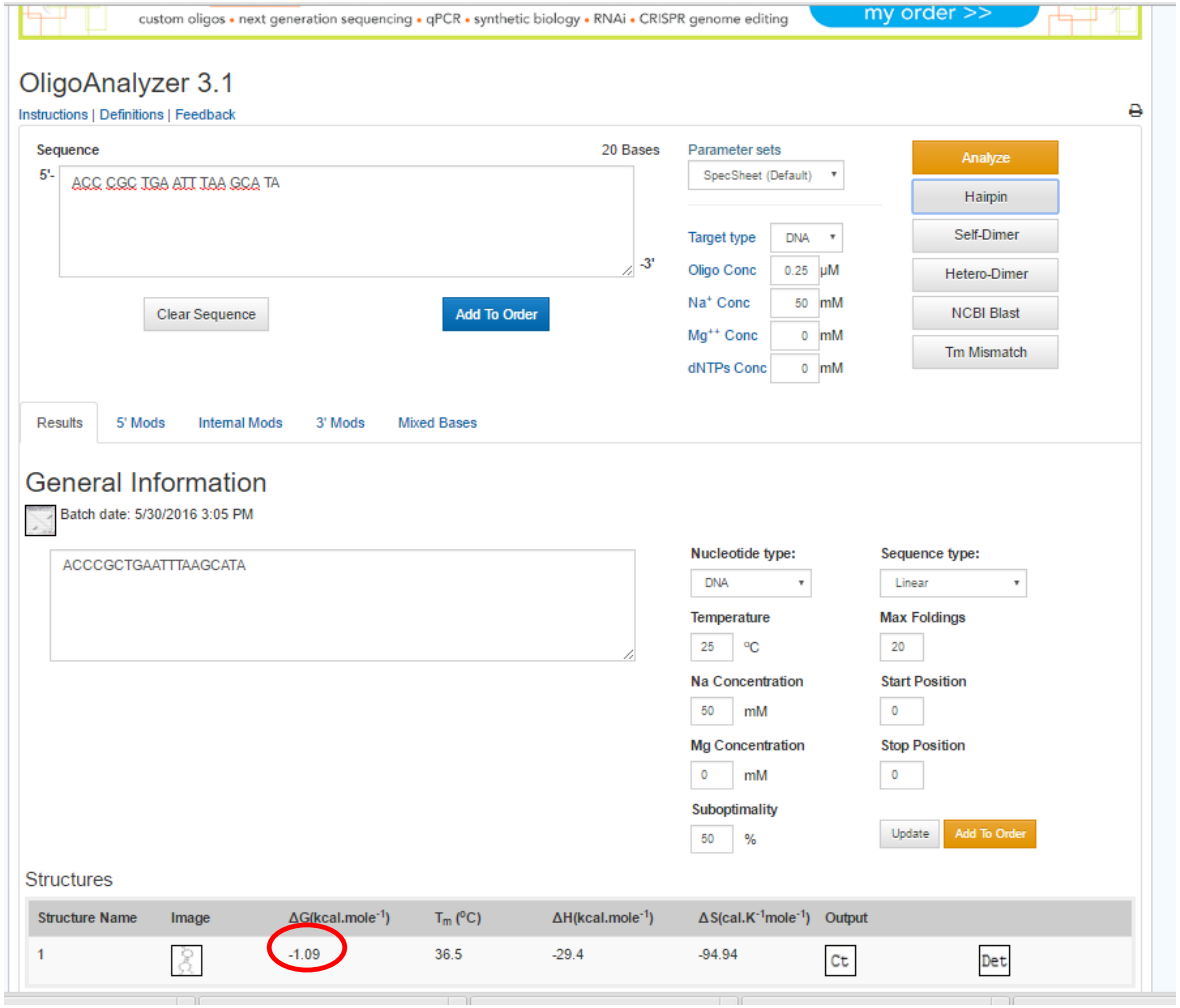
Primer design

Primer Design using Software- analyse primers



Primer design

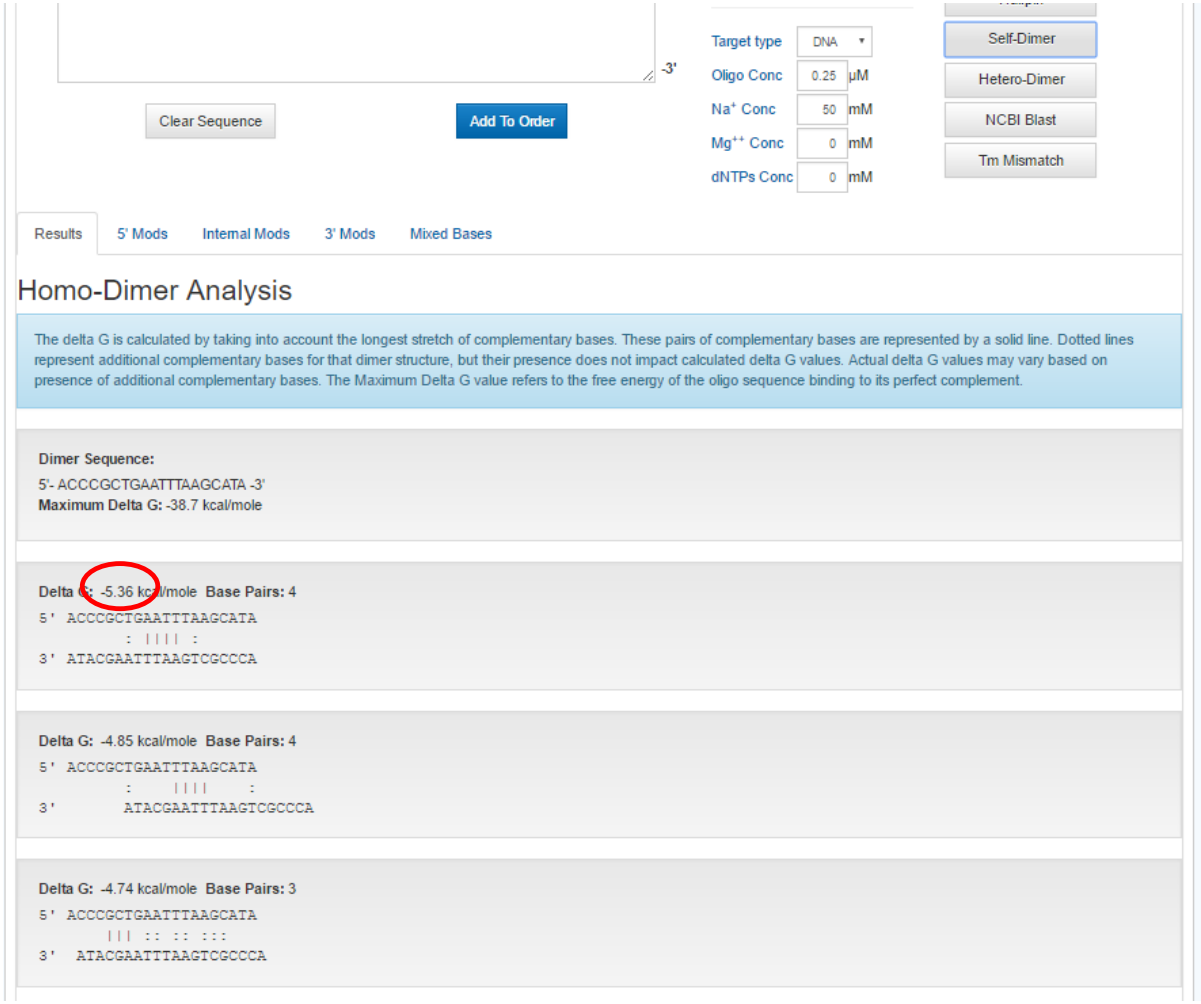
Primer Design using Software- analyse primers



Lenght: 20bp (18-22)
GC%: 40 (40-60)
Harpin: -1 (~-3)

Primer design

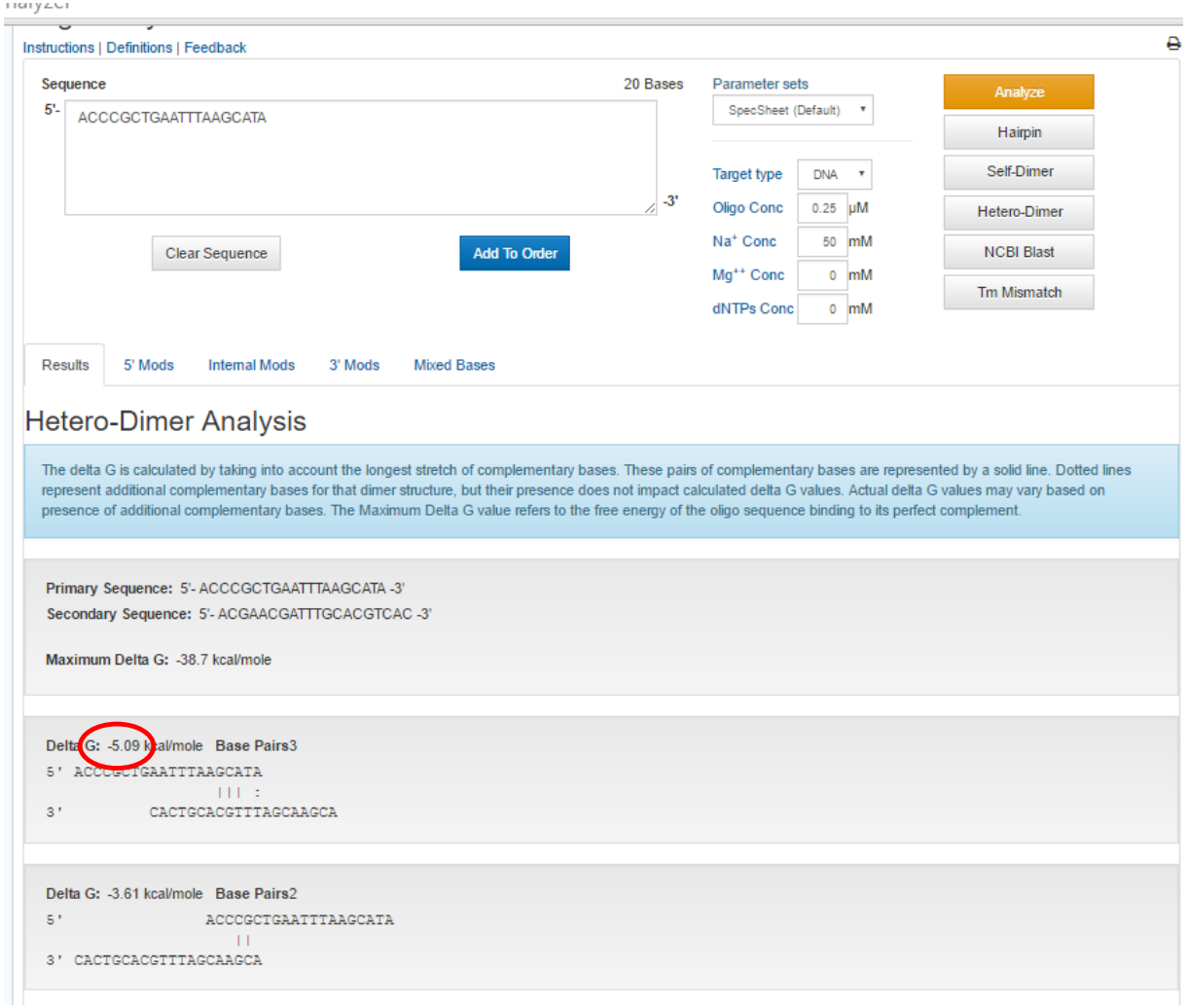
Primer Design using Software- analyse primers



GC%: 40 (40-60)
Harpin: -1 (~-3)
Self-dimer-: -5 (-5)

Primer design

Primer Design using Software- analyse primers

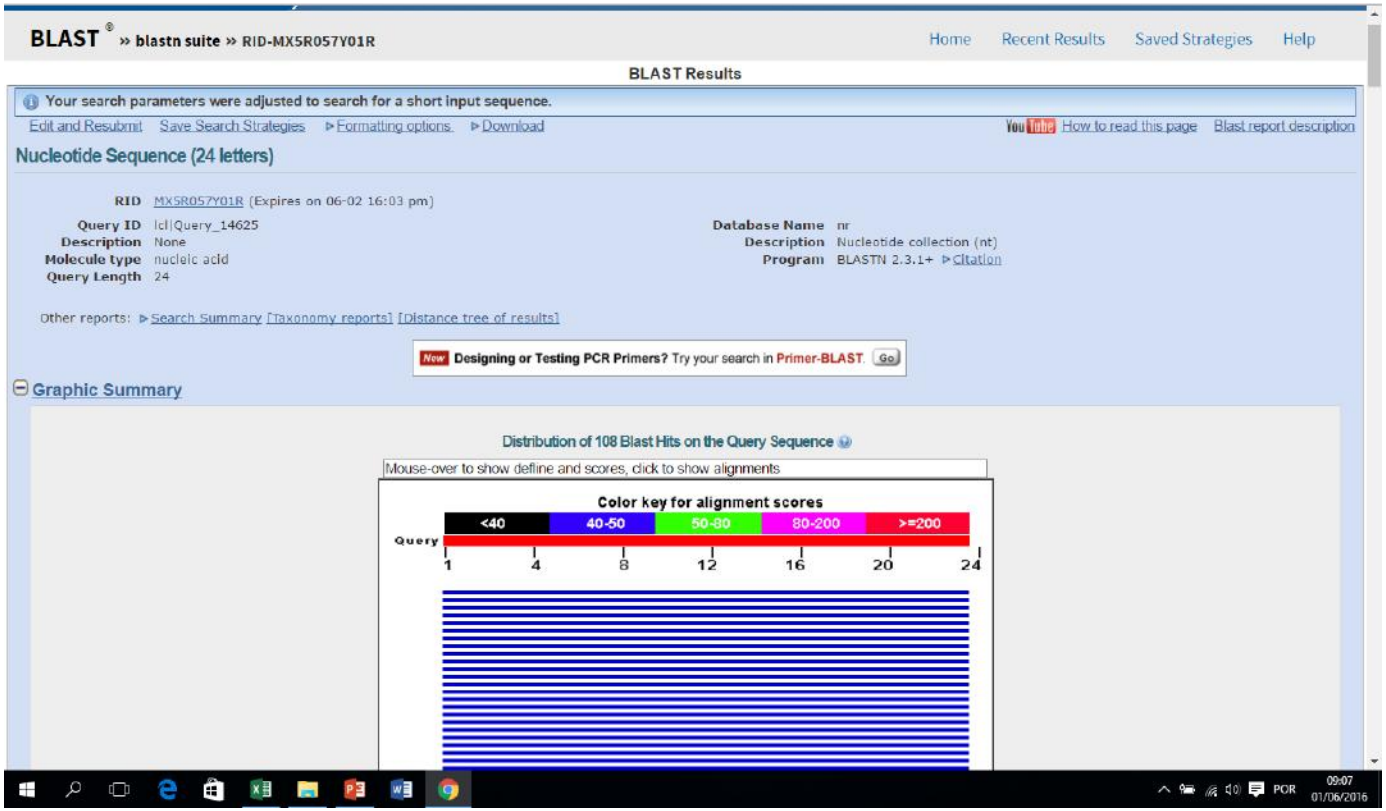


GC%: 40 (40-60)
Harpin: -1 (~-3)
Self-dimer-: -5 (-5)
Hetero-dimer: -5 (-5)
Tm (D1R): 52ºC
(52-58 ºC)
Tm (D3Ca): 56 ºC
(diferença < 5ºC)

Primer design

Primer Design using Software- analyse primers (especificidade)

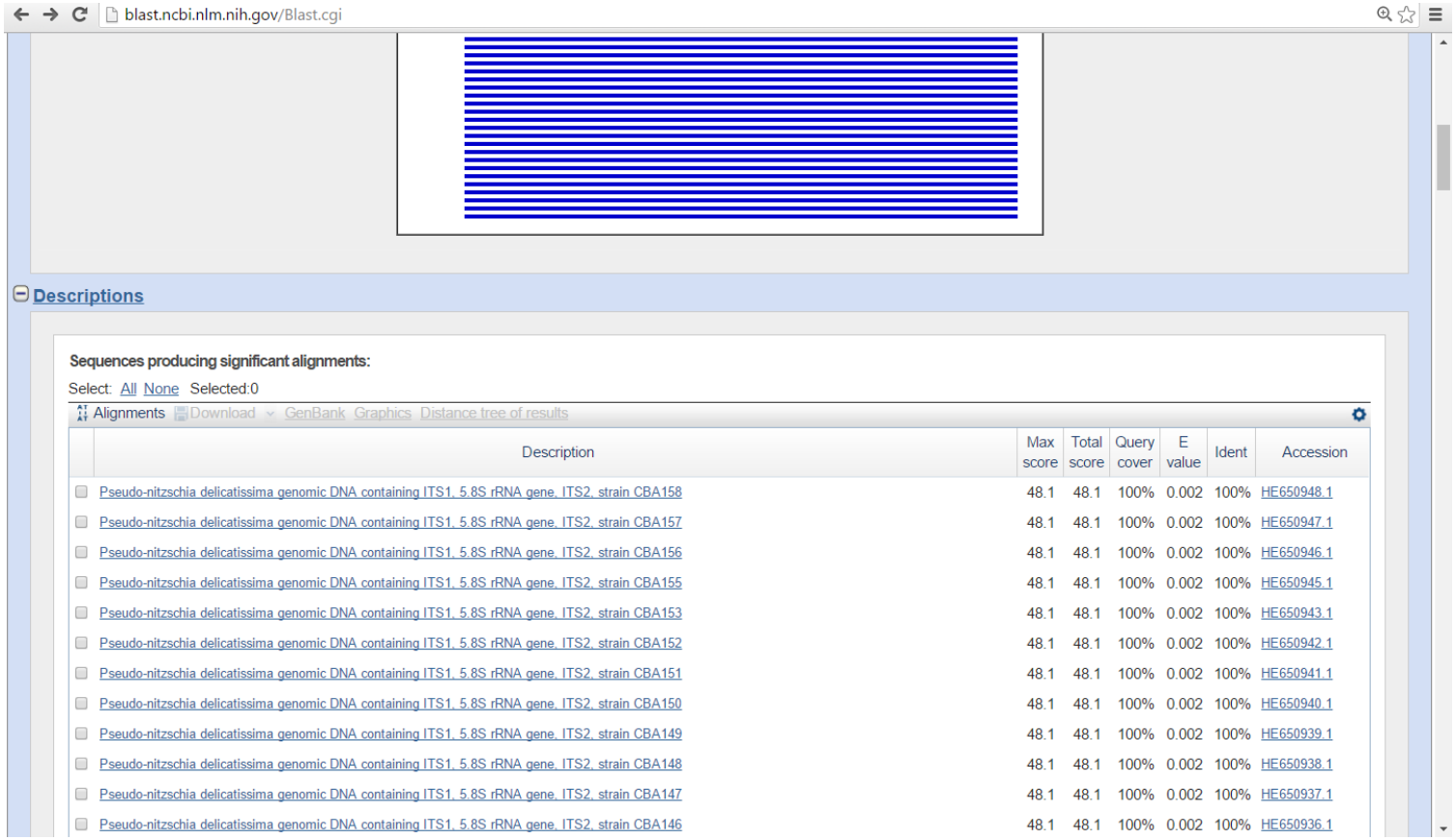
GTGCAATACTTTGCTTGGGTTTCG



Primer design

Primer Design using Software- analyse primers

GTGCAATACTTTGCTTGGGTTTCG

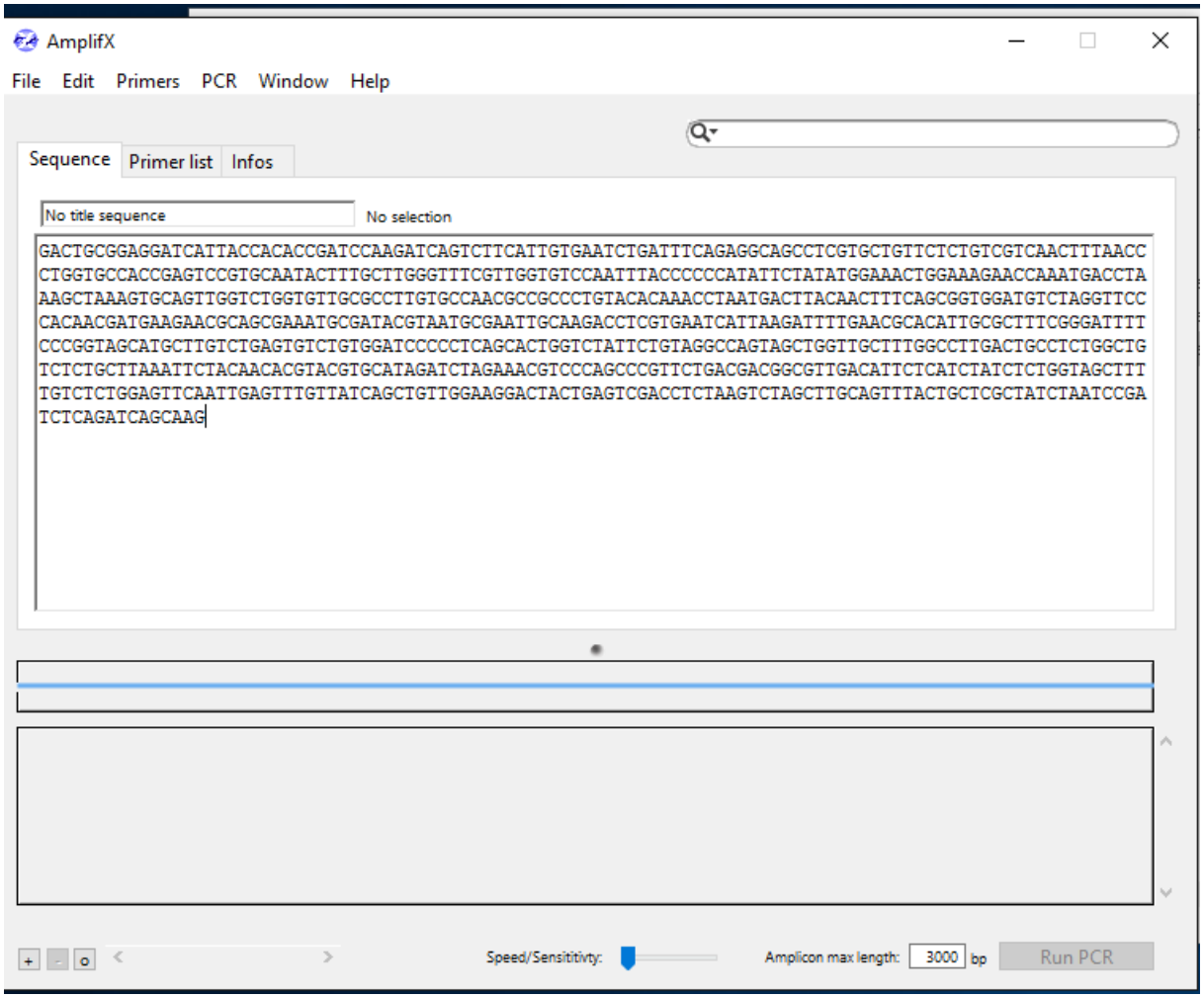


Description	Max score	Total score	Query cover	E value	Ident	Accession
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA158	48.1	48.1	100%	0.002	100%	HE650948.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA157	48.1	48.1	100%	0.002	100%	HE650947.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA156	48.1	48.1	100%	0.002	100%	HE650946.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA155	48.1	48.1	100%	0.002	100%	HE650945.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA153	48.1	48.1	100%	0.002	100%	HE650943.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA152	48.1	48.1	100%	0.002	100%	HE650942.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA151	48.1	48.1	100%	0.002	100%	HE650941.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA150	48.1	48.1	100%	0.002	100%	HE650940.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA149	48.1	48.1	100%	0.002	100%	HE650939.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA148	48.1	48.1	100%	0.002	100%	HE650938.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA147	48.1	48.1	100%	0.002	100%	HE650937.1
Pseudo-nitzschia delicatissima genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain CBA146	48.1	48.1	100%	0.002	100%	HE650936.1

Primer design

Primer Design using Software- analyse primers

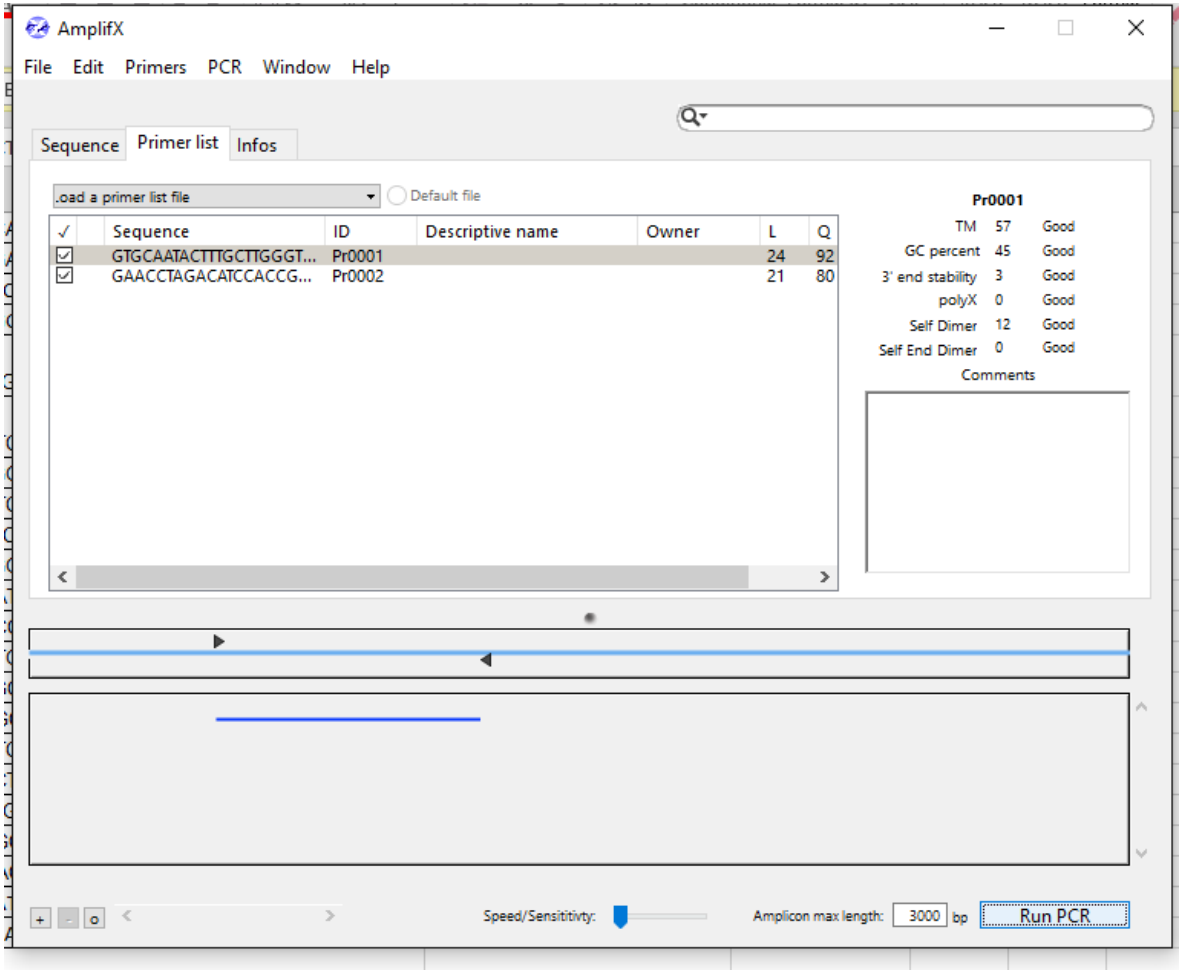
Virtual PCR



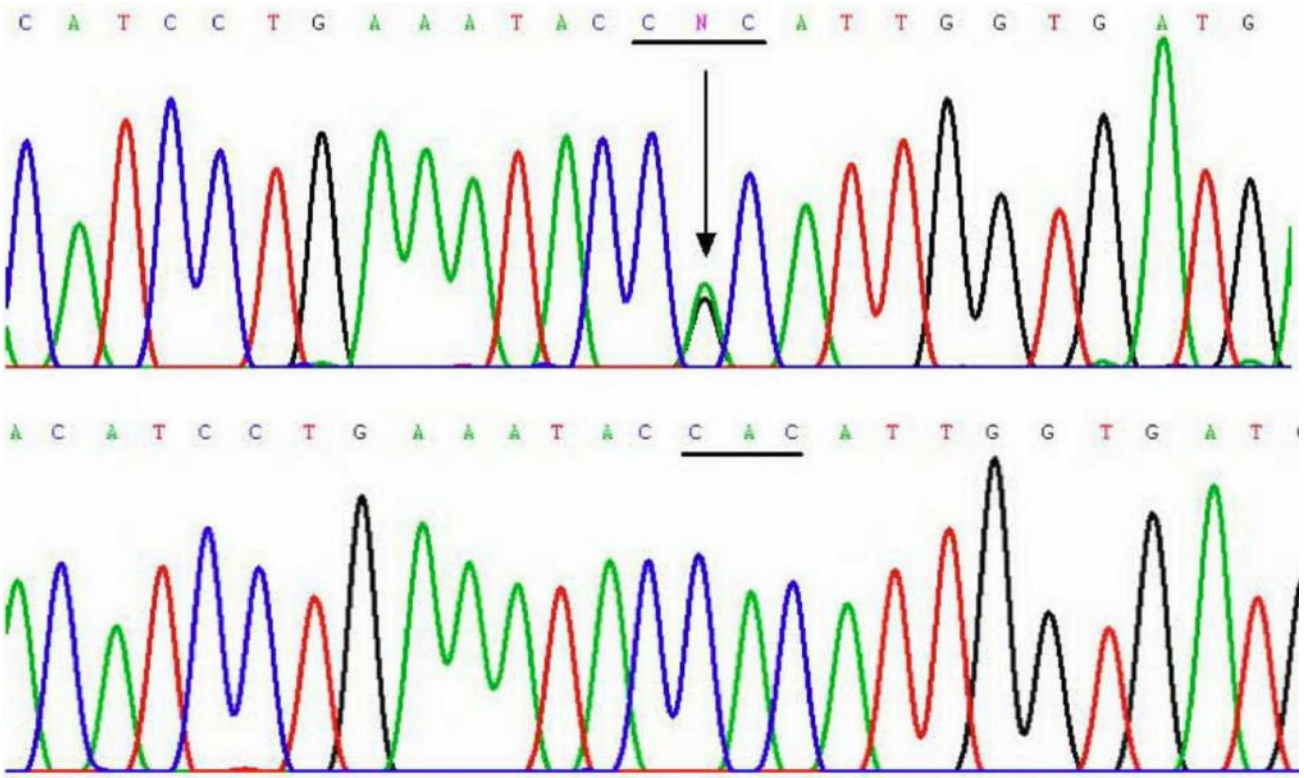
Primer design

Primer Design using Software- analyse primers

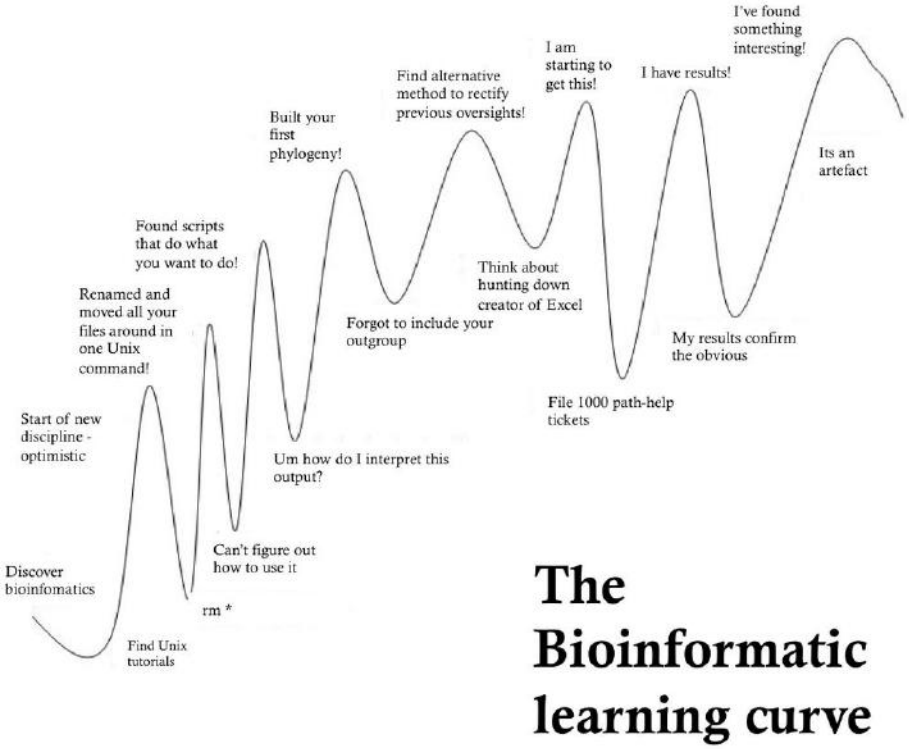
GTGCAATACTTTGCTTGGGTTTCG



Sequencing Results



Learning Curve



Further reading

Swofford et al. 1996. Phylogenetic Inference. In Hillis, Moritz, & Mable [Eds.],

Molecular Systematics. Sinauer Associates, Sunderland, M.A.

Foster, 2007. Inferring phylogenetic relationships from sequence data. In Dear [Ed.],

Bioinformatics. Scion.

Lewis, 2001. Phylogenetic systematics turns of a new leaf. *Trends in Ecology and*

Evolution, 16: 30-37.

Felsenstein 2004. *Inferring Phylogenies*. Sinauer Associates, Sunderland, M.A.

Obrigada

Curso BIOMAR PT
2016

Formadores
Bárbara Frazão
João Paulo Machado

INTRODUÇÃO À BIOLOGIA MOLECULAR E BIOINFORMÁTICA:

Bioinformatics

Lisboa, 12-14 October

João Machado

Bárbara Frazão

Bioinformatic

- What is it? Bioinformatics is the creation , development and operation of databases and other computational tools to collect, organize and interpret data

- Data Sources ? They are usually derived from biological data experiences that provide quantitative and qualitative data

Use of databases in bioinformatics as repositories and sources of information

Data Warehouses

- From 1982 databases began to be created for storing information and sequences of nucleotides

Examples

- European Molecular Biology Laboratory: http://www.embl.org/ (Europe)
- National Institutes of Health: http://www.ncbi.nlm.nih.gov (North America)
- DNA Databank (DDBJ): http://www.ddbj.nig.ac.jp/ (Japan)

Data Warehouses

- From 1986 databases of amino acids (proteins)

Exemplos

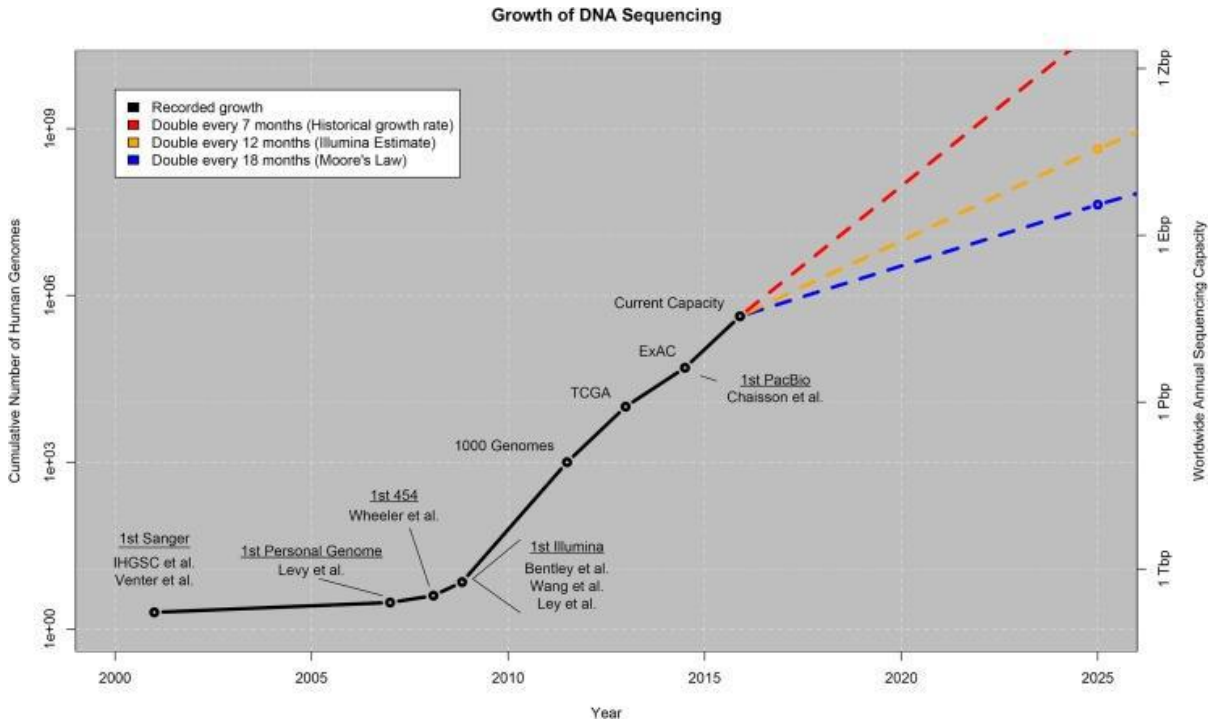
- Swissprot/TrEMBL
- PIR

- In 2002 joined in UniProtKb (http://www.uniprot.org/)

- **UniProtKB/Swiss-Prot** which is manually annotated and is reviewed and
- **UniProtKB/TrEMBL** which is automatically annotated and is not reviewed.

Data growth

- "100-gigabase" in August 2005. 200 billion bp in September 2007. The amount of data doubles every 18 months.



PLoS Biol. 2015 Jul; 13(7): e1002195.

Conventions

GenBank	gb|accession.version
EMBL	emb|accession.version
DDBJ	dbj|accession.version
NCBI	RefSeq ref|accession.version
PDB	pdb|entry|chain
Patents	pat|country|number
NBRF PIR	pir||entry
SWISS-PROT	sp|accession|entry
Protein Research Foundation	prf|name
Local Sequence identifier	lcl|identifier

```
ref|NM_016519.5| Homo sapiens ameloblastin
(AMBN)
```

Conventions

RefSeq categories

Experimentally determined and curated		Genome annotation (computational predictions from DNA)	
NC	Complete genomic molecules		
NG	Incomplete genomic region		
NM	mRNA	XM	Model mRNA
NR	RNA (non-coding)		
NP	Protein	XP	Model protein

```
ref|NM_016519.5| Homo sapiens ameloblastin
(AMBN)
```

Conventions

IUPAC nucleotide ambiguity codes

Symbol	Meaning	Nucleic Acid
A	A	Adenine
C	C	Cytosine
G	G	Guanine
T	T	Thymine
U	U	Uracil
M	A or C	
R	A or G	
W	A or T	
S	C or G	
Y	C or T	
K	G or T	
V	A or C or G	
H	A or C or T	
D	A or G or T	
B	C or G or T	
X	G or A or T or C	
N	G or A or T or C	

Reference:

IUPAC-IUB SYMBOLS FOR NUCLEOTIDE NOMENCLATURE:
Cornish-Bowden (1985) Nucl. Acids Res. 13: 3021-3030.

Conventions

- Gene Names (http://www.genenames.org/)

File Formats

- Fasta files

```
>seq1
----------------------KSKERYKDENGGNYFQLREDWWDANRE
>seq2
---------------YEGLTTANGXKEYYQDKNGGNFFKLREDWWTANRE
>seq3
-----------------------SQRHYKD-DGGNYFQLREDWWTANRH
>seq4
--------------------NVAALKTRYEK-DGQNFYQLREDWWTANYF
```

File Formats

- Phylip interleaved

- The first line of the input file contains the number of species and the number of characters separated by blanks. The information for each species follows, starting with a ten-character species name (which can include punctuation marks and blanks), and continuing with the characters for that species. Phylip format files can be interleaved, as in the example below, or sequential.

```
4 123
seq1 ---------- ---------- ---KSKERYK DENGGNYFQL REDWWDANRE
seq2 ---------- -----YEGLT TANGXKEYYQ DKNGGNFFKL REDWWTANRE
seq3 ---------- ---------- ----SQRHYK D-DGGNYFQL REDWWTANRH
seq4 ---------- ---------- NVAALKTRYE K-DGQNFYQL REDWWTANRA

 TVWKAITCNA --GGGKYFRN TCDG--GQNP TETQNNCRCIG---------
 TVWKAITCGA P-GDASYFHA TCDSGDGRGG AQAPHKCRCD G---------
 TVWEAITCSA DKGNA-YFRR TCNSADGKSQ SQARNQCRC- --KDENGKN-
 TIWEAITCSA DKGNA-YFRA TCNSADGKSQ SQARNQCRC- --KDENGXN-
```

File Formats

- Phylip sequencial

```
4 123
seq1 ---------- ---------- ---KSKERYK DENGGNYFQL REDWWDANRE
TVWKAITCNA --GGGKYFRN TCDG--GQNP TETQNNCRCI G---------
seq2 ---------- -----YEGLT TANGXKEYYQ DKNGGNFFKL REDWWTANRE
TVWKAITCGA P-GDASYFHA TCDSGDGRGG AQAPHKCRCD G---------
seq3 ---------- ---------- ----SQRHYK D-DGGNYFQL REDWWTANRH
TVWEAITCSA DKGNA-YFRR TCNSADGKSQ SQARNQCRC- --KDENGKN-
seq4 ---------- ---------- NVAALKTRYE K-DGQNFYQL REDWWTANRA
TIWEAITCSA DKGNA-YFRA TCNSADGKSQ SQARNQCRC- --KDENGXN-
```

File Formats

- Nexus

```
#NEXUS
BEGIN DATA;
     DIMENSIONS NTAX=10 NCHAR=22;
     FORMAT MISSING=? DATATYPE=DNA GAP=- EQUATE="0=A 1=C";
     OPTIONS GAPMODE=MISSING;
MATRIX

                         [0000000000000000000000]
                         [0000000000000000000000]
                         [0000000001111111111222]
                         [1234567890123456789012]

TaxonA                    AAAAAAAAAAAAAAAA000000
TaxonB                    AA-----AAA--AAAA1--100
TaxonC                    AAA--AAAAAA--AAA010010
TaxonD                    AAAGAA-AAAAGAA-A001001
TaxonE                    ACGTACGTACGTACGT000000
TaxonF                    AAAAAAAAAAAAAAAA000000
TaxonG                    AA-----AAA--AAAA1--100
TaxonH                    AAA--AAAAAA--AAA010010
TaxonI                    AAAGAA-AAAAGAA-A001001
TaxonJ                    ACGTACGTACGTACGT000000
;
END;

[ Indel Character      Sequence Region ]
[ ---------------      --------------- ]
[                                      ]
[ 17              3-7          ]
[ 18              4-5          ]
[ 19              7-7          ]
[ 20              11-12          ]
[ 21              12-13          ]
[ 22              15-15          ]
```

File Formats

GenBank

```
LOCUS       AF023787                618 bp    DNA     linear   PLN 02-MAY-1998
DEFINITION  Bryum stenotrichum small ribosomal protein 4 (rps4) gene,
            chloroplast gene encoding chloroplast protein, partial cds.
ACCESSION   AF023787
VERSION     AF023787.1  GI:3098167
KEYWORDS    .
SOURCE      chloroplast Bryum stenotrichum
  ORGANISM  Bryum stenotrichum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Bryophyta;
            Moss Superclass V; Bryopsida; Bryidae; Bryanae; Bryales; Bryaceae;
            Bryum.
REFERENCE   1  (bases 1 to 618)
  AUTHORS   Cox,C.J. and Hedderson,T.A.J.
  TITLE     Phylogenetic relationships among the ciliate arthrodontous mosses:
            evidence from chloroplast and nuclear DNA sequences
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 618)
  AUTHORS   Cox,C.J. and Hedderson,T.A.J.
  TITLE     Direct Submission
  JOURNAL   Submitted (11-SEP-1997) Dept. of Botany, School of Plant Sciences,
            University of Reading, Whiteknights, Reading, Berkshire RG6 6AS,
            United Kingdom
FEATURES             Location/Qualifiers
     source          1..618
                     /organism="Bryum stenotrichum"
                     /organelle="plastid:chloroplast"
                     /mol_type="genomic DNA"
                     /db_xref="taxon:66994"
     gene            <1..573
                     /gene="rps4"
     CDS             <1..573
                     /gene="rps4"
                     /codon_start=1
                     /product="small ribosomal protein 4"
                     /protein_id="AAC15532.1"
                     /db_xref="GI:3098168"
                     /translation="RRLGSLPGLTNKTPQLKTNSINQSISNKKISQYRIRLEEKQKLR
                     FHYGITERQLLNYVRIARKAKGSTGEVLLQLLEMRLDNVIFRLGMAPTIPGARQLVNH
                     RHILVNDRIVNIPSYRCKPEDSITIKDRQKSQAIISKNLNLYQKYKTPNHLTYNFLKK
                     KGLVNQILDRESIGLKINELLVVEYYSRQA"
ORIGIN
        1 cgccgtttag gatctttacc aggactaact aataaaacac cccagttaaa aactaattcg
       61 atcaatcaat caatatctaa taaaaaaatt tctcaatatc gcattcgttt ggaagaaaaa
      121 caaaaattac gttttcatta tggaataaca gagcgacaat tacttaatta tgtacgtatt
      181 gctagaaaag ctaaagggtc aacaggtgaa gtcttattac aattacttga aatgcgctta
      241 gataacgtta tttttcgatt aggtatggct cctacaattc ctggagcaag gcaactagta
      301 aatcatagac atattttagt taatgatcgt atagtaaata taccaagtta tcggtgtaaa
      361 cctgaggatt ctattactat aaaagatcga caaaaatctc aggctataat tagtaaaaat
      421 ttaaatttgt atcaaaaata taaaacacca aatcatttaa cttataattt tttaaaaaaa
      481 aaaggattgg ttaatcaaat actagatcgt gaatccattg gtttaaaaat aaatgaatta
      541 ttagttgtag aatattattc tcgccaagct taattaacaa ctaagagtat ttgtaattat
      601 atacataata aaaatttg
//
```

File Convertions

- Software (e.g. Seaview, Mega, Mesquite, Bioedit, etc)
  - Requires instalation
- Scripts (generally in Python or Perl)
  - Several are freely distributed in github (https://github.com/)
- Web-based tool (easy)

Hands-On 1

- https://goo.gl/7DgwFF

1) Descarregar as sequencias na pasta hands_on_1

2) Usar o site: http://www.ebi.ac.uk/Tools/sfc/emboss_seqret/

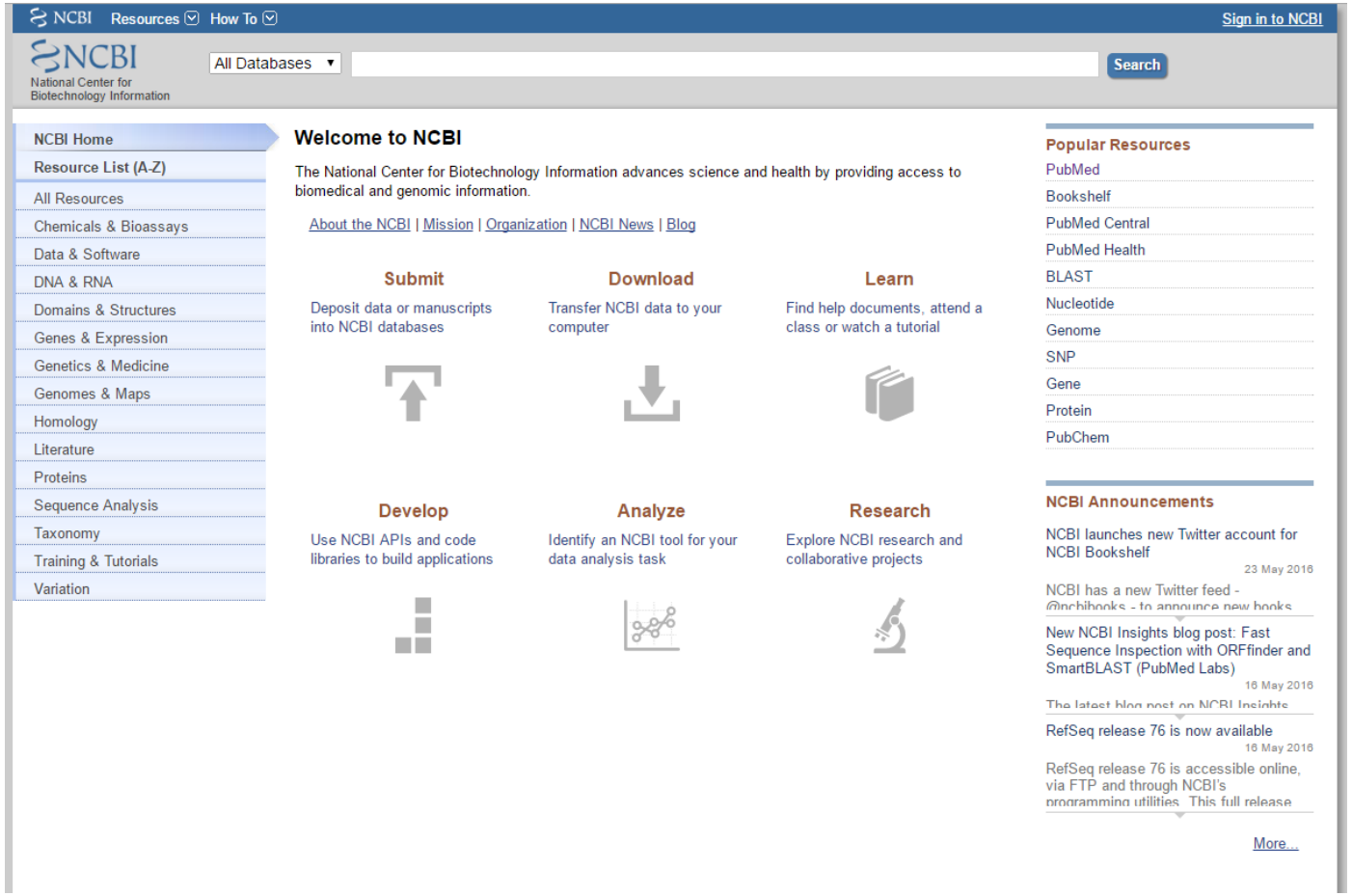
3) Analisar o formato original das sequencias

4) Converter as sequencias para Fasta

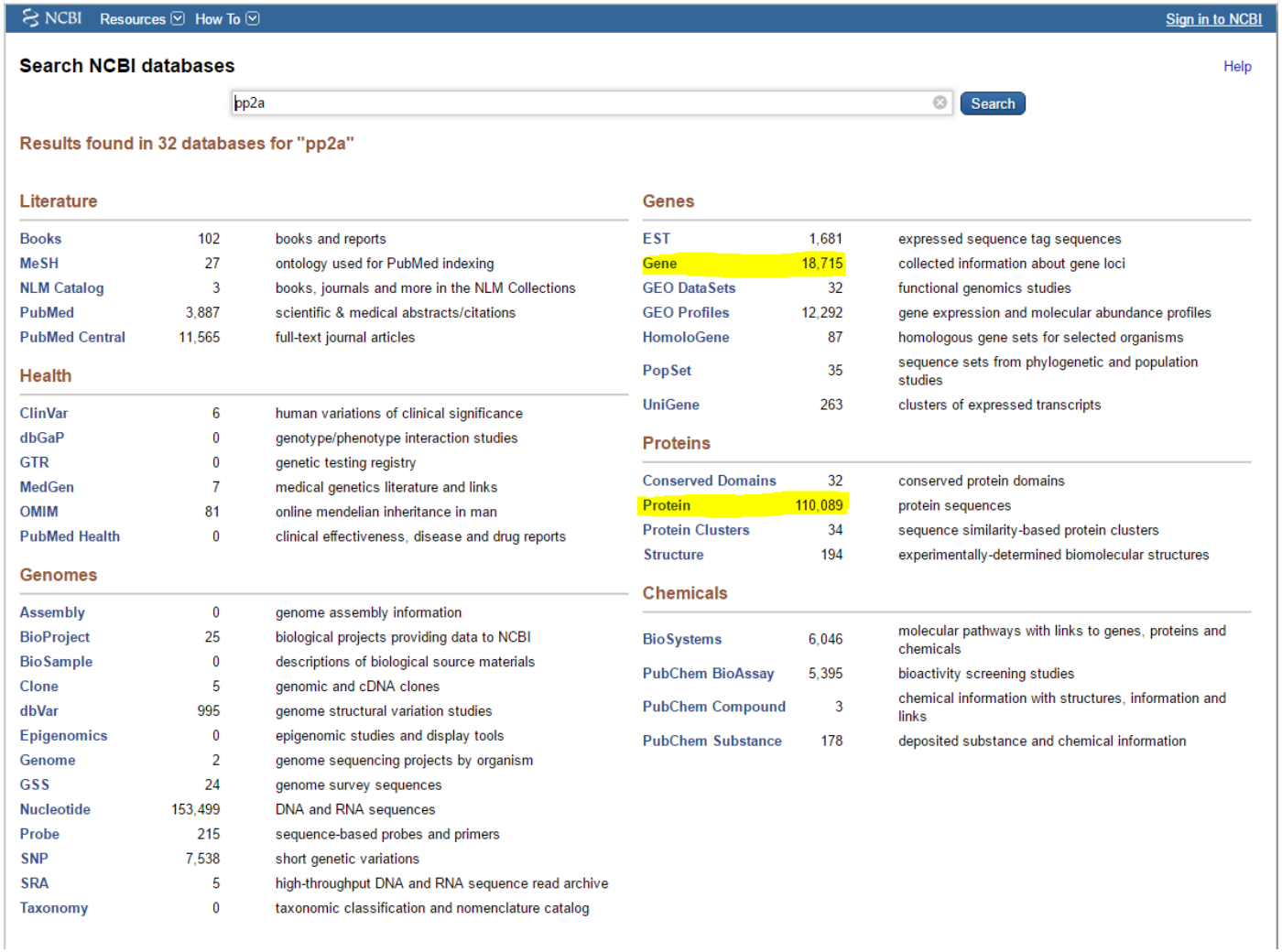
5) Adicionar num ficheiro separado usando editor de texto

Search Methods

Search NCBI


NCBI Resources How To
Sign in to NCBI
NCBI
National Center for Biotechnology Information
All Databases
Search
NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation
Welcome to NCBI
The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.
About the NCBI | Mission | Organization | NCBI News | Blog
Submit
Deposit data or manuscripts into NCBI databases
Download
Transfer NCBI data to your computer
Learn
Find help documents, attend a class or watch a tutorial
Develop
Use NCBI APIs and code libraries to build applications
Analyze
Identify an NCBI tool for your data analysis task
Research
Explore NCBI research and collaborative projects
Popular Resources
PubMed
Bookshelf
PubMed Central
PubMed Health
BLAST
Nucleotide
Genome
SNP
Gene
Protein
PubChem
NCBI Announcements
NCBI launches new Twitter account for NCBI Bookshelf
23 May 2016
NCBI has a new Twitter feed - @ncbibooks - to announce new books
New NCBI Insights blog post: Fast Sequence Inspection with ORFfinder and SmartBLAST (PubMed Labs)
16 May 2016
The latest blog post on NCBI Insights
RefSeq release 76 is now available
16 May 2016
RefSeq release 76 is accessible online, via FTP and through NCBI's programming utilities. This full release
More...

Search NCBI



NCBI Resources How To Sign in to NCBI

Search NCBI databases Help

pp2a Search

Results found in 32 databases for "pp2a"

Literature

Books	102	books and reports
MeSH	27	ontology used for PubMed indexing
NLM Catalog	3	books, journals and more in the NLM Collections
PubMed	3,887	scientific & medical abstracts/citations
PubMed Central	11,565	full-text journal articles

Health

ClinVar	6	human variations of clinical significance
dbGaP	0	genotype/phenotype interaction studies
GTR	0	genetic testing registry
MedGen	7	medical genetics literature and links
OMIM	81	online mendelian inheritance in man
PubMed Health	0	clinical effectiveness, disease and drug reports

Genomes

Assembly	0	genome assembly information
BioProject	25	biological projects providing data to NCBI
BioSample	0	descriptions of biological source materials
Clone	5	genomic and cDNA clones
dbVar	995	genome structural variation studies
Epigenomics	0	epigenomic studies and display tools
Genome	2	genome sequencing projects by organism
GSS	24	genome survey sequences
Nucleotide	153,499	DNA and RNA sequences
Probe	215	sequence-based probes and primers
SNP	7,538	short genetic variations
SRA	5	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	1,681	expressed sequence tag sequences
Gene	18,715	collected information about gene loci
GEO DataSets	32	functional genomics studies
GEO Profiles	12,292	gene expression and molecular abundance profiles
HomoloGene	87	homologous gene sets for selected organisms
PopSet	35	sequence sets from phylogenetic and population studies
UniGene	263	clusters of expressed transcripts

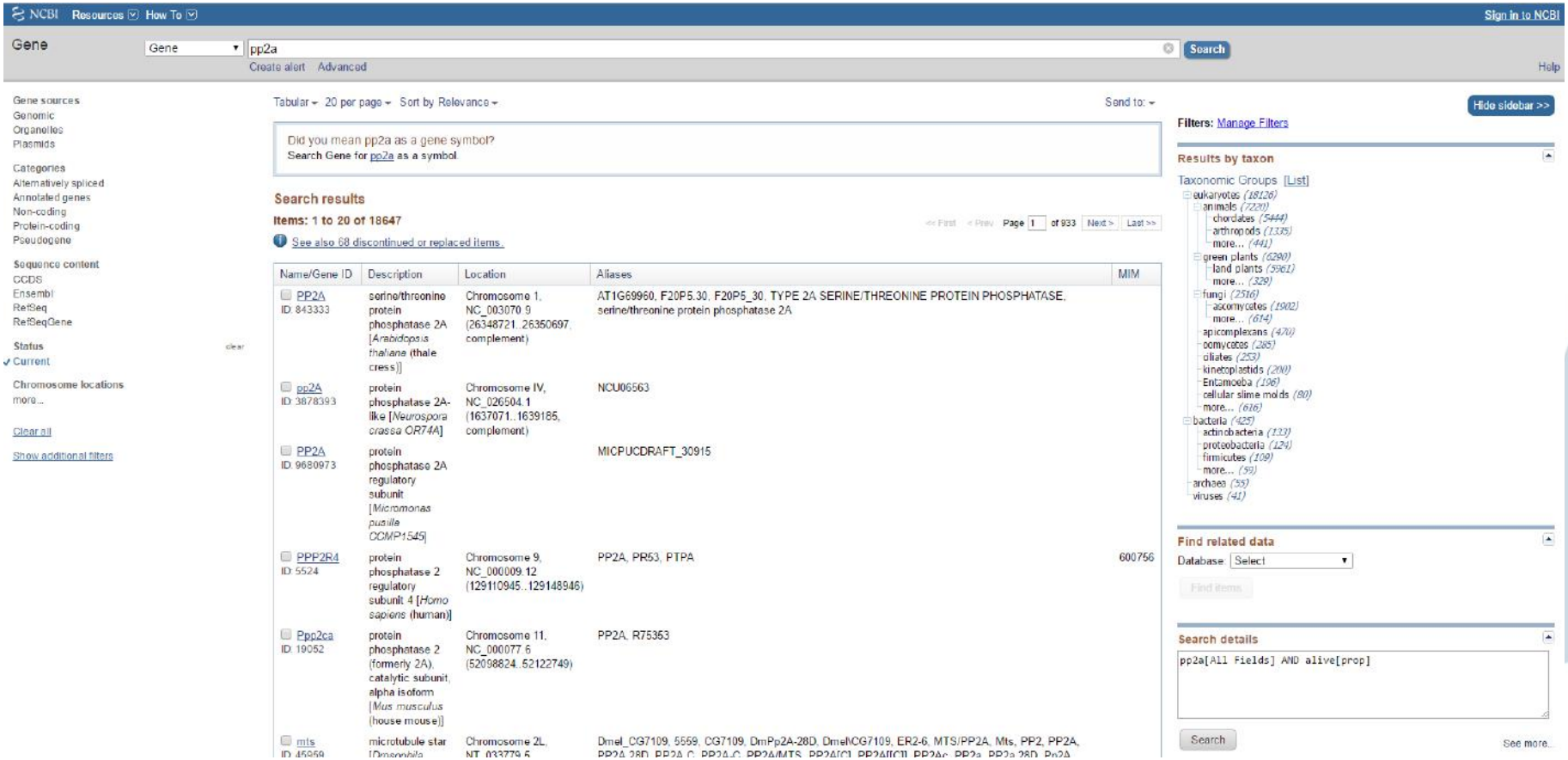
Proteins

Conserved Domains	32	conserved protein domains
Protein	110,089	protein sequences
Protein Clusters	34	sequence similarity-based protein clusters
Structure	194	experimentally-determined biomolecular structures

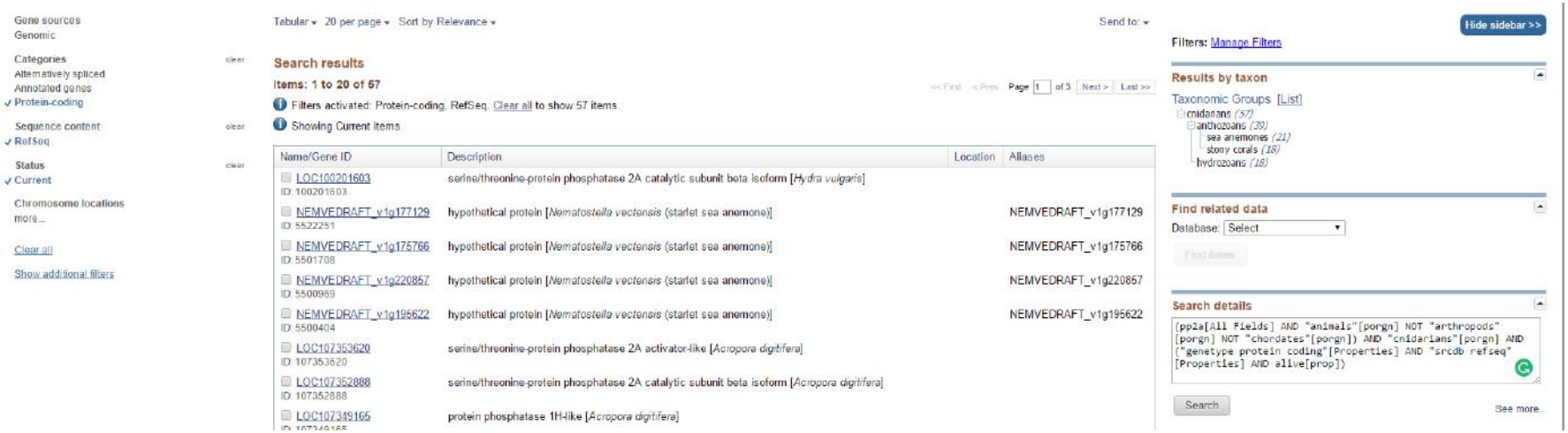
Chemicals

BioSystems	6,046	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	5,395	bioactivity screening studies
PubChem Compound	3	chemical information with structures, information and links
PubChem Substance	178	deposited substance and chemical information

Search NCBI


NCBI Resources How To
Sign in to NCBI
Gene
Gene
pp2a
Search
Create alert Advanced
Help
Gene sources
Genomic
Organelles
Plasmids
Categories
Alternatively spliced
Annotated genes
Non-coding
Protein-coding
Pseudogene
Sequence content
CCDS
Ensembl
RefSeq
RefSeqGene
Status
Current
Chromosome locations
more...
Clear all
Show additional filters
Tabular 20 per page Sort by Relevance
Send to:
Hide sidebar >>
Filters: Manage Filters
Did you mean pp2a as a gene symbol?
Search Gene for pp2a as a symbol
Search results
Items: 1 to 20 of 18647
Page 1 of 933 Next > Last >>
See also 68 discontinued or replaced items.
Name/Gene ID
Description
Location
Aliases
MIM
PP2A ID: 843333
serine/threonine protein phosphatase 2A [Arabidopsis thaliana (thale cress)]
Chromosome 1, NC_003070.9 (26348721..26350697, complement)
AT1G69960, F20P5.30, F20P5_30, TYPE 2A SERINE/THREONINE PROTEIN PHOSPHATASE, serine/threonine protein phosphatase 2A
pp2A ID: 3878393
protein phosphatase 2A-like [Neurospora crassa OR74A]
Chromosome IV, NC_026504.1 (1637071..1639185, complement)
NCU06563
PP2A ID: 9680973
protein phosphatase 2A regulatory subunit [Micromonas pusilla CCMP1545]
MICPUCDRAFT_30915
PPP2R4 ID: 5524
protein phosphatase 2 regulatory subunit 4 [Homo sapiens (human)]
Chromosome 9, NC_000009.12 (129110945..129148946)
PP2A, PR53, PTPA
600756
Ppp2ca ID: 19052
protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform [Mus musculus (house mouse)]
Chromosome 11, NC_000077.6 (52098824..52122749)
PP2A, R75353
mts
microtubule star
Chromosome 2L, NT_033779.5
Dmel_CG7109, 5559, CG7109, DmPp2A-28D, DmelCG7109, ER2-6, MTS/PP2A, Mts, PP2, PP2A,
Results by taxon
Taxonomic Groups [List]
eukaryotes (18126)
animals (7220)
chordates (5444)
arthropods (1335)
more... (441)
green plants (6290)
land plants (5961)
more... (329)
fungi (2516)
ascomycetes (1902)
more... (614)
apicomplexans (470)
oomycetes (285)
ciliates (253)
kinetoplastids (200)
Entamoeba (196)
cellular slime molds (80)
more... (616)
bacteria (425)
actinobacteria (133)
proteobacteria (124)
firmicutes (109)
more... (59)
archaea (55)
viruses (41)
Find related data
Database Select
Find items
Search details
pp2a[All Fields] AND alive[prop]
Search
See more...

Search NCBI


Gene sources
Genomic
Categories
Alternatively spliced
Annotated genes
Protein-coding
Sequence content
RefSeq
Status
Current
Chromosome locations
more...
Clear all
Show additional filters
Tabular
20 per page
Sort by Relevance
Send to:
Search results
Items: 1 to 20 of 57
Page 1 of 3
Next >
Last >>
Filters activated: Protein-coding, RefSeq. Clear all to show 57 items.
Showing Current items.
Name/Gene ID
Description
Location
Aliases
LOC100201603
ID: 100201603
serine/threonine-protein phosphatase 2A catalytic subunit beta isoform [Hydra vulgaris]
NEMVEDRAFT_v1g177129
ID: 5522251
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g177129
NEMVEDRAFT_v1g175766
ID: 5501708
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g175766
NEMVEDRAFT_v1g220857
ID: 5500969
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g220857
NEMVEDRAFT_v1g195622
ID: 5500404
hypothetical protein [Nematostella vectensis (starlet sea anemone)]
NEMVEDRAFT_v1g195622
LOC107353620
ID: 107353620
serine/threonine-protein phosphatase 2A activator-like [Acropora digitifera]
LOC107352888
ID: 107352888
serine/threonine-protein phosphatase 2A catalytic subunit beta isoform [Acropora digitifera]
LOC107349165
protein phosphatase 1H-like [Acropora digitifera]
Hide sidebar >>
Filters: Manage Filters
Results by taxon
Taxonomic Groups [List]
cnidarians (57)
anthozoans (39)
sea anemones (21)
stony corals (18)
hydrozoans (18)
Find related data
Database: Select
Search details
(pp2a[All Fields] AND "animals"[porgn] NOT "arthropods"[porgn] NOT "chordates"[porgn]) AND "cnidarians"[porgn] AND ("genetype protein coding"[Properties] AND "srcdb refseq"[Properties] AND alive[prop])
Search
See more...

Search NCBI

LOC107353620 serine/threonine-protein phosphatase 2A activator-like [*Acropora digitifera*]

Gene ID: 107353620, updated on 16-Apr-2016

Summary

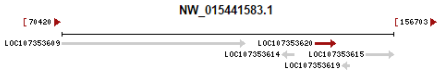
Gene symbol	LOC107353620
Gene description	serine/threonine-protein phosphatase 2A activator-like
Gene type	protein coding
RefSeq status	MODEL
Organism	Acropora digitifera
Lineage	Eukaryota; Metazoa; Cnidaria; Anthozoa; Hexacorallia; Scleractinia; Astrocoeniina; Acroporidae; Acropora

Genomic context

Location: chromosome: Un — See LOC107353620 in Genome Data Viewer

Exon count: 6

Annotation release	Status	Assembly	Chr	Location
100	current	Adig_1.1 (GCF_000222465.1)	Unplaced Scaffold	NW_015441583.1 (136387..141496)



Genomic regions, transcripts, and products

Genomic Sequence: NW_015441583.1 Unplaced Scaffold Reference Adig_1.1 Primary Assembly

Go to reference sequence details

Go to nucleotide: Graphics FASTA GenBank

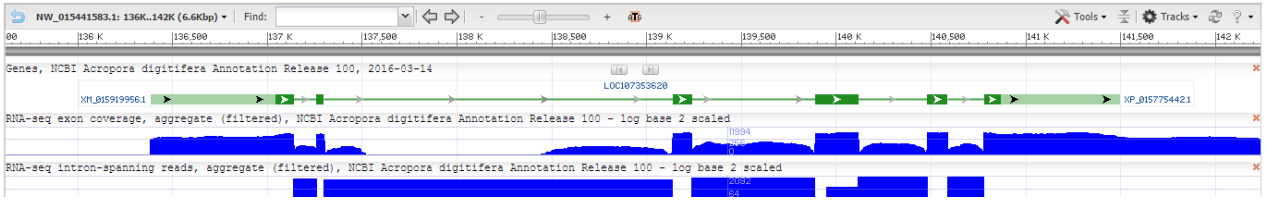


Table of contents

Summary
Genomic context
Genomic regions, transcripts, and products
Bibliography
General protein information
NCBI Reference Sequences (RefSeq)
Related sequences

Genome Browsers

Genome Data Viewer

Related information

BioProjects
Conserved Domains
Gene neighbors
Nucleotide
Protein
RefSeq Proteins
RefSeq RNAs
Taxonomy

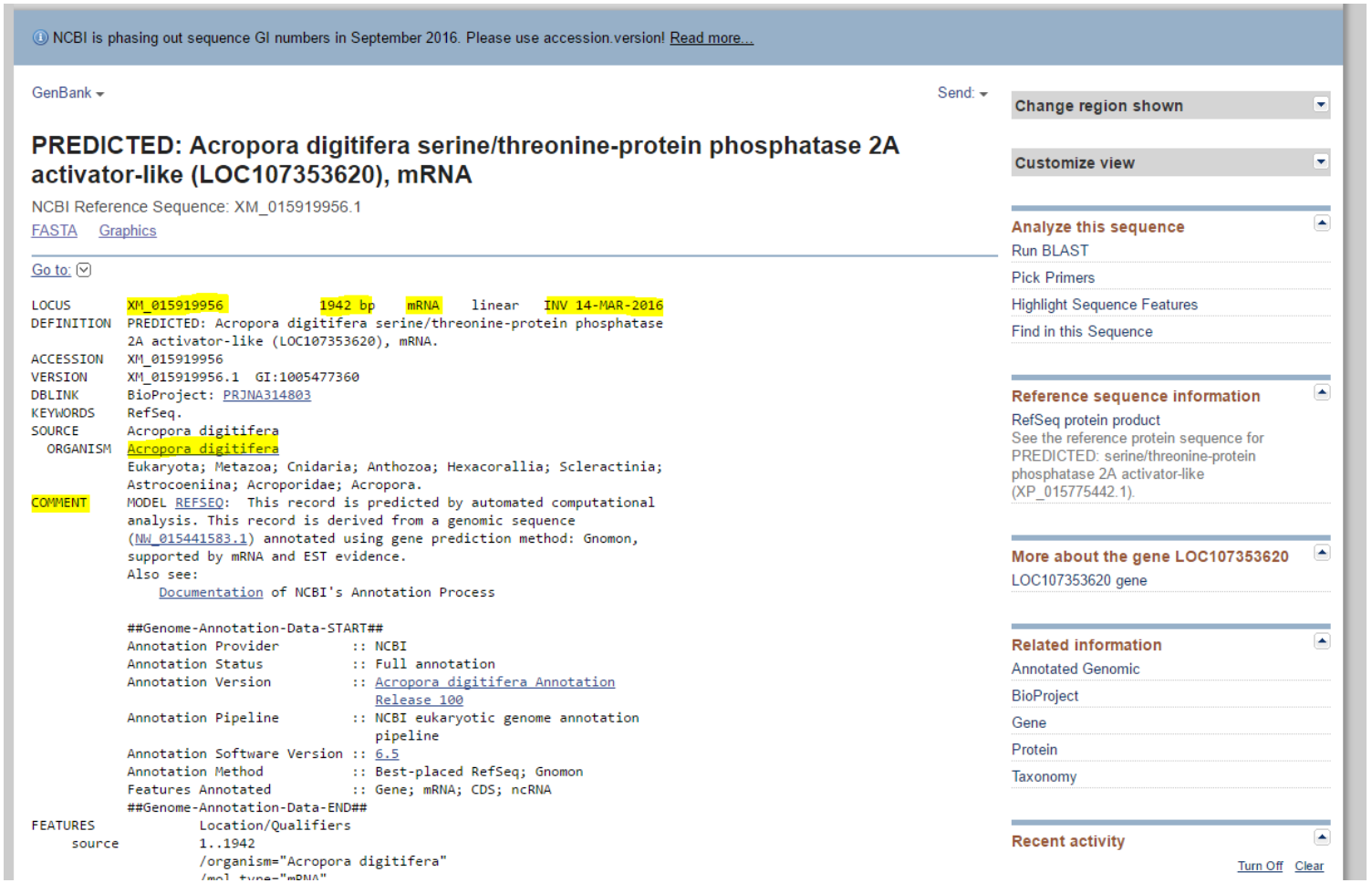
General information

About Gene
FAQ
FTP site
Help
My NCBI help
NCBI Handbook
Statistics

Related sites

BLAST

Search NCBI



NCBI is phasing out sequence GI numbers in September 2016. Please use accession.version! Read more...

GenBank ▾ Send: ▾

PREDICTED: Acropora digitifera serine/threonine-protein phosphatase 2A activator-like (LOC107353620), mRNA

NCBI Reference Sequence: XM_015919956.1

FASTA Graphics

Go to:

```
LOCUS       XM_015919956           1942 bp    mRNA    linear   INV 14-MAR-2016
DEFINITION  PREDICTED: Acropora digitifera serine/threonine-protein phosphatase
            2A activator-like (LOC107353620), mRNA.
ACCESSION   XM_015919956
VERSION     XM_015919956.1  GI:1005477360
DBLINK      BioProject: PRJNA314803
KEYWORDS    RefSeq.
SOURCE      Acropora digitifera
  ORGANISM  Acropora digitifera
            Eukaryota; Metazoa; Cnidaria; Anthozoa; Hexacorallia; Scleractinia;
            Astrocoeniina; Acroporidae; Acropora.
COMMENT     MODEL REFSEQ:  This record is predicted by automated computational
            analysis. This record is derived from a genomic sequence
            (NW_015441583.1) annotated using gene prediction method: Gnomon,
            supported by mRNA and EST evidence.
            Also see:
                Documentation of NCBI's Annotation Process

            ##Genome-Annotation-Data-START##
            Annotation Provider         :: NCBI
            Annotation Status           :: Full annotation
            Annotation Version          :: Acropora digitifera Annotation
                                           Release 100
            Annotation Pipeline         :: NCBI eukaryotic genome annotation
                                           pipeline
            Annotation Software Version :: 6.5
            Annotation Method           :: Best-placed RefSeq; Gnomon
            Features Annotated          :: Gene; mRNA; CDS; ncRNA
            ##Genome-Annotation-Data-END##
FEATURES             Location/Qualifiers
     source          1..1942
                     /organism="Acropora digitifera"
                     /mol_type="mRNA"
```

Change region shown

Customize view

Analyze this sequence
- Run BLAST
- Pick Primers
- Highlight Sequence Features
- Find in this Sequence

Reference sequence information

RefSeq protein product
See the reference protein sequence for PREDICTED: serine/threonine-protein phosphatase 2A activator-like (XP_015775442.1).

More about the gene LOC107353620

LOC107353620 gene

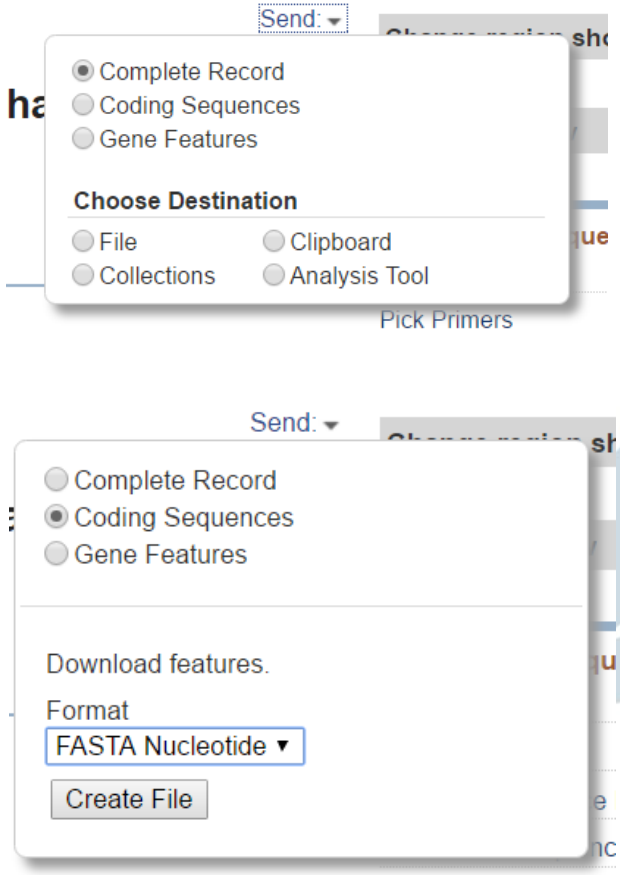
Related information
- Annotated Genomic
- BioProject
- Gene
- Protein
- Taxonomy

Recent activity

Turn Off Clear

Search NCBI

```
FEATURES             Location/Qualifiers
     source          1..1942
                     /organism="Acropora digitifera"
                     /mol_type="mRNA"
                     /db_xref="taxon:70779"
                     /chromosome="Unknown"
                     /country="Japan:Okinawa, Kunigami, Oku"
     gene            1..1942
                     /gene="LOC107353620"
                     /note="Derived by automated computational analysis using
                     gene prediction method: Gnomon. Supporting evidence
                     includes similarity to: 6 mRNAs, 3 ESTs, 25 Proteins, and
                     100% coverage of the annotated genomic feature by RNAseq
                     alignments, including 25 samples with support for all
                     annotated introns"
                     /db_xref="GeneID:107353620"
     CDS             659..1315
                     /gene="LOC107353620"
                     /codon_start=1
                     /product="serine/threonine-protein phosphatase 2A
                     activator-like"
                     /protein_id="XP_015775442.1"
                     /db_xref="GI:1005477361"
                     /db_xref="GeneID:107353620"
                     /translation="MPLQQSVHSLVQPLLPDKFLGAAIELTAYLKDAFGNKTRIDYGT
                     GHEASFAAFLCCLFKLRVLDQSDCAAIVFKVFQRYLELMRRLQLTYRMEPAGSQGVWG
                     LDDFQFLPFIWGSAQLIGHTSLEPQHFTCEKNVEEHHNKYMFLGCIRFINQMKRGPFA
                     EHSNTLWGISSVKTWEKVNSGLMKMYKAEVLSKFPVIQHFVFGTLMSIKEGETFKKPL
                     "
ORIGIN
        1 tcttgagtac tgtagtcttt cccagcattg actgacctag gctggtgaac aatgtttttt
       61 ttttttttt ttttttcat ttgtttctaa gggtaggaga aactactatg aaaggctggg
      121 aaagtcaatt ttatttcata cattaacttg tcactatata agtatgcttg tcactataaa
      181 ctgaaaaatt aaatgtgatt cttttaattc cattttcttt tgtttttttac attttgcaaa
      241 gtcctcaaaa agggtgatat agcaaacact tttctcattc acagctttcg cacatccttg
      301 gttattgtga cttggccaaa agataggaaa atttttcctg ctccagggag aaatataata
      361 ttgcttgatt ctcagaatgg cctgcttata atctgagaaa ttgatcattt ctggtaatta
      421 aggtattgcc ttaatttatt tgtaatggca attggatttt gcttgatgca gcttattaag
      481 aaagccatga attagaagag tccacatcat ttggttgcag ttaagtaatt tccatagttt
```

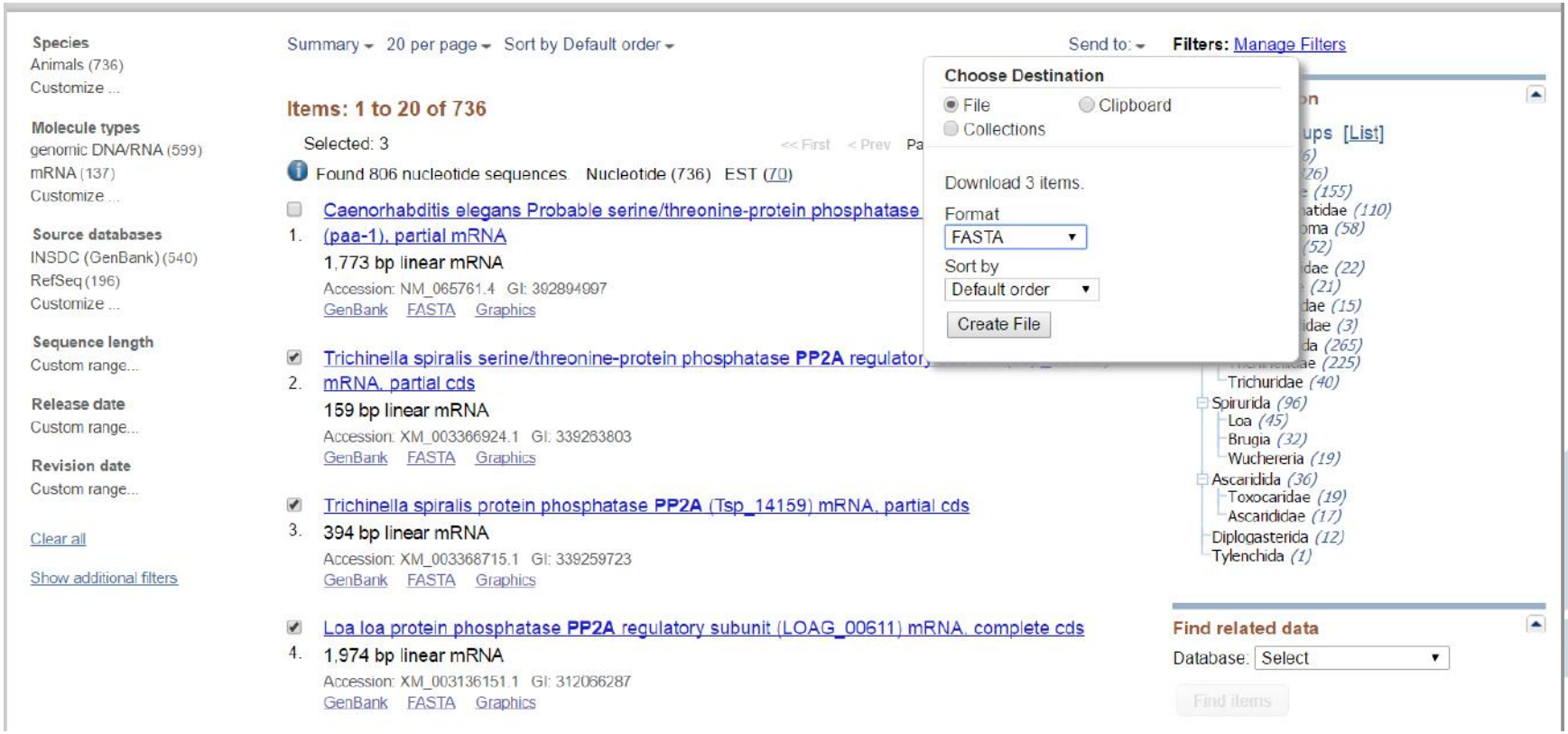


Search NCBI

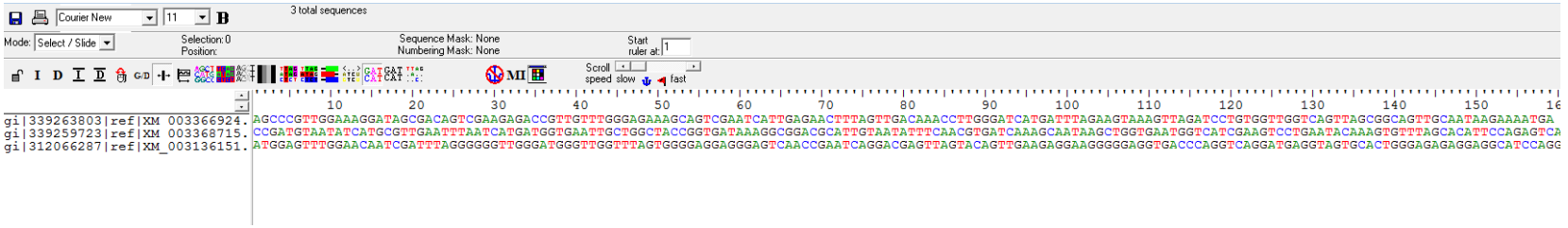
sequence.txt

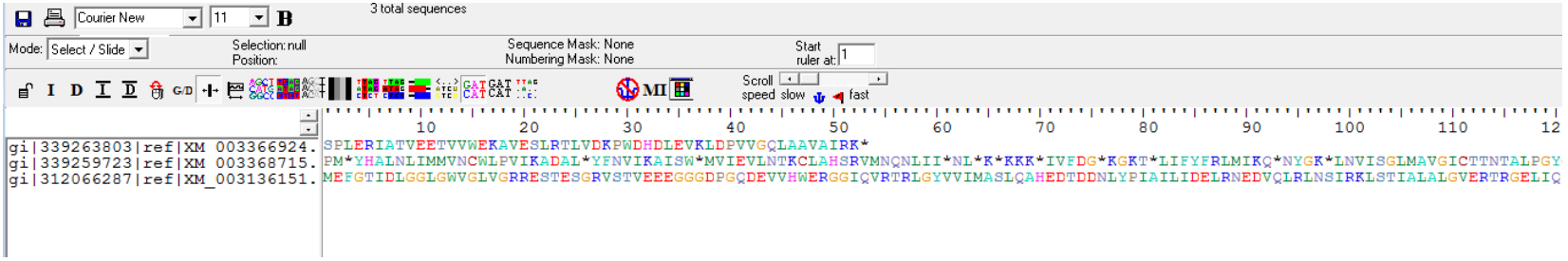
```
>lcl|XM_015919956.1_cds_XP_015775442.1_1 [gene=LOC107353620] [protein=serine/threonine-protein phosphatase 2A activator-like] [protein_id=XP_015775442.1] [location=659..1315]
ATGCCTCTTCAACAGAGTGTTCACTCGTTAGTTCAACCTCTTCTACCAGACAAGTTCCTTGGGGCTGCCA
TTGAATTAACTGCATATTTAAAAGATGCCTTTGGAAATAAAACAAGAATAGACTATGGAACAGGTCATGA
AGCTTCCTTTGCTGCATTTCTTTGTTGTTTATTCAAGCTCAGAGTATTGGACCAAAGTGACTGTGCTGCT
ATTGTGTTCAAGGTTTTTCAGAGGTATTTAGAACTGATGAGACGATTGCAGCTCACTTACAGAATGGAAC
CAGCTGGCAGTCAGGGTGTGTGGGGGCTGGATGATTTTCAGTTTCTCCCTTTCATTTGGGGAAGTGCTCA
GCTGATAGGCCATACAAGTCTAGAGCCACAGCACTTCACTTGTGAAAAAAACGTAGAGGAGCATCATAAC
AAGTACATGTTCCTGGGCTGCATCCGTTTTATAAACCAAATGAAAAGAGGACCCTTTGCAGAACATTCCA
ACACTTTGTGGGGAATAAGCTCTGTTAAAACATGGGAAAAAGTAAACTCTGGTTTGATGAAAATGTATAA
AGCTGAGGTTCTATCCAAGTTCCCAGTCATTCAGCATTTTGTTTTTGGTACATTAATGTCTATAAAAGAA
GGAGAAACGTTTAAAAAACCCCTGTAA
```

Search NCBI

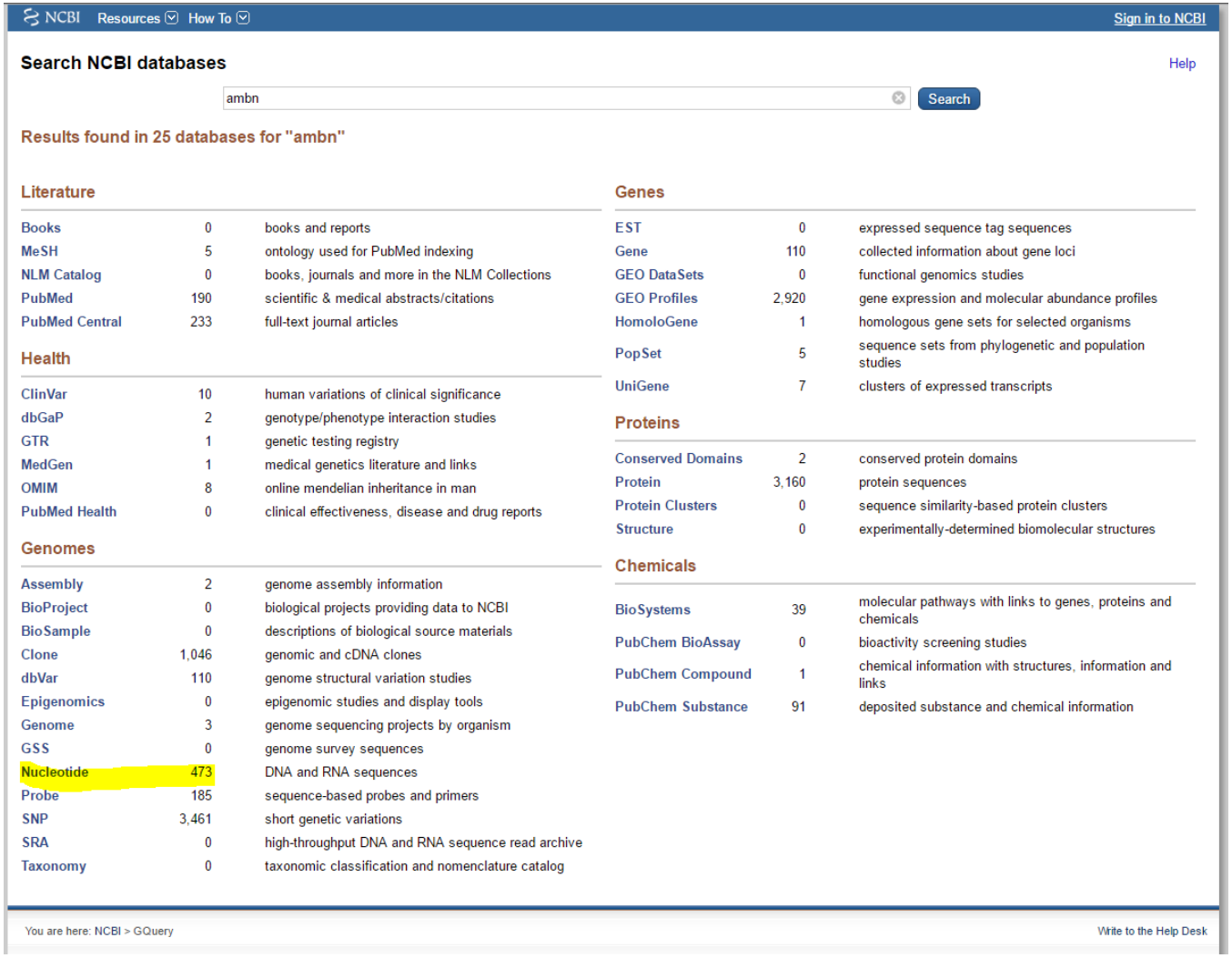

Species
Animals (736)
Customize ...
Molecule types
genomic DNA/RNA (599)
mRNA (137)
Customize ...
Source databases
INSDC (GenBank) (540)
RefSeq (196)
Customize ...
Sequence length
Custom range...
Release date
Custom range...
Revision date
Custom range...
Clear all
Show additional filters
Summary 20 per page Sort by Default order
Send to: Filters: Manage Filters
Items: 1 to 20 of 736
Selected: 3
Found 806 nucleotide sequences Nucleotide (736) EST (70)
1. Caenorhabditis elegans Probable serine/threonine-protein phosphatase (paa-1), partial mRNA
1,773 bp linear mRNA
Accession: NM_065761.4 GI: 392894997
GenBank FASTA Graphics
2. Trichinella spiralis serine/threonine-protein phosphatase PP2A regulator mRNA, partial cds
159 bp linear mRNA
Accession: XM_003366924.1 GI: 339263803
GenBank FASTA Graphics
3. Trichinella spiralis protein phosphatase PP2A (Tsp_14159) mRNA, partial cds
394 bp linear mRNA
Accession: XM_003368715.1 GI: 339259723
GenBank FASTA Graphics
4. Loa loa protein phosphatase PP2A regulatory subunit (LOAG_00611) mRNA, complete cds
1,974 bp linear mRNA
Accession: XM_003136151.1 GI: 312066287
GenBank FASTA Graphics
Choose Destination
File Clipboard
Collections
Download 3 items.
Format
FASTA
Sort by
Default order
Create File
Trichuridae (40)
Spirurida (96)
Loa (45)
Brugia (32)
Wuchereria (19)
Ascaridida (36)
Toxocaridae (19)
Ascarididae (17)
Diplogasterida (12)
Tylenchida (1)
Find related data
Database: Select
Find items

Search NCBI


3 total sequences
Courier New
11
Mode: Select / Slide
Selection: 0
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
gi|339263803|ref|XM_003366924.
gi|339259723|ref|XM_003368715.
gi|312066287|ref|XM_003136151.


3 total sequences
Courier New
11
Mode: Select / Slide
Selection: null
Position:
Sequence Mask: None
Numbering Mask: None
Start ruler at: 1
Scroll speed slow fast
gi|339263803|ref|XM_003366924.
gi|339259723|ref|XM_003368715.
gi|312066287|ref|XM_003136151.

Nucleotide Search



NCBI Resources How To Sign in to NCBI

Search NCBI databases Help

ambn Search

Results found in 25 databases for "ambn"

Literature

Books	0	books and reports
MeSH	5	ontology used for PubMed indexing
NLM Catalog	0	books, journals and more in the NLM Collections
PubMed	190	scientific & medical abstracts/citations
PubMed Central	233	full-text journal articles

Health

ClinVar	10	human variations of clinical significance
dbGaP	2	genotype/phenotype interaction studies
GTR	1	genetic testing registry
MedGen	1	medical genetics literature and links
OMIM	8	online mendelian inheritance in man
PubMed Health	0	clinical effectiveness, disease and drug reports

Genomes

Assembly	2	genome assembly information
BioProject	0	biological projects providing data to NCBI
BioSample	0	descriptions of biological source materials
Clone	1,046	genomic and cDNA clones
dbVar	110	genome structural variation studies
Epigenomics	0	epigenomic studies and display tools
Genome	3	genome sequencing projects by organism
GSS	0	genome survey sequences
Nucleotide	473	DNA and RNA sequences
Probe	185	sequence-based probes and primers
SNP	3,461	short genetic variations
SRA	0	high-throughput DNA and RNA sequence read archive
Taxonomy	0	taxonomic classification and nomenclature catalog

Genes

EST	0	expressed sequence tag sequences
Gene	110	collected information about gene loci
GEO DataSets	0	functional genomics studies
GEO Profiles	2,920	gene expression and molecular abundance profiles
HomoloGene	1	homologous gene sets for selected organisms
PopSet	5	sequence sets from phylogenetic and population studies
UniGene	7	clusters of expressed transcripts

Proteins

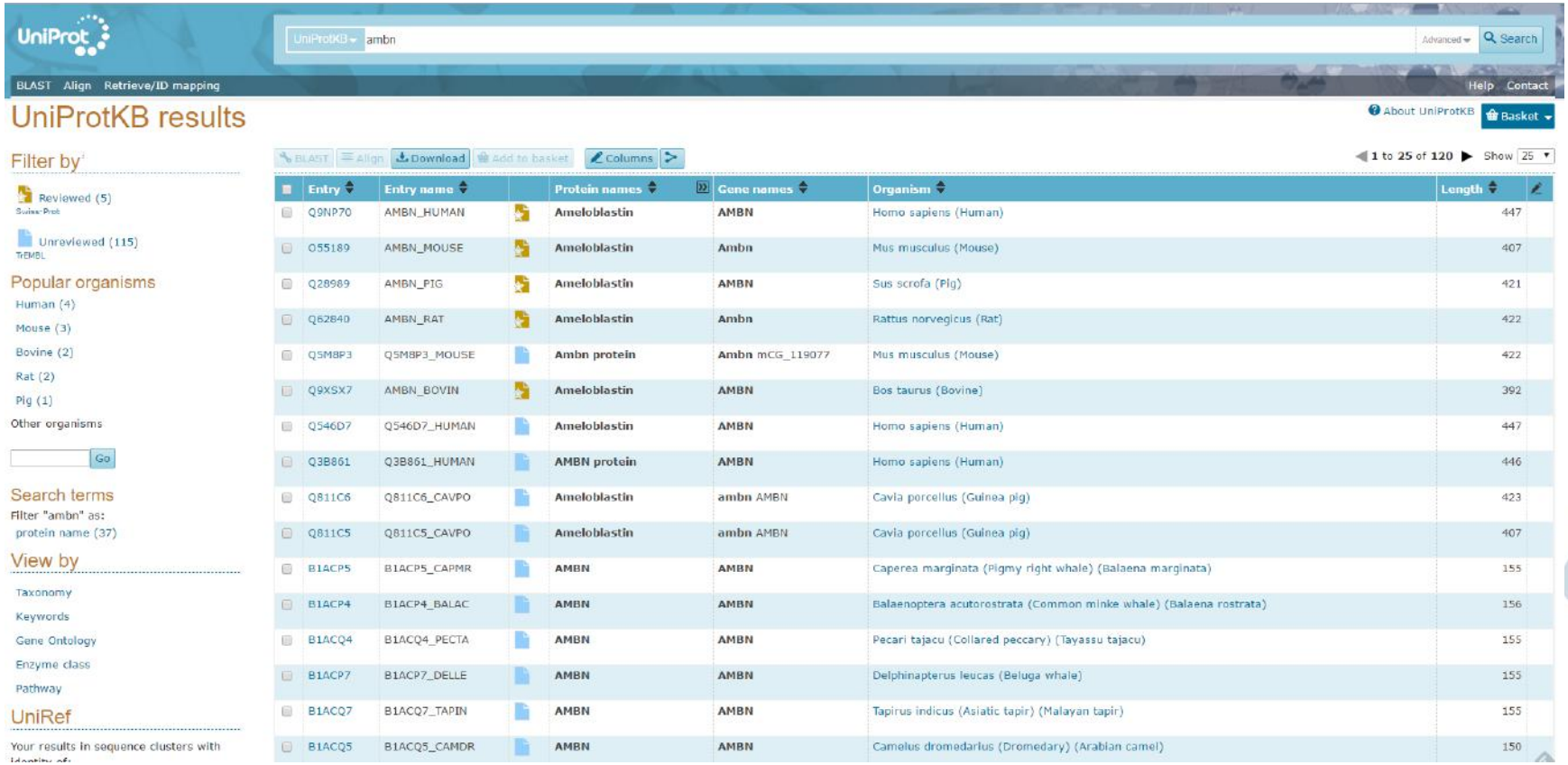
Conserved Domains	2	conserved protein domains
Protein	3,160	protein sequences
Protein Clusters	0	sequence similarity-based protein clusters
Structure	0	experimentally-determined biomolecular structures

Chemicals

BioSystems	39	molecular pathways with links to genes, proteins and chemicals
PubChem BioAssay	0	bioactivity screening studies
PubChem Compound	1	chemical information with structures, information and links
PubChem Substance	91	deposited substance and chemical information

You are here: NCBI > GQuery Write to the Help Desk

UniProt



Entry	Entry name	Protein names	Gene names	Organism	Length
Q9NP70	AMBN_HUMAN	Ameloblastin	AMBN	Homo sapiens (Human)	447
O55189	AMBN_MOUSE	Ameloblastin	Ambn	Mus musculus (Mouse)	407
Q28989	AMBN_PIG	Ameloblastin	AMBN	Sus scrofa (Pig)	421
Q62840	AMBN_RAT	Ameloblastin	Ambn	Rattus norvegicus (Rat)	422
Q5M8P3	Q5M8P3_MOUSE	Ambn protein	Ambn mCG_119077	Mus musculus (Mouse)	422
Q9XSX7	AMBN_BOVIN	Ameloblastin	AMBN	Bos taurus (Bovine)	392
Q546D7	Q546D7_HUMAN	Ameloblastin	AMBN	Homo sapiens (Human)	447
Q3B861	Q3B861_HUMAN	AMBN protein	AMBN	Homo sapiens (Human)	446
Q811C6	Q811C6_CAVPO	Ameloblastin	ambn AMBN	Cavia porcellus (Guinea pig)	423
Q811C5	Q811C5_CAVPO	Ameloblastin	ambn AMBN	Cavia porcellus (Guinea pig)	407
B1ACP5	B1ACP5_CAPMR	AMBN	AMBN	Caperea marginata (Pigmy right whale) (Balaena marginata)	155
B1ACP4	B1ACP4_BALAC	AMBN	AMBN	Balaenoptera acutorostrata (Common minke whale) (Balaena rostrata)	156
B1ACQ4	B1ACQ4_PECTA	AMBN	AMBN	Pecari tajacu (Collared peccary) (Tayassu tajacu)	155
B1ACP7	B1ACP7_DELLE	AMBN	AMBN	Delphinapterus leucas (Beluga whale)	155
B1ACQ7	B1ACQ7_TAPIN	AMBN	AMBN	Tapirus indicus (Asiatic tapir) (Malayan tapir)	155
B1ACQ5	B1ACQ5_CAMDR	AMBN	AMBN	Camelus dromedarius (Dromedary) (Arabian camel)	150

UniProt

PTM / Processing

Molecule processing

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Signal peptide	1 – 26	26	Sequence analysis			Add BLAST
Chain	27 – 447	421	Ameloblastin		PRO_0000001192	Add BLAST

Amino acid modifications

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Modified residue	37 – 37	1	Hydroxyproline By similarity			
Modified residue	43 – 43	1	Phosphoserine By similarity			
Glycosylation	112 – 112	1	O-linked (GalNAc...) By similarity			

Keywords - PTM

Glycoprotein, Hydroxylation, Phosphoprotein

Proteomic databases

PaxDb	Q9NP70.
PRIDE	Q9NP70.

PTM databases

PhosphoSite	Q9NP70.

Expression

Tissue specificity

Ameloblast-specific. Located at the Tomes processes of secretory ameloblasts and in the sheath space between rod-interrod enamel.

Gene expression databases

UniProt

Interaction

GO - Molecular function
- growth factor activity Source: BHF-UCL

Protein-protein interaction databases

BioGrid	106756. 2 interactions.
STRING	9606.ENSP00000313809.

Structure

3D structure databases

ProteinModelPortal	Q9NP70.
ModBase	Search...
MobiDB	Search...

Family & Domains

Domains and Repeats

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Repeat	189 – 201	13	1			Add BLAST
Repeat	202 – 214	13	2			Add BLAST

Sequence similarities
Belongs to the ameloblastin family. Curated

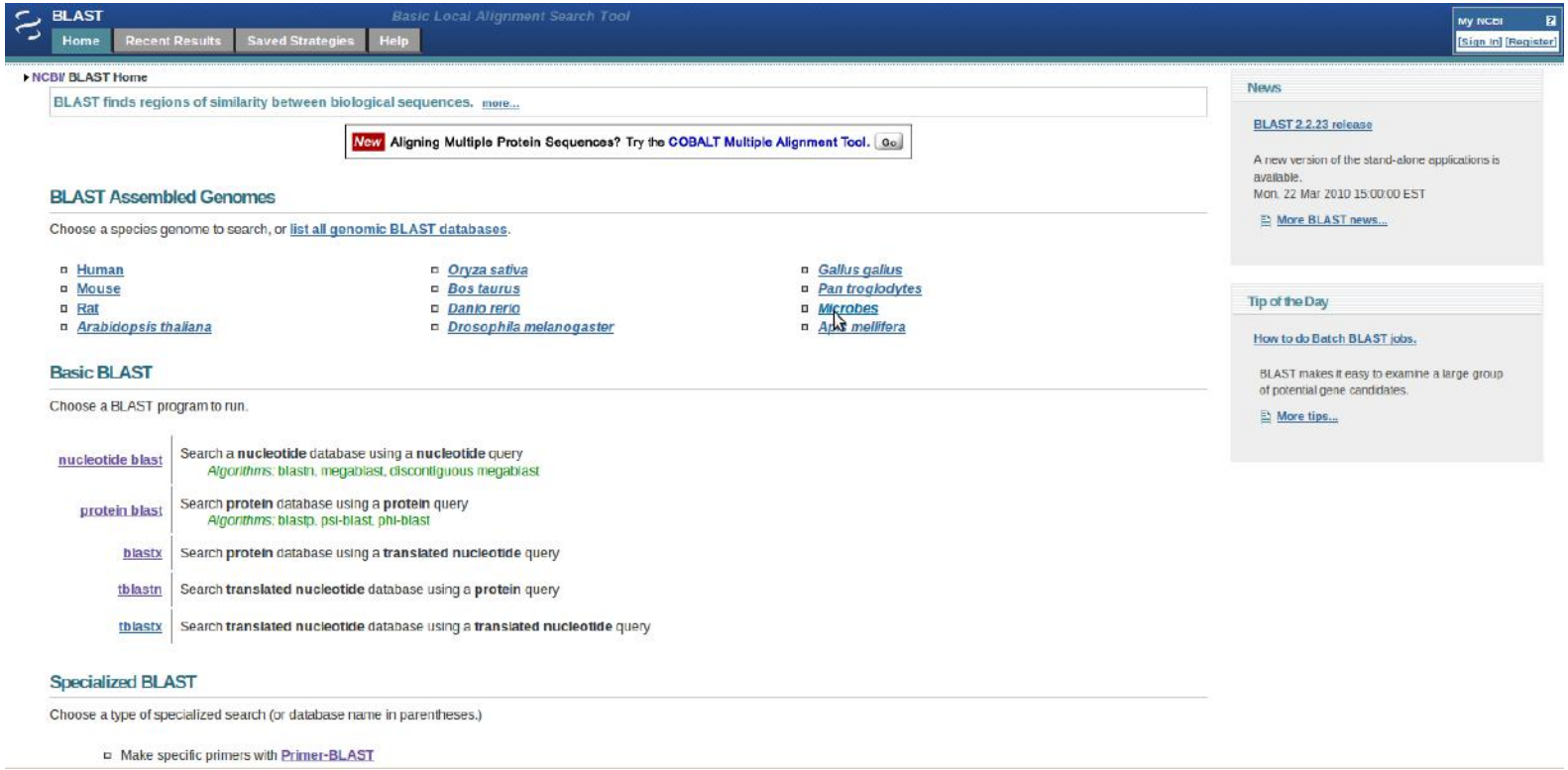
Keywords - Domain
Repeat, Signal

Hands-On 2

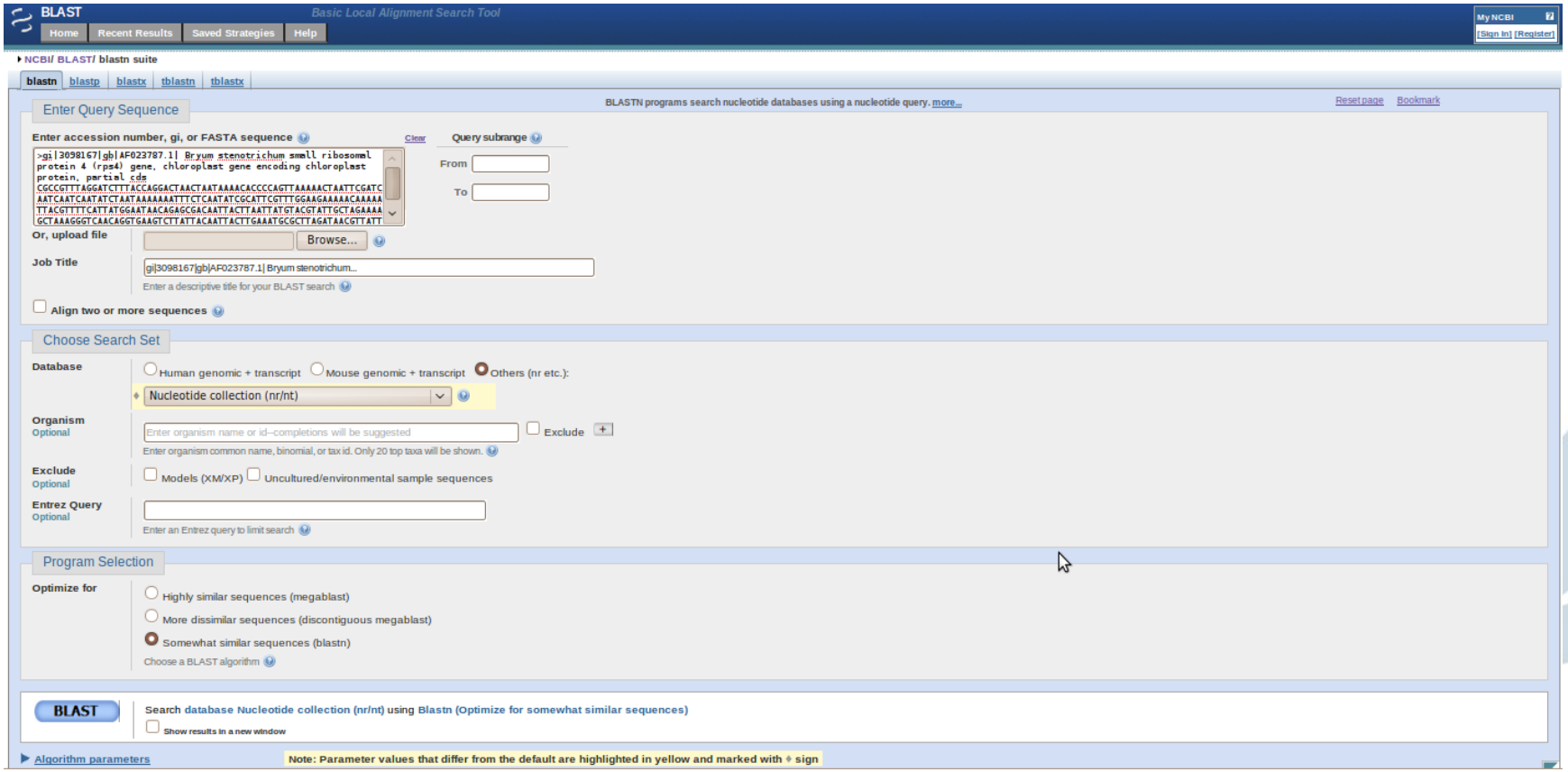
1. Escolher um gene
   - Ameloblastin (AMBN)
   - Enamelin (ENAM)
   - Hemopexin (HPX)

2. Ir à página do NCBI e descarregar sequencias de 5 espécies

NCBI – BLAST (Basic Local Alignment Serach Tool)



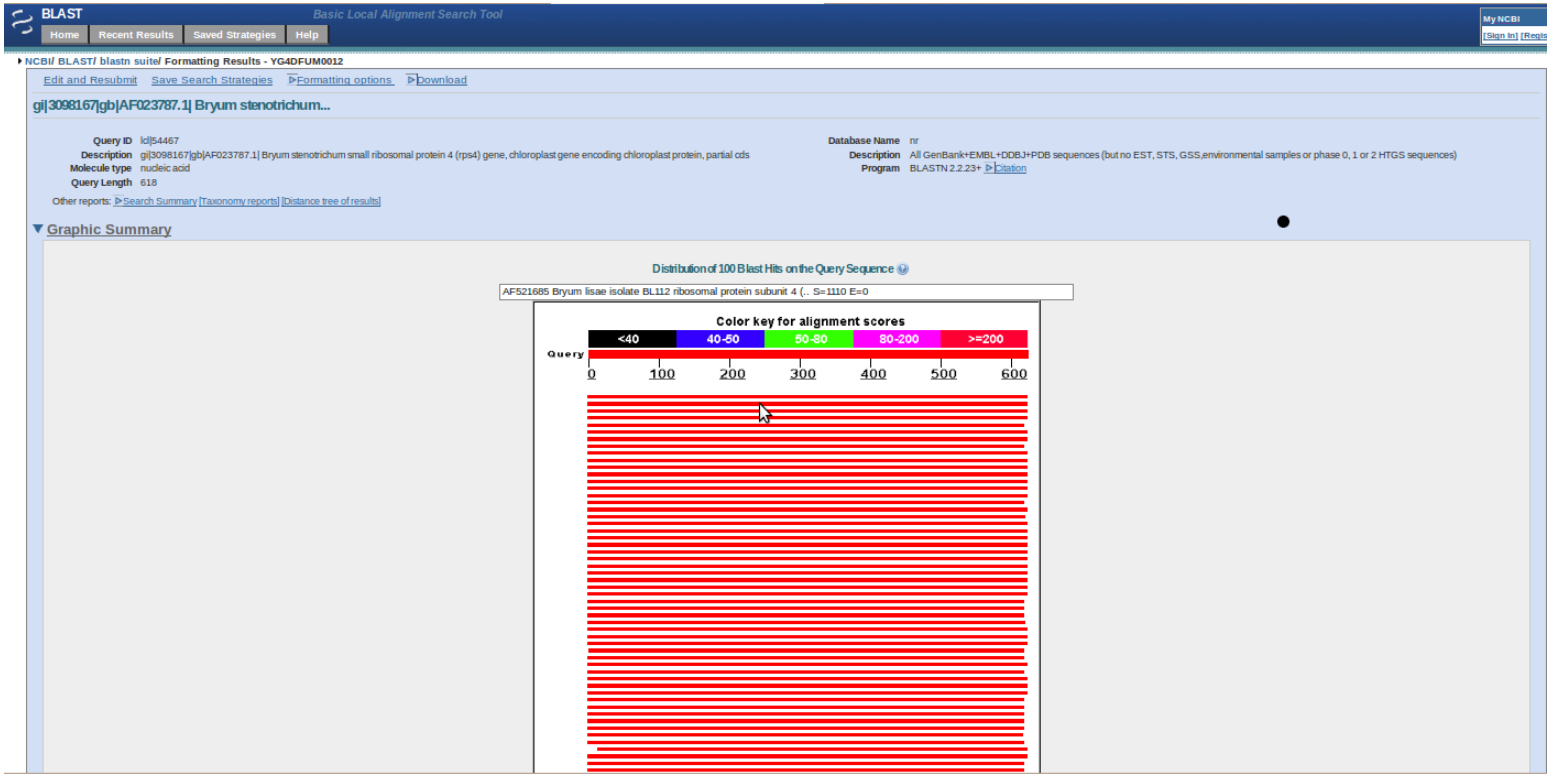
Blast Search



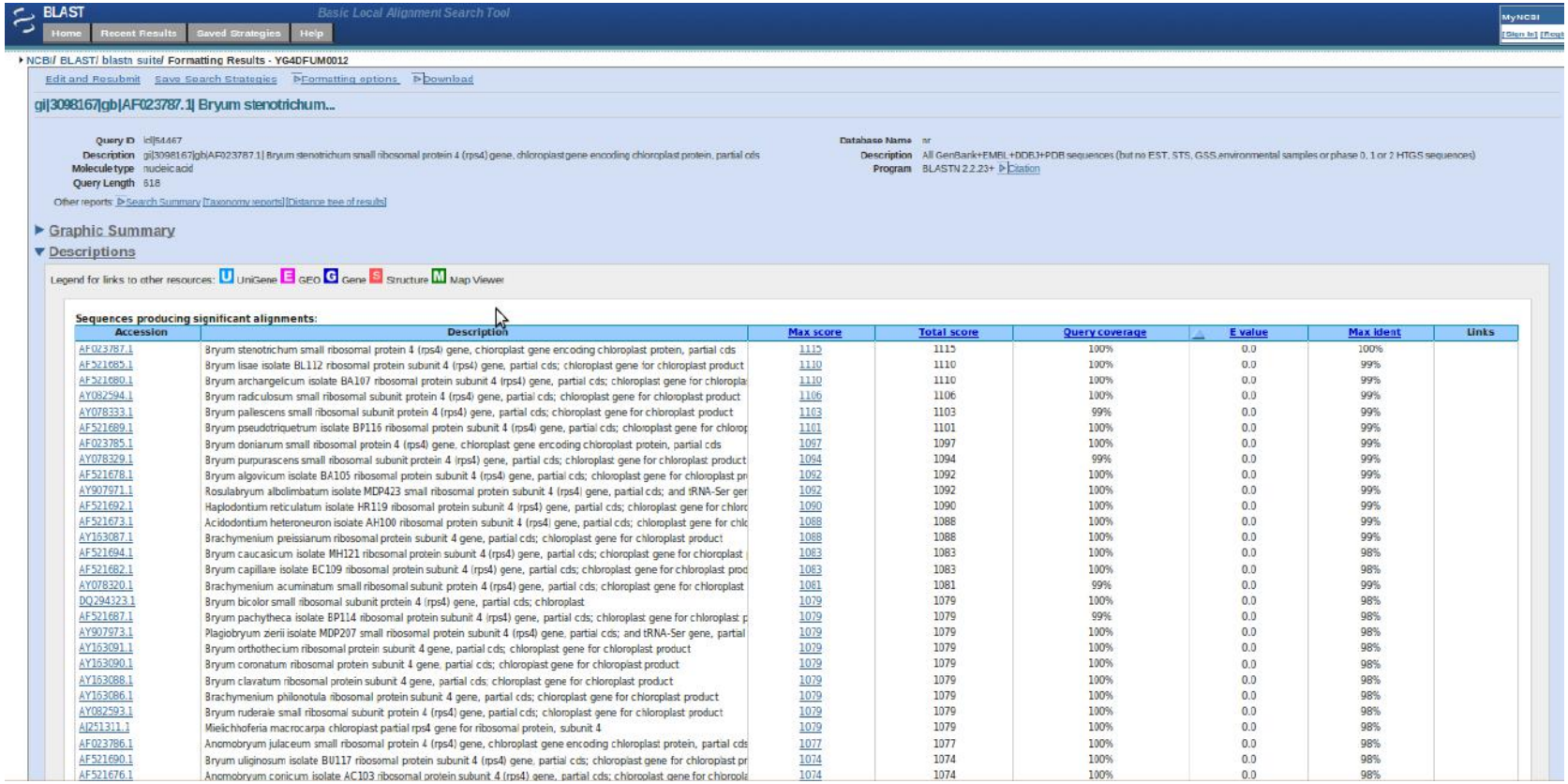
Blast methods

- blastn
- blastp
- blastx
- tblastn
- tblastx

NCBI – nucleotide BLAST result



NCBI – nucleotide BLAST result



E-values: 10^{-4} ***often considered*** good enough for an assumption of homology

```
Query  961   GGAGGTGCACAAGGCTCCCCTATGCCGGAGGCCAACCCAGACAATCTAGAAAACCCAGCT  1020
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1062  GGAGGTGCACAAGGCTCCCCTATGCCGGAGGCCAACCCAGACAATCTAGAAAACCCAGCT  1121

Query  1021  TTCCTTACAGAGCTAGAACCTGCTCCCCACGCAGGGCTCCTTGCTCTCCCTAAGGATGAC  1080
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1122  TTCCTTACAGAGCTAGAACCTGCTCCCCACGCAGGGCTCCTTGCTCTCCCTAAGGATGAC  1181

Query  1081  ATTCCCGGCCTGCCAAGGAGCCCTTCAGGGAAGATGAAGGGACTCCCCAGCGTCACCCCA  1140
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1182  ATTCCCGGCCTGCCAAGGAGCCCTTCAGGGAAGATGAAGGGACTCCCCAGCGTCACCCCA  1241

Query  1141  GCAGCTGCTGACCCACTGATGACCCCTGAATTAGCTGATGTTTATAGGACCTACGATGCT  1200
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1242  GCAGCTGCTGACCCACTGATGACCCCTGAATTAGCTGATGTTTATAGGACCTACGATGCT  1301

Query  1201  GACATGACCACATCCGTGGATTTCCAGGAAGAAGCAACCATGGATACCACGATGGCCCCA  1260
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1302  GACATGACCACATCCGTGGATTTCCAGGAAGAAGCAACCATGGATACCACGATGGCCCCA  1361

Query  1261  AACTCTCTGCAAACATCCATGCCAGGAAACAAAGCCCAGGAGCCCGAGATGATGCATGAC  1320
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1362  AACTCTCTGCAAACATCCATGCCAGGAAACAAAGCCCAGGAGCCCGAGATGATGCATGAC  1421

Query  1321  GCATGGCATTTCCAAGAGCCCTG  1343
              |||||||||||||||||||||||
Sbjct  1422  GCATGGCATTTCCAAGAGCCCTG  1444
```

Range 2: 633 to 671 GenBank Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
53.6 bits(58)	0.011	35/39(90%)	0/39(0%)	Plus/Plus

```
Query  610  CTCCCAGGATTGGATTTTGCTGATCCACAAGGTTCAACA  648
            |||||||| | ||||||||||||||||||| || ||
Sbjct  633  CTCCCAGGAGTAGATTTTGCTGATCCACAAGGTCCATCA  671
```

Range 3: 711 to 749 GenBank Graphics ▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
53.6 bits(58)	0.011	35/39(90%)	0/39(0%)	Plus/Plus

```
Query  532  CTCCCAGGAGTAGATTTTGCTGATCCACAAGGTCCATCA  570
            |||||||| | ||||||||||||||||||| || ||
Sbjct  711  CTCCCAGGATTGGATTTTGCTGATCCACAAGGTTCAACA  749
```

Warnings

- Predictions methods can fail and sometimes accurancy is not available
- Databases can contain incorrect data
- Avoid overvaloration of results

Hands-On 3

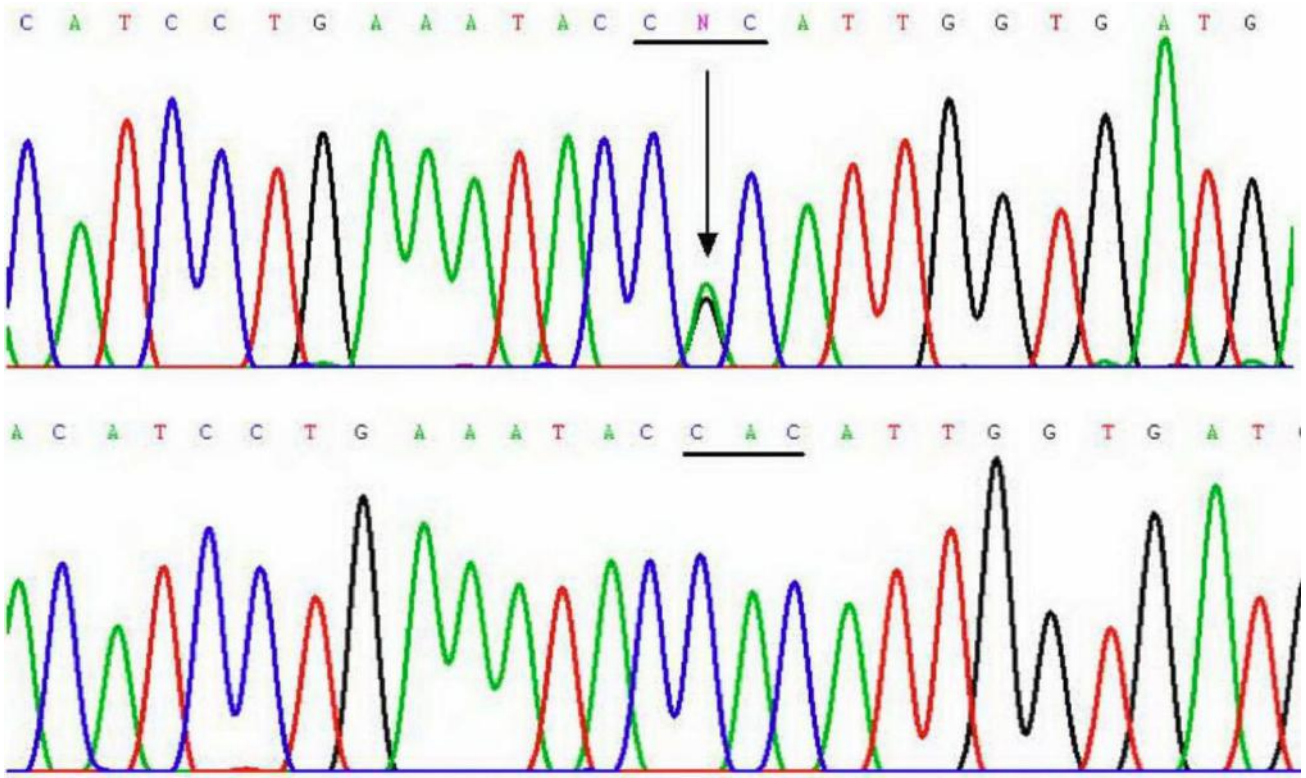
1) Descarregar sequencias da pasta hands_on_3

2) Fazer o “blast” para as sequencias

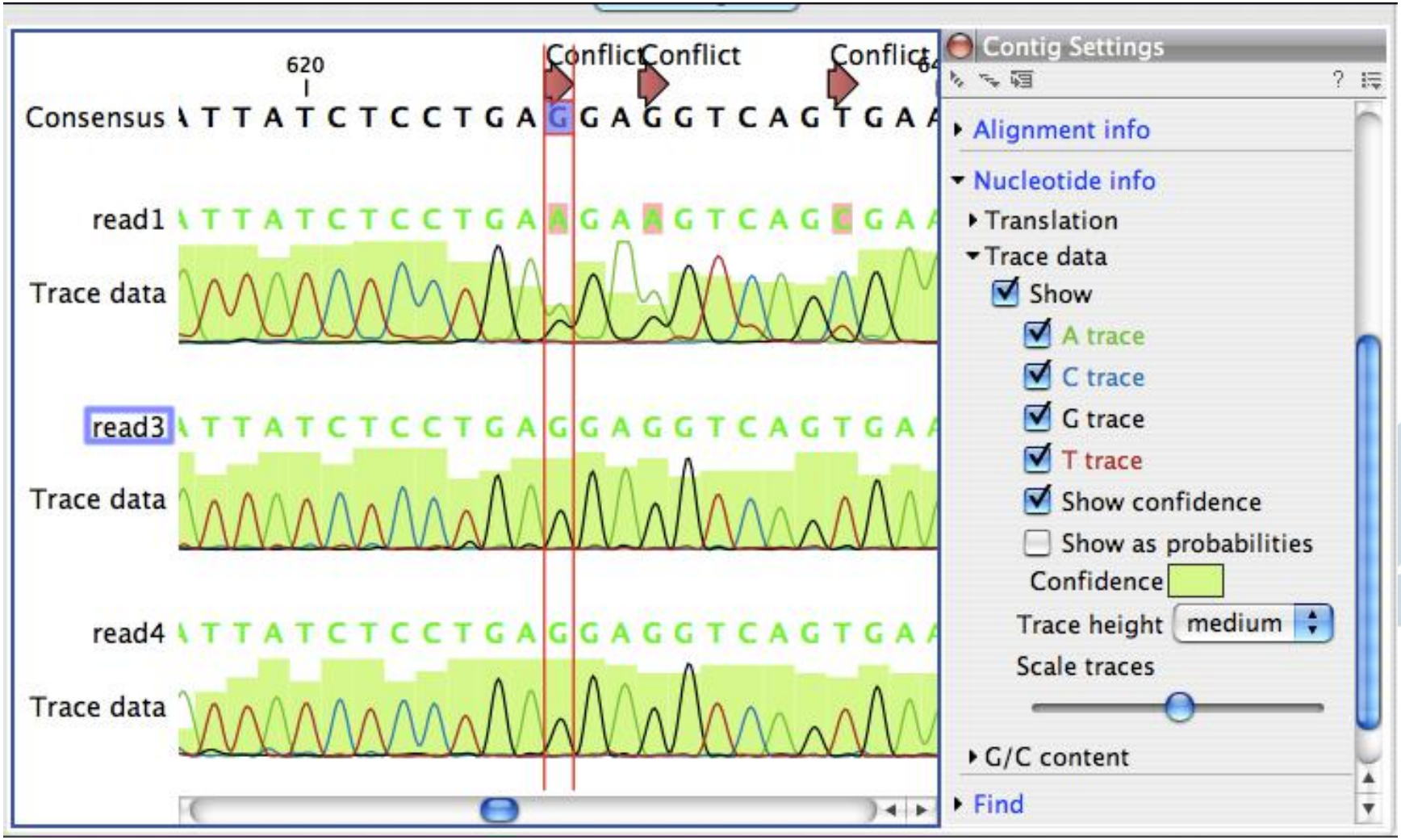
3) Identificar gene e espécies

4) Descarregar 3 top hits

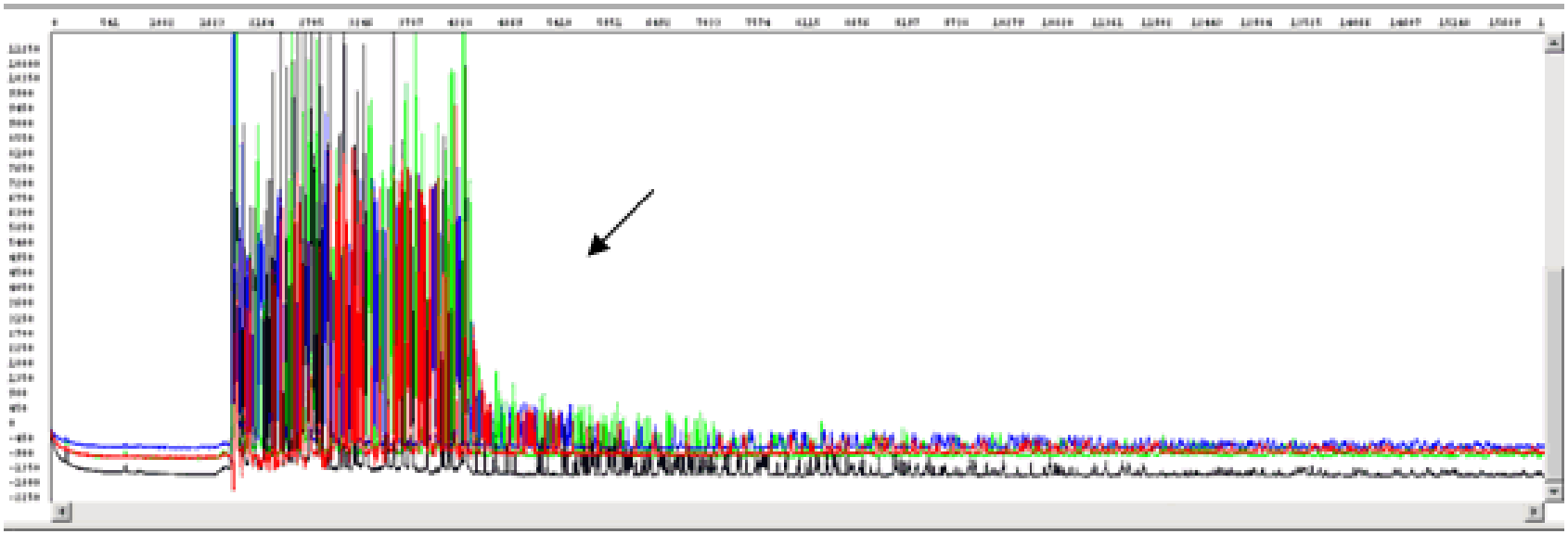
Sequencing Results



Sequencing Results



Sequencing Results



Sequencing Results

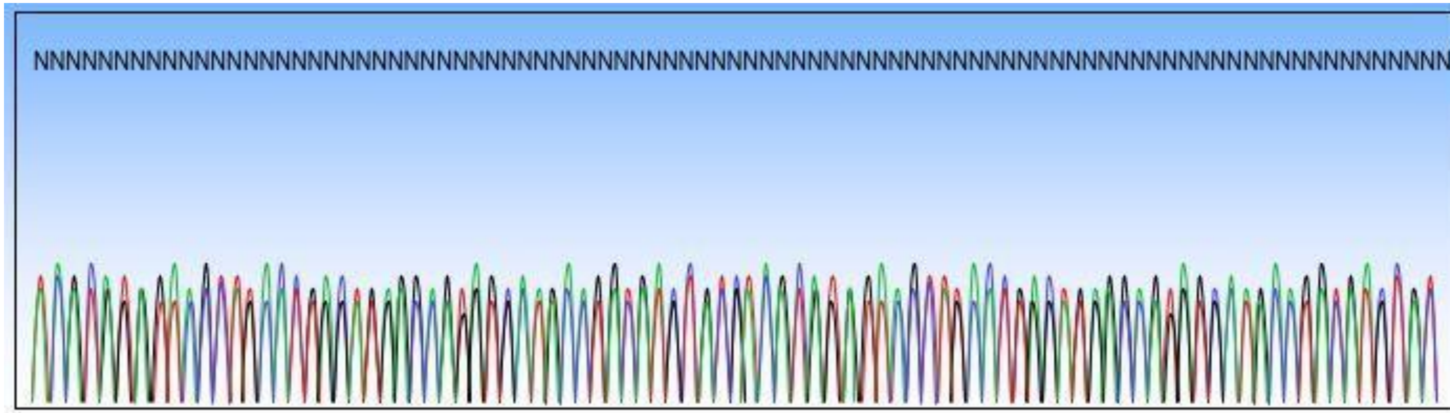
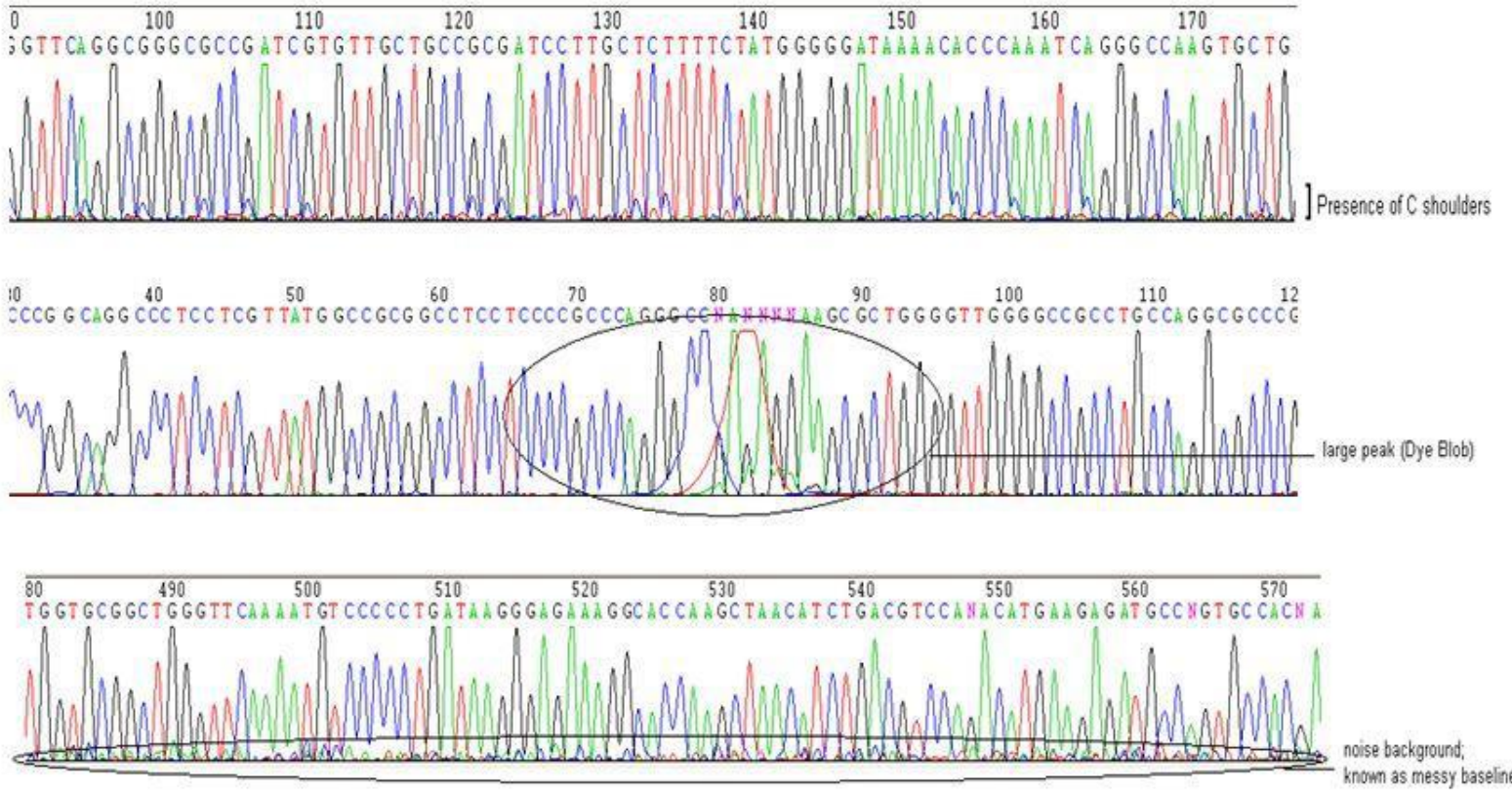


Figure 2: Double Priming Causes Double Sequence

A sample that has two annealing sights for a primer often show double sequence from the beginning of the result. In cases where both priming sights are closely located on the same strand of DNA, presence of the downstream primer could terminate extension of the upstream primer. The result is a single set of peaks located downstream, a positive indicator for double priming. The priming sights do not necessarily need to be identical as long as the 3 prime bases of the primer matches either annealing sight.

Sequencing Results



Methods for multiple sequence alignments

Multiple Sequence Alignments (MSA)

- Web-based (e.g. http://translatorx.co.uk/)
- Local (software)
- Scripts (e.g. http://raven.iab.alaska.edu/~ntakebay/teaching/programming/perl-scripts/perl-scripts.html)

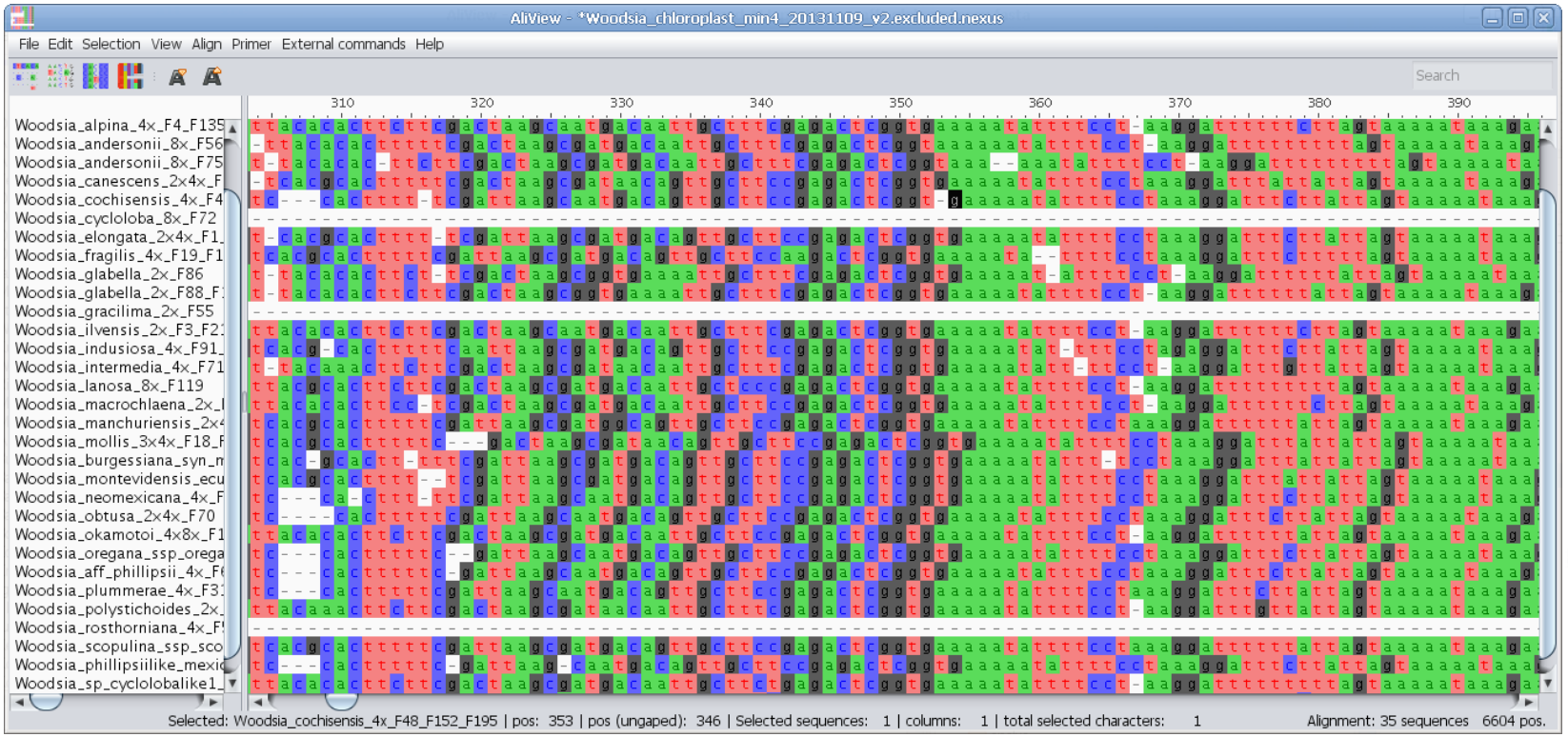
MSA

- Model
- Can be biased
- Quality can be checked (e.g. http://guidance.tau.ac.il/ver2/)
- Parameters:
  - Gap penalties
  - Mismatch
  - Iterations
  - Guiding tree

MSA Algorithms

- ClustalW
- ClustalOmega
- Muscle
- T-Coffee
- MAFT
- Prank

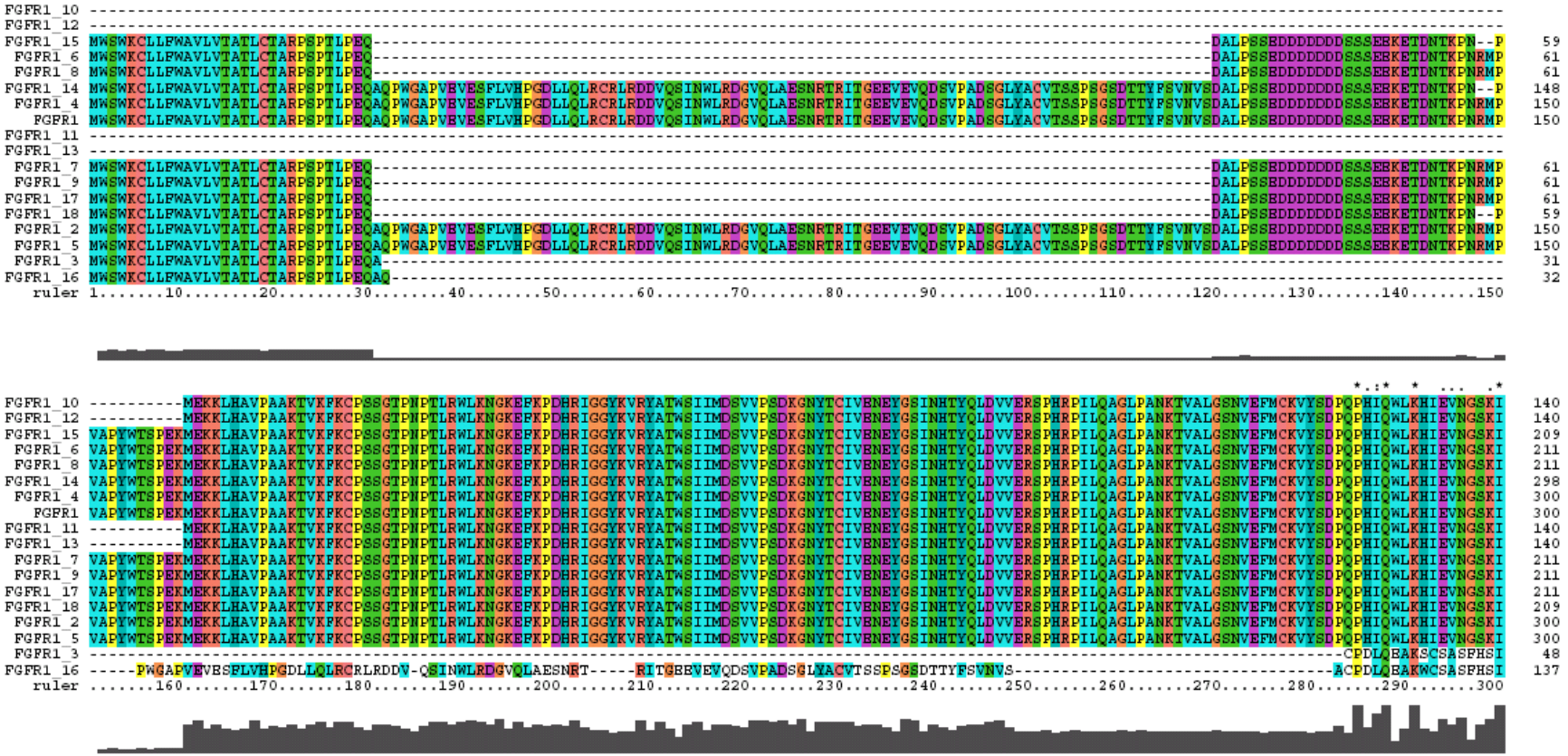
MSA


AliView - *Woodsia_chloroplast_min4_20131109_v2.excluded.nexus
File Edit Selection View Align Primer External commands Help
Search
310 320 330 340 350 360 370 380 390
Woodsia_alpina_4x_F4_F135
Woodsia_andersonii_8x_F56
Woodsia_andersonii_8x_F75
Woodsia_canescens_2x4x_F
Woodsia_cochisensis_4x_F4
Woodsia_cycloloba_8x_F72
Woodsia_elongata_2x4x_F1
Woodsia_fragilis_4x_F19_F1
Woodsia_glabella_2x_F86
Woodsia_glabella_2x_F88_F
Woodsia_gracilima_2x_F55
Woodsia_ilvensis_2x_F3_F2
Woodsia_indusiosa_4x_F91
Woodsia_intermedia_4x_F71
Woodsia_lanosa_8x_F119
Woodsia_macrochlaena_2x_
Woodsia_manchuriensis_2x
Woodsia_mollis_3x4x_F18_F
Woodsia_burgessiana_syn_m
Woodsia_montevidensis_ecu
Woodsia_neomexicana_4x_F
Woodsia_obtusa_2x4x_F70
Woodsia_okamotoi_4x8x_F1
Woodsia_oregana_ssp_orega
Woodsia_aff_phillipsii_4x_F
Woodsia_plummerae_4x_F3
Woodsia_polystichoides_2x_
Woodsia_rosthorniana_4x_F
Woodsia_scopulina_ssp_sco
Woodsia_phillipsiilike_mexi
Woodsia_sp_cyclolobalike1_
Selected: Woodsia_cochisensis_4x_F48_F152_F195 | pos: 353 | pos (ungaped): 346 | Selected sequences: 1 | columns: 1 | total selected characters: 1
Alignment: 35 sequences 6604 pos.

MSA


AliView - *Woodsia_chloroplast_min4_20131109_v2.excluded.nexus
File Edit Selection View Align Primer External commands Help
Search
590 600 610 620 630 640 650 660 670
Woodsia_alpina_4x_F22_F8
Woodsia_alpina_4x_F4_F13
Woodsia_andersonii_8x_F56
Woodsia_andersonii_8x_F75
Woodsia_canescens_2x4x_F
Woodsia_cochisensis_4x_F4
Woodsia_cycloloba_8x_F72
Woodsia_elongata_2x4x_F1_
Woodsia_fragilis_4x_F19_F1
Woodsia_glabella_2x_F86
Woodsia_glabella_2x_F88_F
Woodsia_gracilima_2x_F55
Woodsia_ilvensis_2x_F3_F2
Woodsia_indusiosa_4x_F91_
Woodsia_intermedia_4x_F71
Woodsia_macrochlaena_2x_
Woodsia_manchuriensis_2x
Woodsia_mollis_3x4x_F18_
Woodsia_burgessiana_syn_
Woodsia_montevidensis_ecu
Woodsia_neomexicana_4x_F
Woodsia_obtusa_2x4x_F70
Woodsia_okamotoi_4x8x_F1
Woodsia_oregana_ssp_orega
Woodsia_aff_phillipsii_4x_F
Woodsia_plummerae_4x_F3
Woodsia_polystichoides_2x_
Woodsia_rosthorniana_4x_F
Woodsia_scopulina_ssp_sco
Woodsia_phillipsiilike_mexi
Alignment: 34 sequences 6564 pos.

MSA


FGFR1_10
FGFR1_12
FGFR1_15
FGFR1_6
FGFR1_8
FGFR1_14
FGFR1_4
PGFR1
FGFR1_11
FGFR1_13
FGFR1_7
FGFR1_9
FGFR1_17
FGFR1_18
FGFR1_2
FGFR1_5
FGFR1_3
FGFR1_16
ruler

Hands-On 4

1) Requisitos: Seaview e/ou Mega6

2) Descarregar sequencias da pasta Hands_on_4

3) Importar ficheiro para o Mega ou Seaview

4) Traduzir para aminoácidos

5) Alinhar usando Muscle e ou ClustalW

6) Filtrar resultados alinhamento usando o http://translatorx.co.uk/)

Q & A

INTRODUÇÃO À BIOLOGIA MOLECULAR E BIOINFORMÁTICA:

Bioinformatics 2

Lisboa, 12-14 October

João Machado

Bárbara Frazão

Phylogenetic trees

Check List

- Recognize 5 file formats
- Perform a gene oriented search
- Perform a blast search
- Perform a MSA

The tree of Life

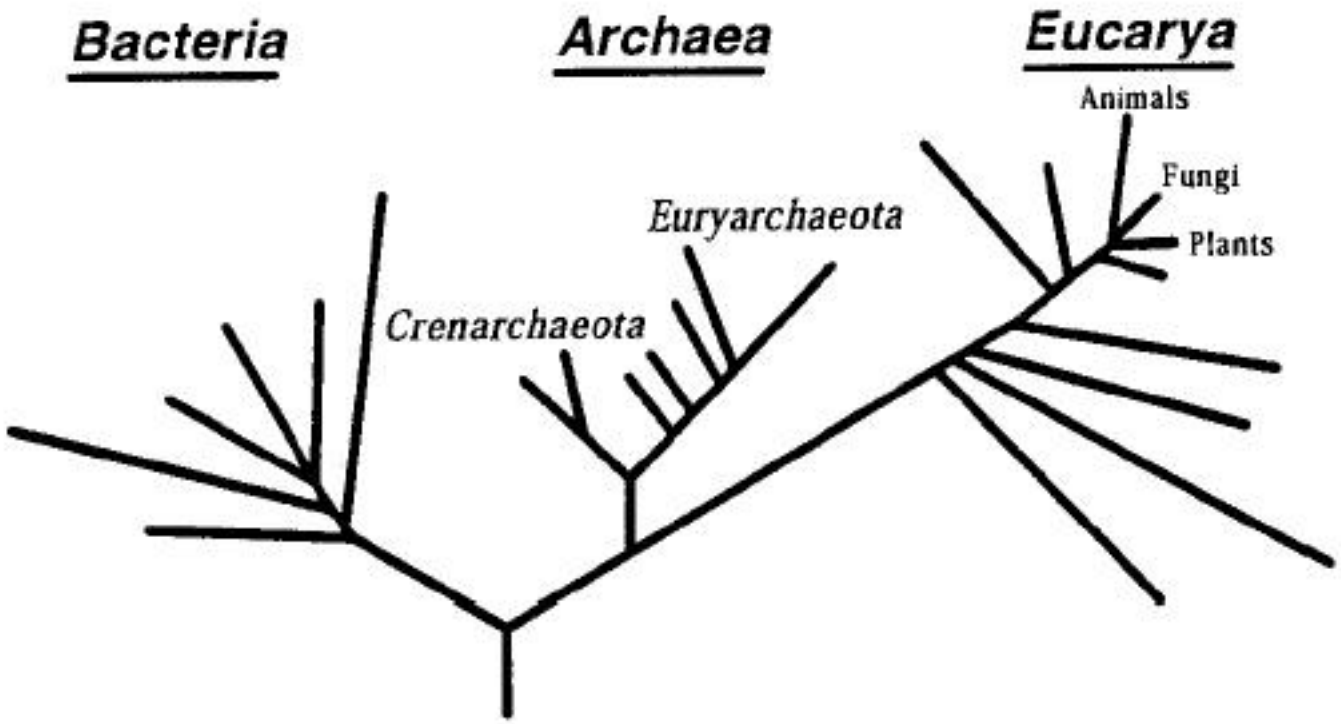
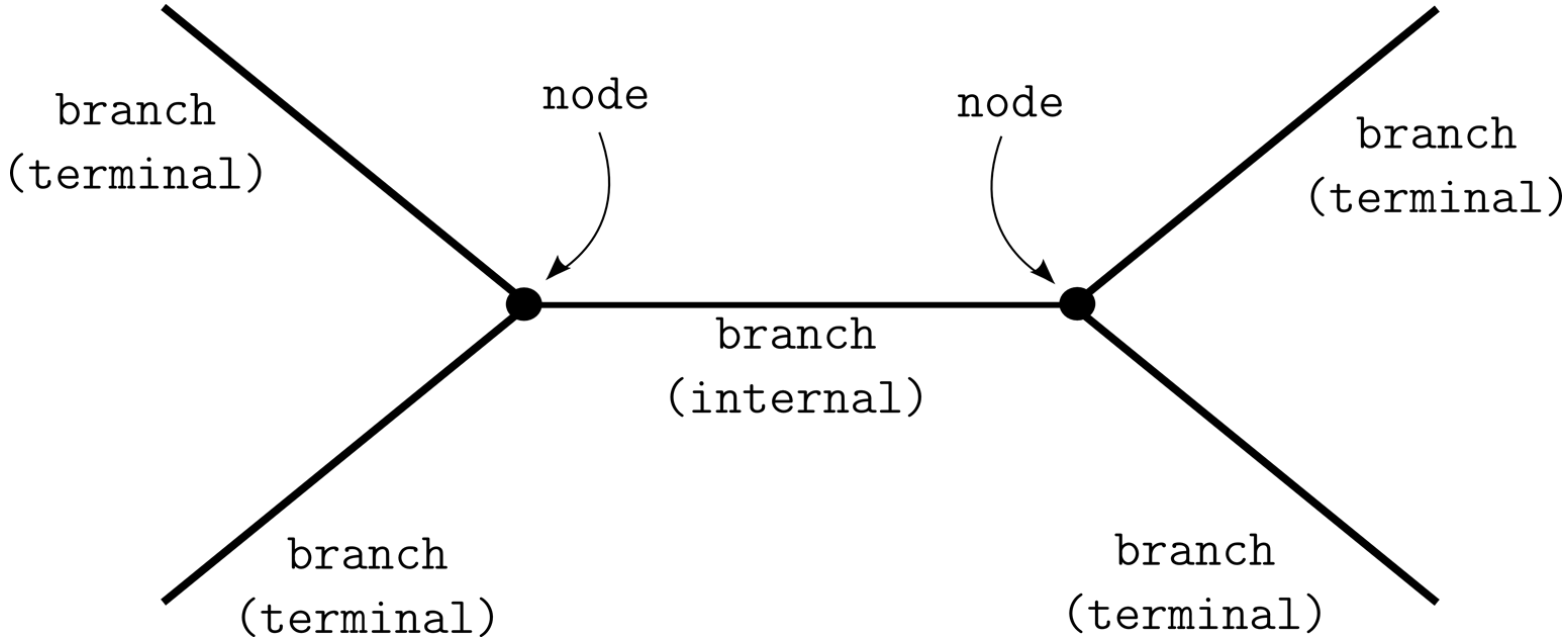


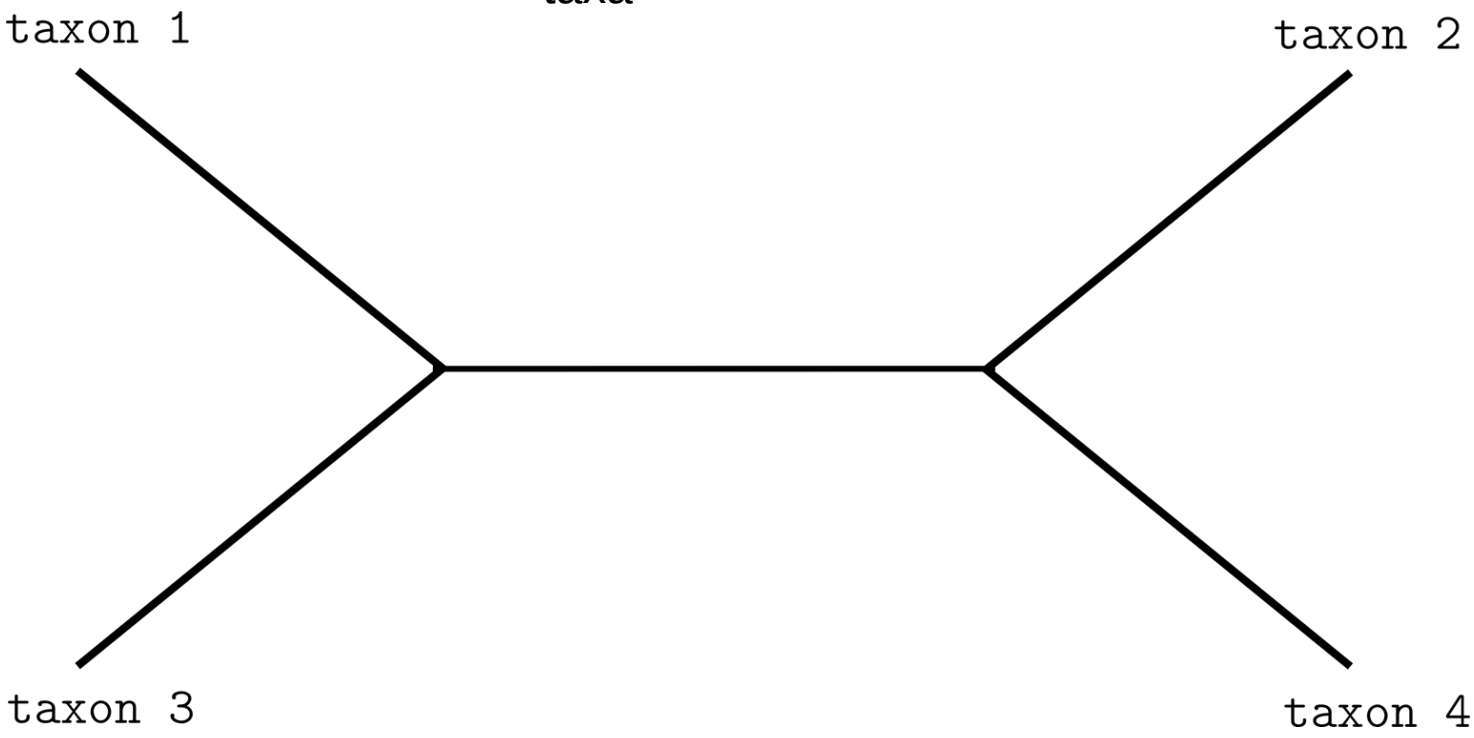
Fig. 1. The basal universal phylogenetic tree inferred from comparative analyses of rRNA sequences (4, 5). The root has been determined by using the paralogous gene couple EF-Tu/EFG (6).

Reading Trees



Reading Trees

Terminals are taxa



Rooted / Unrooted Tree

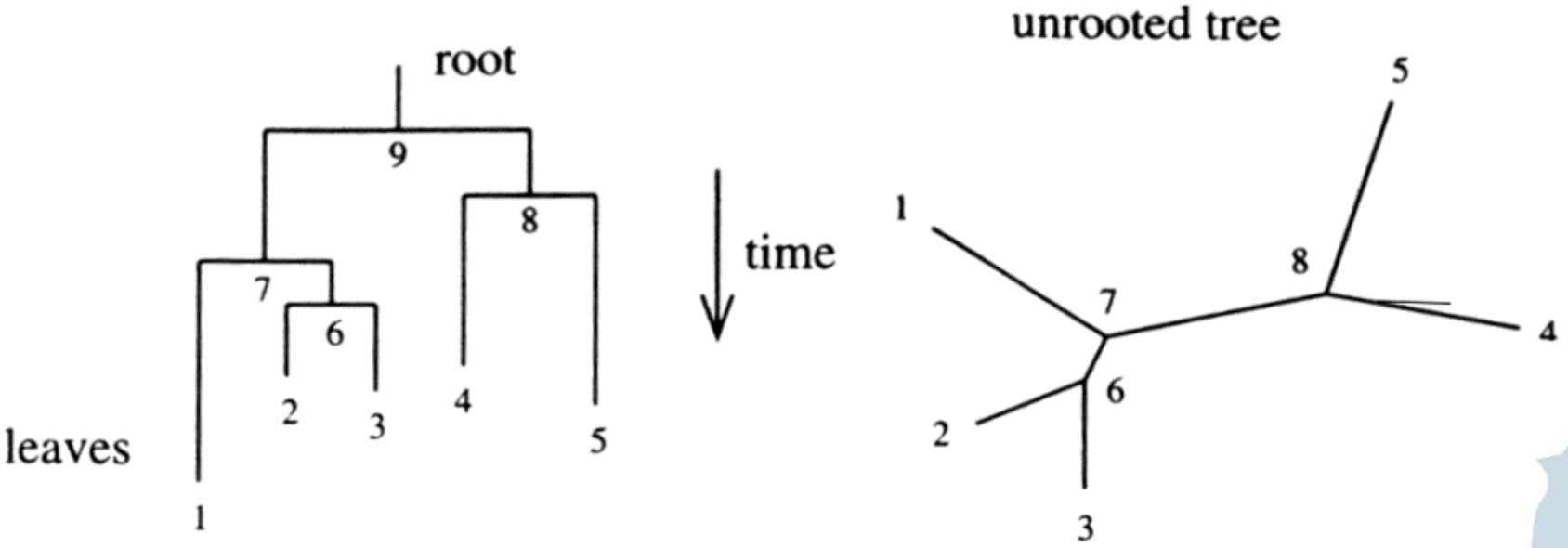
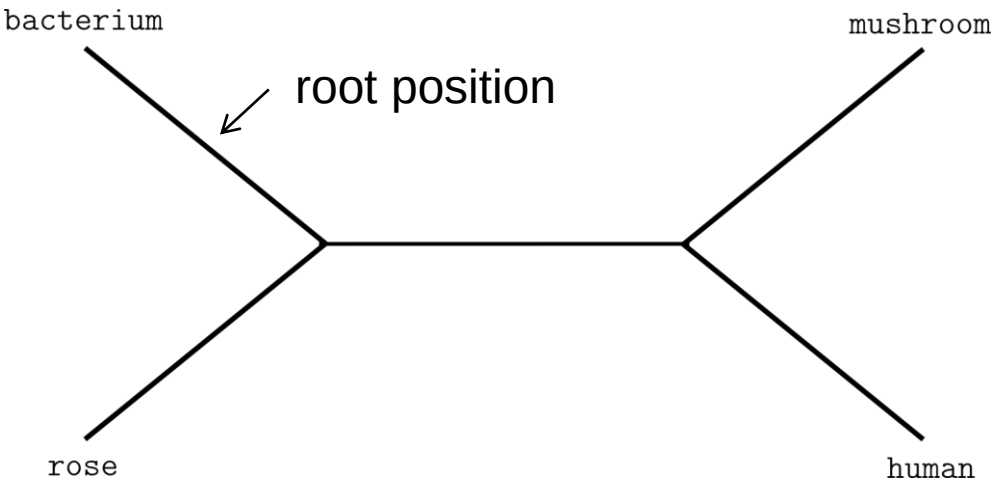


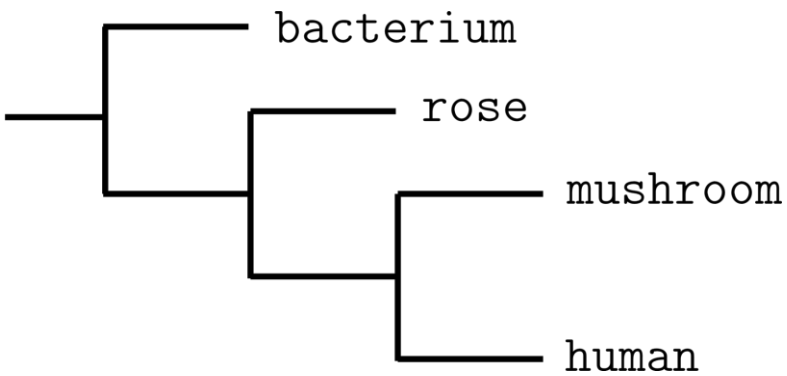
Figure 7.2 *An example of a binary tree, showing the root and leaves, and the direction of evolutionary time (the most recent time being at the bottom of the figure). The corresponding unrooted tree is also shown; the direction of time here is undetermined.*

Rooted / Unrooted Tree

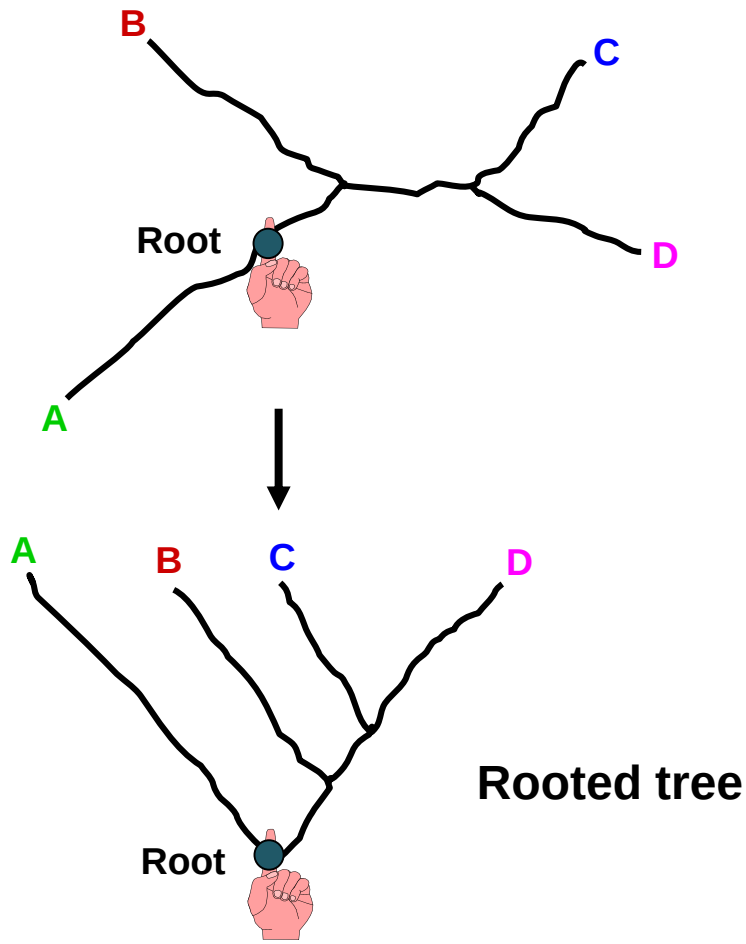
unrooted tree



rooted phylogenetic tree



Rooted / Unrooted Tree



Note that in this rooted tree, taxon A is no more closely related to taxon B than it is to C or D.

Counting Trees

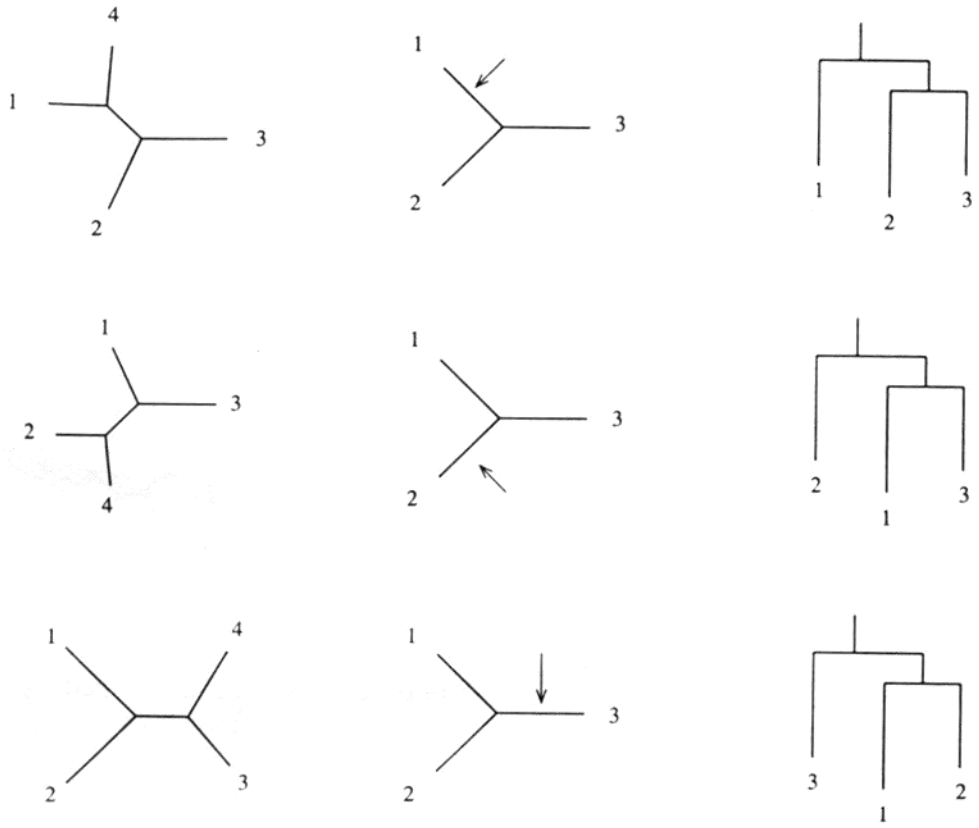
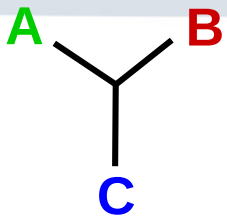
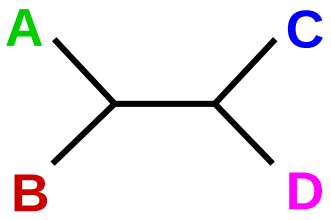
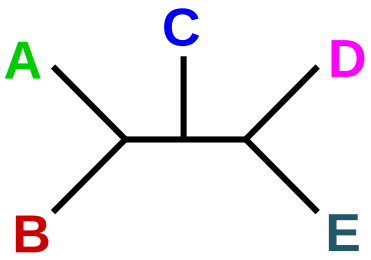


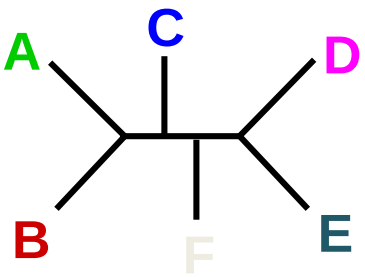
Figure 7.3 *The rooted trees (right-hand column) derived from the unrooted tree for three sequences by picking different edges as positions for the root (arrows).*

Counting Trees





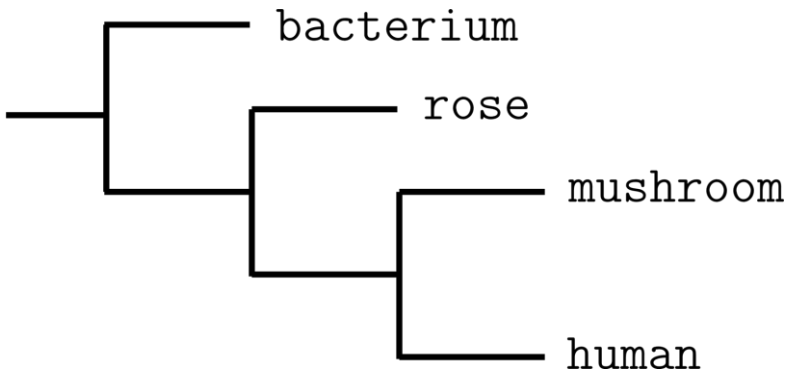


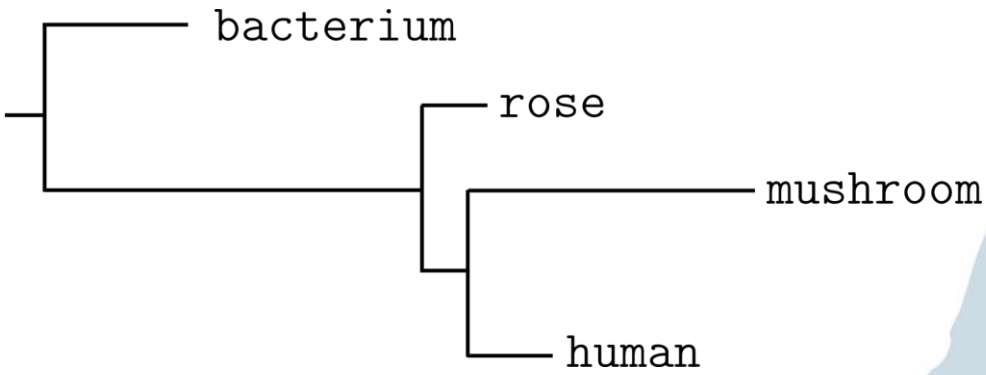


# Taxa (N)	# Unrooted trees
3	1
4	3
5	15
6	105
7	945
8	10,935
9	135,135
10	2,027,025
.	.
.	.
.	.
.	.
30	≈3.58 x 10^{36}

(2N - 5)!! = # unrooted trees for N taxa
(2N- 3)!! = # rooted trees for N taxa

Cladogram vs Phylogram



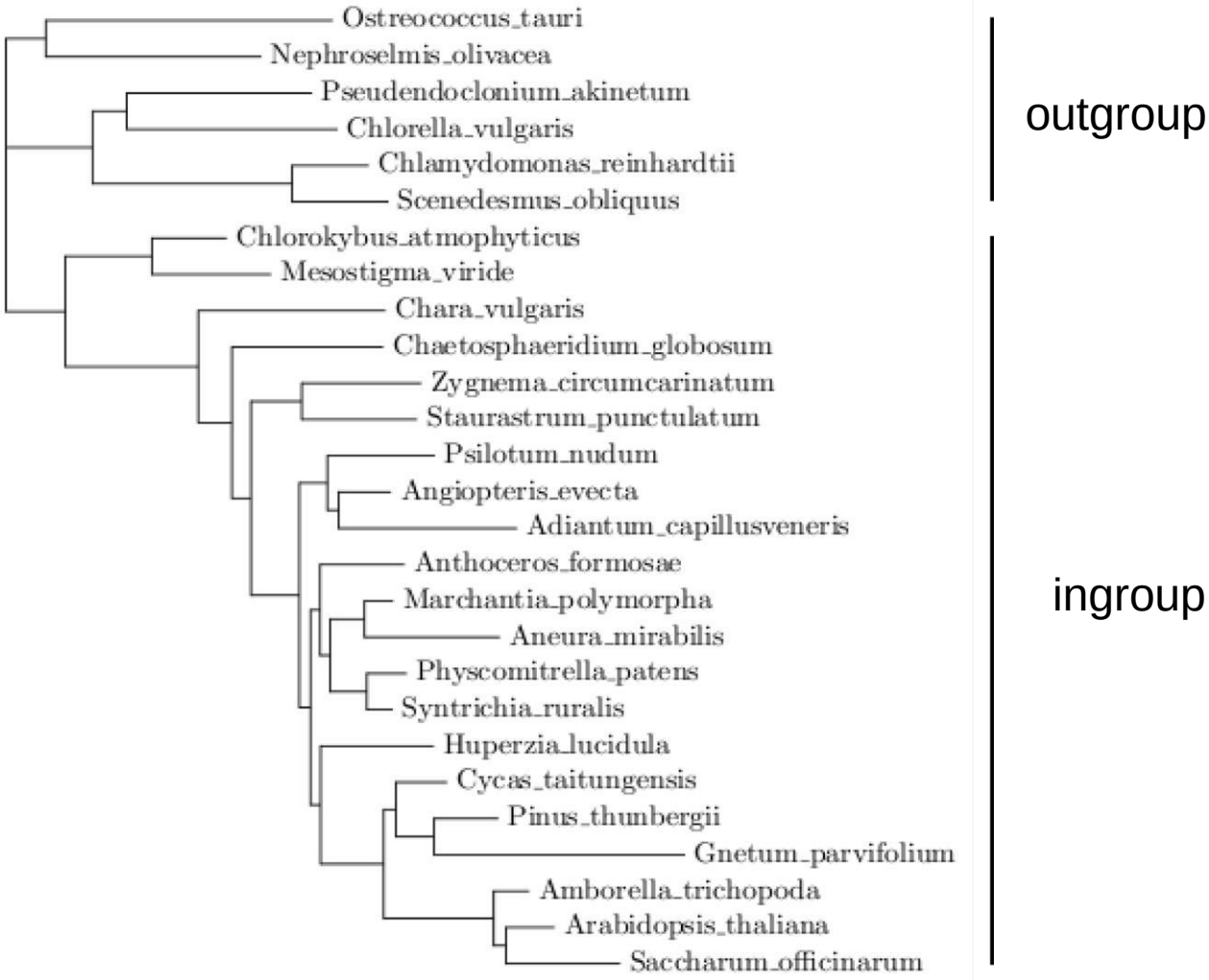


cladogram – arbitrary length branches

phylogram – branch length proportional

to some measure of genetic distance

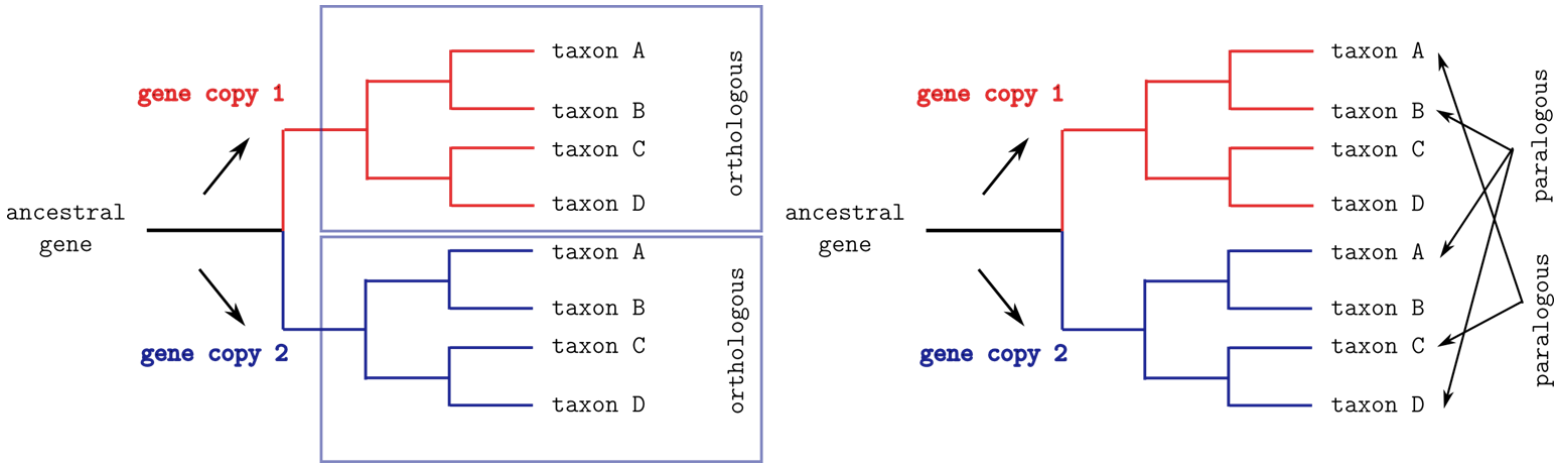
Outgroups


Ostreococcus_tauri
Nephroselmis_olivacea
Pseudendoclonium_akinetum
Chlorella_vulgaris
Chlamydomonas_reinhardtii
Scenedesmus_obliquus
Chlorokybus_atmophyticus
Mesostigma_viride
Chara_vulgaris
Chaetosphaeridium_globosum
Zygnema_circumcarinatum
Staurastrum_punctulatum
Psilotum_nudum
Angiopteris_evecta
Adiantum_capillusveneris
Anthoceros_formosae
Marchantia_polymorpha
Aneura_mirabilis
Physcomitrella_patens
Syntrichia_ruralis
Huperzia_lucidula
Cycas_taitungensis
Pinus_thunbergii
Gnetum_parvifolium
Amborella_trichopoda
Arabidopsis_thaliana
Saccharum_officinarum
outgroup
ingroup

Orthologs and Paralogs

Orthology – homologous gene sequences

Paralogy – gene sequences separated by a gene duplication event



Rooting

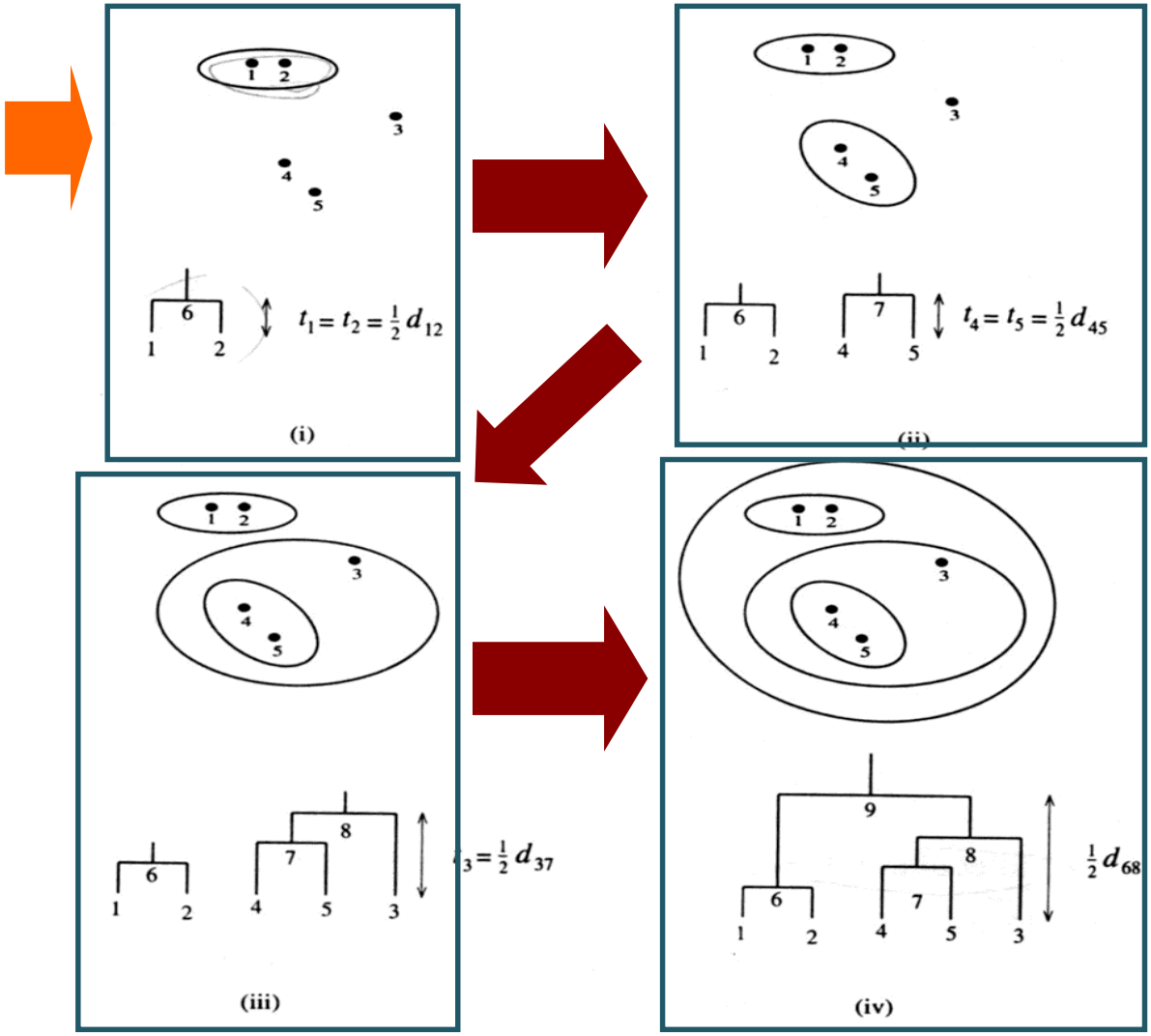
- A phylogenetic analysis most often results in an formally unrooted network
- For phylogenetic analyses can be included an “outgroup” which will be used to root the tree
- The taxa of interested in are called the “ingroup”
- The assumption is that the ingroup taxa are more closely related to each other than any is to the outgroup
- If this assumption is wrong, then the interpretations of the phylogenetic tree will be wrong!

Methods

- Distance-based methods:
- Neighbor-joining
- UPGMA

- Character-based methods:
- Maximum parsimony
- Model-based methods:
  - Maximum likelihood
  - Bayesian inference

UPGMA


1 2
3
4
5
6
1 2
$t_1 = t_2 = \frac{1}{2} d_{12}$
(i)
1 2
3
4 5
6
1 2
7
4 5
$t_4 = t_5 = \frac{1}{2} d_{45}$
(ii)
1 2
3
4 5
6
1 2
7
4 5
8
3
$t_3 = \frac{1}{2} d_{37}$
(iii)
1 2
3
4 5
9
6
1 2
8
7
4 5
3
$\frac{1}{2} d_{68}$
(iv)

UPGMA

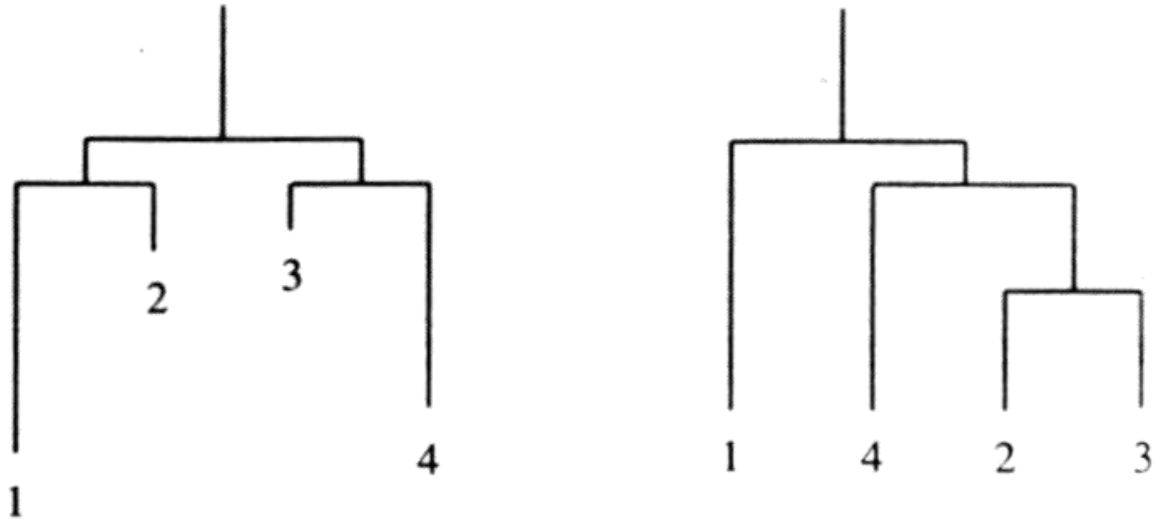
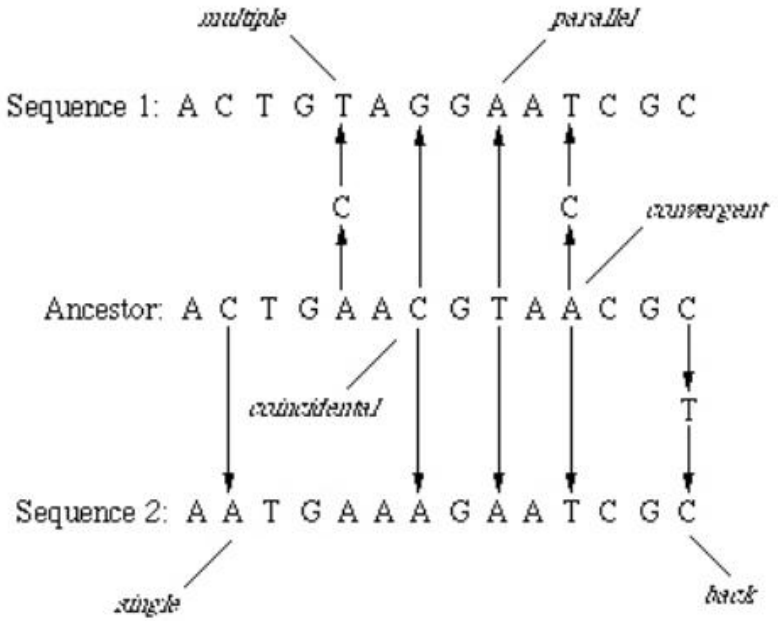


Figure 7.5 *A tree (left) that is reconstructed incorrectly by UPGMA (right).*

Neighbor Joining

- Very popular method
- Does not make molecular clock assumption : modified distance matrix constructed to adjust for differences in evolution rate of each taxon
- Produces unrooted tree
- Assumes additivity: distance between pairs of leaves = sum of lengths of edges connecting them

Neighbor Joining



Use models of substitution to correct these values

File Formats

- Newick (.nwk)

((species1:BranchLength,species2)Bootstrap,species 3);

Distance methods

- Advantages:
  - easy to perform
  - quick calculation
  - fit for sequences having high similarity scores
- Disadvantages:
  - the sequences are not considered as such (loss of information)
  - all sites are generally equally treated (do not take into account differences of substitution rates)
  - not applicable to distantly divergent sequences.

Hands On 5

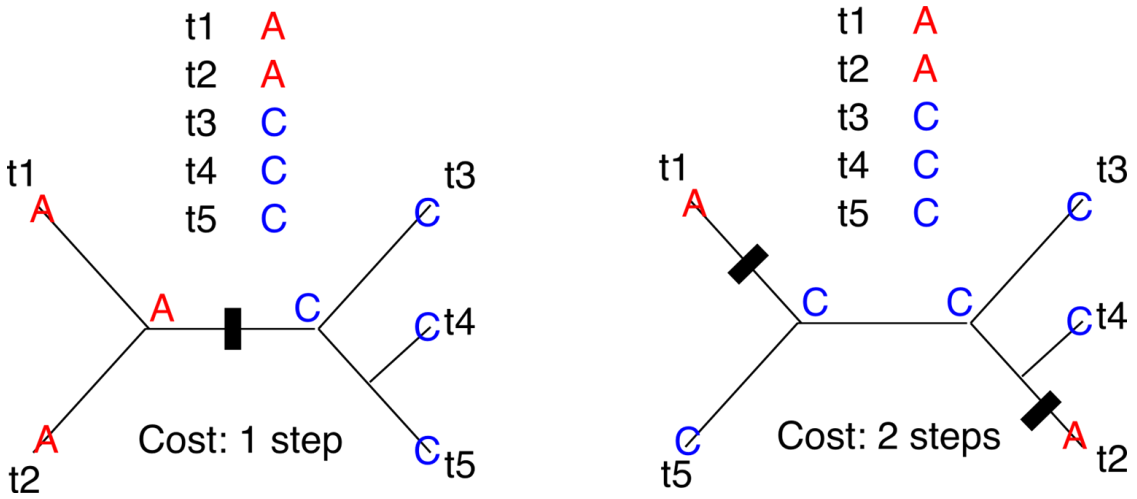
1. Requisitos Seaview e Mega
2. Descarregar sequencias na pasta hands_on_5
3. Alinhar usando Muscle em Aminoácidos
4. Fazer uma NJ de nucleótidos usando os parâmetros por omissão, 1000 bootstrap
5. Repetir o passo fazendo uma árvore de aminoácidos

Characters based tree

Maximum parsimony

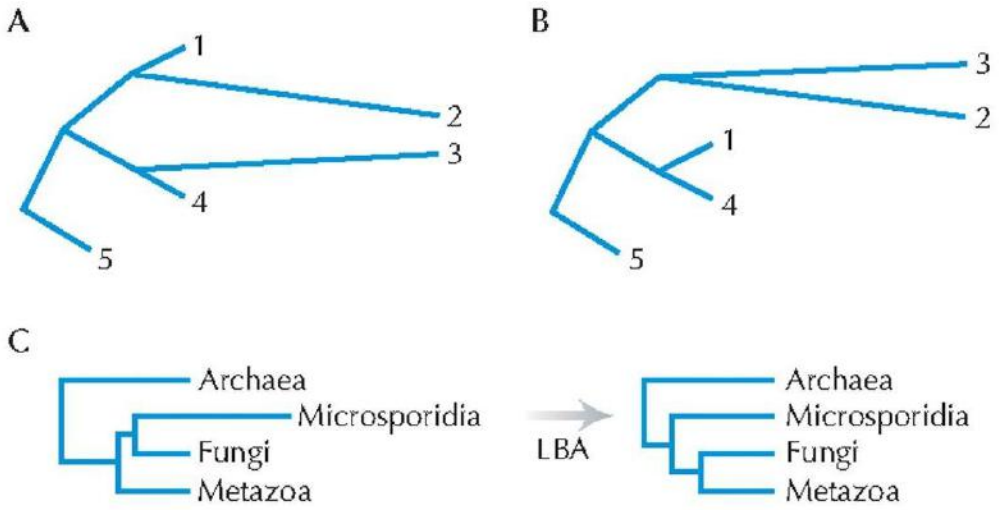
- Object is to minimise the number of changes necessary for the evolution of all characters on a tree.
- Character changes are *typically* treated as equally-weighted ie. the "cost" of changing from one state to another is the same between all states, but various weighting schemes can be applied
- Can be used with both morphological and molecular data, morphological characters may be ordered and polarised
- The tree with the fewest changes/steps is the MP tree. Might find many most- parsimonious solutions, which are often presented as a 50% majority-rule tree

Maximum parsimony



- Fitch's algorithm can be used to determine the most-parsimonious character reconstruction on any tree, the total score (or length) of a tree is the number of steps (changes) required by the most-parsimonious reconstructions of all characters, and the tree (or trees) with the lowest total score is the MP tree (or MP trees)

Long Branch Attraction



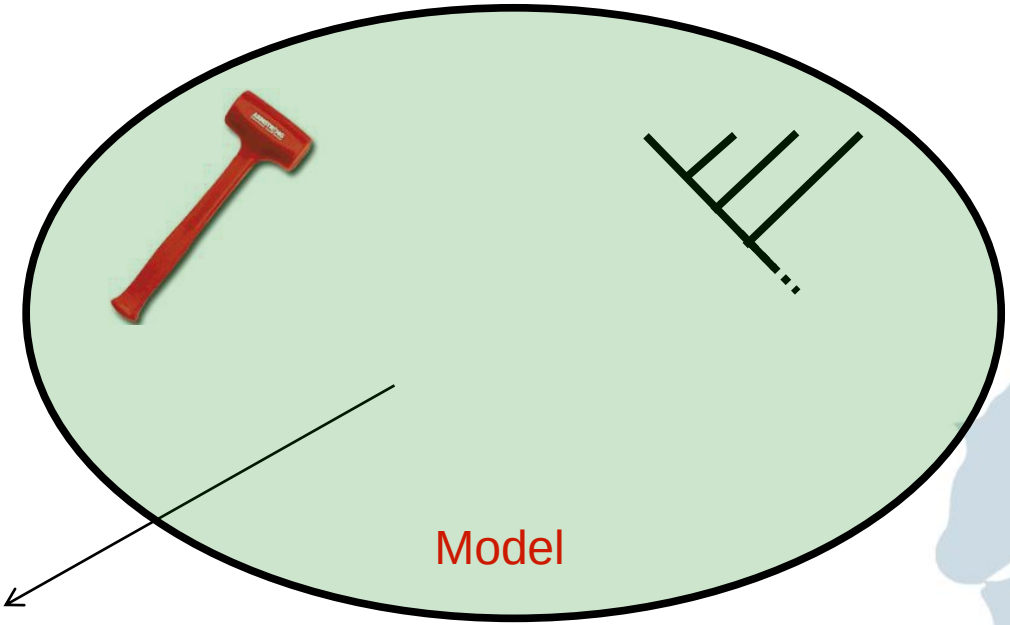
Maximum likelihood and Bayesian inference

- Both use explicit models of character change that are evaluated on a tree using the likelihood function $\propto \mathcal{P}rob(\mathcal{D} \mid \mathcal{H})$

- They differ in their use of statistical paradigms

Model as mechanism of change and tree

Data

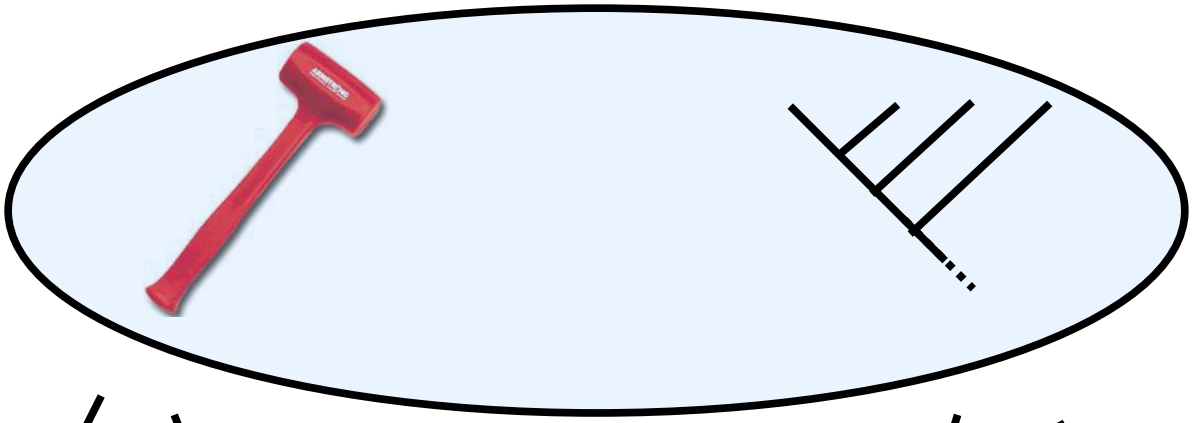


likelihood $\propto Prob(\mathcal{D} \mid \mathcal{H})$

The likelihood is proportional to the probability of *data* given the hypothesis

(a model of character change plus tree topology)

Model as mechanism of change and tree



model

tree

Composition

Substitution rates

Topology

Branch lengths

Instantaneous rate matrix

e.g. general time-reversible model

Models

- 1) the base composition:
- π_a, π_c, π_g, & π_t
  - The base composition frequency parameters remain constant over time (i.e. they are at equilibrium)
  - They express the rate at which changes ***to*** each base occur
  - Hence, the rate of change to a rare base would be low, whereas the change to a common base would be high
    - could be equal: 0.25, 0.25, 0.25, 0.25
    - or not: 0.3, 0.4, 0.2, 0.1
    - perhaps values are estimated from the data

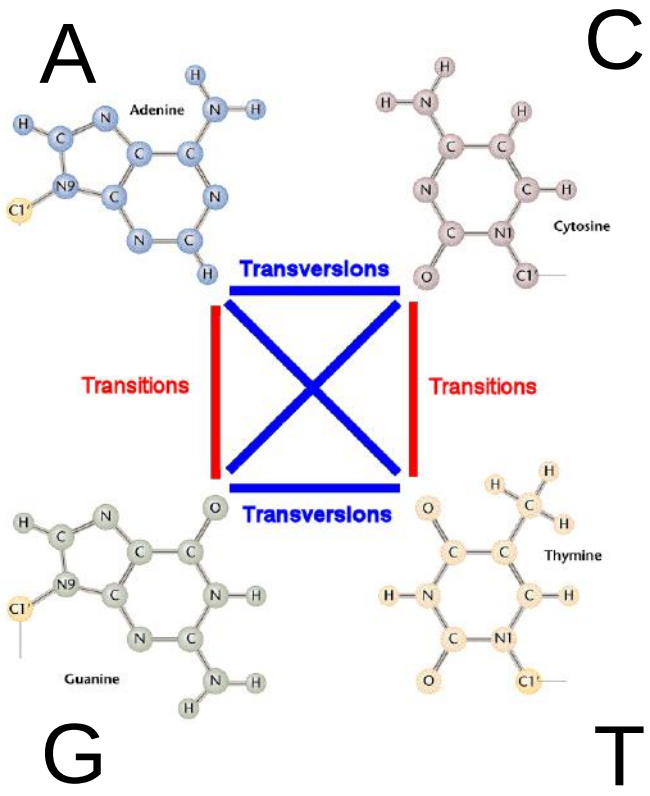
Models

2) Substitution rates:

$$\mathcal{R} = \begin{array}{c} \begin{array}{cccc} \text{A} & \text{C} & \text{G} & \text{T} \end{array} \\ \begin{pmatrix} - & r_{A\to C} & r_{A\to G} & r_{A\to T} \\ r_{C\to A} & - & r_{C\to G} & r_{C\to T} \\ r_{G\to A} & r_{G\to C} & - & r_{G\to T} \\ r_{T\to A} & r_{T\to C} & r_{T\to G} & - \end{pmatrix} \end{array} \begin{array}{c} \text{A} \\ \text{C} \\ \text{G} \\ \text{T} \end{array}$$

Transitions and transversions

purines



pyrimidines

Model GTR

$$\mathcal{Q} = \begin{pmatrix} - & \mu r_{A\to C}\pi_C & \mu r_{A\to G}\pi_G & \mu r_{A\to T}\pi_T \\ \mu r_{C\to A}\pi_A & - & \mu r_{C\to G}\pi_G & \mu r_{C\to T}\pi_T \\ \mu r_{G\to A}\pi_A & \mu r_{G\to C}\pi_C & - & \mu r_{G\to T}\pi_T \\ \mu r_{T\to A}\pi_A & \mu r_{T\to C}\pi_C & \mu r_{T\to G}\pi_G & - \end{pmatrix}$$

$$\mathcal{GTR} = \begin{array}{c} \\ A \\ C \\ G \\ T \end{array} \begin{array}{c} \begin{array}{cccc} A & C & G & T \end{array} \\ \begin{pmatrix} - & \mu r_i\pi_C & \mu r_j\pi_G & \mu r_k\pi_T \\ \mu r_i\pi_A & - & \mu r_l\pi_G & \mu r_m\pi_T \\ \mu r_j\pi_A & \mu r_l\pi_C & - & \mu r_n\pi_T \\ \mu r_k\pi_A & \mu r_m\pi_C & \mu r_n\pi_G & - \end{pmatrix} \end{array}$$

GTR derived

Model derived from the *GTR* model – few have been implemented in phylogenetics

- GTR – unequal base frequencies and 6 substitution types
- SYM – equal base frequencies and 6 substitution types
- HKY85 – unequal base frequencies and 2 substitution types (transitions and transversion)
- F81 – unequal base frequencies and single substitution type
- JC – equal base frequencies and single substitution type

Model selection

2) The Akaike Information Criterion: $AIC_i = -2logL_i + 2p_i$

where: i is the hypothesis (model + tree), and

p is the number of free parameters

- Does not requite models to be nested
- Calculate AIC for each model
- Choose model with lowest AIC score
- To be preferred over the LRT

Model selection

1) The Likelihood Ratio Test (LRT):

where: L_0 is a restricted (simpler) version of L_1

e.g.:

Null model = HKY+G	-lnL0 = 7918.9556
Alternative model = GTR+G	-lnL1 = 7907.3330
2(lnL1-lnL0) = 23.2451	df = 4
P-value = 0.000113	

Tools

- Modeltest conducts the LRT/AIC (and others) among a set of nested models
  – 14 substitution matrices with and without a *pinvar* and *gdasrv*
- Uses a crude distance tree to calculate the likelihoods of the models
- MrModeltest calculates similar for 24 models (those used by MrBayes)
- For amino acid models use ProtTest or ModelGenerator

Models parameters

- It is commonly recognised that not all sites evolve at the same rate – some may constrained by selection. This can be incorporated into the model:

1. *Site-specific rate categories* - defined *a priori*, e.g. first, second, third codon positions of a protein-coding gene.

2. *Proportion of invariant sites* (pinvar) - assumes some proportion of sites is incapable of changing and all other sites vary at the same rate.

3. *Gamma distributed among site rate variation* (gdasrv) - uses a number of discrete categories of rates that partitions a gamma distribution - the shape of the distribution is described by the parameter α

Amino acid substituion models

- Poisson model – (equiv. to JC) equal substitution rates and frequencies
- Proportional model – as Poisson but with unequal (empirically observed) frequencies

Empirically observed transition matrices:

- Dayhoff - derived from Dayhoff, et al.'s (1978) empirical substitution matrix
- JTT - Jones, Taylor, Thornton
- WAG - Whelan and Goldman

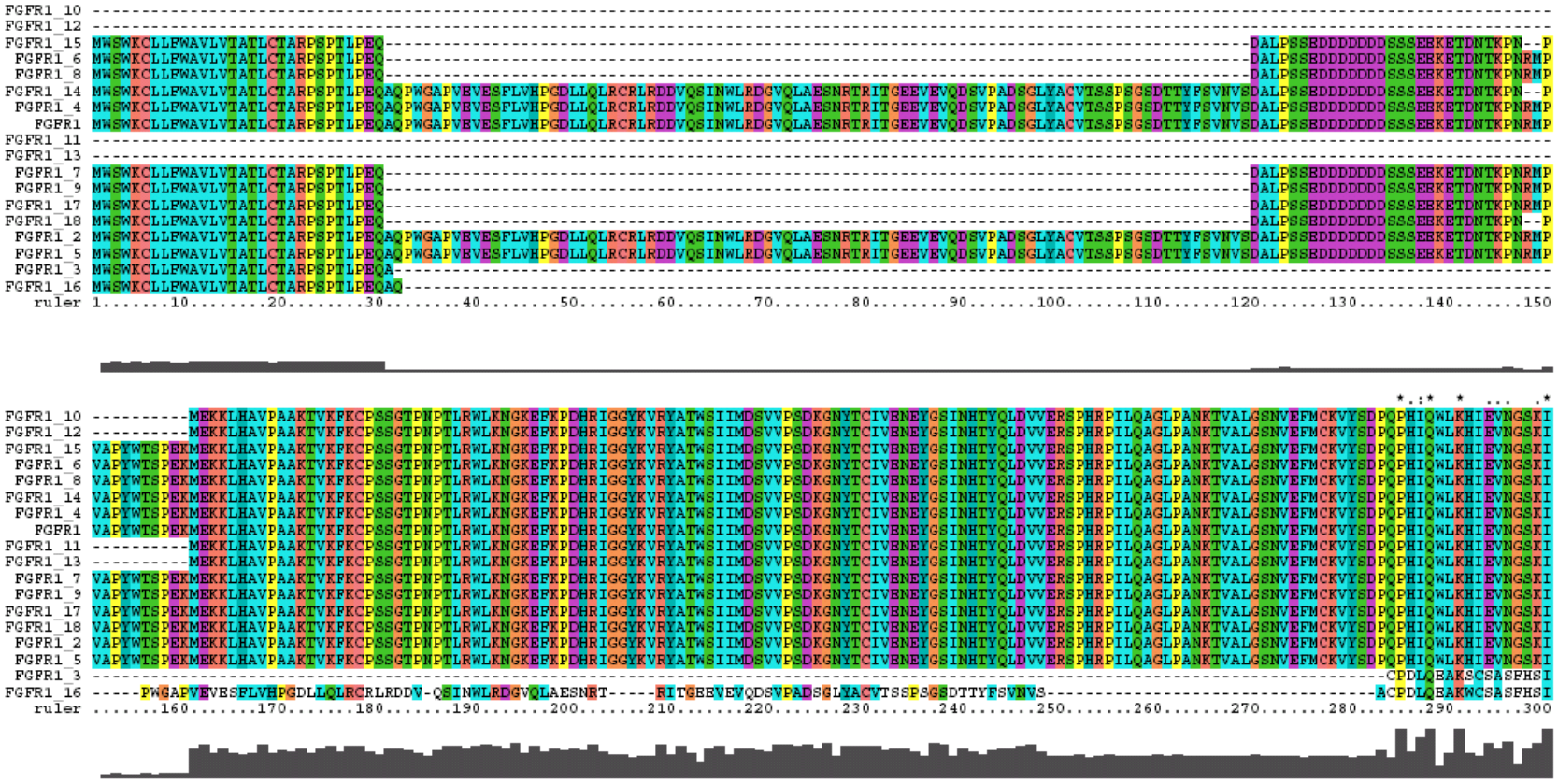
Bootstrap

- The bootstrap is a statistical method that is designed to test the reliability of the result by using pseudo-replicates drawn from the original data

- Draw characters/sites from the original data, with replacement, from the original data set to make a new one the same size. Repeat the phylogenetic analysis on this bootstrap replicate and repeat the process many times (100-1000).

- Interpretation of the bootstrap is difficult. It is known to be biased, but for a particular support values it not known where it is biased up or down. Its usually reckoned that 70% is statistically significant.

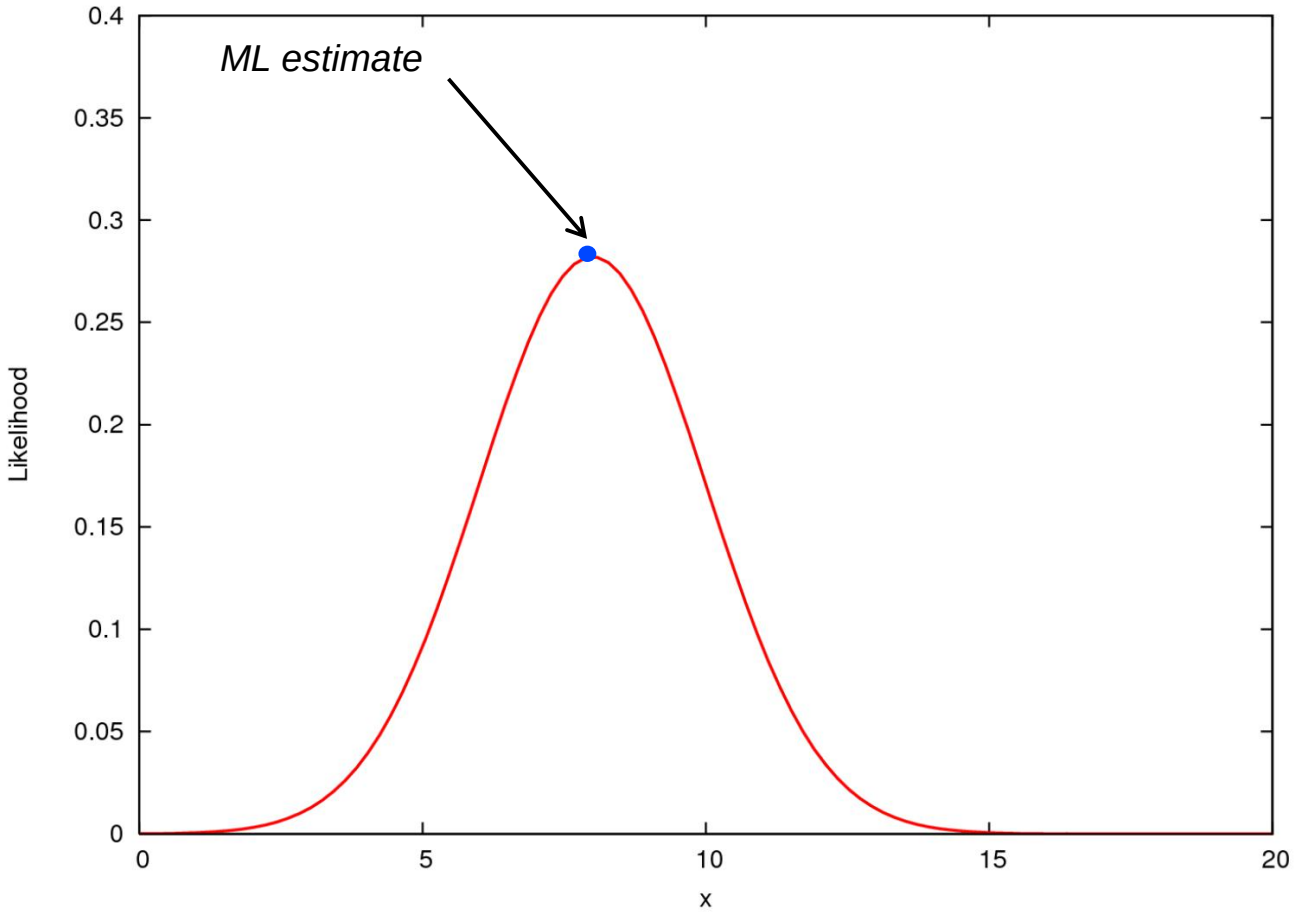
Bootstrap


FGFR1_10
FGFR1_12
FGFR1_15
FGFR1_6
FGFR1_8
FGFR1_14
FGFR1_4
FGFR1
FGFR1_11
FGFR1_13
FGFR1_7
FGFR1_9
FGFR1_17
FGFR1_18
FGFR1_2
FGFR1_5
FGFR1_3
FGFR1_16
ruler
1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150
1.....160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300

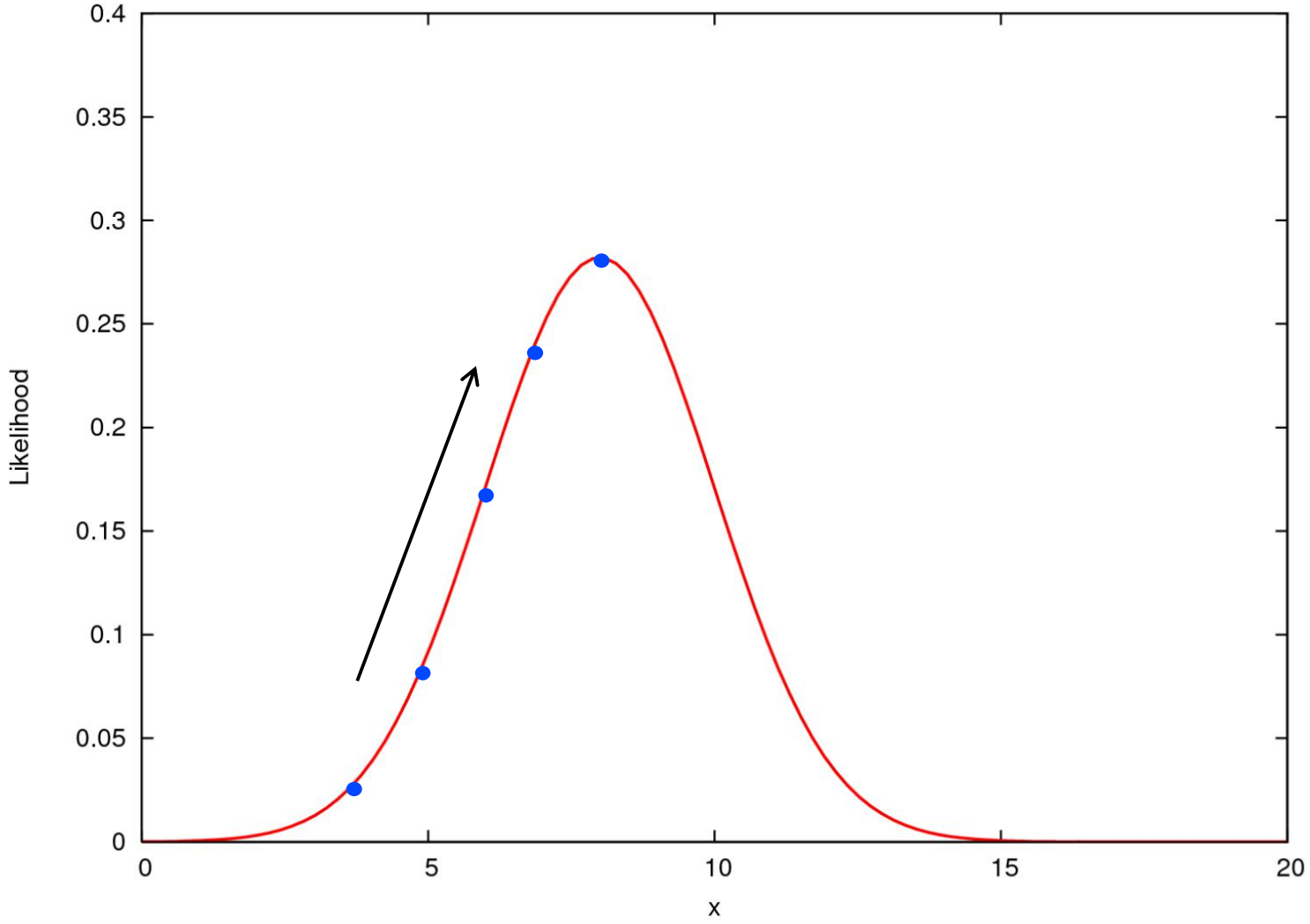
Hands On 6

1. Requisitos Seaview+jModelTest e/ou Mega
2. Alinhar usando MUSCLE
3. Determinar modelo evolucionário
4. Fazer uma ML usando 100 de bootstrap e o modelo determinado no passo 3

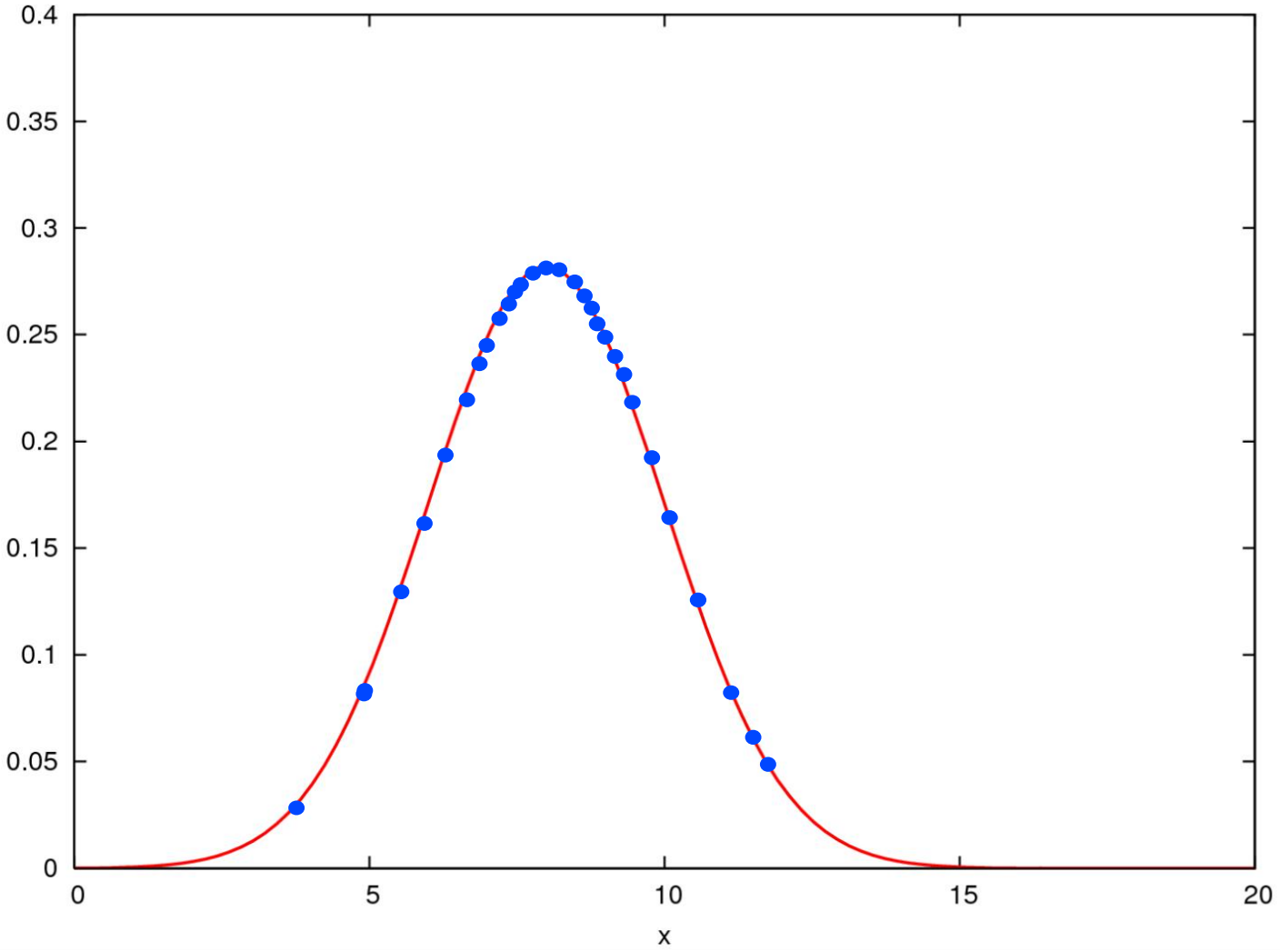
ML and PP density



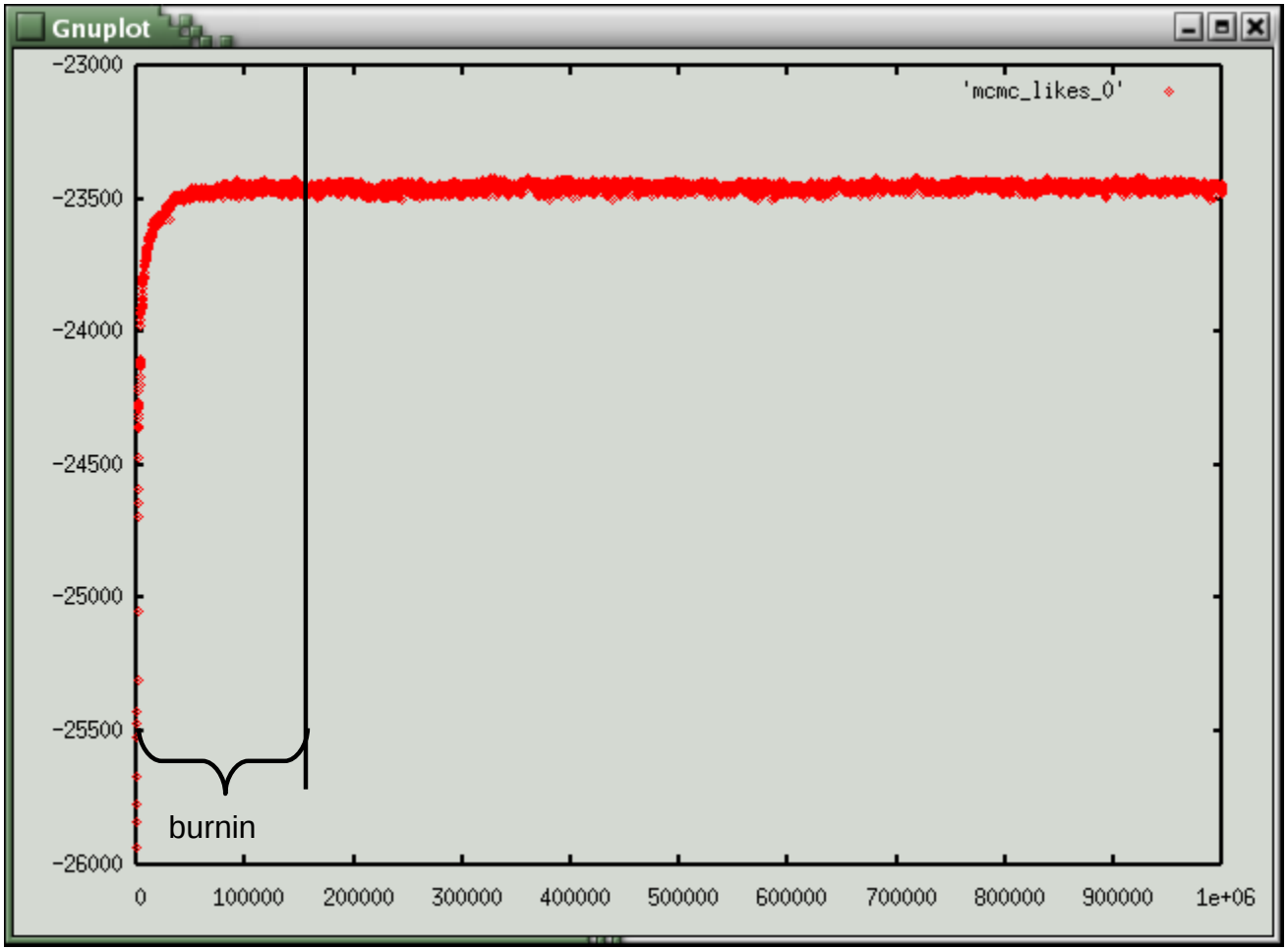
ML and PP density



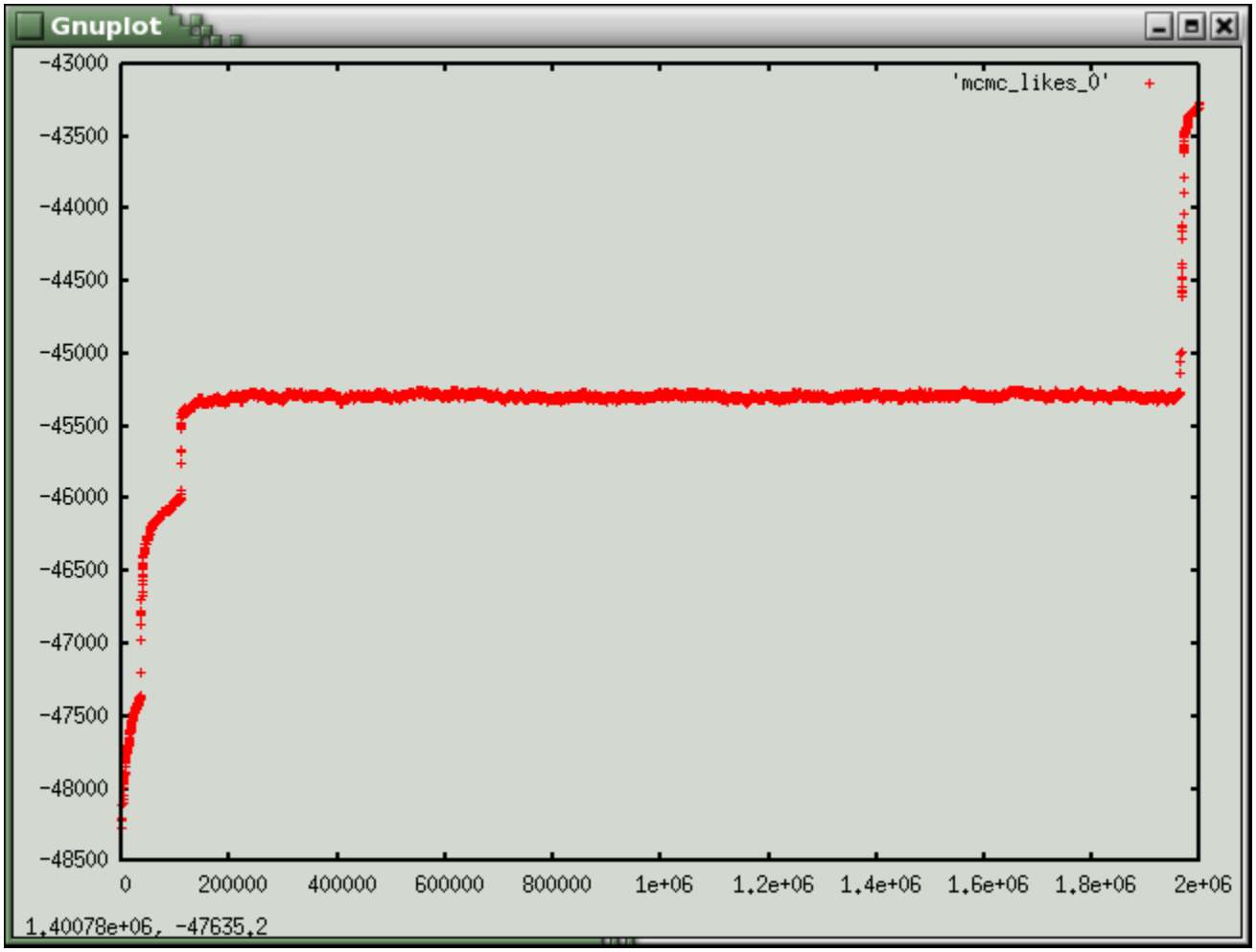
ML and PP density



Likelihood plot



Likelihood plot



Mcmc output

Composition *Substitution Rate* *gdasrv*

gens	*A*	*C*	*G*	*T*	*A-C*	*A-G*	*A-T*	*C-G*	*C-T*	*G-T*	*alpha*
100	0.22515466	0.24584661	0.30733981	0.22165891	0.11970749	0.23917488	0.08580185	0.14260800	0.32394051	0.08876728	0.874116
200	0.22581011	0.23935950	0.28160072	0.25322967	0.11361513	0.22751384	0.10471647	0.16653072	0.26859341	0.11903044	1.930917
300	0.23172551	0.27706732	0.27019486	0.22101230	0.12093299	0.20611349	0.08865832	0.15770240	0.28720945	0.13938334	4.380087
400	0.22999880	0.26063025	0.28697633	0.22239461	0.09333566	0.24749557	0.12001019	0.14071384	0.29772513	0.10071961	10.893358
500	0.24616074	0.25080719	0.27680877	0.22622330	0.10333398	0.20508574	0.10527309	0.16562894	0.31464847	0.10602977	14.875529
600	0.21219225	0.28281963	0.28884274	0.21614538	0.11325671	0.25977835	0.12678584	0.11935713	0.28575591	0.09506605	8.521777

etc...

```
tree t_100 = [&U] (((1:0.263151, 2:0.0564195):0.206267, 3:0.223034):0.73243, 4:0.107335, 5:0.0742962);
tree t_200 = [&U] (((5:0.115119, 4:0.0936513):1.14124, 2:0.0686334):0.206054, 3:0.338046, 1:0.223061);
tree t_300 = [&U] ((2:0.0799976, (4:0.0892171, 5:0.115119):1.10016):0.174534, 3:0.461212, 1:0.236187);
tree t_400 = [&U] ((2:0.0800969, (5:0.126667, 4:0.09186):1.39515):0.21926, 3:0.445261, 1:0.335234);
tree t_500 = [&U] (((4:0.100586, 5:0.149979):1.92335, 2:0.0800969):0.268639, 1:0.335234, 3:0.556183);
tree t_600 = [&U] (((3:0.671303, 1:0.413087):0.347891, 2:0.178729):2.18893, 5:0.0845881, 4:0.143907);
```

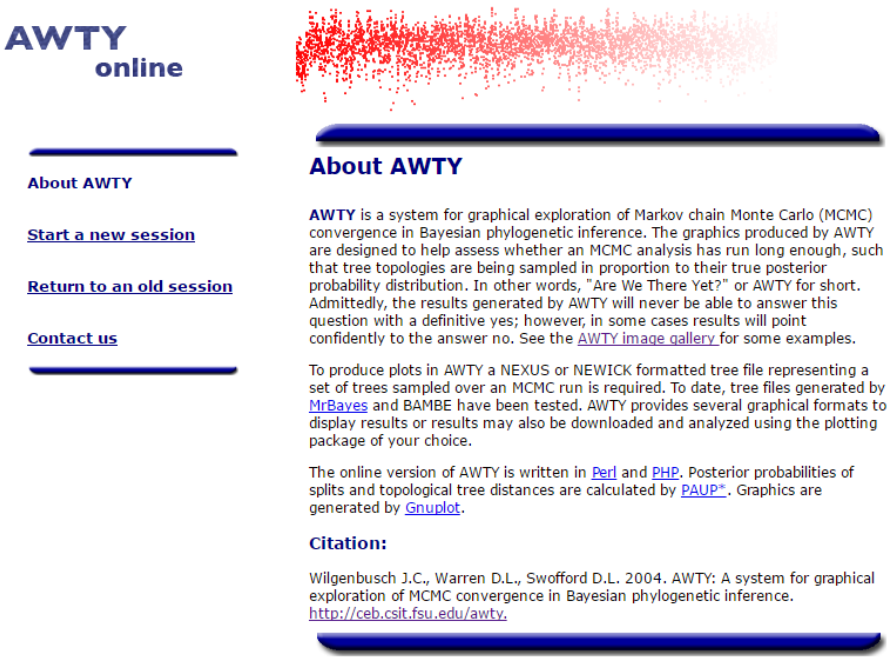
etc...

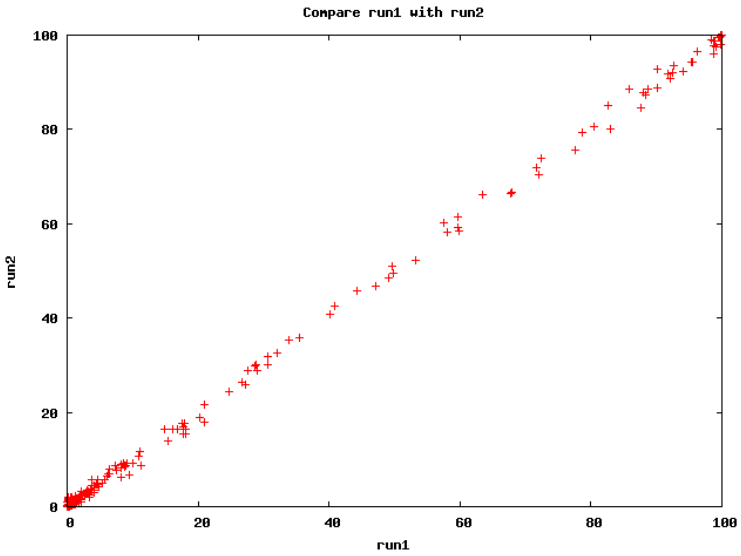
- *Note that at each generation the parameter values are known (i.e. they are the current values of the chain) hence the likelihood is easy and quick to calculate, this makes BA a relatively quick method when compared to ML*

Likelihood plot

- Are we there yet?

AWTY is a system for the graphical exploration of MCMC convergence, written by Jim Wilgenbusch, Dan Warren, and David Swofford.

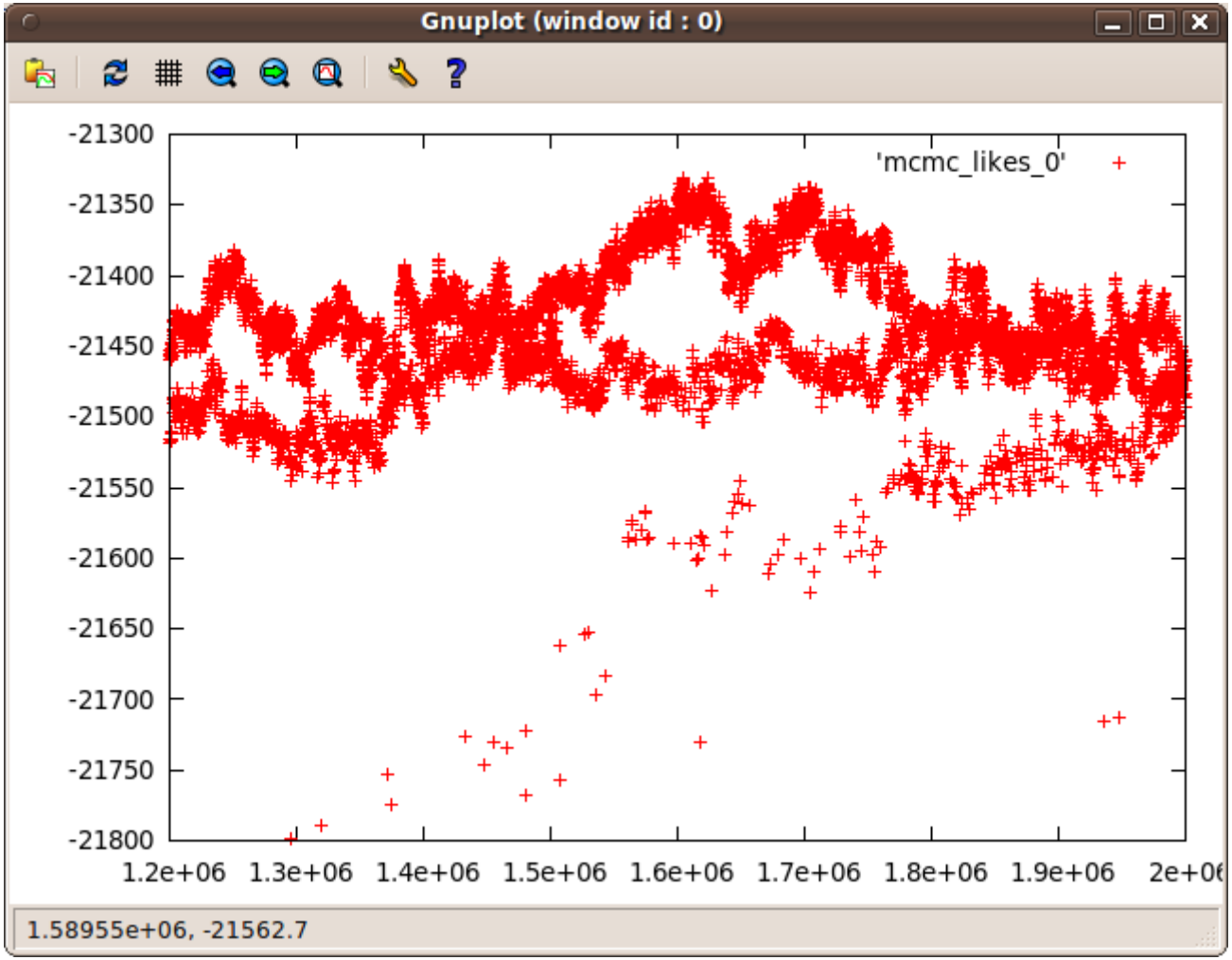




Convergence

- Run the analysis more that once and check that the separate runs give similar results
- Monitor the average standard deviation of split support between two separate runs (MrBayes does this by default)

Likelihood plot



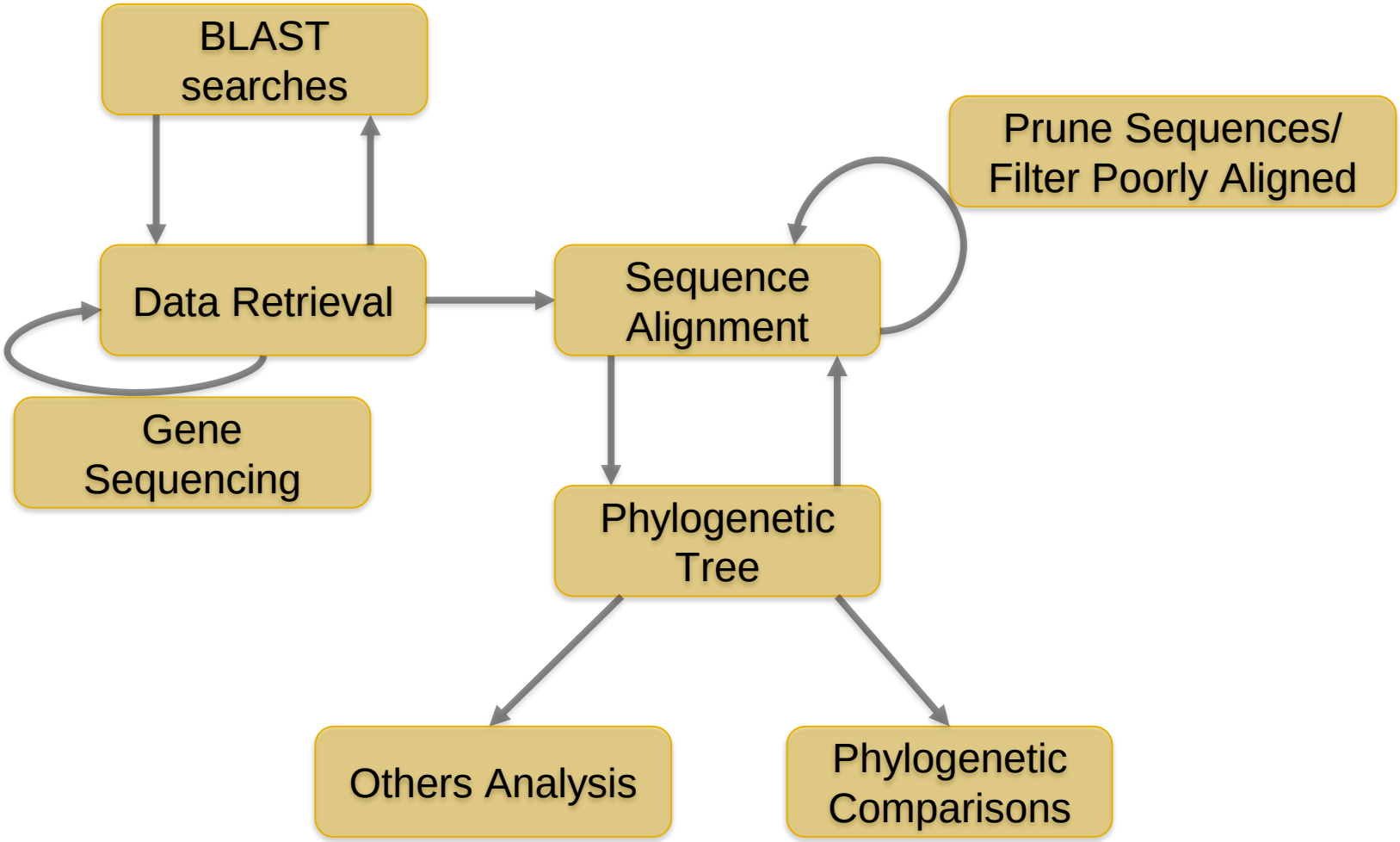
Alternatives

- http://www.thines-lab.senckenberg.de/simba/
- http://phylemon.bioinfo.cipf.es/
- http://phylogeny.lirmm.fr/phylo_cgi/index.cgi

Hands On 7

1. Requisitos Mr. Bayes e TreeGraph
2. Abrir executável do Mr.Bayes (32 ou 64 bits)
3. “Execute nomedoficheiro.nex”
4. “help lset”
5. “lset nst=6 rates=invgamma” (modelo GTR+I+G)
6. “help mcmc”
7. “mcmc ngen=200 000”
8. “sump” (confirmar parâmetros)
9. “sumt conformat=simple”
10. Executar TreeGraph e abrir ficheiro .con gerado no Mr. Bayes

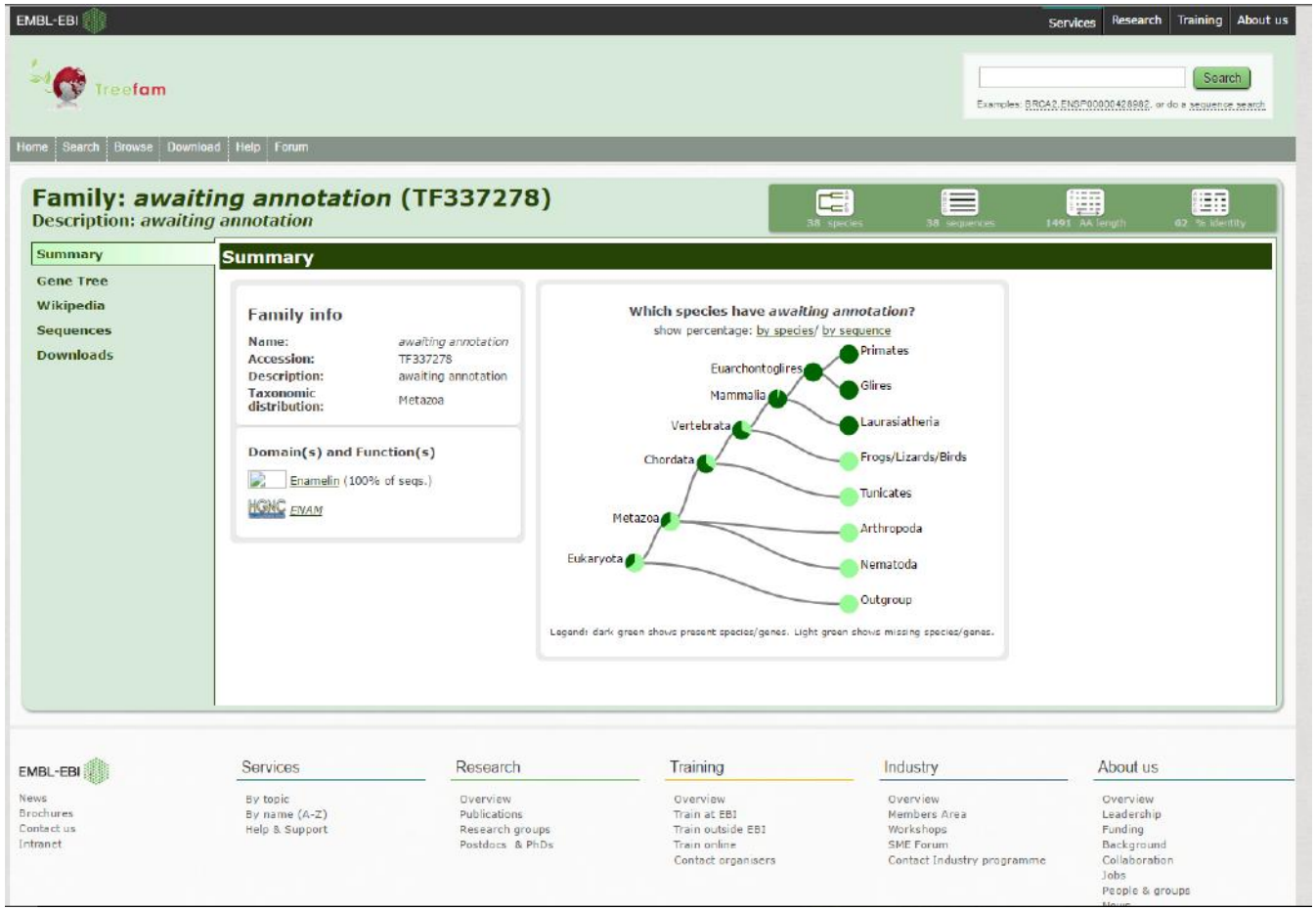
Typical Workflow



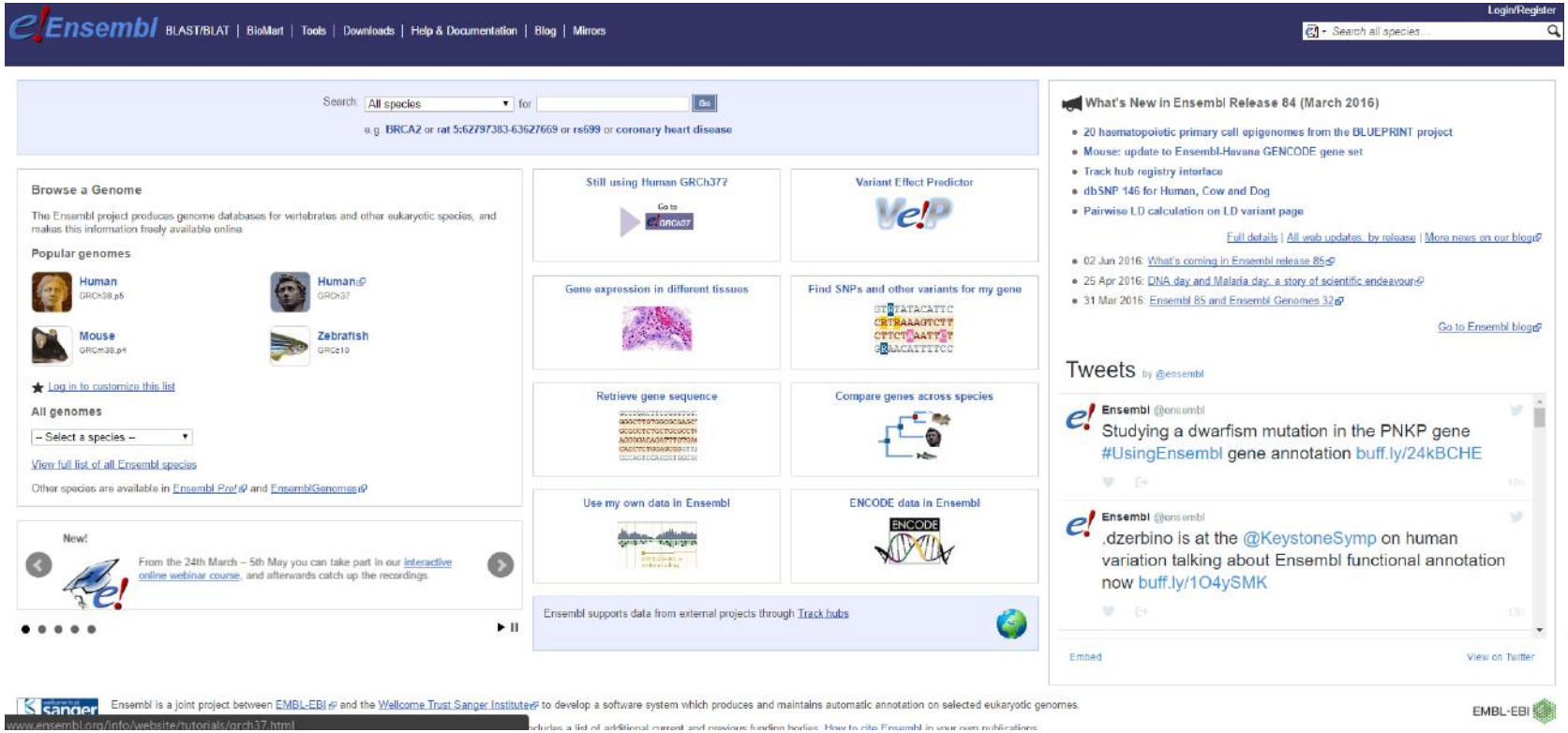
Hands On 8

1. Requisitos Tree-Puzzle
2. Abrir puzzle-windows-mingw.exe
3. k + enter -> K Tree search procedure? Evaluate user defined trees
4. m + enter até ao modelo GTR
5. w + enter até -> w Model of rate heterogeneity? Mixed (1 invariable + 4 Gamma rates)
6. y + enter
7. my_trees.txt (ficheiros com as árvores)
8. Abrir ficheiro .puzzle
9. No final do ficheiro aparece a comparação entre as 3 árvores “COMPARISON OF USER TREES (NO CLOCK)”

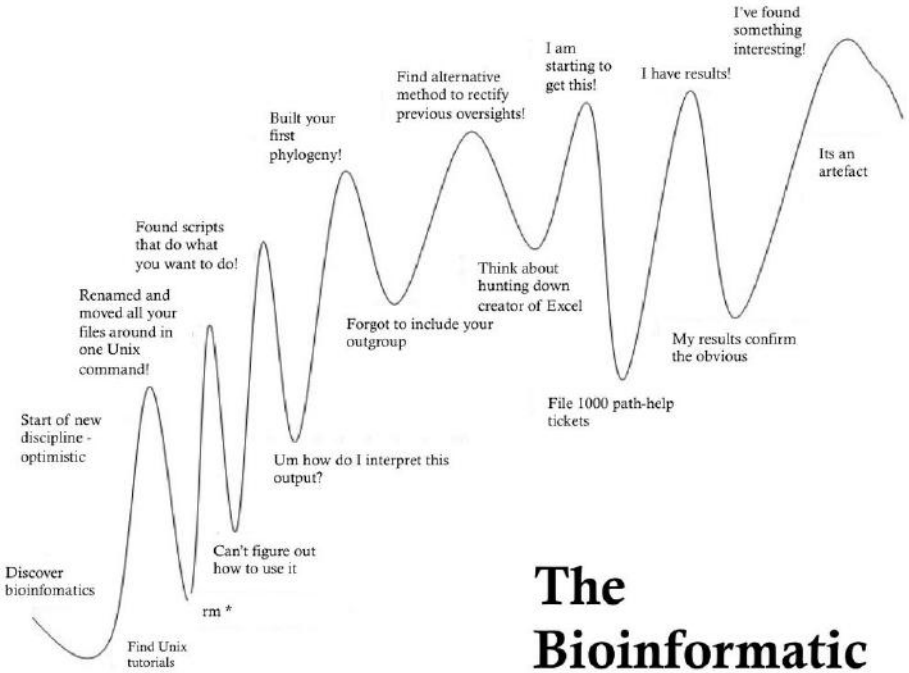
Data Warehouses Again



Data Warehouses Again



Learning Curve



Q & A

Further reading

Swofford et al. 1996. Phylogenetic Inference. In Hillis, Moritz, & Mable [Eds.],

Molecular Systematics. Sinauer Associates, Sunderland, M.A.

Foster, 2007. Inferring phylogenetic relationships from sequence data. In Dear [Ed.],

Bioinformatics. Scion.

Lewis, 2001. Phylogenetic systematics turns of a new leaf. *Trends in Ecology and*

Evolution, 16: 30-37.

Felsenstein 2004. *Inferring Phylogenies*. Sinauer Associates, Sunderland, M.A.