Jessica Beardsley

October 18, 2011

Fall 2011

Pool N58, RNA, Lysozyme

**Progress Report 1**

**Progress, Results, and Discussion**

Lysozyme: Practice Round 1A

Complete RNA bead selection against lysozyme began on September 1, 2011. The goal of this practice round was to get acquainted with the bead based selection protocol, learn new lab techniques, and possibly produce high affinity binders against lysozyme. On September 1, the target lysozyme was immobilized onto beads and selected against. I completed three washes (labeled W0-W3), a mix pool binding reaction (containing RNA from the old N58 pool) and an elution (labeled E1), using 2 volumes of 1X selection buffer for each wash. On September 6, ethanol precipitation and reverse transcription were performed on washes W0, W3, and E1. The reactions were stored in the freezer until September 8, when they were used for cycle course PCR and gel electrophoresis. No negative control was made. 5 uL aliquots of each wash were taken during cycles seven, nine, twelve, fifteen, and twenty to use for gel electrophoresis. The results of the gel can be found in **Figure 1**. The wells were filled according to the following **Table 1**:

|  |  |  |  |
| --- | --- | --- | --- |
| 1. Ladder | 7. Nothing | 13. W3- Cycle 7 | 19. E1- Cycle 7 |
| 2. W0- Cycle 7 | 8. Nothing | 14. W3- Cycle 9 | 20. E1- Cycle 9 |
| 3. W0- Cycle 9 | 9. Nothing | 15. W3- Cycle 12 | 21. E1- Cycle 12 |
| 4. W0- Cycle 12 | 10. Nothing | 16. W3- Cycle 15 | 22. E1- Cycle 15 |
| 5. W0- Cycle 15 | 11. Nothing | 17. W3- Cycle 20 | 23. E1- Cycle 20 |
| 6. W0- Cycle 20 | 12. Nothing | 18. Ladder | 24. Nothing |

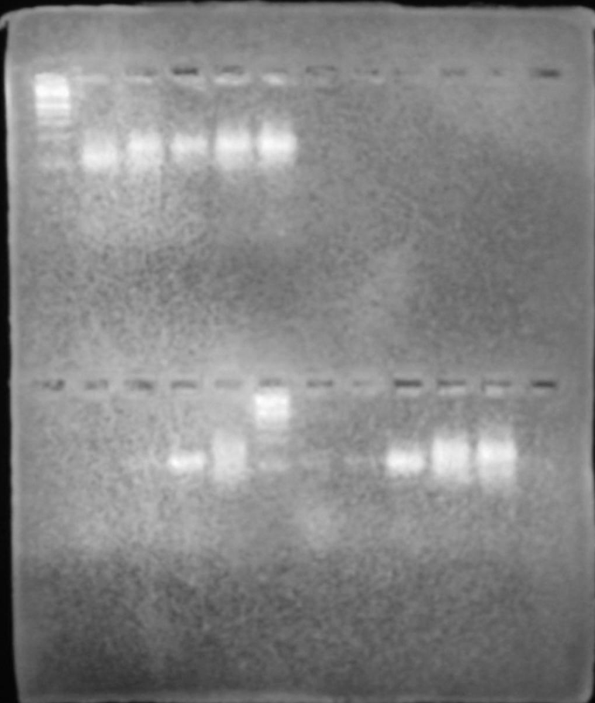
13 14 15 16 17 18 19 20 21 22 23 24

W3-7 3-9 3-12 3-15 3-20 L E1-7 E-9 E-12 E-15 E-20

1 2 3 4 5 6 7 8 9 10 11 12

L W0-7 0-9 0-12 0-15 0-20

**12 cycles for E1 during lsPCR**



**Figure 1: Lysozyme, N58, Round 1A.** The first gel I ran after cycle course PCR in Round 1A is depicted above and appears to have been successful. W0, the positive control, over-amplified as expected because this wash contained all the RNA strands that did not bind to the target. Also, E1, which contains potential aptamers against lysozyme, showed bands before the last wash (W3), suggesting that all possible binders were collected and all weak or nonbinding proteins were removed by the last wash. A well defined band for E1 (before over-amplification) occurred at cycle 12. E1 for the twelfth cycle is boxed in red above. This clear band for E1 determines the number of cycles that will be used during large scale PCR. The resolution is not of the best quality because the gel had to be frozen overnight due to camera malfunctions.

Large scale PCR was then performed on September 20 to make enough E1 template for transcription. Six 100 uL reactions were made, each containing E1 from reverse transcription. A negative control was also made for practice using the same components for the six reactions (found in the “Bead Based RNA Selection Protocol”), but instead of using E1, more diH2O was added. These six reactions were run for twelve cycles as determined by gel electrophoresis after cycle course PCR. On September 30, we combined and ethanol precipitated the reactions. During this step, two errors were made. After the reaction was centrifuged for ten minutes and the supernatant decanted, 300 uL of chilled 70% EtOH was not added to the reaction. This was not realized until after transcription was underway. The second error was made at the end of ethanol precipitation. When combing the pellets of what were initially two 300 uL large scale reactions, 20 uL of diH2O was accidentally added to each reaction instead of 10 uL. This made the total precipitated dsDNA volume 40 uL instead of 20 uL. Transcription was then performed on this diluted reaction, and on October 1 a PAGE gel was run. The results of PAGE appeared to be successful. Ethanol precipitation was performed on the gel elution on October 4, but after extended periods of chilled centrifugation, there was no visible pellet. The gel had not been as successful as initially assumed, most likely because of the errors during ethanol precipitation.

Lysozyme: Practice Round 1B

A second practice round against lysozyme, also using complete RNA bead selection, began on October 6, 2011. The main objective of this second practice round was to perfect my selection techniques and complete a full round by myself. Target immobilization was performed using buffers I had made the day before, 1X PBS, 10X PBS Selection buffer with MgCl2, and 1X PBS selection buffer. A mix pool binding reaction was made using RNA from the new N58 pool. I completed three washes (labeled W0-W3) and an elution (labeled E1) for this round, using 2 volumes of 1X selection buffer for each wash. On October 11, they were ethanol precipitated. Each precipitation reaction initially contained 100 uL of reaction from washes W0, W3, and E1,10 uL 3M NaAc, 3 uL Glyco-blue, and 1110 uL 100% EtOH. I only added what was available in the lowest volume wash (W0, ~100 uL), having forgotten that I had needed to add enough diH2O so that each wash reaction ended up being 400 uL. I quickly corrected this so that the new precipitation components were 400 uL of reaction from washes W0, W3, and E1, 40 uL 3M NaAc, 3 uL Glyco-blue, and 1110 uL 100% EtOH. Ethanol precipitation was continued normally. Reverse transcription was then performed on the precipitated washes. Each reaction totaled 20 uL and their components can be found in the “Reverse Transcription” section of the “Complete RNA Bead-Based Selection Protocol.” On October 13, cycle course PCR, gel electrophoresis, and large scale PCR were performed. For cycle course PCR, the same components were used in Round 1A, except this time a negative control was made using all of the reaction components except for ssDNA from reverse transcription. diH2O was used instead. 5 uL aliquots of each wash were taken during cycles six, nine, twelve, fifteen, and twenty to use for gel electrophoresis. The results of the failed gel can be found in **Figure 2**. The wells were filled according to **Table 2**:

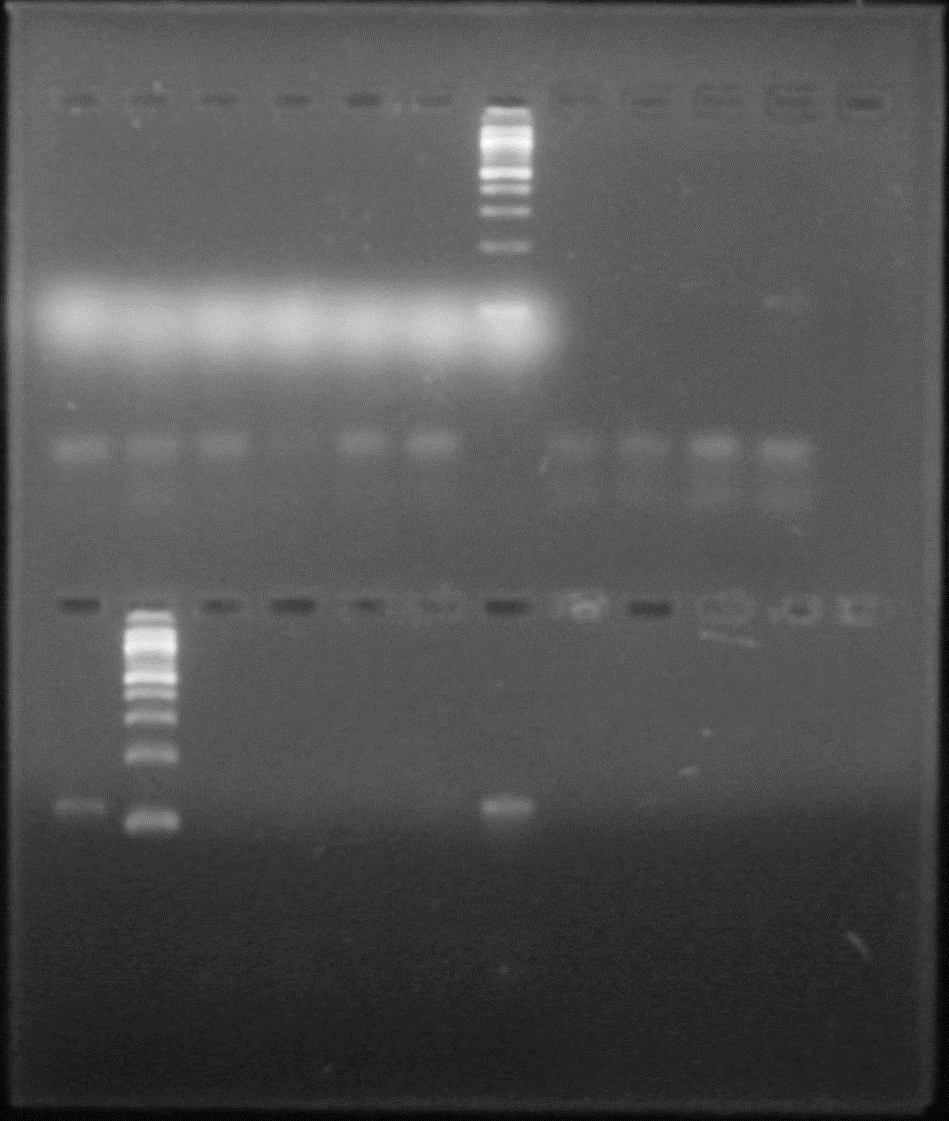
|  |  |  |  |
| --- | --- | --- | --- |
| 1. W0- Cycle 6 | 7. Ladder | 13. Negative Control | 19. E1- Cycle 20 |
| 2. W3- Cycle 6 | 8. W3- Cycle 9 | 14. Ladder | 20. Nothing |
| 3. W0- Cycle 9 | 9. W3- Cycle 12 | 15. E1- Cycle 6 | 21. Nothing |
| 4. W0- Cycle 12 | 10. W3- Cycle 15 | 16. E1- Cycle 9 | 22. Nothing |
| 5. W0- Cycle 15 | 11. W3- Cycle 20 | 17. E1- Cycle 12 | 23. Nothing |
| 6. W0- Cycle 20 | 12. Nothing | 18. E1- Cycle 15 | 24. Nothing |

13 14 15 16 17 18 19 20 21 22 23 24

Neg L E1-6 E-9 E-12 E-15 E-20

1 2 3 4 5 6 7 8 9 10 11 12

W0-6 W3-6 0-9 0-12 0-15 0-20 L W3-9 3-12 3-15 3-20



**Figure 2: Lysozyme, N58, Round 1B.** The gel after my second practice cycle course was a complete failure. There were no apparent bands W0, which should have the most and earliest over-amplification. There were also no bands seen under W3, or E-1 until the cycle 20. There appears to be some bands at the very bottom of the top portion for washes W0 and W3, but bands should not appear that far down. It is unclear what these bands are. Also, there is the possibility of contamination because the negative control, which lacks ssDNA, showed a band. So what did appear in the well for E1-Cycle 20 is not reliable, and must not be used to determine the amount of cycles used for lsPCR. The failure is possibly due to errors made during reverse transcription.

Although the gel was a complete failure, I decided to continue on with the round to work on my selection techniques. Large scale PCR was performed on the E1 ssDNA from reverse transcription. The components of large scale were the same as they were for Round 1A. After large scale, the reactions were placed in the freezer.

**Problems Encountered**

Lysozyme: Practice Round 1A

During the first selection round of lysozyme, three errors were made. The first, which may not technically be an error but should be done, was made during cycle course PCR. A negative control was not made, so the results that were seen cannot be trusted. There is a possibility that contamination had occurred, especially since many of the RNA pools were producing bands when used in no template negative controls.

The second and third errors made during Round 1A occurred during ethanol precipitation after large scale PCR. First, chilled 70% EtOH was not added after the first centrifugation. This most likely had a major impact on the purification and removal of dsDNA (made during lsPCR) from the solution. Not adding this component may have prevented the dsDNA from fully “crashing” out of the solution. Also, at the end of ethanol precipitation, twice the amount of diH2O was added than normal, further diluting the concentration of “crashed” aptamer dsDNA.

Lysozyme: Practice Round 1B

No apparent mistakes had been made during Round 1B until after cycle course PCR when the gel failed. The exact reason for why the gel failed is unknown. It was suggested that errors had to have been made during or right before reverse transcription, and it is possible that a major component might not have been added to the reverse transcription reactions. Also, a band appeared under the negative control, suggesting that there was possible contamination from the N58 primers. This, too, had some impact on the outcome of the gel.

**Conclusion and Future Work**

The purpose of these two practice rounds was to get me familiar with the protocol, allow me to quickly acquire new lab skills, and better understand the research I am doing. My first two rounds have taken longer than usually, but being new to the stream this semester, I feel that I have learned a lot and am catching on quickly. I only made a few mistakes during each practice round, but these mistakes were critical to the development of my practice project. As stated before, I will continue on with Round 1B, even though I had a failed cycle course PCR gel. I plan on completing transcription and a PAGE gel within the next week. I will most likely use someone else’s large scale and transcription products during PAGE, precipitation, and quantitation. By the end of this week or the beginning of next week, I plan on starting selection against my real target, BCL-2. By the next report, I plan on completing three full washes.