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Progress Report Prepared for

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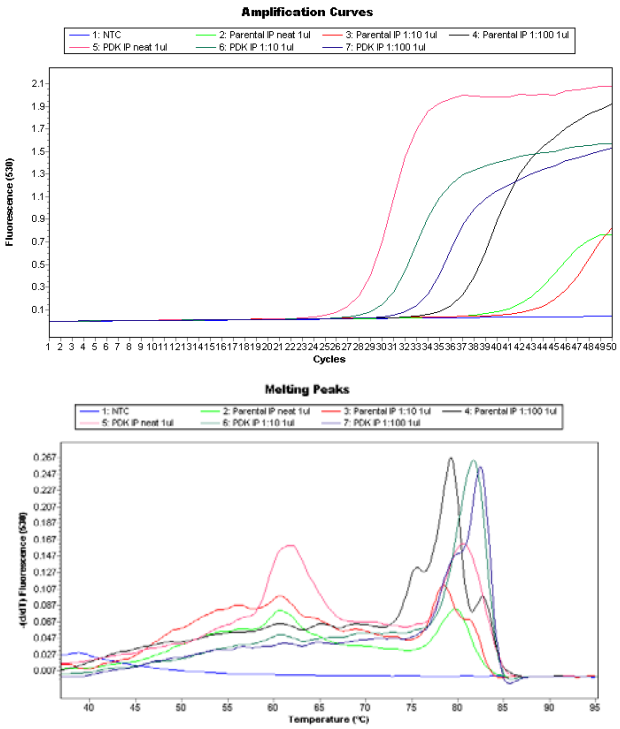

Fig. 1. PCR amplification of Comma-D PDK and Comma-D parental immunoprecipitated material. This is a repeat of the last experiment shown in the August 2009 progress report top further validate the specificity of aptamers to PDK1. Pink, Comma-D PDK immunoprecipitated (IP) material undiluted; dark green, IP material diluted 1:10; blue, IP material, 1:100. The other samples, which show some level of amplification, amplified much later. The no-template control was also clean. The melting curve peak observed near 62° is a heteroduplex artifact. These data are remarkably similar to those observed the when an identical experiment was performed earlier in this project. Ref: [Notebook NC 6-113].

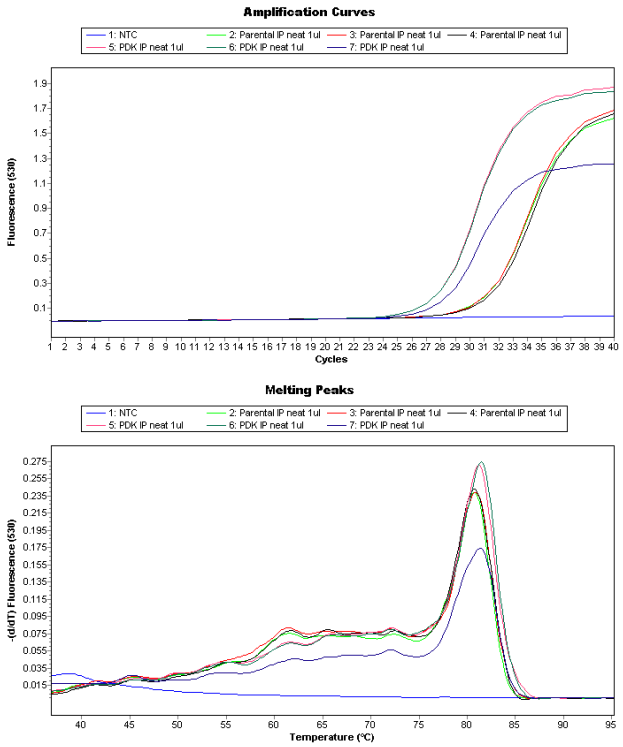

Fig. 2. Real-time PCR amplification of enriched aptamer library for affinity assessment. This experiment is a smaller-scale variant of the experiment described in Fig. 1. Three identical samples from Comma-D parental IP material and three identical samples from Comma-D PDK cells. All three Comma-D PDK samples amplified earlier than the samples from the parental Comma-D cell line. This experiment was performed to ensure reproducibility of data prior to moving onto the last set of experiments associated with this project. Ref: [Notebook NC 6-130]

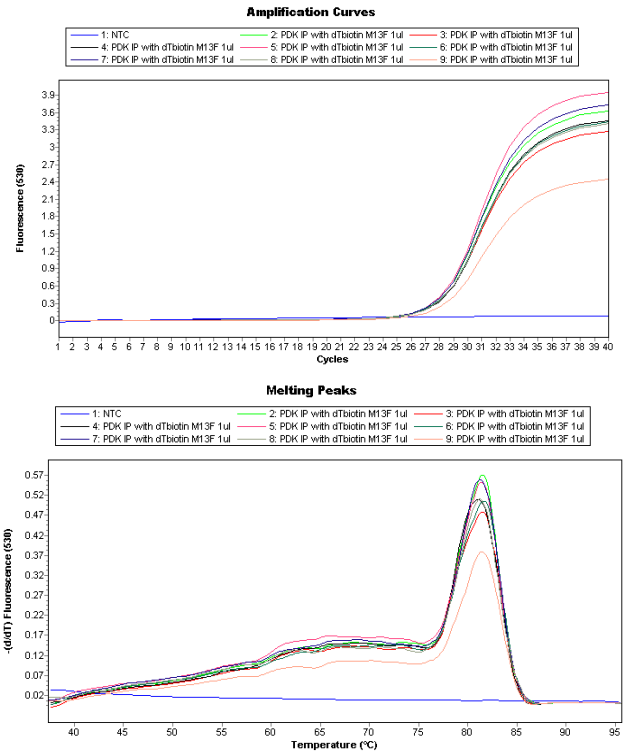

Fig. 3. Large-scale amplification of enriched aptamer library for affinity assessment. Eight identical samples were PCR-amplified using biotinylated primers to facilitate magnetic capture on streptavidin-magnetic beads. Also included was a non-template control (blue) which did not produce any signal. Ref: [Notebook NC 6-119]

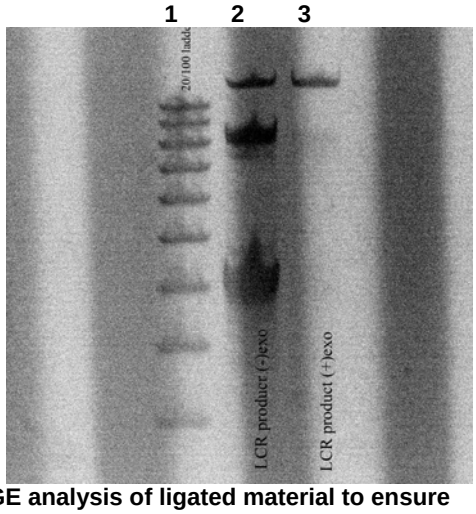

Fig. 4. PAGE analysis of ligated material to ensure circularization with biotinlyated aptamer library. Following ligation and exonuclease treatment, the single band observed in lane three indicates successful circularization. This material is now being used to validate the PDK-specific nature of the enriched aptamer pool. The samples were run on a 10% detnaturing gel. Lane 1, MW ladder; lane 2, ligated material, exonuclease (-); lane 3, ligated material, exonuclease (+). Ref: [Notebook NC 7-35]

Discussion

It was reported in the previous progress report that transitioning into the final phase of this project was imminent. In order to ensure an absolutely accurate characterization of the enriched aptamer library, the experiment shown in Fig. 5 of the August progress report "PCR amplification of Comma-D PDK and Comma-D parental immunoprecipitated material" was repeated, and, as shown in Fig. 1 in this report, was very successful.

Next, large-scale amplification of the enriched aptamer library was performed (Fig. 2) using biotinylated primers. This will facilitate capture of biotin-labeled aptamers with streptavidin-coupled magnetic beads in the final experiments associated with this project, as described below.

In order to further ensure reproducibility and reliability of the enriched pool of aptamers, three samples of immunoprecipitated (IP) material from Comma-D PDK cells and three samples of IP material from Comma-D parental cells were amplified by real-time PCR (Fig. 3). It is clear from the amplification profile that the Comma-D PDK samples amplified earlier than those from the parental samples, thereby verifying that an enriched pool of PDK-specific aptamers has indeed been generated.

The PCR amplified material was then circularized using the ligase chain reaction (LCR). As shown in Fig. 4, the reaction itself was robust and the subsequent exonuclease treatment to remove all but the circularized material was very successful. The circularized library was then incubated with streptavidin-magnetic beads for 30min, after which the beads were sequestered with a magnet. The supernatant was removed from the tube and was examined spectrophotometrically to ensure complete capture on the streptavidin matrix. The majority of the biotinylated material was captured by the beads (data not shown).

The above-referenced streptavidin beads with the library attached have been exposed to a whole-cell lysate of Comma-D cells transfected with PDK, and run in parallel with a lysate from the parental Comma-D cells. Additionally, streptavidin beads without library were run in an identical fashion as a sham control. After lysate incubation and washing, the beads were heat-eluted in an SDS running buffer and dot-blotted onto supported nitrocellulose using a vacuum manifold. The filter will be incubated with the anti-FLAG antibody (M2) and will be developed with an HRP-conjugated, antimouse Ig secondary antibody using chemiluminescence. These results are pending completion this week and will be forward as soon as they become available.