

Notes on Oyster Nanostring Codeset Selection

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Lessons Learned From Round 1

- Genomic DNA needs to be cut with a frequent cutting enzyme in addition to HpaII/MspI to target fragment size <1000nt for efficient hybridization – **for next round cut with *AluI* *in silico* before designing probes to pick CCGG sites on fragments 100 – 750bp**
- Some probes still show counts after MspI digestion. This could be due to cross-hyb at another sequence, a SNP in the CCGG cut site or outer cytosine methylation at CCGG – **only proactive step to take here is to ensure specificity of probes against the known sequence databases (EST and BAC).**

Summary of design for Round 2

- Rich performed first round of *in silico* probe design using EST and BAC databases. In summary:
 - sequences were *in silico* digested targeting 100 – 750nt *AluI* fragments
 - fragments were filtered to only contain a single MspI site located at least 50nt from both the 5' and 3' ends of the fragment to ensure that the probes have full targets
 - 100nt target sequences were constructed centered around the MspI site
 - A modified version of the probe design pipeline was run and regions that passed were forwarded to Roberts Lab
- Roberts Lab was asked to narrow down the targets. We narrowed the 1521 'transcriptomic seq' probes and 672 'genomic seq' probes to about 80. The 80 probes were selected based on:
 - ESTs:
 - must be annotated
 - cover a range of predicted methylation status and biological functions
 - Genomic targets:
 - selected ALL probes on annotated genes (safest)
 - selected 2 BAC clones (with lots of protein hits – most informative for us)
- Rich checked these 80 targets for uniqueness and sent Roberts Lab a table with # of blast hits. Roberts lab selected final probes. Rationale for selection:
 - we selected the top 8 that worked well from the first code set
 - new probes were selected if only 1 hit from the blast table
 - BAC probes that had no hits to other BACs (we went back to the original 672 genomic probes and blasted them to find these).