Protocol S1: Microarray Probe Recovery by "Scratching"

Supplement to Urisman et al., *PLOS Pathogens* 2006 "Identification of a Novel Gammaretrovirus in Prostate Tumors of Patients Homozygous for R462Q *RNASEL* Variant."
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This protocol describes the recovery of hybridized material from spotted oligonucleotide glass microarrays. In our laboratory, we typically recover material that has been amplified using the Round A/B/C Random Amplification Protocol (see Wang et al., *PLOS* 2003). Thus DNA molecules hybridized to the microarray have defined sequences at each end (Primer B) that can be used to further amplify the material following recovery from the microarray.

Protocol

1. Manually spot 70mers of interest from the original hybridization onto blank microarray slides (poly-lysine coated) using a P2 pipette tip. The locations of each 70mer can be identified by physically marking the backside of the slide with a diamond pen. Post-process arrays according to the standard protocol.
2. Perform the hybridization and scan the array as usual.
3. For each spot of interest, carefully "scratch" the spot with a new sterile syringe needle by dragging it across the spot area 5 to 10 times.
4. Dip the probe tip directly into a PCR tube filled with 100 ul of PCR mix (see below) to seed the reaction.
5. Amplify recovered material using 30-40 cycles of PCR with standard Round B conditions.

<u>PCR Mix</u>

50 mM $MgCl_2$	4.0ul
10X PCR Buffer	10.0ul
25 mM dNTP	1.0ul
Primer B (100pmol/ul)	1.0ul
Taq Polymerase	1.0ul
Water	83.0ul

6. Clone the amplified material into a vector such as pCR2.1 TOPO-TA (Invitrogen).
9. Miniprep and sequence individual plasmids.

Primer B:
5'-GTTTCCCAGTCACGATC