PCR and IVT protocol for Luminex detection 08/09/06

PCR:
Use Primers ASC11 and ASC100 for PCR, stocks are at 100uM.

Forward:
ASC11- 5'-gagggcctatttcccatgat

Reverse:
ASC100- 5'GTAATACGACTCACTATAGGGC**CTCTTCGGAGATCAGCTTC**

The forward primer is in the U6 promotor (upstream of the hairpin)
The reverse primer is downstream of the barcode

PCR cycle:
94ºC for 4 min

94ºC for 45 sec
56ºC for 45 sec
72ºC for 45 sec
Repeat last 3 steps for desired number of cycles.

72ºC for 15 min

PCR: Use Invitrogen Platinum taq
(We sometimes get contamination so you may want to consider setting up the reaction in the hood or use RNA and DNA-free water.)

1.5ul MgCl2
5ul buffer
1.5 10mM dNTP
1ul of 10mM R/F primer mix
1ul taq
5ul DNA
34.5ul H20
0.5ul of DMSO

Purify PCR using Zymo DNA Clean & Concentrator 5 purification kit and elute in 10 ul water. You can also use Qiagen PCR purification kit instead but more PCR product may be needed as template for the IVT reaction

Do 25 to 28 cycles of PCR. If IVT reaction does not work then can increase the cycle number to 33. (I run parallel samples for 35 to 38 cycles to check gel and make sure this worked and that I have no contamination in the water sample.)

IVT: Use Ambion Megascript Short kit -Cat # 1354

2uL ATP
2uLGTP
1.5uL CTP
1.5uL UTP
1.75uL bioCTP
1.75uL bioUTP
2uL enzyme mix
2uL buffer
3.5uL water
2uL PCR product.

Set this reaction up at room temperature.

6 hours at 37ºC then 4ºC hold.
(Original protocol called for double the amount of bioCTP and bioUTP but we have found that it is not necessary. Bionucleotides purchased through Enzo Life sciences. Bio-16-UTP cat # 42814B and Bio-11-CTP cat # 42818B)

Clean up using Qiagen RNeasy kit and measure A260 to determine concentration.

Use 100ng of RNA to anneal to Luminex Beads.