Hybrid Capture protocol 3

Post-hyb PCR for array and solution capture eluates

This protocol is for post-elution amplification of captured DNA from an array or solution capture (i.e. following on from Hybrid Capture Protocol 2a or 2b).

PCR primers:
PE.1 = 5′ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T 3′
PE.2 = 5′ CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATC*T 3′

* indicates phosphorothioate. Both primers are HPLC purified.

1. Prepare the PCR master mix for 1 sample:

10 x PCR Buffer	5 µl
25 mM $MgCl_2$	4 µl
2.5 mM dNTPs	4 µl
10 µM PE.1	2.5 µl
10 µM PE.2	2.5 µl
Platinum®*Pfx* DNA Polymerase (Invitrogen)	0.4 µl
Sample (from capture)	31.6 µl

The total volume of the final reaction should be 50 µl.

2. Vortex briefly and spin down.

3. Run on thermocycler with the following program:

94 °C for 5 min

94 °C for 15 sec
58 °C for 30 sec x 18 cycles
72 °C for 30 sec

72 °C for 5 min
4 °C indefinitely

4. Transfer PCR product into a 1.5ml Lo-Bind tube. Run the sample on an Agilent DNA 1000 chip on a Bioanalyzer 2100. If PCR was successful, proceed to the SPRI clean up:

SPRI bead cleanup

Allow SPRI beads to come to room temperature for at least 30 minutes. Reagents need to be mixed well prior to use and should appear homogeneous and consistent in colour.

1. Take 90 µl of SPRI beads and add them to the 50 µl of PCR sample in a 1.5 ml Lo-Bind tube.

2. Vortex and hold at room temperature for 5 minutes.

3. Place tube in the magnetic rack and leave for 5 minutes or until sample is clear.

4. Carefully remove the clear solution from the tubes and discard.

5. Dispense 500 µl of 70 % ethanol into each tube while in the magnetic rack taking care not to disturb the magnetic beads. Aspirate and discard ethanol.

6. Repeat the ethanol wash once again. Total of two washes.

7. Dry the samples on a heat block (keep the lid of the tube open) at 37 °C for 5 – 10 minutes or until the residual ethanol has evaporated.

8. Add 50 µl of molecular biology grade water, vortex and incubate at room temperature (20 °C) for 2 minutes.

9. Place tubes into the magnetic rack and leave for 2-3 minutes or until sample is clear.

10. Carefully remove the water and retain in a new 1.5 ml Lo-Bind tube.

11. Repeat step 9 -12 once more, retaining the water in the same 1.5 ml Lo-Bind tube. Total volume of elute should be 100 µl.

12. Put the tube into the magnetic tool for 10 min.

13. Transfer the sample to a new 1.5 ml Lo-Bind tube leaving behind any precipitated beads.

After SPRI clean up, quantify by qPCR [1] and sequence.

References

1. Quail M.A., Swerdlow H. & Turner D.J. Improved protocols for the Illumina genome analyzer sequencing system. *Curr Protoc Hum Genet* **Chapter 18**, Unit 18 12 (2009).