ExoSeq: Protocols - Exon Sequencing

Overview:
PCR products provided by the PCR team are sequenced with specific primers in both directions to produce double-stranded sequence. The throughput of exon sequencing is over 1 million reads per month.

Method:
Dilution and re-arraying of primers:

1. Sense and antisense primers for sequencing are received in 96-well plates at a concentration of 28 ng/µl. Dispense 5 µl of sterile double distilled water (DDW) into all of the wells of a PCR plate (green for sense, red for antisense). Using the EP3 pipetting robot (details of robot method available on request), dispense 5 µl of primer into each quadrant of 384-well Eppendorf plate to give a final concentration of 15 ng/µl.

Dilution of PCR product:

1. Add 15 µl DDW to each well of the PCR plate using a microdrop.

Sequencing:

1. Make up the sequencing mix. One bottle of sequencing mix contains:
    - BigDye (v3.1) 10.0 ml
    - Sanger BigDye reaction buffer (v2) 112.5 ml
    - ddH_2O 37.5 ml
    - dGTP BigDye v3.0 3.2 ml
2. Add the reagents for the sequencing reaction into a new plate using the PE Minitrak5 robot. Each 5 µl reaction contains 2 µl sequencing mix, 2 µl diluted primer, 1 µl diluted PCR product. Use blue plates for the sense primers and yellow for the antisense primers.
3. Heat seal the plates containing the sequencing reaction, and centrifuge briefly to bring the contents of each well to the bottom of the well. Place on the MJ Thermocyclers.
4. Cycling conditions are as follows:
    - 96℃ for 30 seconds

    45 cycles of:
    - 92℃ for 8 seconds
    - 50℃ for 8 seconds
    - 60℃ for 2 minutes

    Keep reactions at 10℃ until ready to proceed with the next precipitation step.
5. Add 30 µl of standard sequencing precipitation mix (770 ml Ethanol, 16 ml 3 M sodium acetate, 188 ml DDW) to each well using a Multidrop. Centrifuge at 4,000 rpm at 4℃ for 30 minutes. It is not necessary to pre-chill the centrifuge. Do not exceed the centrifugation time. Spin upside down on an absorbent pad for 1 minute at 400 rpm to remove all supernatant.
6. Add 30 µl of 80% ethanol to each 384 plate using a Multidrop. Centrifuge at 4000 rpm /4℃ for 5 minutes. Spin upside down on an absorbent pad for 1 minute at 400 rpm, to remove all supernatant. Allow the plate to dry thoroughly before loading on the sequencing machines.