ExoSeq: Protocols – Standard ExoSeq DNA Panel Preparation

Overview:

The standard ExoSeq DNA Panel consists of 48 individuals from the CEPH families supplied by Coriell Cell Repositories. DNA samples are diluted to 1 ng/µl and pre-aliquoted into 384-well plates for PCR in groups of 48. Details of the DNA Panel can be found here: http://www.sanger.ac.uk/resources/downloads/human/exoseq.html.

Methods:

Sample arrival:

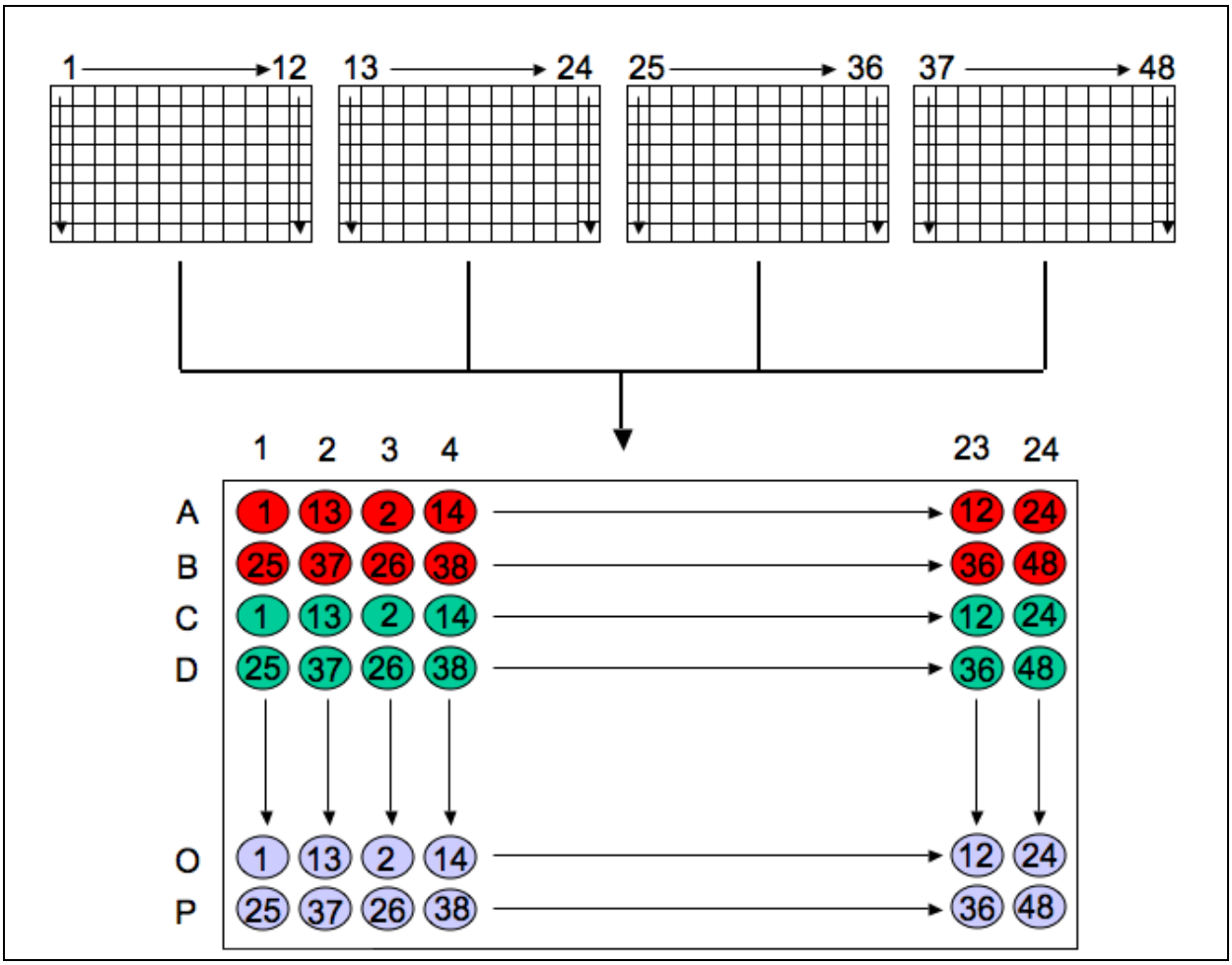
1. On arrival of a new batch of CEPH DNA, enter the lot number, date extracted and supplied concentration into a new worksheet on the CEPH DNA tracking spreadsheet. Use the spreadsheet to calculate the amount of DNA and T0.1E needed for 50 ml of a 1 ng/µl dilution for each sample.

Preparation of the diluted stocks:

1. Print barcodes for each of the new 50 ml dilutions required.
2. Make 3 separate 50 ml dilutions of 1 ng/µl each sample in 50 ml falcon tubes using the volumes as calculated from the spreadsheet. Use the pump dispenser to add the T0.1E followed by a Gilson pipette (using a barrier tip) to add the DNA. Vortex gently to mix.
3. Store the diluted samples at 4°C until needed.

Preparation of the deep-well working dilution boxes:

1. Label 4 deep-well boxes with CEPH DNA Box 1 A, B, C or D. Scan the 12 barcodes on the 50 ml dilution falcon tubes for samples 1 - 12 to produce bar codes for CEPH DNA Box 1.
2. Pour the contents of the first falcon tube for sample 1 into a sterile trough.
3. Use a multichannel pipette with barrier tips to add 1500 µl sample 1 into each well of the first column of each of the 4 deep-well boxes.
4. Repeat steps 2 and 3 for samples 2 - 12 putting 1500 µl of DNA into each well of columns 2 - 12 respectively.
5. Seal the boxes and store at 4°C until needed.
6. Label 4 deep-well boxes with CEPH DNA Box 2 A, B, C or D. Scan the 12 barcodes on the 50 ml dilution falcon tubes for samples 13 - 24 to produce bar codes for CEPH DNA Box 2.
7. Pour the contents of the first falcon tube for sample 13 into a sterile trough.
8. Repeats steps 3 - 5 for samples 13 - 24.
9. Label 4 deep-well boxes with CEPH DNA Box 3 A, B, C or D. Scan the 12 barcodes on the 50 ml dilution falcon tubes for samples 25 - 36 to produce bar codes for CEPH DNA Box 3.
10. Pour the contents of the first falcon tube for sample 25 into a sterile trough.
11. Repeats steps 3 - 5 for samples 25 - 36.
12. Label 4 deep-well boxes with CEPH DNA Box 4 A, B, C or D. Scan the 12 barcodes on the 50 ml dilution falcon tubes for samples 37 - 48 to produce bar codes for CEPH DNA Box 4.
13. Pour the contents of the first falcon tube for sample 37 into a sterile trough.
14. Repeats steps 3 - 5 for samples 37 - 48.
15. Group together all the CEPH DNA Box As to form the first set of working dilution boxes of 48 DNA samples, then the Bs to form the second set etc.



96-well and 384-well DNA plate layouts. The four 96 deep-well stock dilution plates (shown at the top of the figure) are combined to produce one 384-well DNA plate ready for PCR (shown at the bottom of the figure). The numbers in the circles representing the wells of the 384 well plate refer to the DNA sample in that well. The coloured rows group together the wells which are amplified by an individual STS in PCR.

Creation of DNA Plates for PCR:

1. Select the appropriate method on the Beckmann FX robot . Place the 4 deep-well boxes containing the 48 DNA samples, 2 sets of 5 Eppendorf 384-well white-skirted PCR plates and tips on the flat bed of the robot as directed by the layout diagram displayed on the robot PC.
2. Aliquot 7.5 µl of each DNA sample into each of 4 wells of the 384-well plate in each of the 4 quadrants of the plate. Further details of the method used by the robot are available on request.
3. When the robot has finished aliquoting the DNA samples, heat seal the PCR plates, spin briefly in a centrifuge and store at -20℃ until required.