ExoSeq: Protocols - Standard ExoSeq PCR

Overview:

For standard ExoSeq, amplification is routinely performed on 48 DNA samples with 8 STSs in a single experiment. This ensures that individual primers amplify all of the DNA samples under the same conditions thus reducing plate-to-plate variability. The plate layout showing the positions of DNA samples and primers in the 384-well PCR plate is shown in the figure at the end of the protocol. Additional formats are available if needed (96 STS x 4 DNA samples; 1 STS x 384 DNA samples). Current PCR throughput is 30 boxes of 96 STSs across 48 DNA samples per week. This equates to 2,880 STSs and 138, 240 PCR products to be sequenced per week. Each stage of the process is extensively tracked using barcoding supported by an underlying Oracle database.

Method:

PCR:

- 1. DNA is pre-aliquoted at a concentration of 1 ng/μl, 7.5 μl per well, into 384-well plates and stored frozen until required (for more details see the ExoSeq DNA Panel Preparation Protocol). Remove the required number of DNA plates from the freezer, thaw, mix gently and spin briefly in a plate centrifuge to bring the contents of the plates to the bottom of the wells. Twelve DNA plates are needed for each box of 96 STSs.
- 2. The mixed primers are also pre-aliquoted into 96-well plates at a concentration of 10 ng/μl, 180 μl per well, and stored at 4°C until required (for more details see ExoSeq Pre-Screening Protocol). Remove the required primer plates, mix gently and spin briefly in a plate centrifuge to bring the contents of each plate to the bottom of the wells.
- 3. Make a premix of 8,850 μl 10X Buffer (as supplied with the enzyme), 8,850 μl 1 mM dNTPs, 531 μl HotStart Taq, 8,400 μl DDW.
- 4. Add 270 μl of premix to each well of the mixed primer plate using a multi-channel pipette.
- 5. Use an 8-channel Tecan robot to add 7.5 μl of this mix to the appropriate wells of the DNA plates (details of the robot method available on request).
- 6. Heat seal each plate and spin briefly in a centrifuge to bring the contents of the plates to the bottom of the wells. Place the plates on the MJ Thermocyclers.
- 7. PCR cycling conditions are as follows for the standard Exohot60 reaction on an MJ thermocycler:
 - 95°C for 15 minutes

Followed by 39 cycles of:

- 95°C for 30 seconds
- 60 °C for 30 seconds
- 72°C for 30 seconds

Followed by:

- 95°C for 30seconds
- 60°C for 30 seconds
- 72°C for 10 minutes
- 8. After PCR, remove 2 μ l of product from the first column of each PCR plate to a new plate, add 10 μ l of cresol red/sucrose solution to each well and run the products on a 2.5% agarose gel containing ethidium bromide to check that amplification has been successful (it is impractical to check all of the PCR products).
- 9. Visualise the results after electrophoresis, photograph and record the results in the ExoSeq database.

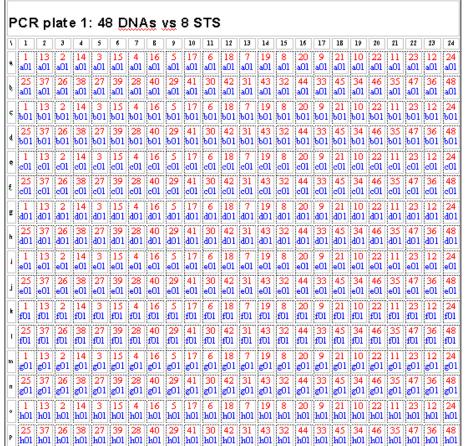
- 10. Make a master mix for PCR product clean-up as follows: (For 12 PCR plates) 3,168μl Shrimp Alkaline Phosphatase (1unit/ul), 3,168 μl 10X reaction buffer (200 mM Tris HCl pH8.0, 10 mM MgCl2), 3,168 μl DDW, 316.8 μl exonuclease (10 units/μl). Add 2 μl of clean-up master mix to each well of each PCR plate using a multichannel pipette. Heat seal the plates and centrifuge briefly. Place the plates onto the MJ Thermocycler.
- 11. Clean-up conditions are as follows: incubate at 37 °C for 30 minutes followed by 80 °C for 15 minutes. Centrifuge the plates briefly and freeze at -20 °C until sequenced.
- 12. Use an 8-channel Tecan robot (details of robot method available on request) to re-array and dilute the sense and antisense primers for sequencing. The volume and concentration of primers depends on the number of sequencing reactions but the plate layout is shown below:

1						
2						
2						
4						
5						
5 6 7						
7						
8						

Plate layout for sense and antisense primers for sequencing – Stock sense and antisense primer plate layout

1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	S	3	3
4	4	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	-5	5	5	5
6	6	6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8	8	8

Plate layout for sense and antisense primers for sequencing – Diluted rearrayed sense and antisense primer



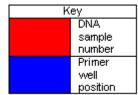


Plate layout of PCR plate 1 for amplification of 48 DNA samples with 8 STSs. The 48 DNA samples each appear 8 times. PCR plate 1 contains primers from column 1 of the mixed primers plate, PCR plate 2 contains primers from column 2 etc.