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℃ 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℃ for 15 minutes

    Followed by 39 cycles of:
    - 95℃ for 30 seconds
    - 60℃ for 30 seconds
    - 72℃ for 30 seconds

    Followed by:
    - 95℃ for 30seconds
    - 60℃ for 30 seconds
    - 72℃ for 10 minutes
8. After PCR, remove 2 µl of product from the first column of each PCR plate to a new plate, add 10 µl 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											
3											
4											
5											
6											
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	3	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 re-arrayed sense and antisense primer

PCR plate 1: 48 DNAs vs 8 STS

\	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
a	1 a01	13 a01	2 a01	14 a01	3 a01	15 a01	4 a01	16 a01	5 a01	17 a01	6 a01	18 a01	7 a01	19 a01	8 a01	20 a01	9 a01	21 a01	10 a01	22 a01	11 a01	23 a01	12 a01	24 a01
b	25 a01	37 a01	26 a01	38 a01	27 a01	39 a01	28 a01	40 a01	29 a01	41 a01	30 a01	42 a01	31 a01	43 a01	32 a01	44 a01	33 a01	45 a01	34 a01	46 a01	35 a01	47 a01	36 a01	48 a01
c	1 b01	13 b01	2 b01	14 b01	3 b01	15 b01	4 b01	16 b01	5 b01	17 b01	6 b01	18 b01	7 b01	19 b01	8 b01	20 b01	9 b01	21 b01	10 b01	22 b01	11 b01	23 b01	12 b01	24 b01
d	25 b01	37 b01	26 b01	38 b01	27 b01	39 b01	28 b01	40 b01	29 b01	41 b01	30 b01	42 b01	31 b01	43 b01	32 b01	44 b01	33 b01	45 b01	34 b01	46 b01	35 b01	47 b01	36 b01	48 b01
e	1 c01	13 c01	2 c01	14 c01	3 c01	15 c01	4 c01	16 c01	5 c01	17 c01	6 c01	18 c01	7 c01	19 c01	8 c01	20 c01	9 c01	21 c01	10 c01	22 c01	11 c01	23 c01	12 c01	24 c01
f	25 c01	37 c01	26 c01	38 c01	27 c01	39 c01	28 c01	40 c01	29 c01	41 c01	30 c01	42 c01	31 c01	43 c01	32 c01	44 c01	33 c01	45 c01	34 c01	46 c01	35 c01	47 c01	36 c01	48 c01
g	1 d01	13 d01	2 d01	14 d01	3 d01	15 d01	4 d01	16 d01	5 d01	17 d01	6 d01	18 d01	7 d01	19 d01	8 d01	20 d01	9 d01	21 d01	10 d01	22 d01	11 d01	23 d01	12 d01	24 d01
h	25 d01	37 d01	26 d01	38 d01	27 d01	39 d01	28 d01	40 d01	29 d01	41 d01	30 d01	42 d01	31 d01	43 d01	32 d01	44 d01	33 d01	45 d01	34 d01	46 d01	35 d01	47 d01	36 d01	48 d01
i	1 e01	13 e01	2 e01	14 e01	3 e01	15 e01	4 e01	16 e01	5 e01	17 e01	6 e01	18 e01	7 e01	19 e01	8 e01	20 e01	9 e01	21 e01	10 e01	22 e01	11 e01	23 e01	12 e01	24 e01
j	25 e01	37 e01	26 e01	38 e01	27 e01	39 e01	28 e01	40 e01	29 e01	41 e01	30 e01	42 e01	31 e01	43 e01	32 e01	44 e01	33 e01	45 e01	34 e01	46 e01	35 e01	47 e01	36 e01	48 e01
k	1 f01	13 f01	2 f01	14 f01	3 f01	15 f01	4 f01	16 f01	5 f01	17 f01	6 f01	18 f01	7 f01	19 f01	8 f01	20 f01	9 f01	21 f01	10 f01	22 f01	11 f01	23 f01	12 f01	24 f01
l	25 f01	37 f01	26 f01	38 f01	27 f01	39 f01	28 f01	40 f01	29 f01	41 f01	30 f01	42 f01	31 f01	43 f01	32 f01	44 f01	33 f01	45 f01	34 f01	46 f01	35 f01	47 f01	36 f01	48 f01
m	1 g01	13 g01	2 g01	14 g01	3 g01	15 g01	4 g01	16 g01	5 g01	17 g01	6 g01	18 g01	7 g01	19 g01	8 g01	20 g01	9 g01	21 g01	10 g01	22 g01	11 g01	23 g01	12 g01	24 g01
n	25 g01	37 g01	26 g01	38 g01	27 g01	39 g01	28 g01	40 g01	29 g01	41 g01	30 g01	42 g01	31 g01	43 g01	32 g01	44 g01	33 g01	45 g01	34 g01	46 g01	35 g01	47 g01	36 g01	48 g01
o	1 h01	13 h01	2 h01	14 h01	3 h01	15 h01	4 h01	16 h01	5 h01	17 h01	6 h01	18 h01	7 h01	19 h01	8 h01	20 h01	9 h01	21 h01	10 h01	22 h01	11 h01	23 h01	12 h01	24 h01
p	25 h01	37 h01	26 h01	38 h01	27 h01	39 h01	28 h01	40 h01	29 h01	41 h01	30 h01	42 h01	31 h01	43 h01	32 h01	44 h01	33 h01	45 h01	34 h01	46 h01	35 h01	47 h01	36 h01	48 h01

Key	
(red)	DNA sample number
(blue)	Primer well position

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.