ExoSeq: Protocols - Pre-Screening

Overview:

Pre-screening is used to determine the optimum amplification conditions of the primers prior to use. An extensive tracking system has been developed which includes a gel scoring system and the ability to cherry pick and re-array primers automatically. Primers are ordered in a 96-well format and are tested initially at 60℃ annealing temperature with a positive control of genomic DNA and a negative control of T0.1E. Primers which fail to amplify a single band at 60℃ annealing temperature are re-arrayed into a new plate and pre-screened again at 60℃ with the addition of 7-deaza dGTP. Successful primers are grouped according to amplification condition and re-arrayed into new 96-well plates. 95% of primers pass pre-screen under one of these two conditions. Primers that fail to amplify a single band at this stage are re-designed.

Methods:

Primer dilution:

Mixed stock primers (containing the sense and antisense primer) are received at a concentration of 100 ng/µl and require dilution to the working concentration of 10 ng/µl (see illustration below).

1. Record the primer plate EP number, range of primer stSG Sanger identification numbers on the lid and the bottom of a round-bottomed microtitre plate.
2. Dispense 90 µl T0.1E into each well of the microtitre plate then add 10 µl of the stock mixed primers using filter tips.

Pre-screening:

1. Using the appropriate method on the Beckman Biomek FX (details of robot method available on request), aliquot the diluted primers into two PCR plates as follows: Transfer 3 µl from wells A1 to H1 of diluted mixed primers to columns 1 and 2 of PCR plate 1. Transfer 3 µl from wells A2 to H2 of diluted mixed primers to columns 3 and 4 of plate 1, from wells A3 to H3 to columns 5 and 6 etc. until PCR plate 1 is full. Transfer 3 µl from wells A7 to H7 of diluted mixed primers to columns 1 and 2 of PCR plate 2. Continue until primers from wells A12 to H12 are transferred into columns 11 and 12 of PCR plate 2.
2. Make a premix of sufficient volume for all reactions plus 10% to allow for dead volumes and pipetting errors. Mix (per reaction): 1.5 µl 10x buffer (supplied with the enzyme), 1.5 µl 1mM dNTPs, 0.09 µl Hotstart Taq, 8.41 µl sterile water (DDW). Add 11.5 µl of premix per well to each of the PCR plates from step 1 using a Beckman Biomek FX robot (details of robot method available on request).
3. Use a multi-channel to add 0.5 µl of genomic DNA at 10 ng/µl to columns 1, 3, 5, 7, 9, 11, of each PCR plate and 0.5 µl of T0.1E to columns 2, 4, 6, 8, 10 and 12 of each PCR plate. This gives a final volume of 15 µl for the PCR.
4. Seal the plates with a heat sealer and place on the MJ thermocycler PCR machine.
5. PCR cycling conditions are as follows for the standard Exohot60 reaction on an MJ thermocycler:
   - 90℃ for 15 minutes

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

   Followed by:
   - 95℃ for 30 seconds

- 60℃ 30 for seconds
- 72℃ for 10 minutes

6. Use the Beckman Biomek FX robot (details of robot method available on request) to add 10 µl of cresol red/sucrose solution to each well prior to loading 20 µl of each sample on a 2.5% agarose gel containing ethidium bromide. The gel loading is staggered to allow for easy reading of results.
7. Use the automatic gel scoring software to score the gel and record the results in the database.

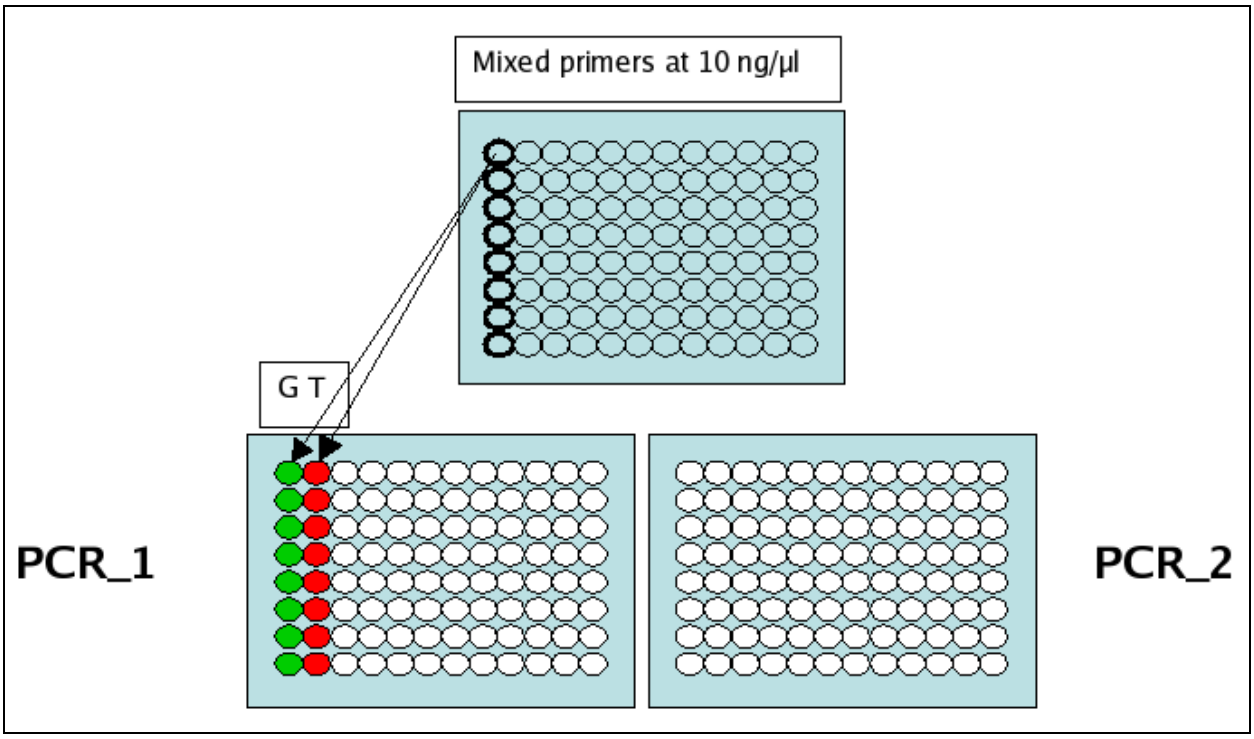

Plate layout for PCR pre-screening. G = genomic DNA, T = T0.1E