Aim

This protocol describes PCR amplification of cDNA plasmid inserts, gel electrophoresis, purification, and storage of PCR products in 384 well format. The automation group at the MPI for Molecular Genetics currently utilizes this procedure for the generation of DNA probes for the generation of DNA microarrays.

Material

- Polymerase mixture (varying suppliers, dependent on actual conditions)
- 10x Polymerase buffer (composition)
- 100 mM dATP, dCTP, dGTP, dTTP (Qbiogene, Germany; #NTACG111)
- MilliQ Water
- 384 Well PCR Plate (Eppendorf #0030 128.508)
- clone selection in Genetix (#X7001) culture plates
- Isopropanol
- primer
- PCR mixture (to be prepared in a clean bench):

Reagent	25µl reaction	400x25µl reactions
ddH2O	21.65µl	8660µl
10xPuffer	2.5µl	1000µl
dNTPs [25 mM each]	je 0.05µl	je 20µl
For23 (125pmol/µl)	0.2 µl	80µl
Rev30 (125pmol/µl)	0.2 µl	80µl
Taq [5U/µl]	0.25µl	100µl
Pfu	0.00125µl	0.5µl

Procedure

A. PCR setup:

1. Fill 25 µl of the PCR mixture to each position of the PCR plate with a multi channel pipette device or preferentially with a 384 well pipette robot (HTP-Unit, MPI-MG).
2. Inoculate each position of the PCR plate with 1µl of the freshly thawed clones from the corresponding position of the clone collection plate (Genetix #X7001) by using an adapted 384 well parallel pipeting device (HTP-Unit, MPI-MG) or alternatively by 384 well replicators (Genetix # X5050).

Note that the clone plate shall not be thawed and refrozen more than three times and used for inoculating PCRs. Prepare suitable copies of the plates in time! After use the clone plates are to be rapidly frozen by placing them on dry ice and store them at –80°C.

3. Seal the PCR plates with suitable tape (HTP-Unit, MPI-MG) spin down all liquid briefly at 3500 rpm, and transfer the inoculated plate into 384 well thermocycler (MJ research PTC 225). And start the following cycling routine using a heated lid.
4. Cycling routine:
    1. 94°C ; 3 min
    2. 94°C; 1min
    3. 54°C; 1min
    4. 72°C; 2min
    5. Go to 2; 40times/ 35times
    6. 72°C; 5min
    7. 15°C; forever
    8. end

    N.B. : the annealing temperature in step 3 has to be adjusted to the optimal annealing temperature of the primer pair used for the amplification of the clone inserts.
5. upon completion spin down the PCR plates and store the PCR products at -20°C until purification/ further use.

B. Purification of the PCR products by isopropanol (IPA) precipitation in 384 well format:

1. Transfer the PCR products from the PCR plate to a Genetix spotting plate (Cat #:X6004).
2. Add 20 µL of isopropanol to each well, pipette up and down for four times, seal the plates; then mix by gentle vortexing. This mixture is not to reach the sealing tape. **N.B.:** In case the mixture reaches sealing tape the isopropanol can interact with the glue of some branches of sealing tape. This can result in a clogging of several well impairing all subsequent steps.
3. Leave at –70°C for two hours or alternatively at –20°C overnight.
4. Place plates at room temperature for 20 min,
5. Spin 3500 rpm at least for two hours at room temperature
6. Carefully pour off IPA by placing a tissue over the plate and inverting it. (the tissue absorbs the IPA by capillary action)
7. Briefly dry pellets in speed Vac, for 20 min without heating.
8. Add 5uL of spotting solution (3X SSC; 1,5 M Betain)
9. Mix by spinning briefly 2000 rpm/20mins/4°C and leave overnight at 4°C.
10. Repeat the spin described above and store plates at –20°C.

11. Quality control the DNA concentration by running out 0.5uL from random wells on a 1% agarose gel.
12. Depending on the quid of each initial PCR this procedure yields a PCR product concentration in average clearly above 100 ng/µl. Otherwise the PCR conditions outlined in 3a have to be optimised for the gene probes to be spotted.

Version	Tracking of changes	Name	Date