

Probe preparation

PCR clone amplification for pGEM®-T Easy (Promega) Libraries.

cDNA libraries are stored in 384-well cell culture plates (Nunc Inc No.:164688) in liquid LB culture media with ampicilin (100 µg/ml). Each plate is divided in to 4 96-well PCR plates.

Each PCR reaction solution consists of:

Component	Amount 1 reaction(µL)
10X PCR Buffer	5
Mg ⁺² (25 mM)	3
SP6 primer (50 mM)	0.2
T7 primer (50 mM)	0.2
dNTP (5mM each)	0.5
CIAT Taq Polymerase	0.4
Nuclease-free Water	35.7

Make a master mix for 100 reactions per 96-well plate. Pipette 45 µl of PCR reaction solution to each well.

Add 5 ul of an over-night *E. coli* culture on LB liquid media from 384-well flat-bottom plates (Nunc No. 164688) to each well of the PCR plate with a multipipettor or with the Liquid Handling robot TECAN Genesis (<http://www.tecan.com>). Do not add *E. coli* culture before PCR reaction solution.

Keep accurate track of the relation of the sequences location in the 96-well PCR plate and the 384-well cell culture plate.

PCR program:

- 94°C X 10 `
- 94°C X 30 ``
- 57°C X 30 ``
- 72°C X 1` 30 ``

35 cycles:

- 94°C X 30 ``
- 52°C X 30 ``
- 72°C X 1` 30 ``

Final extensión: 72°C X 10 `

Check 5 ul of the PCR product of random clones (~10%) on EtBr agarose gels. There should be a single strong band for each clone, and there should be bands of different sizes from around 200bp to 800bp.

Transfer of PCR products to 384-well round-bottom plates

Ready-to-spot probes are stored in 384-well round bottom plates (Genetix, No.:X6004) at -20°C.

Prepare spotting buffer 2X:

SSC * 6x, Betaine 3M. Filtrate it through 0.2 um filter.

*SSC 1X is:	NaCl	0.15 M
	Sodium citrate	0.015 M

Fill a 384-well Genetix plate with 15 ul of 2X spotting buffer in each well.

Add 15 ul of probe-PCR product from 96-well PCR plate to 384-well Genetix plate.

Keep the same location of the sequences in the 384-well genetix plate as in the 384-well cell culture plate.