CENTRE NATIONAL DE RESSOURCES GÉNOMIQUES VÉGÉTALES

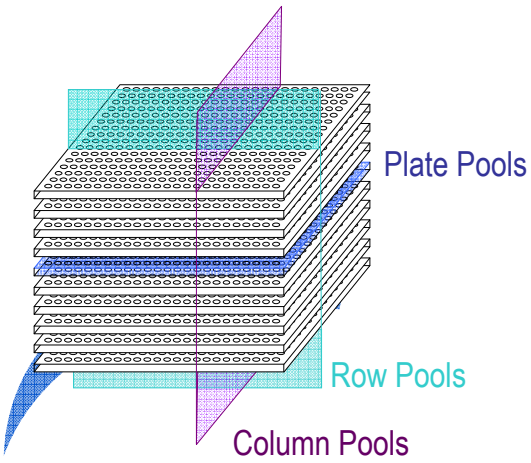
DNA Pools Production

The CNRGV proposes pooling of genomic libraries.
Pools of bacterial clones constitute a powerful tool for the screening of genomic libraries. Bacterial clones are mixed, with the aim of minimizing the number of reactions required to identify a clone containing a sequence of interest.

Procedure

Bacterial clones stored on 96-well or 384-well plates are mixed in a two- or three-dimensional matrix. The approach used depends on the size and depth of the library concerned.

Example of three-dimensional pooling:



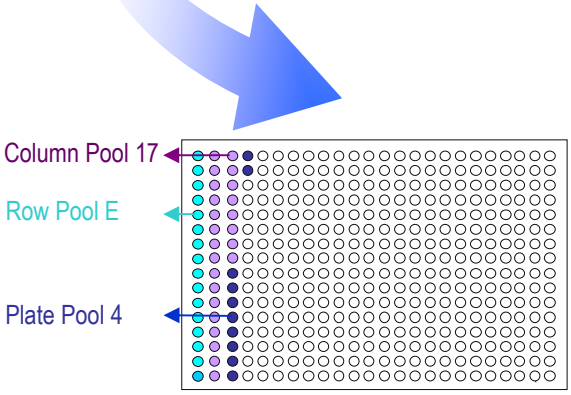
- Production of different pools

Superpool: mix of all of the clones from all the plates

Plate Pools : mix of the 384 clones on each plate

Row Pools : mix of the clones from each row for all the plates: 16 row pools

Column Pools : mix of the clones from each column for all the plates: 24 column pools



Example of a positive clone: plate 4 E17

- Pool transfer

All the plate, column and line pools are reorganized on a 384-well microtiter plate.

During screening, the co-ordinates of the clone containing the sought sequence is directly identified by crossing the column, row and plate co-ordinates.

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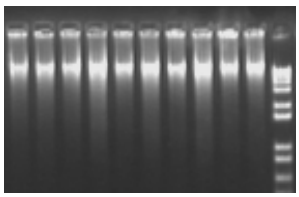
Pool amplification

The mix of clones are amplified with Phi29 DNA polymerase, thereby substantially increasing the amount of DNA available. PCR can then be used for direct screening for clones of interest.

Dean, F.B., Genome research, 2001

Example of a plasmid Phi29 amplification



Agarose gel electrphoresis of Phi29 amplified DNA pools

We send you the amplified pooled DNA on 384-well plates, so that you can carry out your own screening.

Quality control

The quality of all the pools is validated by screening referenced controls. All the mixing and amplification steps are carried out with a high-throughput automated pipette, guaranteeing traceability.

Request for services

The CNRGV has already generated pools for certain genomic libraries. A list of these pools can be obtained directly from our website.
You can also formulate your requests for the production of pools corresponding to your specific needs directly online:

http://cnrgv.toulouse.inra.fr/

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