Pooling strategy and Screening Services

CNRGV PLANT GENOMIC CENTER

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CNRGV produces efficient tools to facilitate the use of BAC libraries in various applications such as genome analysis, physical mapping, map-based cloning and sequencing projects.
We have developed efficient methods to create and screen pools (1-2-3D) of BAC libraries. We describe the construction of pools (of 1-2-3 Dimensions) for BAC libraries using large scale DNA amplification enzyme and PCR screening.
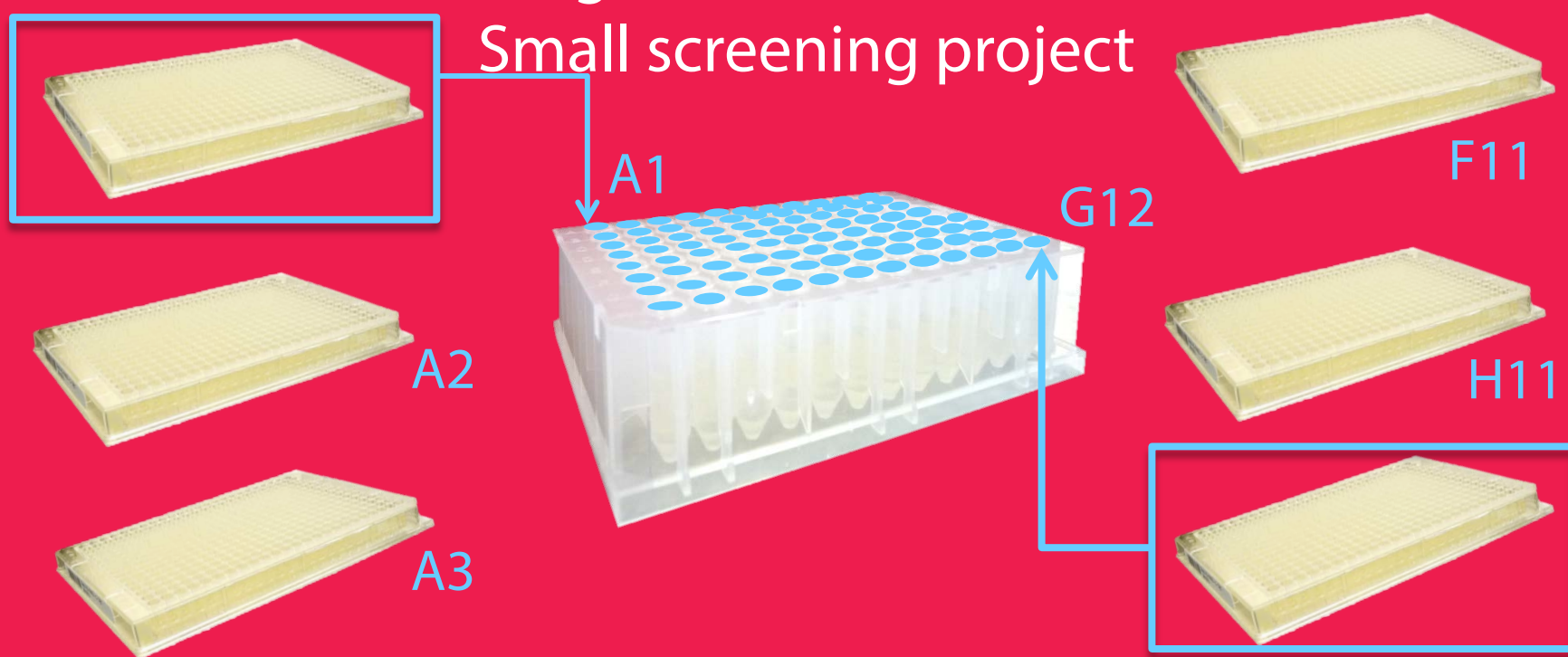
1D-2D-3D Pooling strategy
Number or Groups of plates are defined in order to:
- obtain a specific coverage per block
- mix both large and smaller inserts within all the blocks.

1D Pools

(1 Dimension)
Small genome size < 400 Mb
Small screening project

A1 G12 F11
A2 H11
A3

1. Plate Pools of each microplate (384 wells)

2. 1 PCR to screen 384 clones

3. Identification of clone coordinates on positive plate pool

= 16 row pools
+ 24 column pools

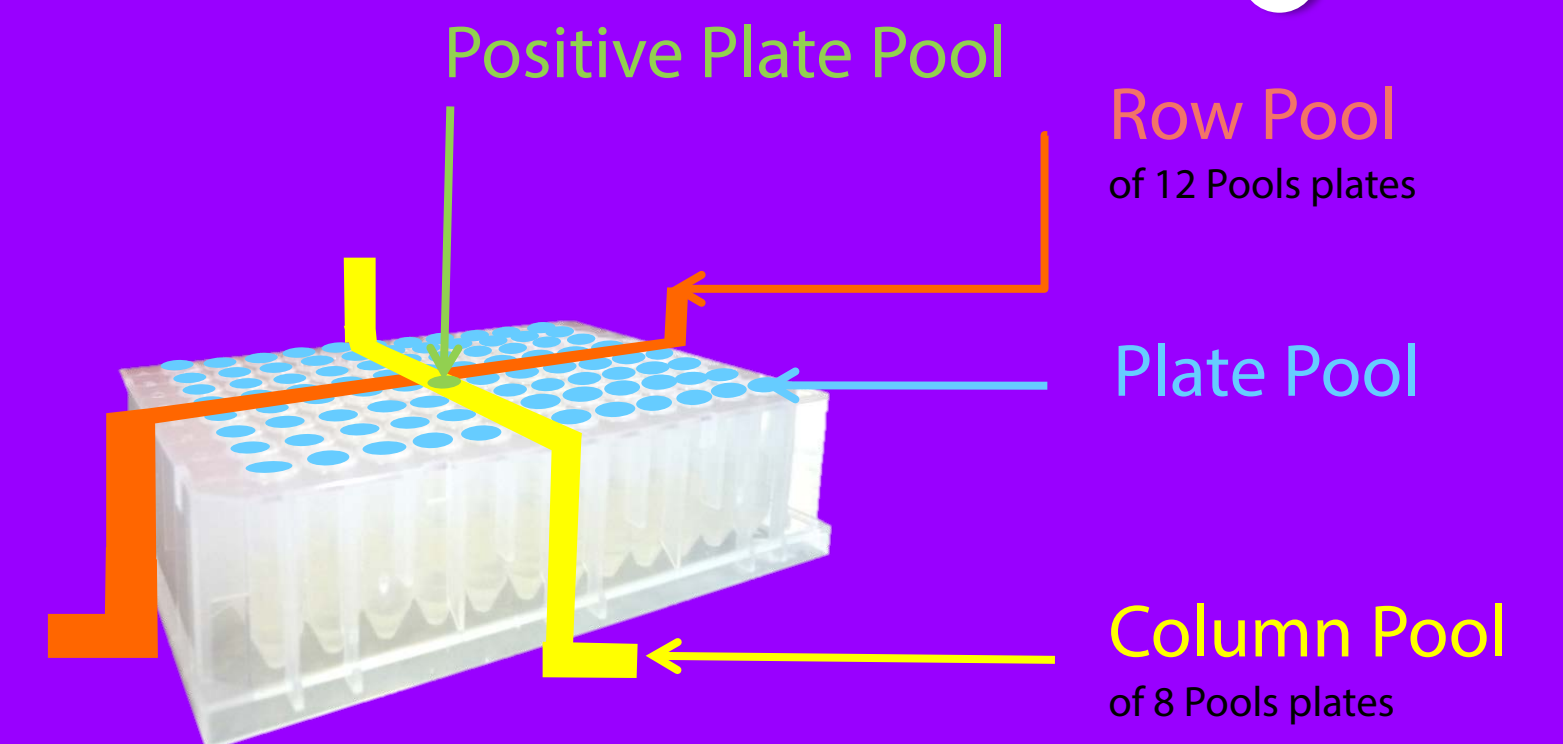= 40 PCR reactions to identify the coordinates of the positive clone on the 384 wells-plate
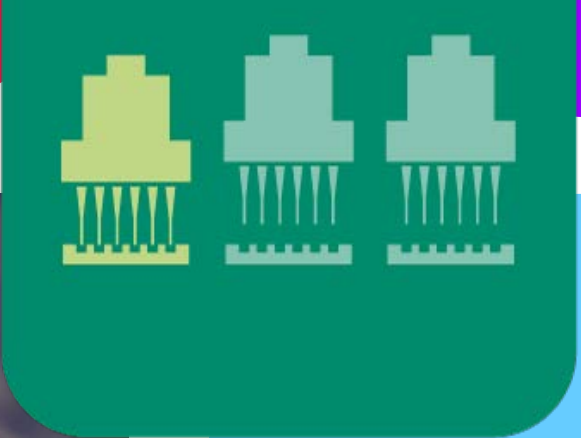
2D Pools

(2 Dimensions)
Genome size > 400 Mb

A1 G12
A2
A3

Positive Plate Pool
Row Pool of 12 Pools plates
Plate Pool
Column Pool of 8 Pools plates
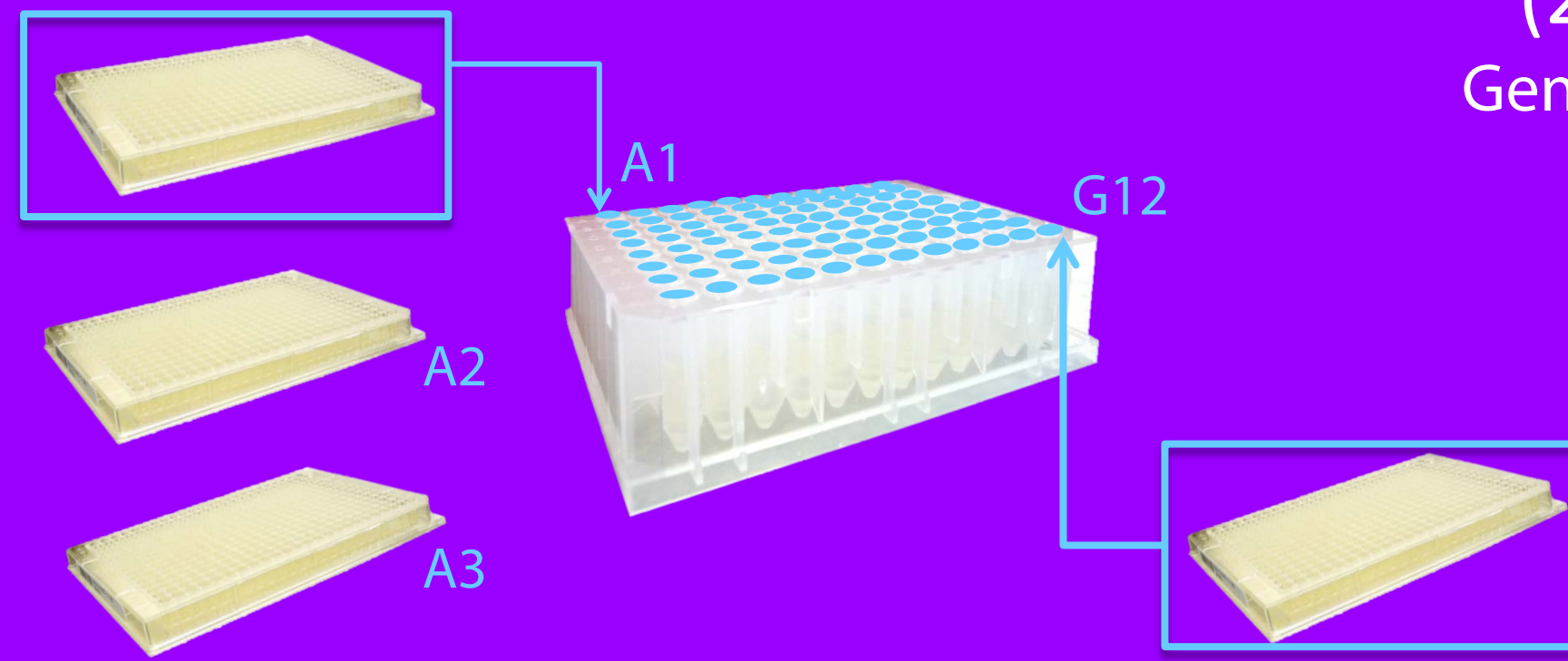
1. Plate Pools of 96 384-wells-microplates
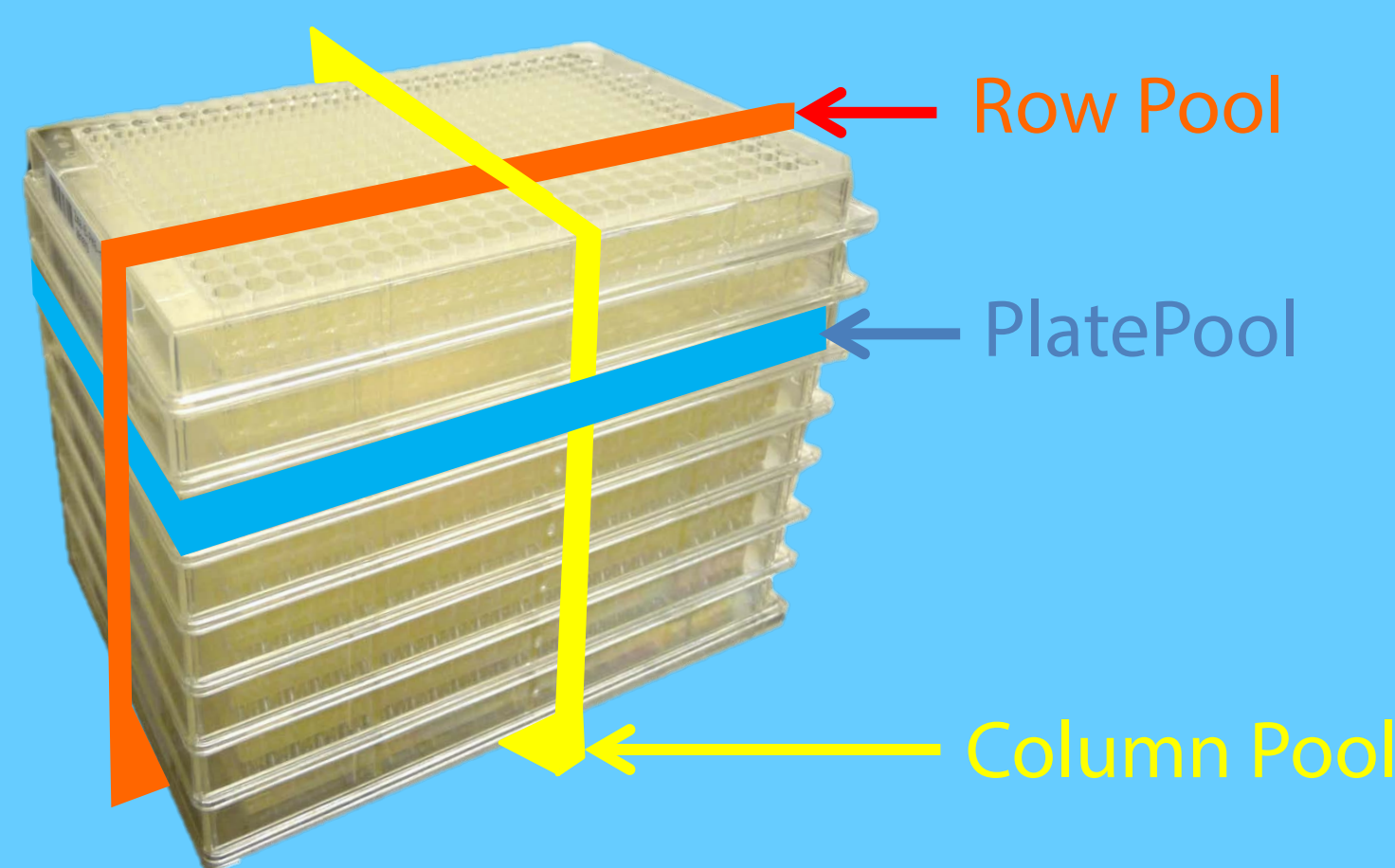
Organization of Plate Pools on 96 well plate

2. Plate Pools mixed in 2D: rows and column
=   8 row pools
+ 12 column pools

= 20 PCR reactions to identify a positive plate among 96 plates

3. Pools of each positive plate
= 16 row pools
+ 24 column pools

= 40 PCR reactions to identify the coordinates of the positive clone

3D Pools

(3 Dimensions)

Row Pool
PlatePool
Column Pool

3D organisation for 8 microplates

For each group of plates corresponding to less that 1 X genome coverage

Plate, column, line-pools and a superpool containing all the clones of the block are generated:

1 Block =   8 Plate Pools
+ 16 Row Pools
+ 24 Column Pools
+   1 SuperPool

= 49 PCR reactions to screen 3072 samples to identify the coordinates of the positive clone

The CNRGV possesses essential equipment for high throughput activities on genomic libraries. The whole process of mixing the clones and transfering the pools in 384 wells-plates is automated and electronically tracked.
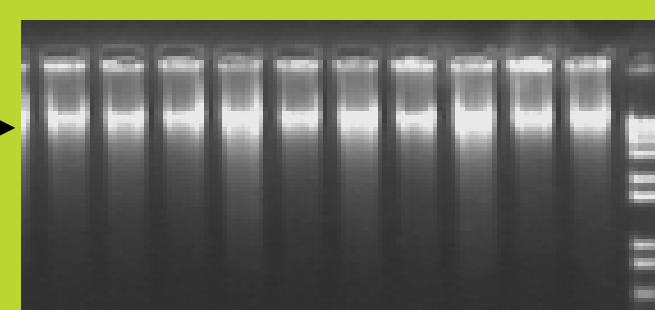
Phi29 global Pools amplification
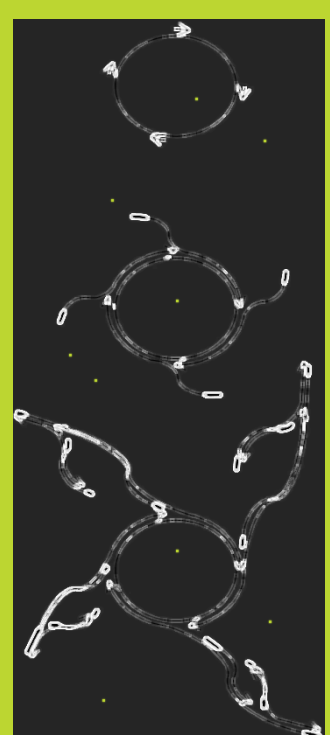
Phi29 enzyme: whole genome DNA polymerase amplifies DNA thanks to random primers, in a rolling-circle mechanism.

This step provides a high quantity of material and saves time by suppressing the long and tedious DNA extraction steps,

Dean, F.B
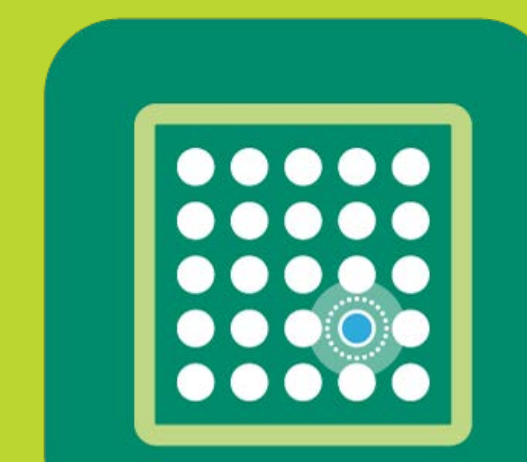Genome research, 2001

Average amplification size ~ 40kb

Agarose gel analysis for the phi29 amplified pool DNA
Amplification Yield : 1000 fold in 2 hours
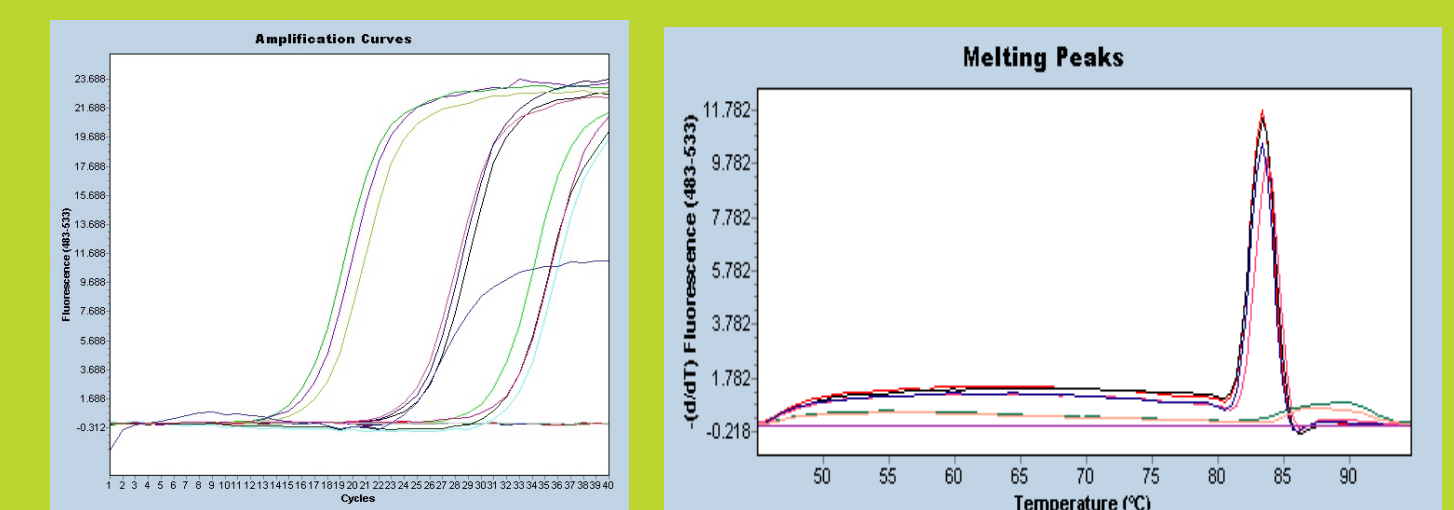~1000 screenings / Phi29 amplification
(on 1µl of each pools)

PCR

Screening DNA Pools by Real Time PCR

SCREENING SERVICES

Use Melting Curves data to discriminate the positive samples