Strategies for improving genomic region isolation using BAC libraries

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French Plant Genomic Resource Center (CNRGV) is a non-for-profit service centre dedicated to plant genomics.
The objectives of the CNRGV are to gather, to **conserve and to manage genomic** libraries but also to **provide high throughput molecular tools** to the scientific community.
The CNRGV is already in charge of more than 7 millions unique samples among more than 50 plant genomic libraries of model and crop plant.

• **Because cloning of genomes into bacterial artificial chromosome (BAC) libraries constitutes an invaluable tool for genomic analysis, CNRGV offers to construct plant BAC libraries.**

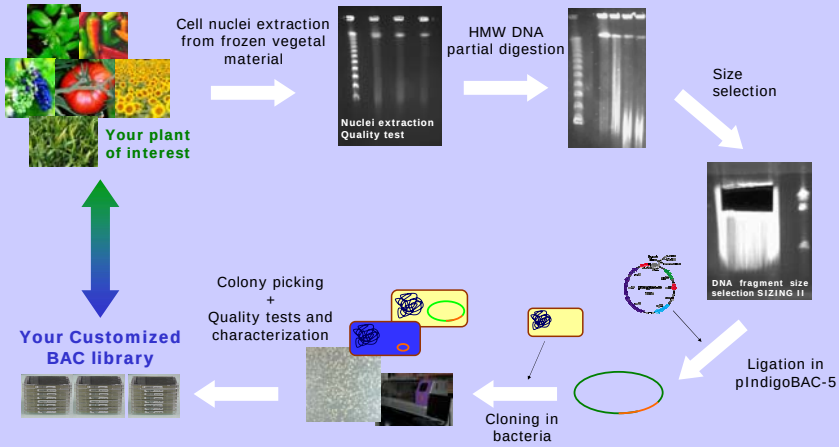
• BAC library construction can be associated to our efficient **3D-pools** production and screening methods. All this steps can be carried out at CNRGV.
This **"3D-pools screening pipeline"** has been developed and validated at CNRGV.

• Fast identification of large DNA regions of interest based on **Non Gridded BAC Libraries** method is set-up at CNRGV. It allows easier access to genic diversity.

Plant BAC library construction service

BAC library constructions carried out in 2009 :

Species	Genotype	Library name	Enzyme	mean Insert size (kb)	Clone number
Medicago truncatula	F83	Mt-B-F83	Hind III	65	55 296
Arabidopsis thaliana	Ita-0	Ath-B-Ita0	Hind III	96	15 360
Arabidopsis lyrata	09A11	Aly-B-09A7	Hind III	73	15 360
Arabidopsis lyrata	05B17-1	Aly-B-05B17	Hind III	89	15 360
Arabidopsis lyrata	05B37-3	Aly-B-05B37	Hind III	102	9 984
Arabidopsis halleri	05B8-1	Aha-B-05B8	Hind III	110	13 824
Arabidopsis halleri	L406	Aha-B-L406	Hind III	115	13 440
Arabidopsis halleri	HF11-4	Aha-B-HF11	Hind III	92	14 592
Arabidopsis halleri	PL22-138	Aha-B-PL22-138	Hind III	84	18 512
Arabidopsis halleri	Bulk III	Aha-B-III	Hind III	94	44 544
Helianthus annuus	XRQ	Han-B-XRQ	Hind III	92	162 000
Cichorium intybus	S1	Cin-B-S1	Hind III	120	81 408
Cichorium intybus	S2	Cin-B-S2	Hind III	90	89 088
Phytophtora parasitica	/	Ppa-B-I	Hind III	134	11 520
Spartina maritima	/	Sma-B-MOB	Hind III	106	44 544
Helianthus annuus	Ha412	Han-B-Ha412	Hind III	140	142 080
Passiflora edulis	Flavicarpa	Pav-B-Flav	Hind III	110	82 944
17 libraries				105	827 856



3D-pools : efficient tool for high-throughput BAC library screening

Schema of 3D-pools produced for the characterization of a quantitative resistance to Downy Mildew (Plasmopara halstedii) in cultivated Sunflower

PROMOSOL Mildew project

Laboratoire Interactions Plantes Microorganismes, UMR 441-2594 INRA/CNRS, Castanet Tolosan
UMR 1095 Amélioration et Santé des Plantes INRA/ Université Blaise Pascal, Clermont Ferrand
UMR 1065 Snaté des plantes INRA/ENITA, Bordeaux
UR 1258 CNRGV–INRA, Castanet Tolosan

BAC library construction on resistant XRQ genotype

170 000 clones
Insert size ~90 kb
5.8 X genome
HindIII digestion

POOLING

- **14 blocks of 32 plates (0.41X/block)**
- **Each block pooled according to :**

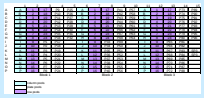
PLATE / LINE / COLUMN

For each block :
32 Plate Pools
16 Line Pools
24 Column pools
1 superpool

Pools of clones amplified by Ph29 polymerase

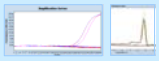
- Simple to perform
- Reliable
- Huge quantity of DNA matrix produced

14 blocks of 32 plates (0.41X/block)
14 PCR reactions + 72/positive block to screen the whole library (5.8X)



- Reduced number of PCR to perform
- Easily usable
- DNA matrix in 384well plates : convenient to send

EFFICIENT screening process
Use of real time PCR for screening



- Fast
- Traceability
- Informative : melting curves

Fast BAC clones isolation in genotypes of interest : Non Gridded BAC Libraries strategy

Your Genotype of interest

Cell nuclei extraction from vegetal material → HMW DNA partial digestion → Size selection → Ligation in pIndigoBAC-5 → Cloning in bacteria → PCR screening of BAC clones pools → Plating of positives pools → Hybridization and recovering of positives BAC clones → **BAC clones containing targeted sequences**

Method derived from :
Direct targeting and rapid isolation of BAC clones spanning a defined chromosome region. Funct Integr Genomics. 2005 Apr;5(2):97-103
Isidore E, Scherrer B, Bellec A, Budin K, Faivre-Rampant P, Waugh R, Keller B, Caboche M, Feuillet C, Chalhoub B

• Our aim is to **avoid time and cost expensive steps** of BAC clones organization in microplates in order to **access efficiently the diversity** among plant cultivars.

Various projects regarding wheat cultivars are already in progress through collaboration with INRA Clermont-Ferrand and CSIRO Canberra.
e.g. Mapping of the Gene SKr gene in Courtot wheat line

for details : Hélène Bergès, "Strategies for Improving Genomic Region Isolation Using BAC Libraries"
Large Insert DNA Libraries and Their Applications Workshop - Sunday Morning, 10 January 2010 - 9:45 am

• We propose the **fast BAC clones isolation method associated to BAC's sequencing services using the 454 technology** available at PlaGe, INRA, Toulouse.

CNRGV is involved in numerous projects on various model and crop species including :

- Wheat and barley improvement: TriticeaeGenome FP7 (1)
- Tomato: Physical mapping for sequencing of chromosome 7 (2)
- Sunflower: SunyFuel et Mildew (3)
- Medicago: Various projects as Medicago Stock Center (4)
- Eucalyptus : Eucalyptus BAC libraries as tools to identify and characterize genomic sequences involved in wood formation (5)
- Pepper : PHYTOSOL-2 Functional confirmation of candidate genes for a broad-spectrum resistance QTL against Phytophthora in Solanaceae (6)
- Rapeseed improvement (7)
- Chicory Genetic and physical mapping of the S-locus (Asteraceae) (8)
- Sugarcane genome sequencing (9)

CNRGV's collaborators:
(1) ETGI / ITMI / Triticeae Genome partners
(1) Génétique, Diversité et Ecophysiologie des Céréales UMR 1095 Clermont-Ferrand - France
(2) Laboratoire de génomique et d'amélioration des fruits INRA/INP-ENSAT Toulouse - France
(3,4) Laboratoire des Interactions Plantes Micro-organismes INRA/CNRS Toulouse –France
(5) Centro das Florestas e Produtos Florestais Lisbonne - Portugal
(6) Caractérisation Fonctionnelle des interactions Plantes Bioagresseurs INRA-UR1052-GAFL Avignon - France
(7) The Australian National University, Canberra - Australia
(7) Amélioration des Plantes et Biotechnologies Végétales UMR118 INRA/AgroCampusOuest/Université de Rennes - France
(8) Stress abiotiques et différenciation des végétaux cultivés, UMR USTL/INRA1281/SADV Lille - France
(9) Departamento de Botânica-IBUSP São Paulo, Brasil
(9) Structure et évolution des génomes CIRAD, UMR 1098 Montpellier, France

The libraries, tools and techniques presented above as well as similar tools produced on numerous plant species are available for scientific community
See more information and make your own request for specific genomic tool or service - or set up of collaborative project - on our web site

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

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