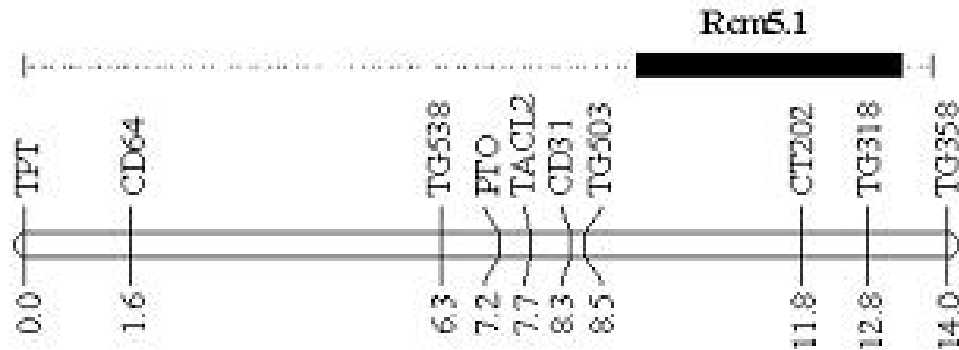


Module 2 – Specific Background for Exercise

During this Module, we will be screening a transformation competent artificial chromosome (TAC) library. The TAC vector is a modified “bacterial artificial chromosome” (BAC), and the vector is described below. The library was constructed from DNA of *L. hirsutum* accession LA 407, a wild relative of cultivated tomato that contains novel genes for resistance to bacterial pathogens. One gene of interest, Rcm5.1, was mapped to chromosome 5. We are interested in identifying clones from this region to facilitate genetic mapping at high resolution and for the eventual cloning of Rcm5.1.



Genetic map of Rcm 5.1 locus

Structural features of the TAC vector

A brief description of the TAC vector is included below, and further information regarding the vector and construction of the library can be found using the following links:

TAC library

<http://www.kazusa.or.jp/en/plant/TAC/TAC.html>

Vector

<http://www.kazusa.or.jp/en/plant/TAC/GeneralINF.html>

Vector modification and use for Rice and Tomato

<http://journals.ohiolink.edu/local-cgi/send-pdf/050304082706329094.pdf>

The TAC vector is a modified BAC vector that can be maintained in *E. coli* and transferred to *Agrobacterium tumefaciens* for plant transformation. The vector contains the P1 bacteriophage replicon, which maintains the vector in a single copy. Single copy maintenance increases the stability of foreign DNA in *E. coli*. The vector also contains the pRiA4 replicon of the *Agrobacterium rhizogenes* Ri plasmid. This origin maintains a single of the vector in *A. tumefaciens*. The kanamycin resistance marker gene (NPTI) allows selection of clones in both *E. coli* and *A. tumefaciens* by culture in the presence of kanamycin. The NPTI gene has been modified by removal of the Hind III site.

Unique Hind III and Bam HI cloning sites for the ligation of genomic DNA are included in the TAC vector between the *sacB* gene and its promoter. Clones with inserts are selected on sucrose-containing agar plates. The insertion of a DNA fragment into the Hind III or Bam HI cloning site prevents the production of levansucrase, which is encoded by the *sacB* gene. The production of levansucrase is lethal for *E. coli* in the

presence of 5% sucrose; culture of transformed cells in the presence of this sugar eliminates those harboring the empty vector.

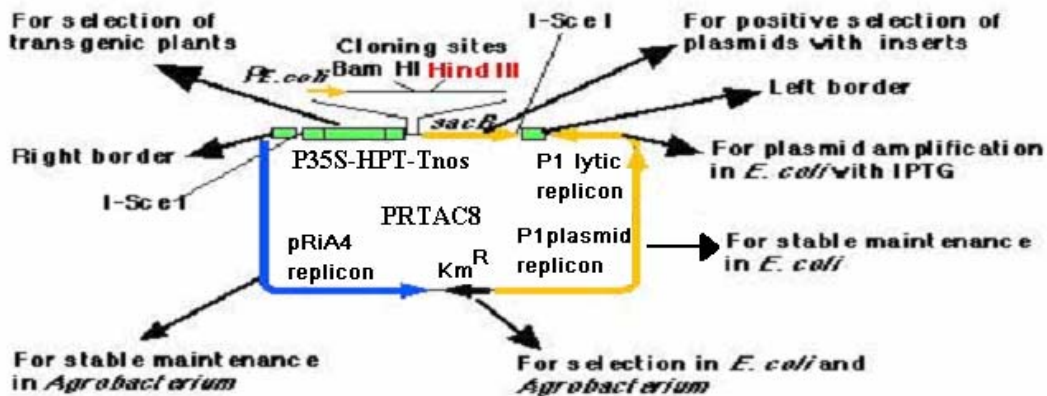
Plasmids containing the P1 replicon have a DNA-yield disadvantage due to single copy maintenance in *E. coli*. The single copy restriction can be overcome by releasing suppression of the P1 lytic replicon with isopropyl-β-D-thiogalactopyranoside (IPTG). The resulting plasmid amplification ensures that several micrograms of TAC plasmid DNA can be obtained from a 3-ml culture grown in the presence of 0.5 mM IPTG (supplemental protocol).

The TAC vector includes TAIL-PCR (thermal asymmetric interlaced polymerase chain reaction) primer sequences (R1, R2, R3, L1, L2, and L3) flanking the cloning sites (see vector map). These primer sequences are included to facilitate the isolation of end fragments from TAC clones.

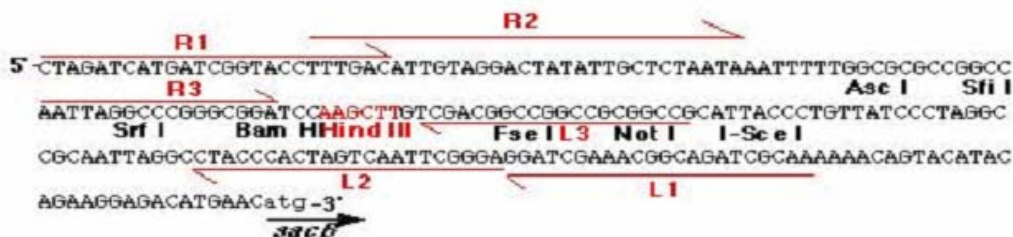
The TAC vector contains features that are required for *Agrobacterium*-mediated gene transfer into plants, including the left and right borders (LB and RB) and all cis sequences. The plant-selectable marker gene HPT, which encodes hygromycin phosphotransferase, is also included in the vector between the LB and RB sequences. HPT is under the control of the nopaline synthase gene (*nos*) promoter (Pnos) and upstream of the *nos* terminator (Tnos).

Unique sites for Asc I, Sfi I, Srf I, Fse I, and Not I, each of which recognizes rare 8-bp sequences, are included in the TAC vector flanking the Hind III and Bam HI cloning sites. These sites can be used to create a series of deletion clones in order to identify the target gene by complementation tests.

Vector map



(b)



Screening The LA 407 TAC Library

The TAC library is stored at -80 C as 48 glycerol stocks of *E. coli* pools. Each pool contains approximately 1,000 to 2,700 distinct clones. DNA has been extracted from these pools and is available as a resource. We will first screen the pools by using the Polymerase Chain Reaction (PCR) using primers for TG318 and CT202. Following this “primary screen”, positive pools will be titrated to determine the appropriate dilution for subsequent steps. We will use the Q-Pix robot at the MCIC to pick *E. coli* colonies containing TAC clones from petri dishes to a 384 well storage plate. The Q-Pix can be used to grid a nylon-based membrane from the 384 well plates and this membrane is used for the secondary screen. A probe is made by radioactive labeling of our genes of interest (TG318 (TG23) and CT202), and hybridized against the membrane. The pattern of hybridization allows us to identify the 384 well plate and the plate address of the positive clone.

Flow sheet:

Library construction

Transformation and dilution plating

Wash colonies from plates and store in pools

Grow pools and extract plasmid DNA

Screen pools using PCR primers specific for genes of interest

http://www.accessexcellence.org/RC/AB/IE/PCR_Xeroxing_DNA.html

<http://www.faseb.org/opa/bloodsupply/pcr.html>

Titrate libraries with a goal of determining appropriate dilution to obtain ~500 colonies per 150 mm plate (xxx how many plates do we need xxx?)

Use Q-Pix robot in the MCIC to pick clones and store them in 384 well plates

Grow *E. coli* containing TAC clones in 384 well plates

Use Q-Pix to grid clones from 384 well plates to nylon membrane

Grow colonies on nylon membrane, lyse cells, affix DNA to membrane.

Hybridize membranes with ^{32}P labeled probe.

Use pattern map to de-convolute the grid and determine which plate(s) and plate address(es) contains the positive clone(s)

Protocols

Tue. April 19	Introduction to DNA libraries Identification of positive pools (PCR reactions)
Thur. April 21	Identification of + pools and titration of pools
Tue. April 26	Picking clones to 384 well plate using the Q-Pix MCIC
Thurs. April 28	Arraying clones from 384 well plate using the Q-Pix MCIC
Tue. May 3	Marker/Mapping lesson I (DNA extraction PCR amplification)
Thur. May 5	Marker/Mapping lesson II (Gel electrophoresis, sequencing)
Tue. May 10	Marker/Mapping lesson IV (analysis)
Thur. May 12	Marker/Mapping lesson IV (analysis)