Plasmids/Vectors and DNA Libraries

Plasmids/Vectors

• **Plasmid/Vector** - Self replicating, extrachromosomal (separate from the large chromosomal DNA) DNA molecules found in all bacterial species.
• Plasmid replication may be independent of the cell cycle.
  – Results in hundreds of plasmid copies per cell.
Plasmids/Vectors

- Most prokaryotic plasmids are double-stranded circular DNA molecules.
- The size of plasmids varies widely, from several kilobases to hundreds of kilobases.
- They are not essential for cellular functions.
- In nature plasmids contain a variety of genes.
  - Antibiotic resistance
  - Production of restriction enzymes
  - Production of toxins
  - Production of amino acids
  - Much, much more…

Plasmids/Vectors

- Molecular Biologists use plasmids/vectors in their research
  - Cloning (making many copies) a gene.
  - Expressing a gene.
  - Moving a gene from one organism to another.
  - Sequencing a gene.
- A variety of plasmids have been engineered and can be purchased through biological supply companies. (www.promega.com)
Plasmids/Vectors

- All plasmids/vectors contain **three common features**
- Replicator (ori or origin of replication) = Where the plasmid begins to make a copy of itself.

Plasmids/Vectors

- **Selectable marker** = Isolates those bacteria which contain the plasmid.
- Provides resistance to an antibiotic (ampicillin, kanamycin, tetracycline, chloramphenicol, etc.).
- Bacteria will grow on medium containing these antibiotics only if the bacteria contain a plasmid with the appropriate selectable marker.
Plasmids/Vectors

- **Multiple Cloning Site (polylinker, MCS)** = Region of the plasmid which has unique restriction sites.
- Area where the plasmid can be cut open using restriction enzymes.
- **Restriction enzymes** cut DNA at specific DNA sequences.
Plasmids/Vectors

- In order to study a DNA fragment (e.g., a gene), it needs to be **amplified (copied)** and eventually purified.
- These tasks are accomplished by inserting the DNA into a **Plasmid/vector**.

![Diagram of DNA amplification process]

**Transformation** - Plasmids can be inserted into bacteria.
- The plasmids replicate hundreds of times in the bacteria.
- The bacteria multiply and make more copies of the plasmids.
- As a result the plasmid with the inserted DNA is **amplified/cloned** many thousands of times.
Plasmids/Vectors

- Not all of the bacteria will take up the plasmid.
- The bacteria which take up the plasmid need to be isolated.
- **Antibiotics** prevent bacteria from growing.
  - *Ampicillin is a common antibiotic.*
- Many plasmids contain a gene for antibiotic resistance as their **selectable marker**.
  - *Ampicillin resistance* is a common selectable marker.

Plasmids/Vectors

- Cells are transformed with a plasmid containing the gene for **antibiotic resistance**.
- The cells are grown on a plate with the growth media LB and an antibiotic such as ampicillin. *(LB-amp)*
- Only the bacterial cells containing the plasmid will grow.
- The bacteria that lack the plasmid will cease to grow or will die.
- This will leave only those bacteria which contain the plasmid.
Plasmids/Vectors

- **Bacterial colony** - A cluster of bacteria which originated from a single cell.
  - All cells in a bacterial colony are genetically identical.

DNA Library

- **DNA Library** - Collection of DNA fragments from an organism stored in vectors and replicated in *E.coli*.
Genomic Library

- **Genomic Library** - Consists of fragments of DNA from the organisms entire genome.
  - Introns
  - Exons
  - Promoters
  - Regulatory regions
  - Contains at least one copy of every DNA sequence
cDNA Library

- **cDNA** (complimentary DNA) - The reverse transcription of mRNA
- A cDNA library represents only the DNA which is expressed as mRNA.
cDNA Library

- Identifies only genes that are expressed.
- Does not contain introns.
- A cDNA library is specific to the mRNA used to make it.
  - Muscle cells, neuronal cells, intestinal cells, embryos, will all express different mRNA and as a result will generate different cDNA libraries.
- Can be used to identify gene splice points

Preparing the *Wolffia* cDNA Library

**Purification of mRNA**

1. Collect and grind up plants in mild denaturing solution
2. Spin out debris (Tissue, membranes, etc)
3. Treat with DNase (removes DNA)
4. Treat with Phenol (removes protein)

   - Isolate mRNA by binding to Oligo (dT) Beads
   - mRNA binds to column
   - rRNA and tRNA flow through column
   - Elute poly-A + mRNA from column
Preparing the *Wolffia* cDNA Library

**Synthesis of cDNA from mRNA**

- Purify mRNA
- Prime with oligo-dT
- Synthesize cDNA with RT
- Degradate mRNA
- Anneal second strand primer
- Synthesize second strand of cDNA
- Digest with SfiI
- Clone into SfiI digested pDNR-Lib vector

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Preparing the *Wolffia* cDNA Library

**SfiI digestion sites of pDNR-Lib**

- **SfiI**
  - Con.:
    - GGCCNNNNNGGCC CCGGNNNNNCCGG ➔ GGCCNNNN NGGCC CCGGNN NNNNCCGG

- **SfiIA**
  - GGCCATTACGGCC CCGTAAATGCCGG ➔ GGCCATTA CGGCC CCGGT AATGCCGG

- **SfiIB**
  - GGCCCGCTCGGCC CCGGCCGAGCCGG ➔ GGCCGCCCT CGGCC CCGGC GGAGCCGG
Preparing the *Wolffia* cDNA Library

**Cloning W.a. cDNA fragments into the pDNR-Lib polylinker**

- Some plasmids/vectors will close without an insert from the cDNA library.
- Bacteria can be transformed by both plasmids with or without an insert and will be antibiotic resistant.
- Both will grow on an LB-amp plate.

Blue/White Screen

- Some plasmids/vectors will close without an insert from the cDNA library.
- Bacteria can be transformed by both plasmids with or without an insert and will be antibiotic resistant.
- Both will grow on an LB-amp plate.
Blue/White Screen

- **Blue/White Screen** = a technique which isolates and identifies those bacteria which contain a plasmid with an insert.
- **β-galactosidase** - An enzyme which breaks down (hydrolyzes) the modified galactose sugar X-gal into the blue pigment 5,5'‐dibromo‐4,4'‐dichloro‐indigo.
- **LacZ** = the gene which codes for the enzyme **β-galactosidase**
- **LacZ** is another type of **selectable marker**.
- **LacZ** is found in the MCS of a plasmid

**Blue colonies do not contain a DNA insert**

**White colonies contain a DNA insert.**