Cloning in bacteria

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DNA cloning involves separating a specific gene or DNA segment from a larger chromosome, attaching it to a small molecule of carrier DNA, and then replicating this modified DNA both an increase in cell number and the creation of multiple copies of the cloned DNA in each cell. A clone is an identical copy.

First proposed by Peter Lobbane et al. 1973 at the Stanford University.
Lobban et al. 1973 "Biochemical Method for Inserting New Genetic Information into DNA of Simian Virus 40: Circular SV40 DNA Molecules Containing Lambda Phage Genes and the Galactose Operon of Escherichia coli".

• Cloning was made possible by the discovery, isolation and application of restriction endonuclease by Werner et al. (1970)
Overview cloning bacteria

Key steps
1. Cutting DNA at precise locations.
2. Cloning vector
3. Joining two DNA fragments covalently
4. Transformation to a host cell
5. Selecting or identifying host cells that contain recombinant DNA
1. How can we cut DNA at specific sites?

**Restriction endonuclease enzymes**

Type II bind to DNA at a specific sequence and make a double-stranded cut at or near that sequence.

Can form **sticky** or ‘cohesive’ ends or **Blunt ends**

Most recognition sequences are **palindromic**

eg: `GAATTC` `CTTAAG`

‘..live not on evil…’

The **sticky ends** are important for annealing. DNA ligase will eventually form a covalent bond btn the sugar-phosphate
2. Cloning vectors

Allow amplification of inserted DNA fragments

- Developed from naturally occurring bacterial plasmids
- Contain an origin of replication (ori)
- Contain numerous restriction sites
- Contain genes that confer resistance to antibiotics, thus allowing selection of bacterial colonies carrying the plasmid
- Introduced into competent bacterial cells by transformation
2. Cloning vectors cont..

Expression vectors
Vectors that can yield the protein products of the cloned genes.

- For active gene expression:
  i) strong promoter
  ii) ribosome binding site near ATG codon

- The main function of an expression vector is to yield the product of a gene, therefore a strong promoter is necessary.

- The more mRNA is produced, the more protein product is made.

Oligohistidine regions like this have a high affinity for metals like nickel, so proteins that have such regions can be purified using nickel affinity chromatography.
3. Joining two DNA fragments covalently

**DNA Ligase**

DNA ligase, much more efficient with sticky ends, for blunt end not efficient.

**Linkers:** short, double-stranded, blunt-ended, DNA fragments. Contain a sticky end restriction site.

**Adaptors.** These are linkers with cohesive ends or a linker digested with RE, before ligation. By adding adaptors to the ends of a DNA, sequences that are blunt can be converted into cohesive ends.

**Homopolymer tailing (TdT).** Terminal deoxynucleotidyl transferase adds single stranded tail in the presence of one nucleotide producing sticky ends.
3. Ligation cont..

What next ......???
4. Transformation a host cell

Most bacteria, including E. coli, only take up a limited amount of DNA. In GE bacteria are treated to increase uptake. Following treatment, cells are said to be competent.

Treating growing *E. coli* cells with solutions (CaCl2 and MgCl2) the cells are made “competent” to take up DNA.

Incubate thawed cells with DNA, then “heat-shock” at 42°C for 30 seconds (DNA is taken up by cells).

Spread plate out on appropriate selection media.

1: Mix competent cells and plasmid vector

2: Keep on ice for 30 min

3: Heat shock at 42°C 30 sec

4: Plate out cells on Ampicillin selective medium
4. Transformation cont...

Only transformed E.Coli will grow on Amplicilin supplemented media

**Transformantion efficiency:**
Can be calculated as number of colonies formed per mg of input DNA.
5: Selecting/Screening

Transformed cells will grow on selective media. Others will not!
5. Selection/screening cont…

Interrupting LacZ

Lac Z gene

pUC18

Beta-galactosidase

X-gal (colorless) → Gal + X (Blue dye)

Blue colonies = β-galactosidase synthesized
X-gal → blue product

White colonies = β-galactosidase not synthesized
X-gal → no blue product

Agar + X-gal + IPTG

Blue colony = non-recombinant
White colony = recombinant
Analysis of recombinants

For analysis of recombinants:

- Colony PCR and resolve on agarose
- Perform restriction digestion analysis
- Sequencing
- Immunoblotting for expression vector
Some cloning applications

• Human insulin, Human growth hormone, interferons, growth factors, blood clotting factors, Plasminogen activator, tumor necrosis factor, novel recombinant antibodies

• Synthetic peptides as recombinant vaccines (Hep B, malaria, rabies, HIV/AIDS)

• Gene Transfer in plants---increased yield, improved tolerance, herbicide res., disease resistance
Aknowledgment

Steven and Danielle

Classmates

All IPMB staff
...je vous remercie de votre attention