GEN2052 Notes

Lectures 1 – 10: Genomics

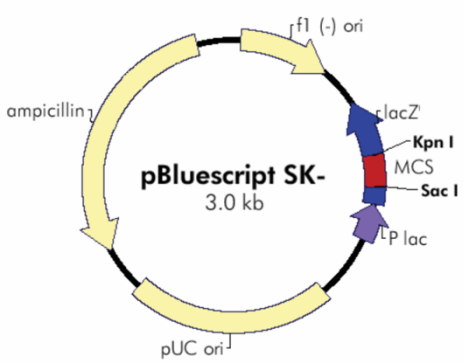
Lecture 1: DNA and RNA Analysis

Introduction

- Uses in vitro molecular techniques to isolate fragments of DNA via recombinant DNA technology
- Cloning is used to make multiple copies of a DNA fragments via bacterial vectors

Plasmid Elements

- Antibiotic Resistance Gene
  - Allows selection for bacteria which contain the plasmid
- MCS (Multiple Cloning Site)
  - Site with many restriction enzyme sites to insert DNA fragments
- Origin of Replication
  - F1 ori: Single strand replication, incorporation into phage
  - pUC ori: High copy number bacterial plasmid origin of replication

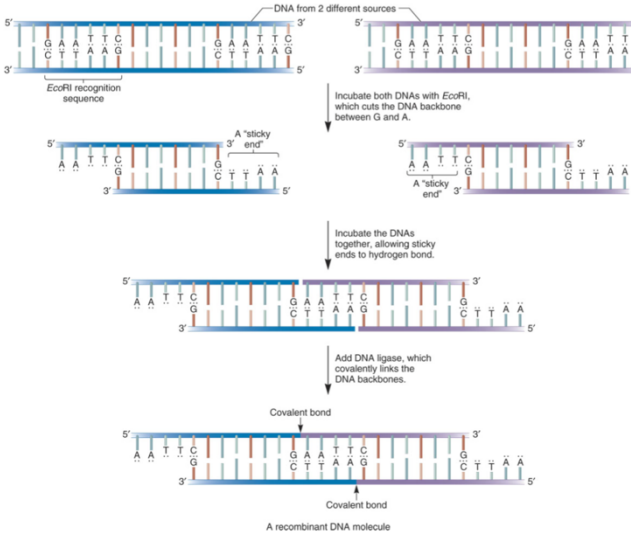


- LacZ (encodes ß-galactosidase)
  - Allows selections of plasmids containing an insert

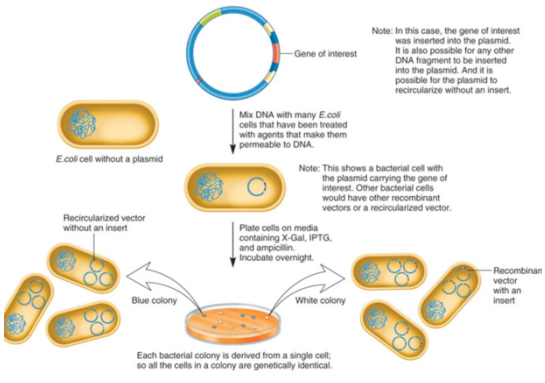
Types of Vectors

- Plasmid
  - Inserts up to 20kb
- Cosmid
  - Inserts up to 50kb
- Bacterial Artificial Chromosome (BAC)
  - Inserts up to 300kb
- Yeast Artificial Chromosome (YAC)
  - Inserts up to 1.5Mb

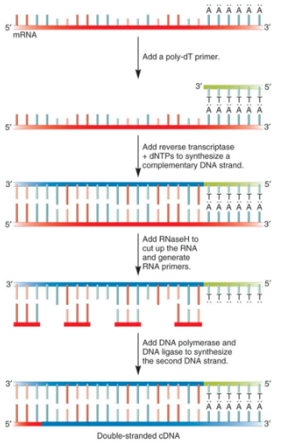
Cloning Genomic DNA



1. Isolate genomic DNA (remove protein content)
2. Break DNA into fragments to clone (sonication/restriction endonucleases)
    - Sonication: High pitched sound that breaks DNA
    - Endonucleases: Cleaves DNA at specific target sequences
3. Bacterial transformation via electroporation or heat shock
    - Electroporation: High voltage shock causes holes in bacterial membrane
    - Heat Shock: Bacteria take up Cl^- and water and create holes in membrane, moved from ice to 42° to ice to allow for entry of DNA
4. Selection of vectors with inserts
    - Only bacteria containing the vector will be able to grow on antibiotic plates
    - Stimulate lacZ gene via IPTG (lactose analogue)
    - X-Gal is added which is colourless but when cleaved by ß-galactosidase produces a blue product



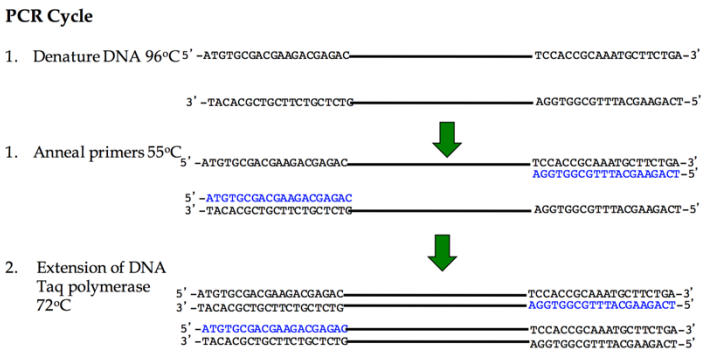
Cloning Complementary DNA (cDNA) (RNA)



1. Generated from an RNA sample
2. Reverse transcriptase (enzyme used by retroviruses)
3. Must use DNA ligase when blunt ends form (or linker sequences)

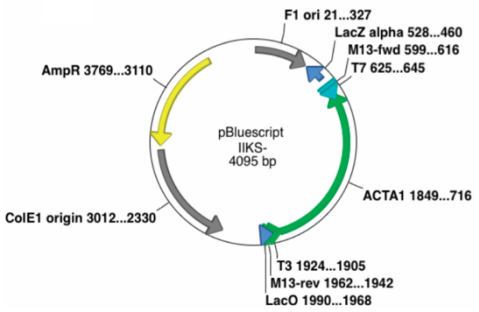
DNA Libraries (PCR)

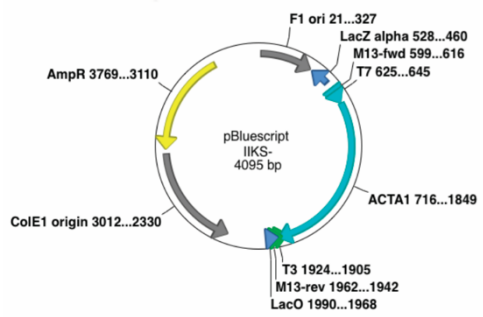
- Cloning of random genomic or cDNA
- Uses PCR for targeted amplification of DNA fragment
- Each round of PCR doubles copies of targeted DNA
- Bacterial cultures are less error prone than PCR



Determining Orientation

- Compare known restriction map to restriction enzymes see where cuts are
- This can then be visualised via electrophoresis to see where the bands are cut
- Can use directional cloning (2 R.E)





Sequencing

- Addition of dideoxy sequences (nucleotides that lack an OH group)
- This means once it is added to a chain of DNA no other nucleotides can be added onto the chain
- Used to determine what the last nucleotide was
- Now uses fluorescence markers to tag different coloured bases

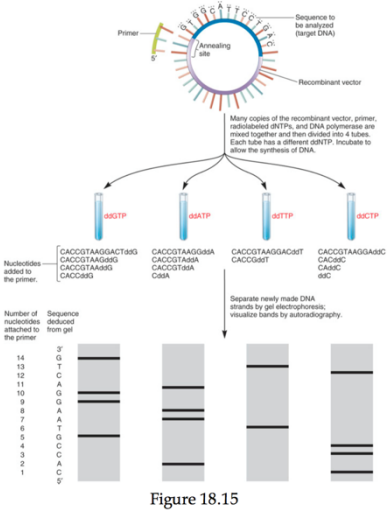
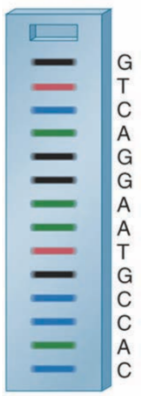


Figure 18.15

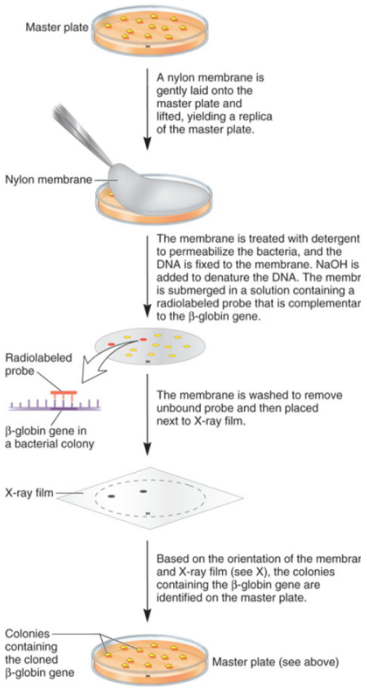


16 (a) Automated sequencing gel

Lecture 2: DNA and RNA Analysis II

A-T Cloning

- Many DNA polymerases produce products with a 3′ adenine overhang
- Create vectors with 3′ thymidine overhang
- Ligate and use restriction mapping to determine orientation



Colony Hybridisation

1. Grow plates of bacteria containing library
2. DNA is transferred to a membrane (cross-linked)
3. Generate a labelled probe for sequence of interest (radiolabelled or bioluminescent)
4. Determine location of probe on membrane
5. Identify bacterial colony and pick form plate

Probes and Screening

- Fragment of gene of interest
- Homologue form another species
- Closely related gene
- Short DNA oligo designed protein sequence
- Synthesis probe by PCR with radiolabelled dNTPs

Libraries

- Gridded Libraries
  - DNA from library is extracted and plated on a grid in a known pattern
  - Can screen filters and then order identified clones
- Pooled Libraries
  - PCR in all wells into rows and columns (and plates)

Subclones

- Clone fragments of larger DNA clone
- Often unknown DNA sequences due to segmentation