

- So which cell has picked up human DNA, and not only plasmid DNA
- Is it white- does it have the insert, and does it have ampicillin resistance

Making a cDNA (copy DNA) probe

- Derive the mRNA sequence from the sequence you are interested in
- You take a precursor as its making a lot of rna being a precursor
- Hijack and purify enzyme of retroviral activity
- So use mRNA to make a single stranded DNA and make it a double stranded DNA via *DNA polymerase*
- So we have a cDNA for the mRNA
- Insert into vector to make a lot of it
- Use a filter to sample the DNA
 - hybridise a specific radioactive probe to the DNA (single strand binds to form double strand DNA on disc)
- Replica of DNA is on disc now. Disc can be screened for correct colony
- Isolate the plasmid
- Isolate the fragment from the plasmid

Summary:

- Each colony has a plasmid
- Plasmid on filter
- Denature so it's single stranded
- Take probe
- Label it
- Single stranded so it floats around
- Only binds where it finds a specific sequence (as single stranded probe and single stranded DNA bind at specific sequence)
- Expose to xray...
- Identify a colony

Restriction enzymes- used to identify changes in DNA sequences

Southern Blot- Agarose gel electrophoresis of digested DNA to separate fragments

1. Denature and transfer to filter
2. Take cloned gene fragment and radioactively label it
3. Add to filter to allow hybridisation
4. Lay on photographic film to visualise bands

Polymerase chain reaction

- Cloning without cells
- Rapidly isolates DNA fragments