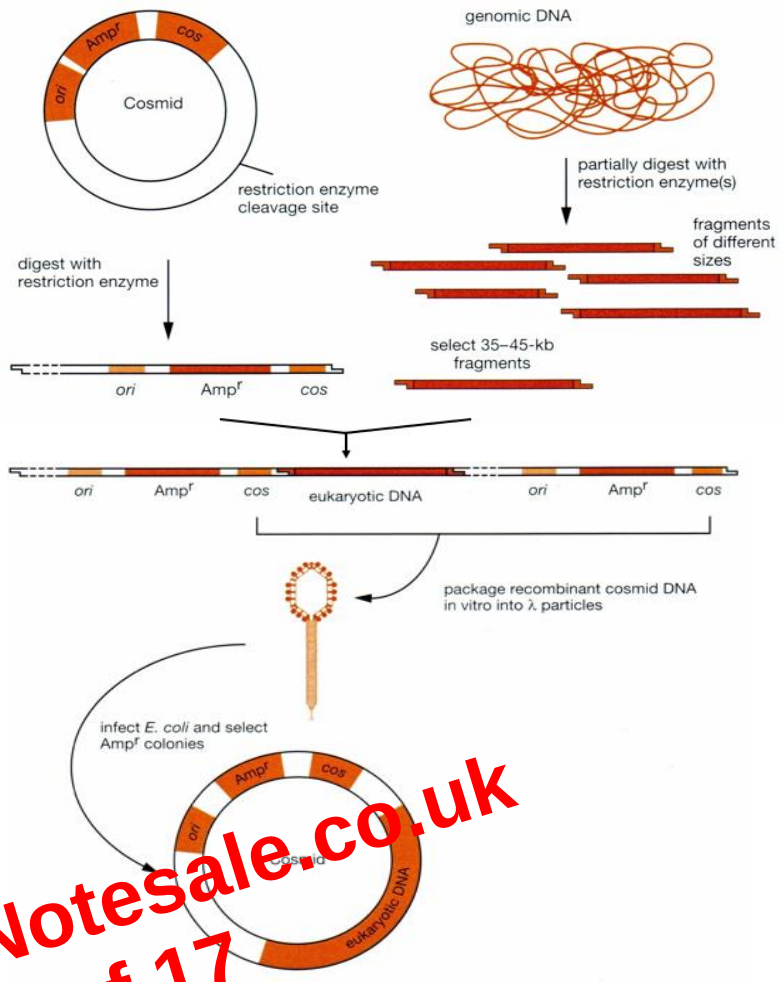
- Adding EcoRI linkers adds restriction sites for both ends, and the 3' one is removed to leave sticky ends for ligation into lambda phage
- cDNA library derived from mRNA (which isn't suitable as it is too easily degraded)
- Phage lambda normally used as it has lytic abilities, but others may be used



- Genomic library construction breaks down into three steps:
  1. Isolation of high quality genomic DNA
  2. Preparation of a compatible vector
  3. Insertion of genomic DNA into the vector
- The isolated genomic DNA is cut with the same enzyme as the cosmid
- They are then ligated together, and recircularised
- Vector is usually phage lambda, or high capacity vectors

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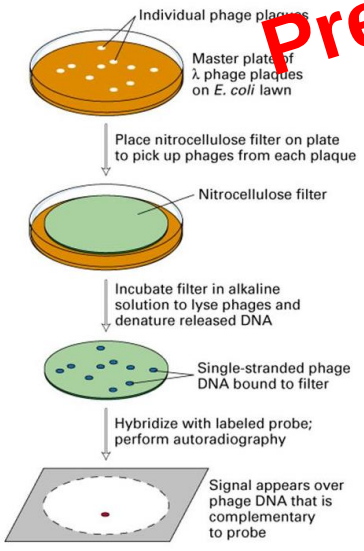
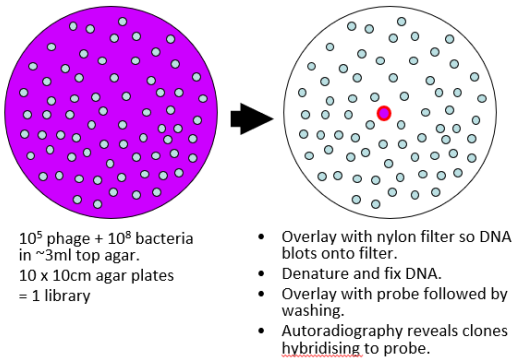
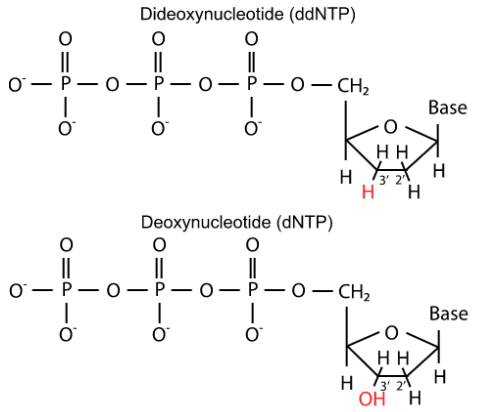
- Describe the basics of library screening
  - When screening for clones you have to make sure there is complete coverage
  - For genomic libraries this means calculating the probability that a given sequence is represented, and using that, the size of your insert, and the size of the total genome, you can calculate the exact number of clones needed
  - For cDNA libraries it is guesswork – but for both it's normally around a million
  - A common method of screening is via radiolabelled specific nucleic acid probes (bits of ssDNA which code for the sequence you want). Plaques form on a lawn where λ has lysed the E.coli, which contain naked recombinant DNA (with lots of different genome fragments). Southern blotting using nylon which binds to the DNA (which is denatured with NaHO), and incubate with a radiolabelled probe. Then X-ray to locate the plate and plaque with the fragment, and isolate the DNA.

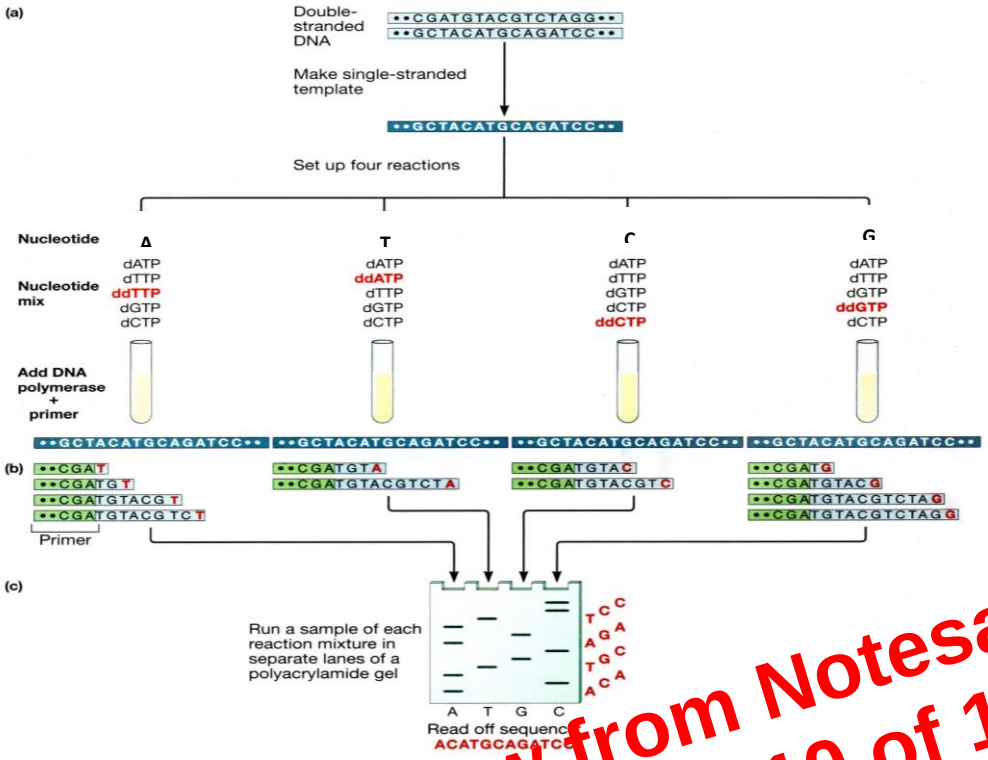




  - Hybridisation of the probe to the fragment is affected by several parameters: G≡C content, fragment length, base-pair mismatch (if using homolog probes), salt concentration (the less ionic a solution, the more hydrophobic bases try to stay hidden

- Sanger or dideoxy DNA sequencing has all of the principles above, with random termination achieved by using dideoxynucleic acids (ddNTPs). It incorporates radiolabelled DNA as a detection method, and templates can be ssDNA, dsDNA, dsDNA plasmids, PCR products etc (basically DNA).
- Dideoxynucleotides have no OH at the 3' position, so polymerases cannot extend from it, terminating the fragment.





- A synthetic primer is annealed to the template (need to know some of the sequence – or can generate like with reverse PCR)
- Four reactions each containing all the dNTPs and DNA polymerase, each with one of the four normal bases labelled
- Complementary ddNTPs to the labelled normal base are added to each reaction (eg ddTTP to the A reaction etc)
- ddNTP incorporation leads to chain termination

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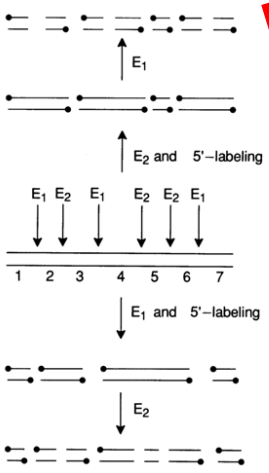
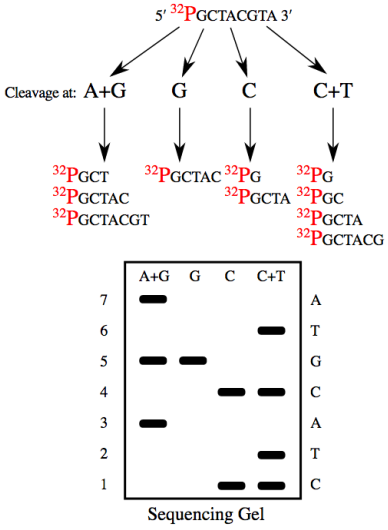
- Maxim-Gilbert chemical sequencing was developed earlier and was more popular, but it relies on cleaving the bases with several dangerous chemicals:
  - Formic acid (purine specific – adenine and guanine)
  - Dimethyl sulphate (guanine only)
  - Hydrazine (pyrimidines - cytosine and thymine)
  - Hydrazine with NaCl (cytosine only)
- Modified DNAs were then cleaved by hot piperidine at the modified base positions
- The modification reactions were set statistically to modify one base per molecule
- DNA fragments needed to be less than 300bp, and were normally prepared from digesting the template (normally end-labelled by T4 PNK, phosphorylating the 5' end)
- Labelled dsDNA species denatured, and strands were separated by denaturing PAGE





- Lots of chemical options to change the cleavage
- Nowadays it is rarely used, except for mapping protein-DNA binding sites – end label the DNA that you think the protein will bind to, and methylate all molecules once. If methylated, the protein cannot bind