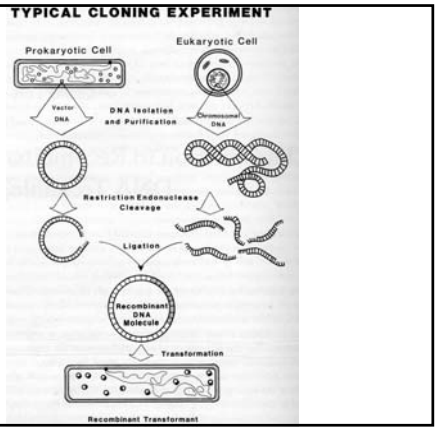
Colony PCR

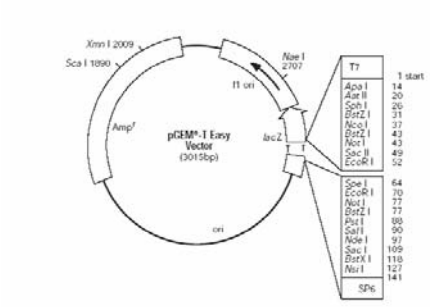
CPSC265 Class 8

Cloning

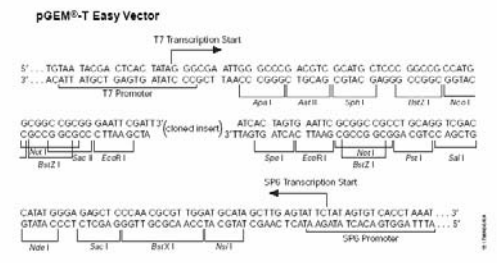
- Cloning is the way in which we can take a single molecule, and make lots of bacterial cells that contain an identical molecule.
- These cells are clones, hence the name
- This used to be the only way to amplify DNA. It is still by far the most accurate.



Plasmid vectors – circular, autonomous bacterial DNA



The vector is made with a "T" overhang



Taq polymerase leaves an "A" overhang

- Taq is the thermostable DNA polymerase from *Thermus aquaticus* we used for PCR.
- When Taq synthesizes a new strand, it always puts an extra "A" at the end
- This can be useful, but note: **other polymerases do not do this, they leave "blunt" ends. Only Taq polymerase leaves 'A' overhangs. 'Blunt' end vectors do not work with Taq, we need a 'T' overhang.**

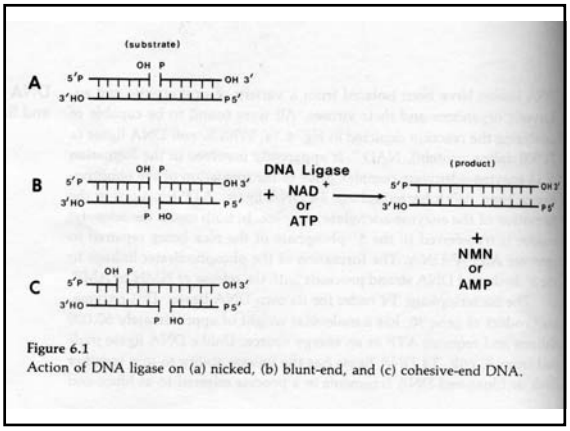


Figure 6.1
Action of DNA ligase on (a) nicked, (b) blunt-end, and (c) cohesive-end DNA.

DNA ligase

- Repairs gaps in the sugar-phosphate backbone of DNA
- Creates phosphodiester bonds
- Does not do anything with the bases

Transformation of bacteria

- Two main methods for transformation
- Chemical / Heat Shock
  As done in last practical, this method gets DNA into the cell by making them porous using CaCl2 and a 42 C heat treatment
- Electroporation
  Makes cells porous using high-voltage electricity

Imperfect science

- Most of the plasmid / insert combinations will not ligate
- Most of the bacteria will not be transformed
- We only need one molecule to get into one bacterium to make one colony.

PCR from clones

- Often clones will religate containing any old DNA (eg primer dimers)..
- The DNA can go in in either orientation
- We can use the PCR to tell which colonies have the insert we want, and which orientation it is in.