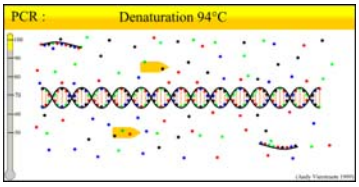
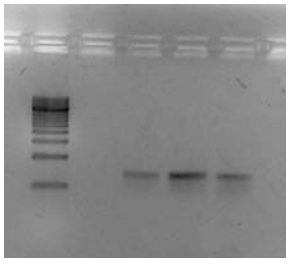
Bacterial cloning

Especially of PCR product DNA

PCR recap

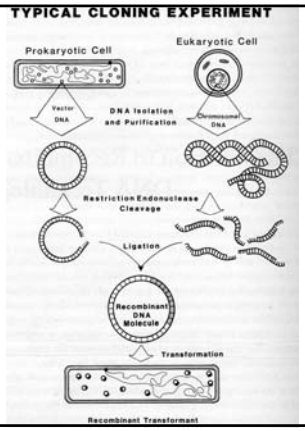


PCR gel product

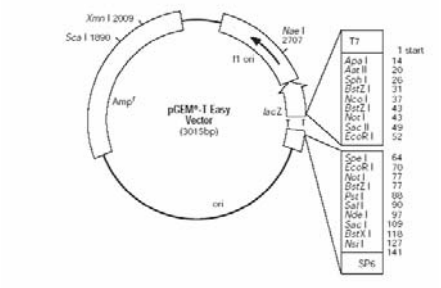


Cloning

- 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.



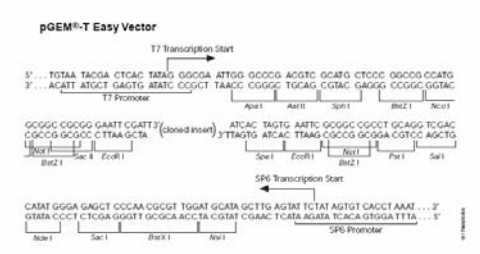
Plasmid vectors – circular, autonomous bacterial DNA



Cloning PCR products

- When we amplify DNA using PCR, it is often necessary to "clone" this DNA
- We do this in order to replicate it without errors
- Also, by cloning a protein coding sequence into *E. coli*, we can then produce the protein in the bacterium.

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, only Taq polymerase**

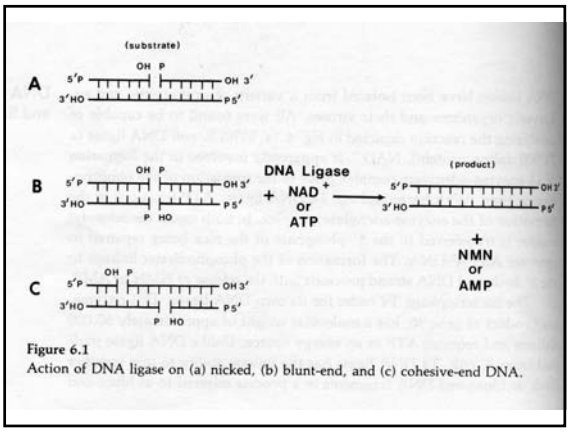


Figure 6.1
Action of DNA ligase on (a) nicked, (b) blunt-end, and (c) cohesive-end DNA.

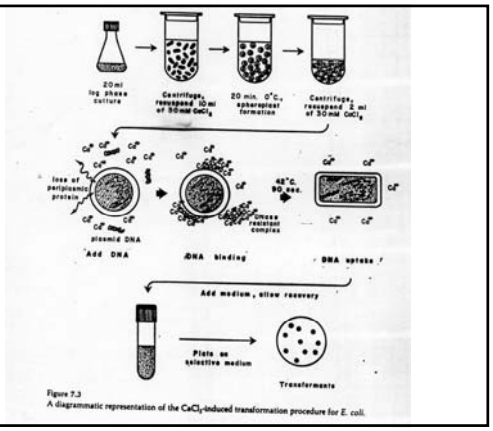


Figure 7.3
A diagrammatic representation of the $CaCl_2$-induced transformation procedure for *E. coli*.