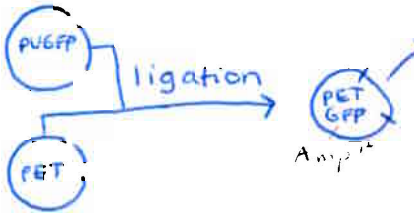
Subcloning Project - Transformation



put into E.coli; → "transformation"
only E.coli w/

* Transformation is 3rd way to move DNA into cell, phage infection + transduction (GEN)

Amp^R on plasmid → DNA must be circular + have origin of replication to be maintained
will grow

- To be transformed, bacteria must be competent (able to take up DNA)
- How to make bacteria competent?
  $CaCl_2$ treatment
- Why do we use AG1111 bacteria?
  very competent - can get a lot of plasmid yield
  Not all bacteria so competent
  Is this the bacteria that will glow? NO Why not? NO T7 polymerase
  Why do we use this then? need very competent for ligation rxn

Transformation protocol

1) mix DNA + cells on ice
2) heat shock
   cells take up DNA
3) add SOC medium (rich growth media)
   all cells grow, replicate
   1 hr → to make Amp^R protein
4) plate on LB-Amp O/N
   selection - only Amp^R
   cells grow

compare 2 rxns →

Ligation Rxns	Colonies	Reason
1) vector + insert	YES	pET-GFP
2) vector alone	YES (small amount) / NO	(-) control, phosphatase complete digestion
3) insert alone	NO	(-) control, pUGFP contam.
4) uncut pET (gel)	YES	(+) control, gel isolation, gel in transformation control
5) diluted pET (uncut)	YES countable	(+) control, transformation calculate transformation efficiency #colonies/micrograms DNA
6) ligation buffer	NO	(-) control - background

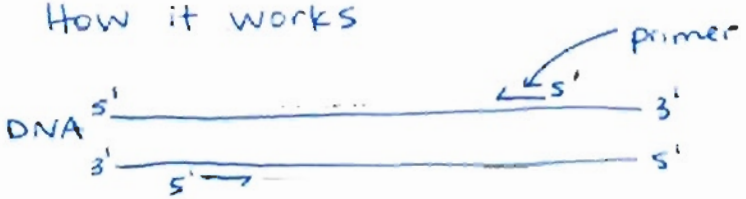
Each bacteria takes up 1 plasmid
each colony same plasmid

PCR - polymerase chain rxn

What does it do? Amplify specific fragment of DNA

What do we use it for? To find out which ara gene is disrupted by insertion

How it works



Primers on both sides of given region,
DNA polymerase replicates that region (between primers) exponentially.

DNA pol only adds to 3'OH (so DNA grows 5'→3')
↑
Carbon

Rxn Components

1) DNA
2) primers (flank region of interest) - forward + reverse
3) DNA polymerase - replicates DNA - Taq **Why Taq?**
4) dNTPs
5) $MgCl_2$ - cofactor
6) buffer

Steps

1) heat rxn 94°C (denaturation) - all strands separate
2) reduce temp to 55-65°C (annealing) - primers bind to DNA **What is temp based on?**
3) temp 72°C (extension) - DNA polymerase copies DNA
4) go to step 1

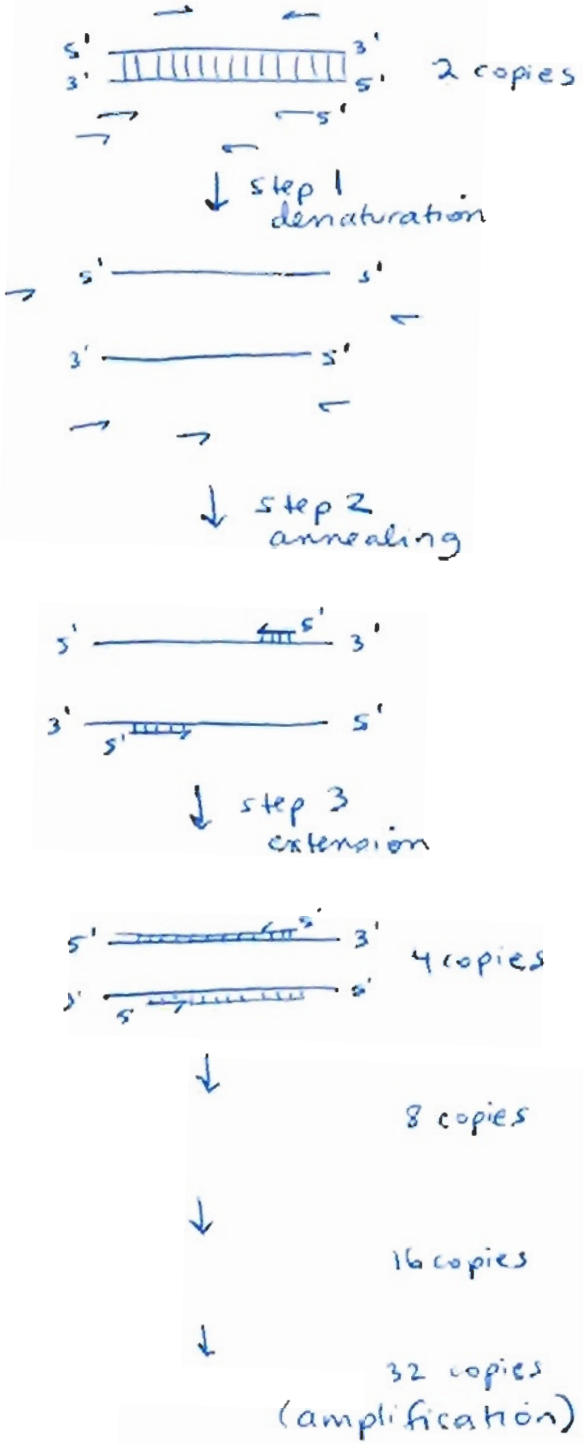
optimal temp for Taq

How fast does Taq work? 1kb/min

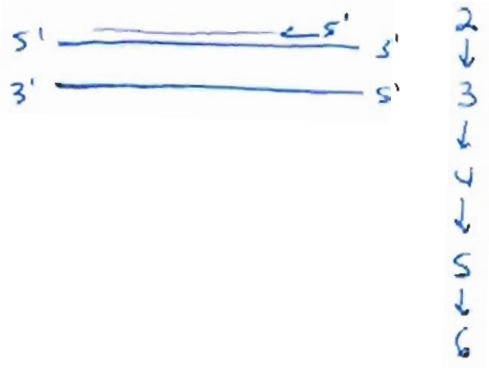
min extension 2.5 min

max size product 2.5 kb

PCR



If only 1 primer



Parameters to optimize

- annealing T (G/C content + length)
- primer sequence
- [Mg]
- extension time (1 kb/min)

PCR similar to primer labeling

After movie,

Rev+For Primers - reference coding strand

DNA 2 strands - 1 is same sequence as mRNA

1 is template