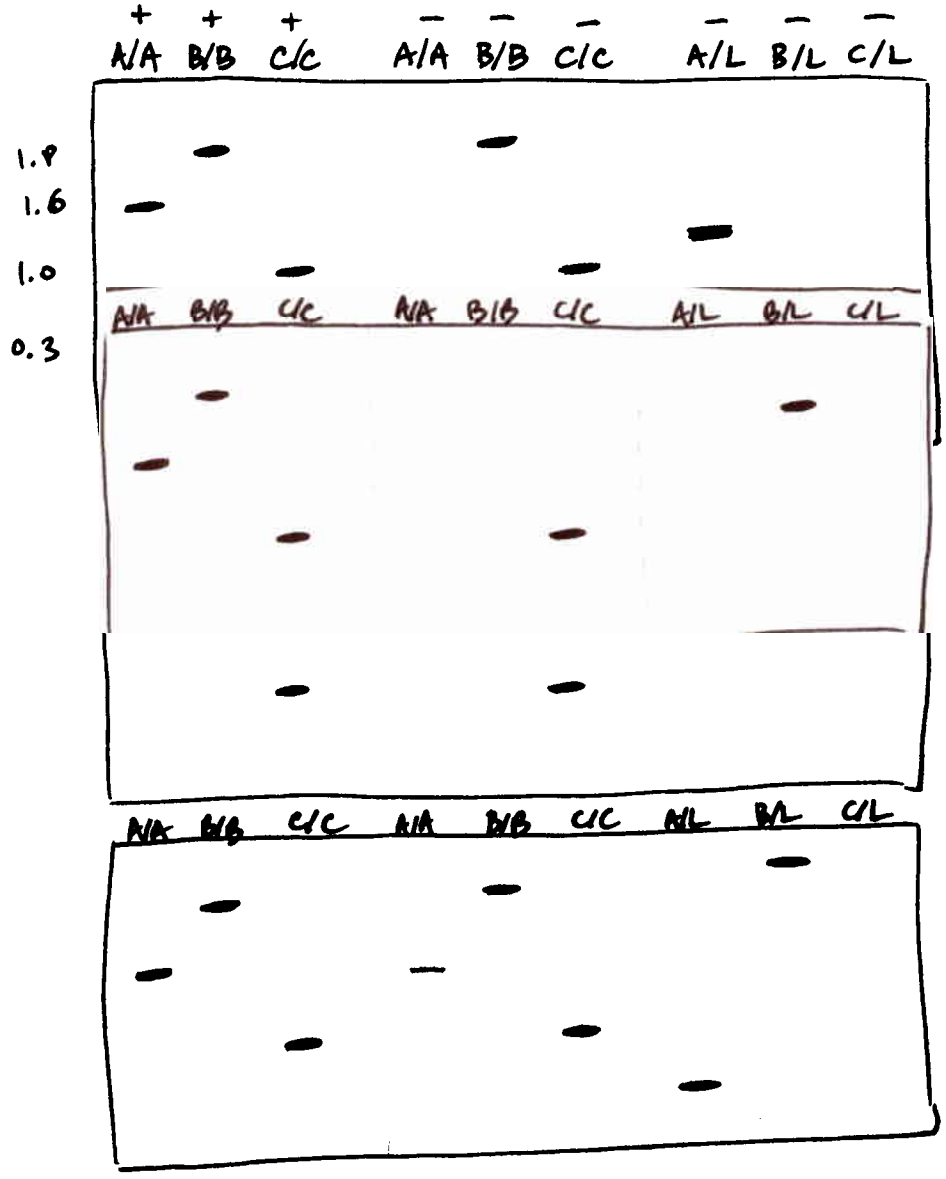
KDM Day 5 Recitation Handout

Examples of gels from PCR analysis of ara mutants

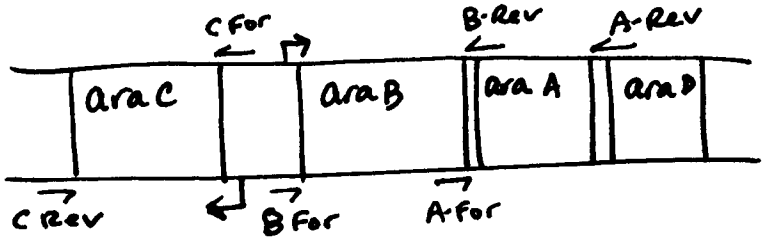


insertion in ara A

insertion in ara B, in 3' end, such that ara A Forward primer can't bind, or binds too far away for exponential amplification of ara A

insertion in ara A, in 5' end such that ara B Forward primer + LacZ primer close enough for exponential amplification.

Band in A/A(−) is fainter + due to contamination from WT DNA



RDM Day 5

Subcloning Project (goal: make pET GFP)

↑ GFP = cDNA (not genomic)

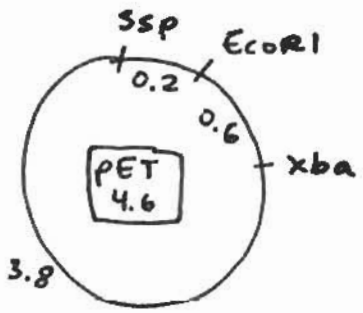
Diagnostic RE digests

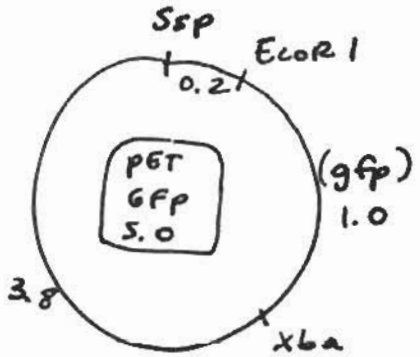
- to find out which plasmids that you miniprepped have the gfp insert

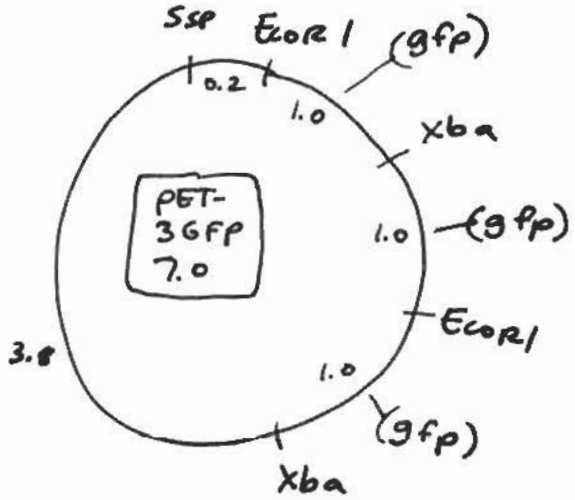
Which enzymes did you use?

1) EcoR1 + Xba
2) Ssp + Xba

Possible Plasmids







Restriction Enzyme	pET 4.6	pET GFP 5.0	pET GFP(3) 7.0
EcoR1 + Xba	0.6 4.0	1.0 4.0	1.0 (brighter) 4.0
Ssp + Xba	0.8 3.8	1.2 3.8	1.2 2.0 3.8

keep in mind:

- Partial digests could happen
- Bright band stuck in wells could be chromosomal DNA from miniprep

- If digests don't work, running uncut will still tell you if you have gfp (pET-GFP larger, runs slower, even supercoiled)