DNA Engineering: M1D3 Lab Talk

20.109 (F11)
09.22.11

Fixing FNT M1D1

would like resubmission on index card

Primer design

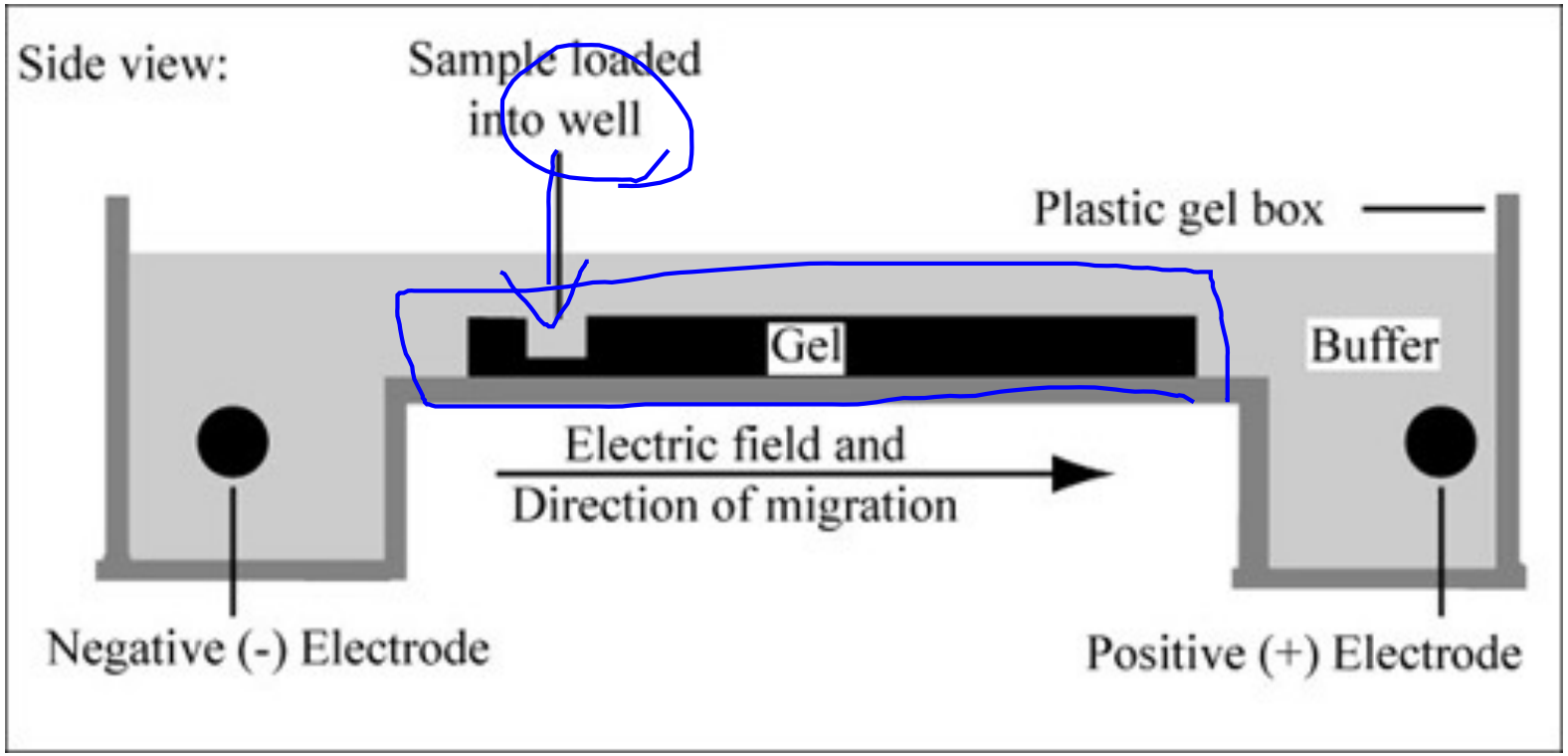
- How to retrieve seq, in gen'l
- How to find relevant part
- Design of landing seq (length? Tm? GC?)
- Design of flap seq (cloning?)
- Hints for reverse primer
- Other things to check

PCReaction details

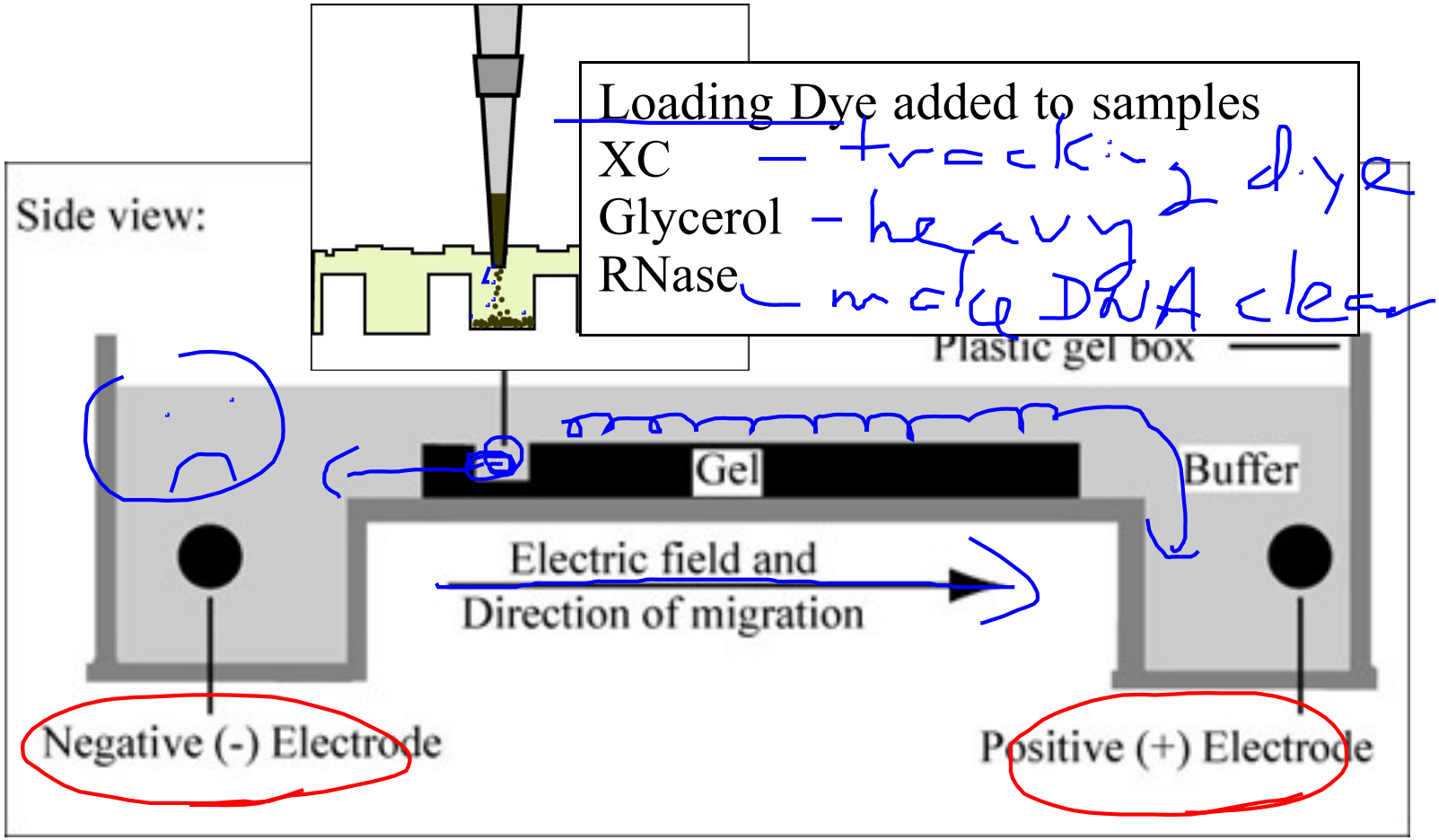
- Components:
  - what?
  - volume, mass or conc?
  - how to assemble?
- Cycling conditions:
  - anneal temp?
  - extension time?

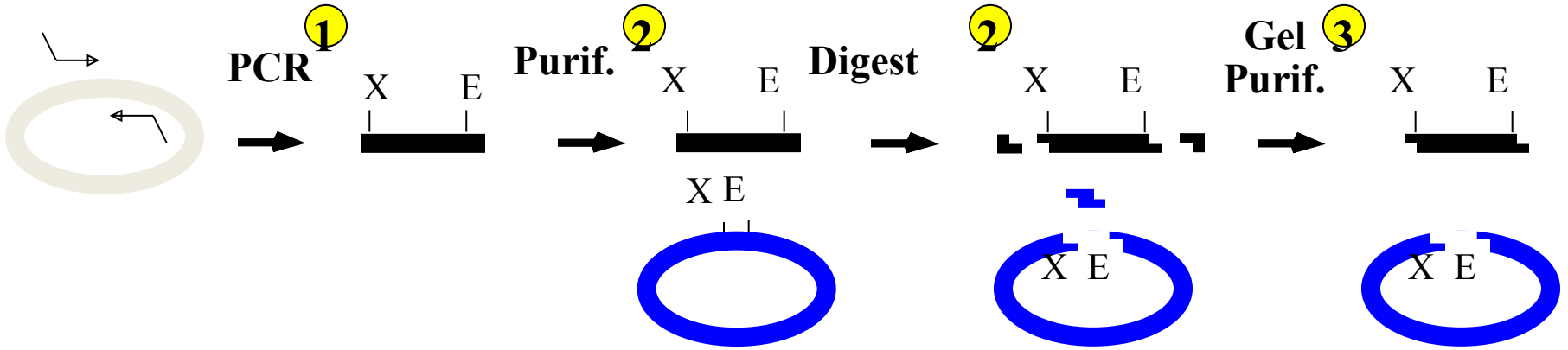
General note: be cautious with wording taken directly from wiki

Agarose Gel Electrophoresis

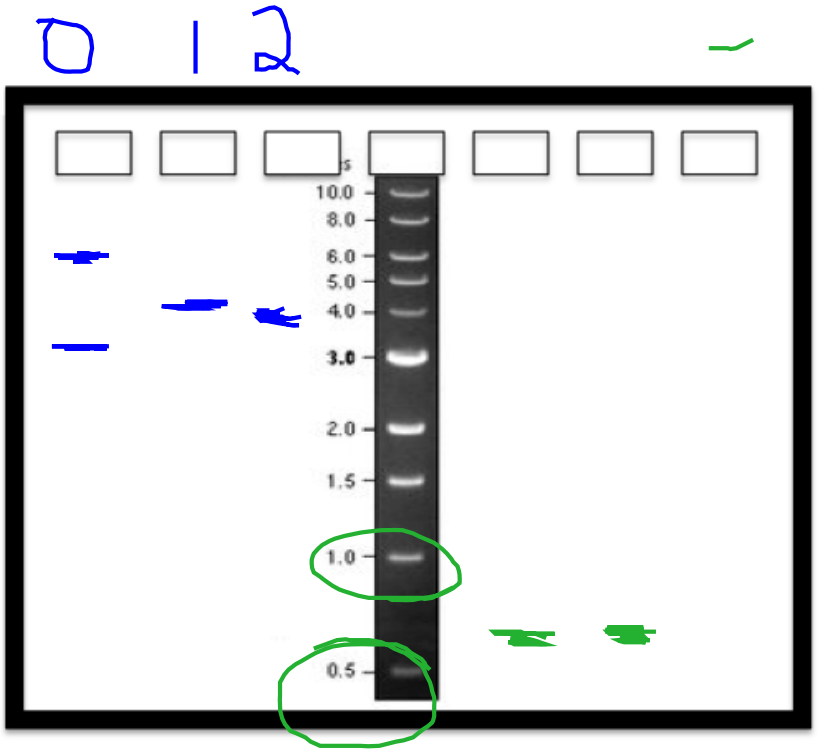


Agarose Gel Electrophoresis





Lane	Sample	Volume to load
1^	Uncut pCX-NNX^	10 µL^
2	pCX-NNX XbaI	5 µL
3	pCX-NNX EcoRI	5 µL
4	pCX-NNX XbaI + EcoRI	25 µL
5	1Kb DNA Ladder	10 µL
6	PCR Product XbaI + EcoRI	25 µL
7	PCR Product Uncut	25 µL
8	PCR no-template-control	25 µL



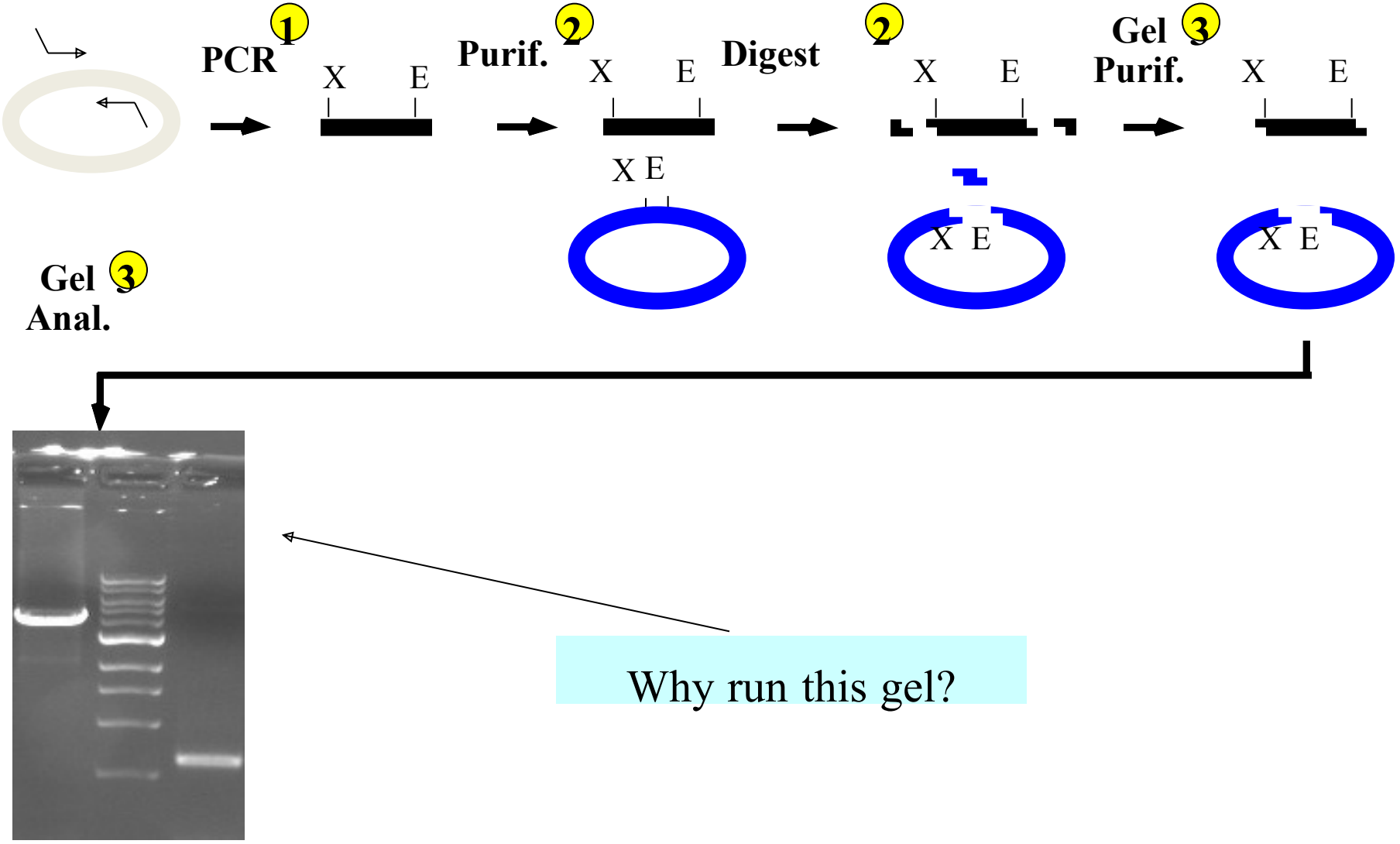
Agarose Gel Electrophoresis

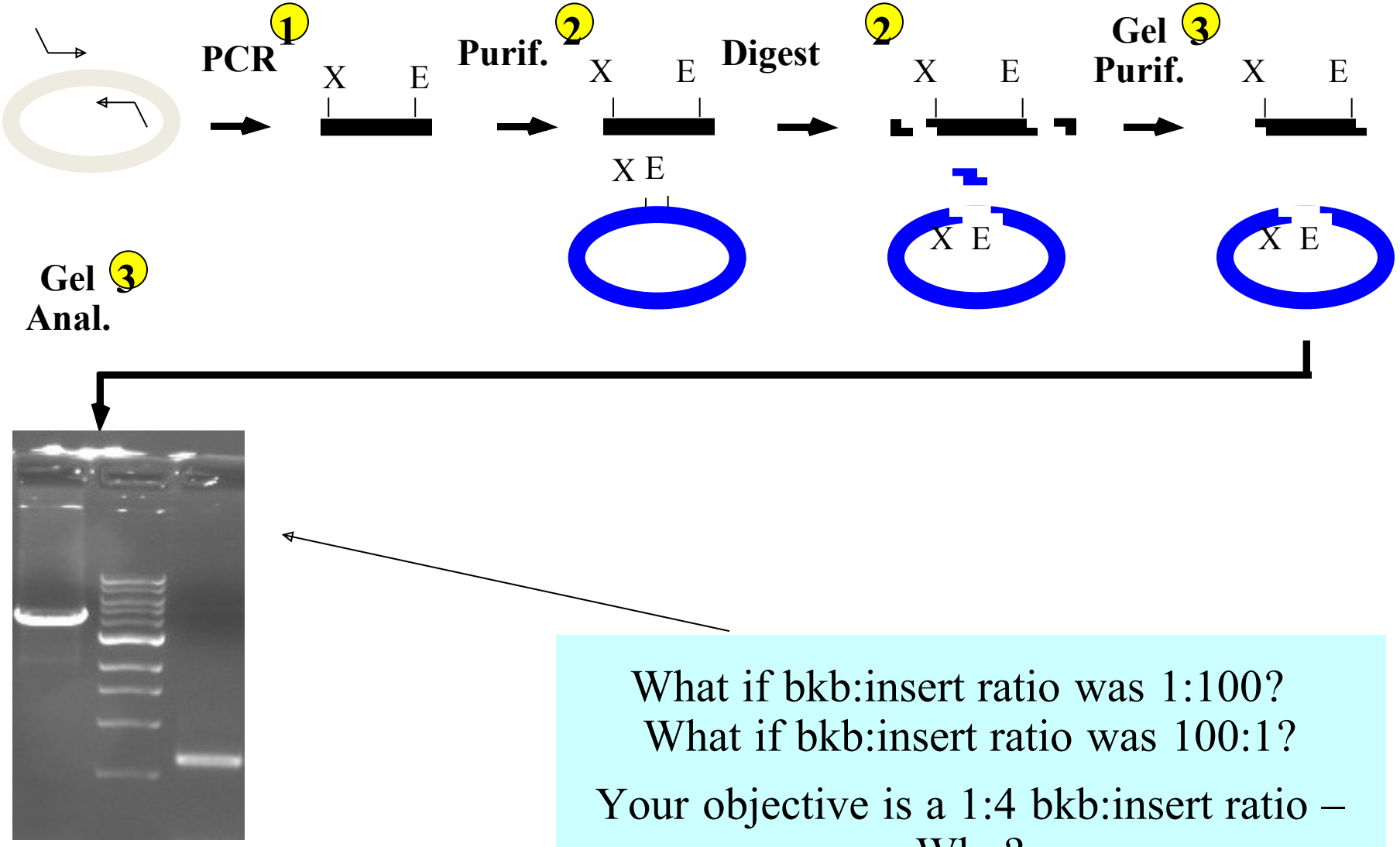
SAFETY NOTES:

- Use nitrile gloves
- Need face shield when excising DNA bands from gel

NEXT STEPS:

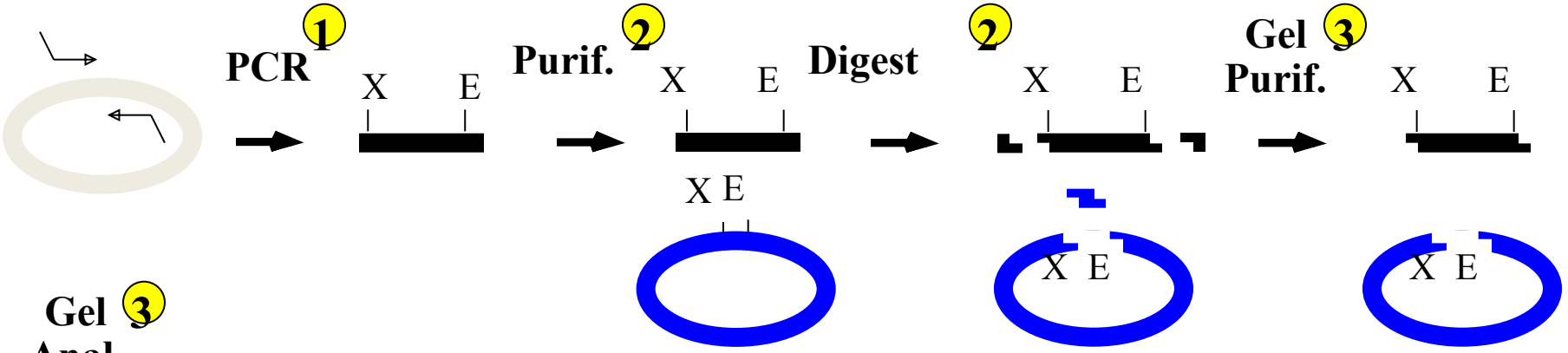
1. Q-kit to melt agarose, isolate DNA
2. Remove aliquot to check recovery on gel

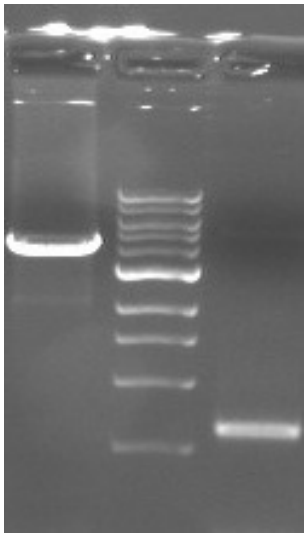

PCR 1
X E
Purif. 2
X E
X E
Digest 2
X E
X E
Gel Purif. 3
X E
X E
Gel Anal. 3
Why run this gel?



What if bkb:insert ratio was 1:100?
What if bkb:insert ratio was 100:1?

Your objective is a 1:4 bkb:insert ratio – Why?





How do you figure out how to get a 1:4 molar ratio?

bkb:

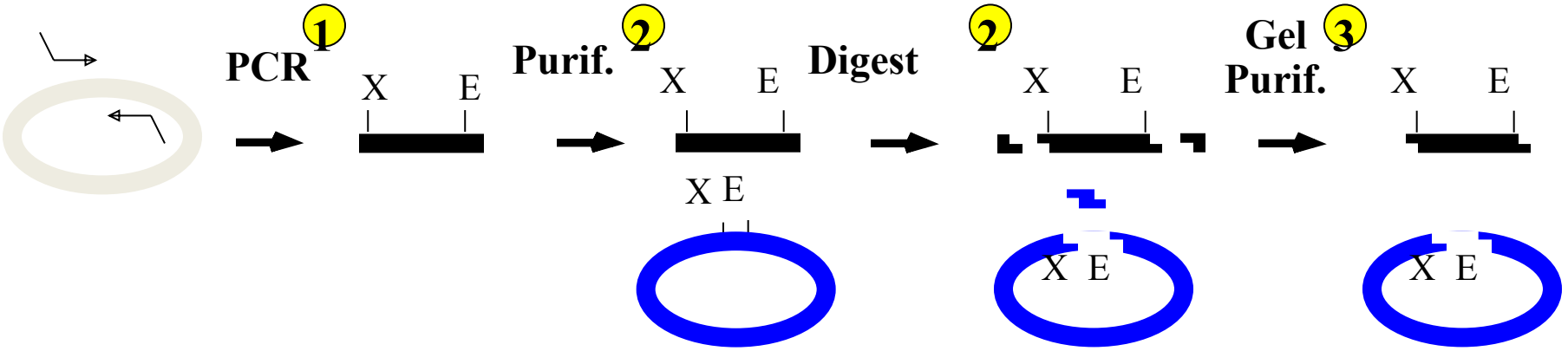
insert:

Plan for today and next week

R Load gel / L S / Excise

T Ligate / EHS / Txn

R Miniprep
Check plasmid / T C



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