PLEASE: Help yourself to cookies in the tea room

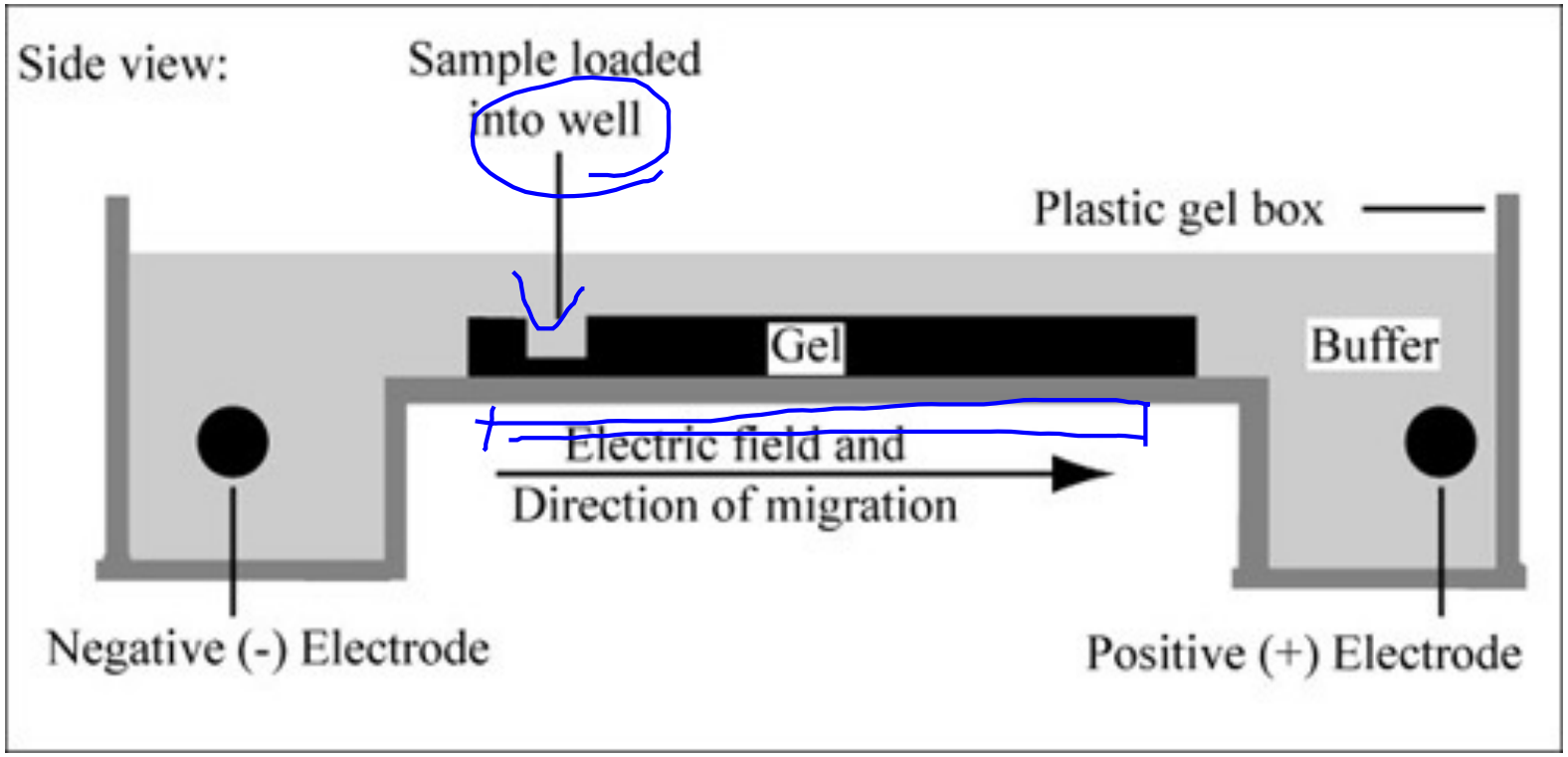
Wish Divya a happy b-day

DNA Engineering: M1D3 Lab Talk

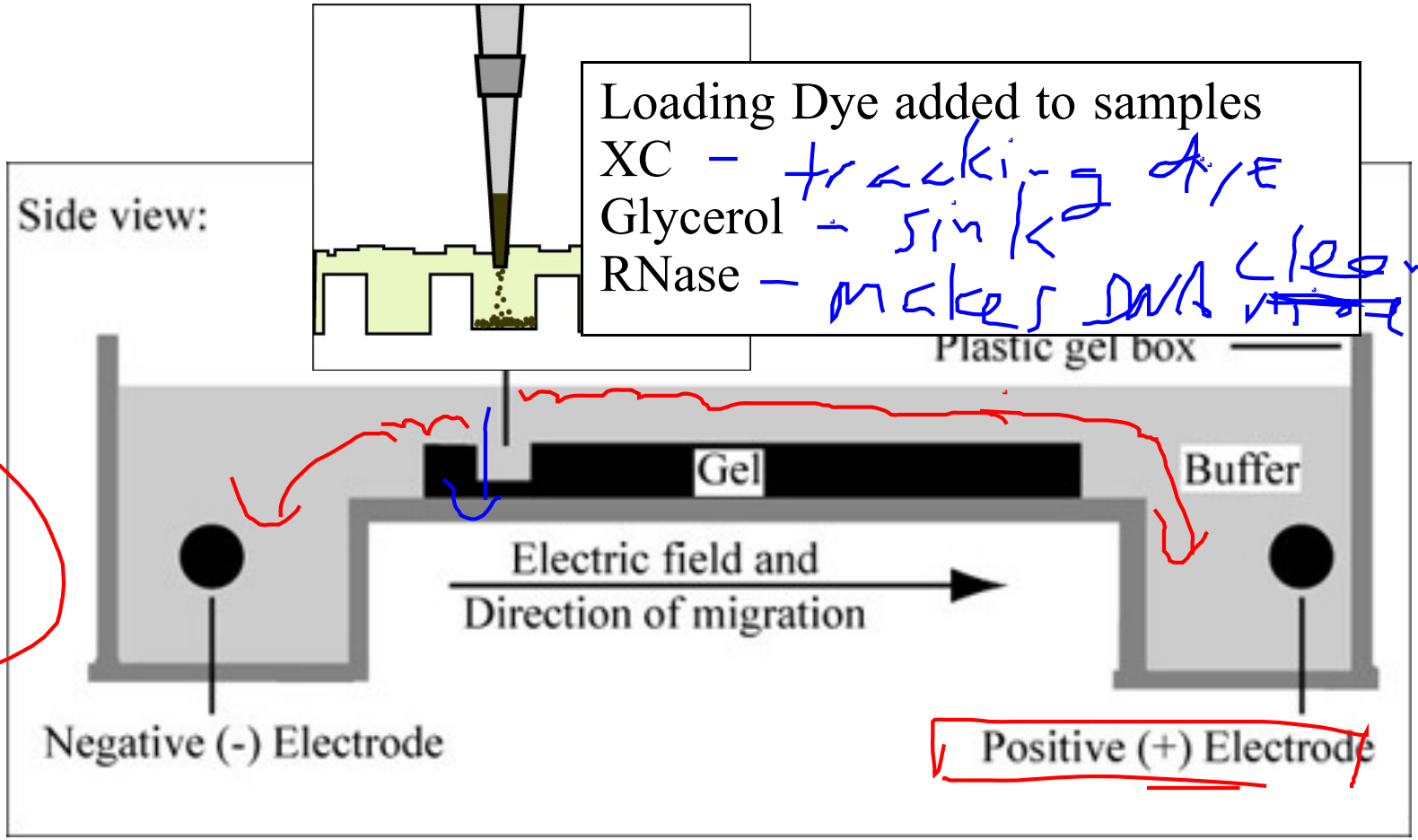
20.109 (F12)
09.18.12

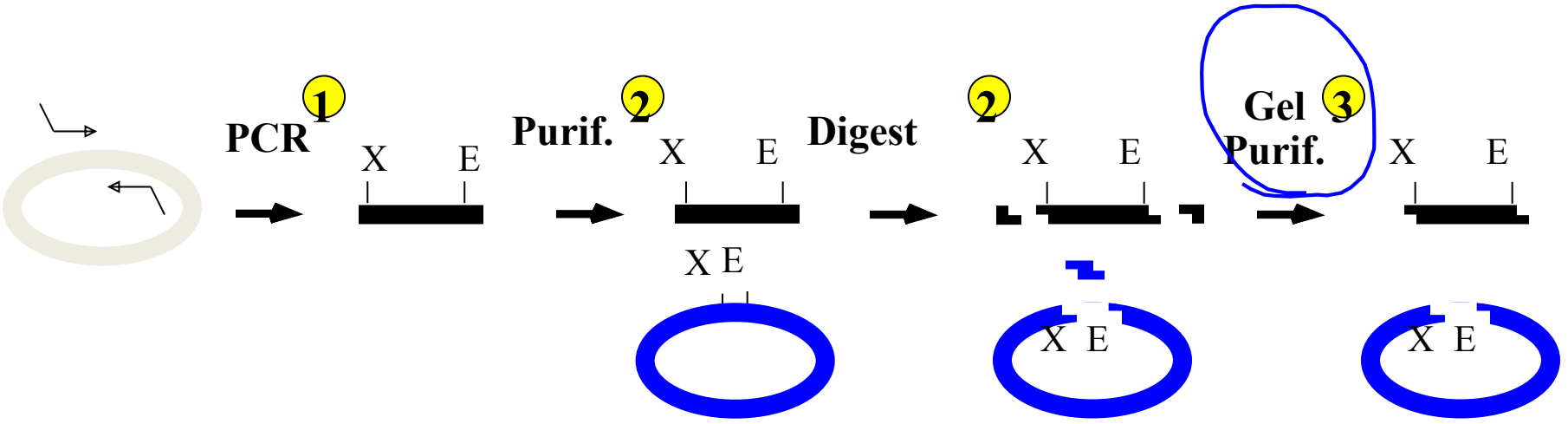
9/24 Drew Endy SBWG 56-614
Noon

Agarose Gel Electrophoresis



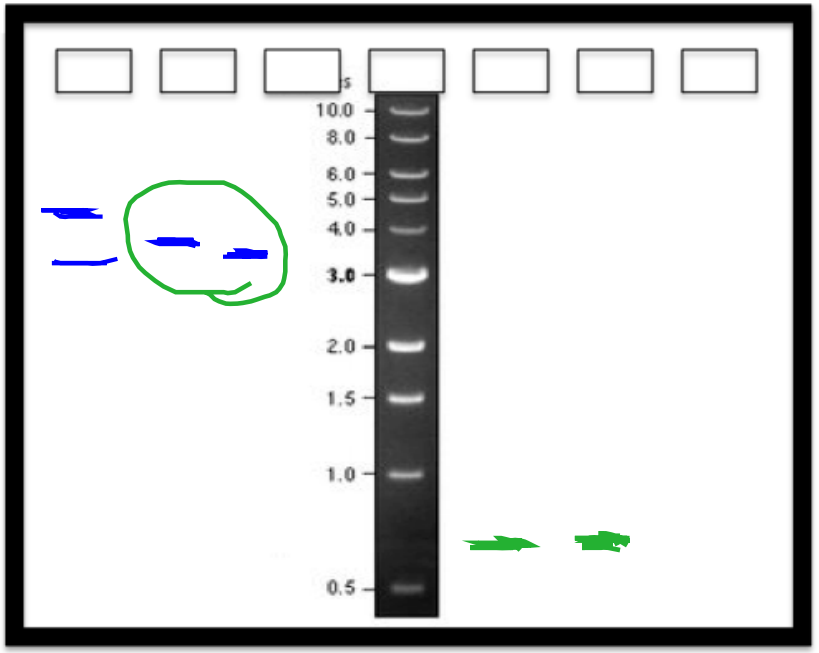
Agarose Gel Electrophoresis





1 2 3 4 6 7 8

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	20 µL
6	PCR Product XbaI + EcoRI	25 µL
7	PCR Product Uncut	25 µL
8	PCR no-template-control	25 µL



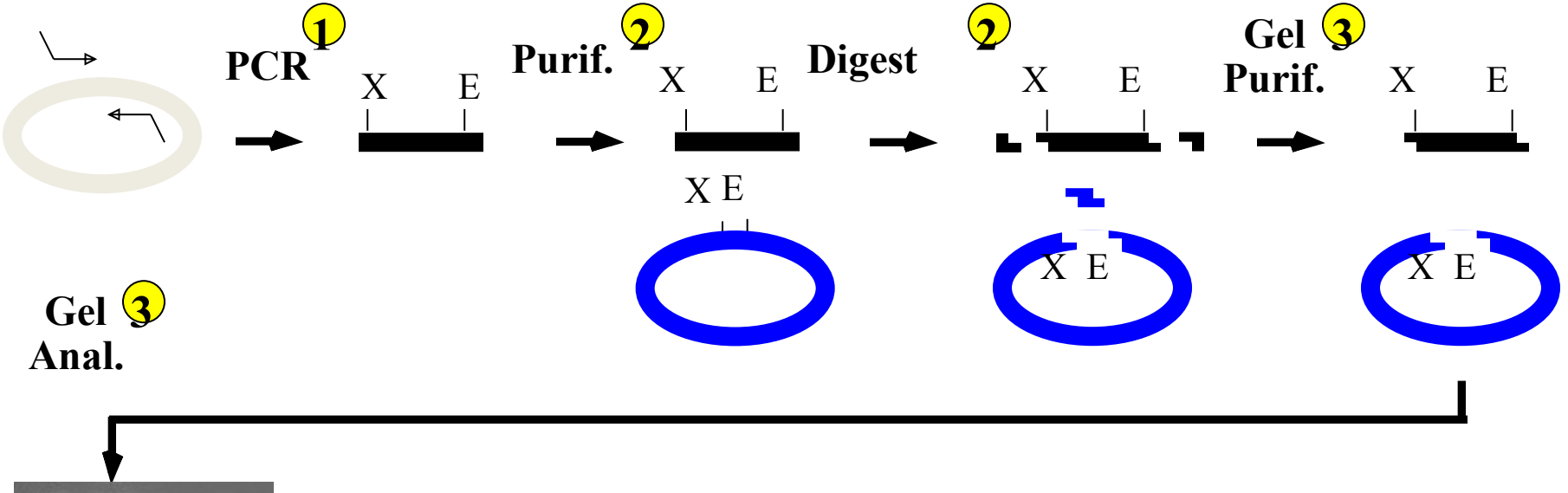
Agarose Gel Electrophoresis

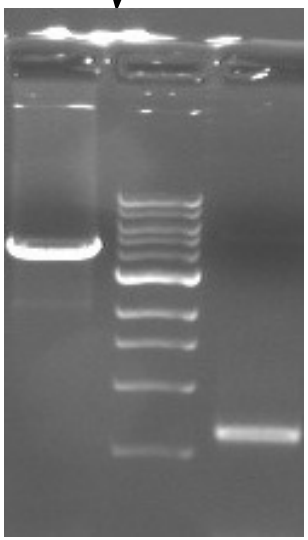
SAFETY NOTES:

- 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

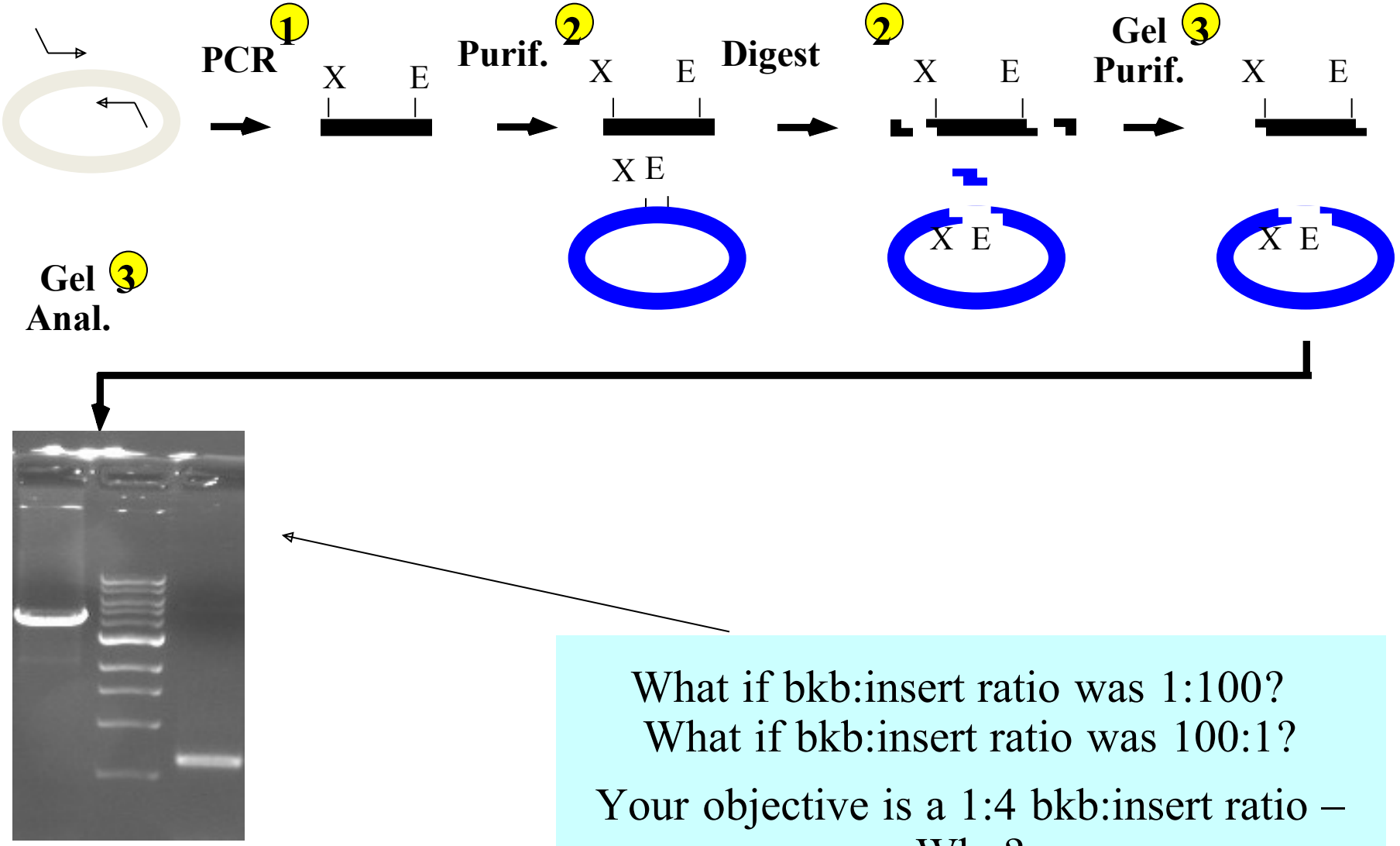




Why run this gel?

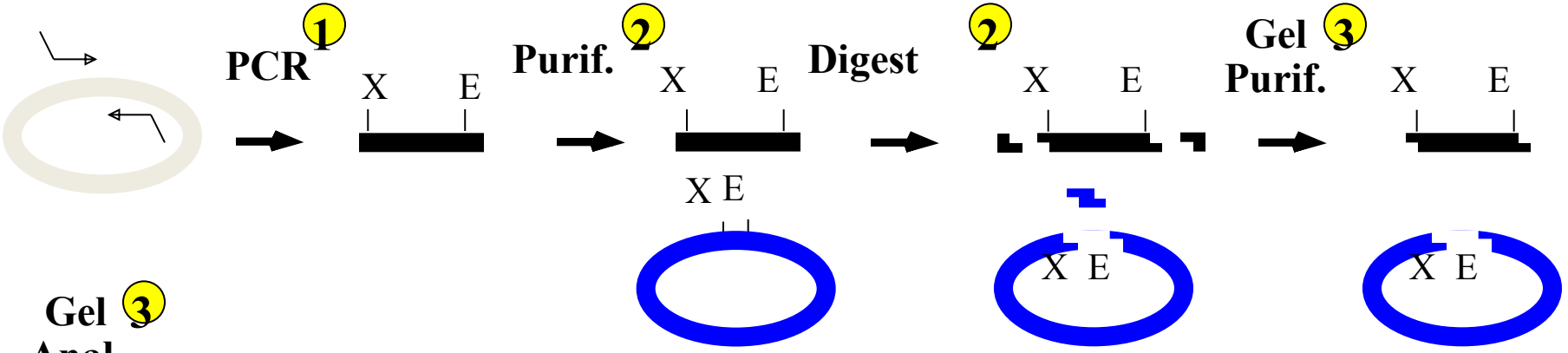
(1) make sure there's DNA

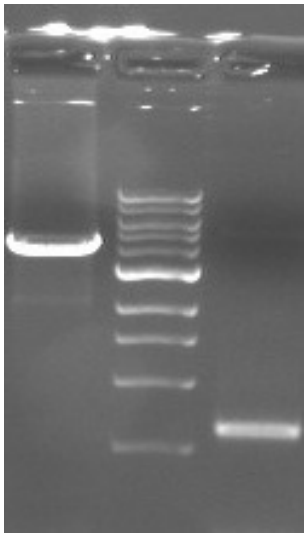
(2) vary ratio



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:

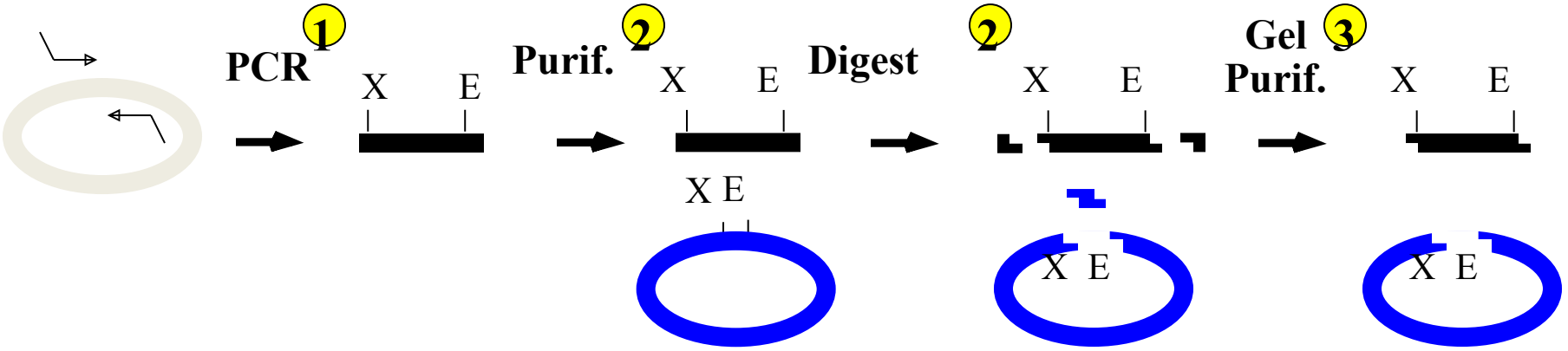
insert:

Plan for today and next week

T Load gel / LR / Excise
Purify
Turn in Yellow sheets.

T Ligate / EHS / Txn

R Miniprep + check / TC.



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