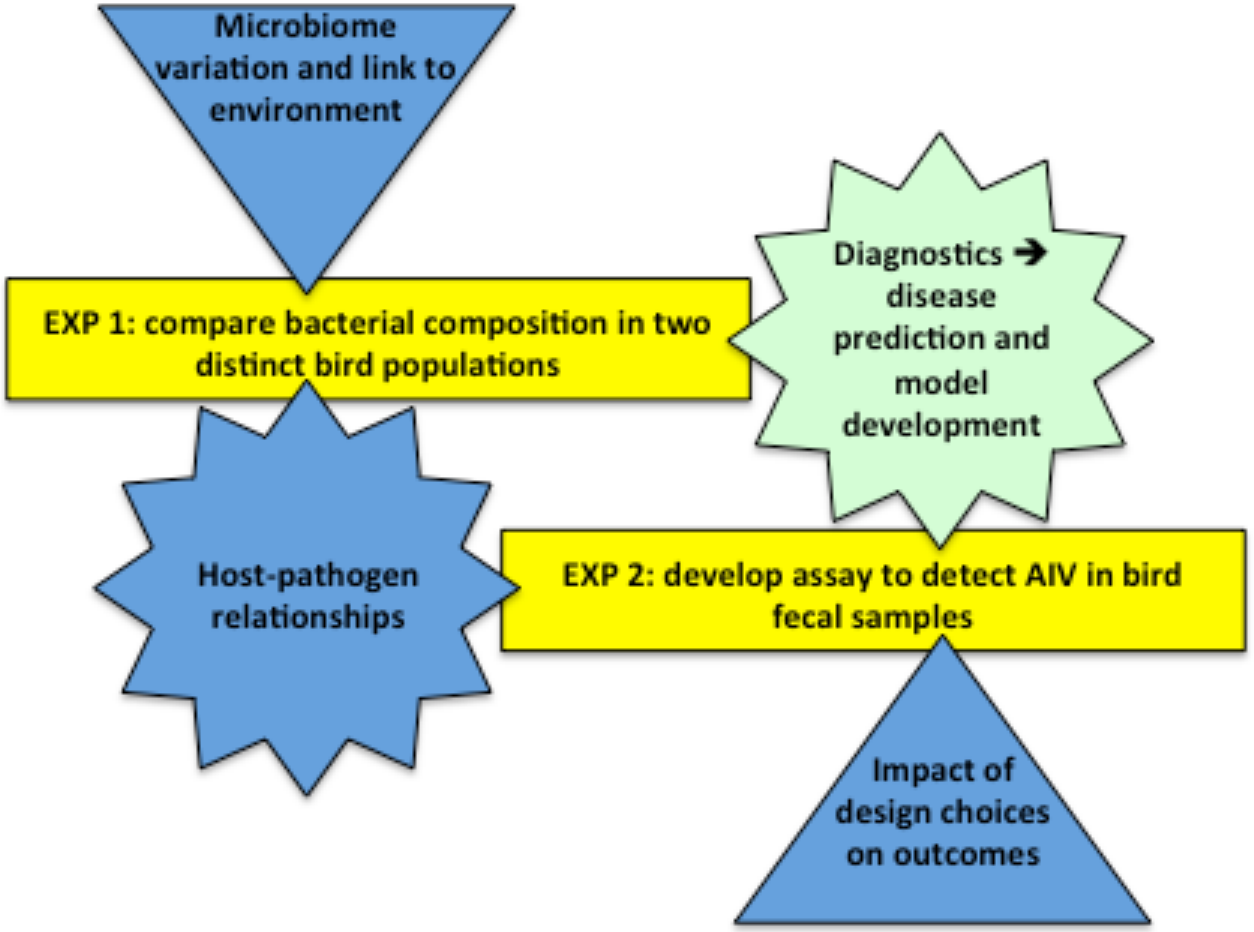
M1D4:
DNA cloning

2/20/15

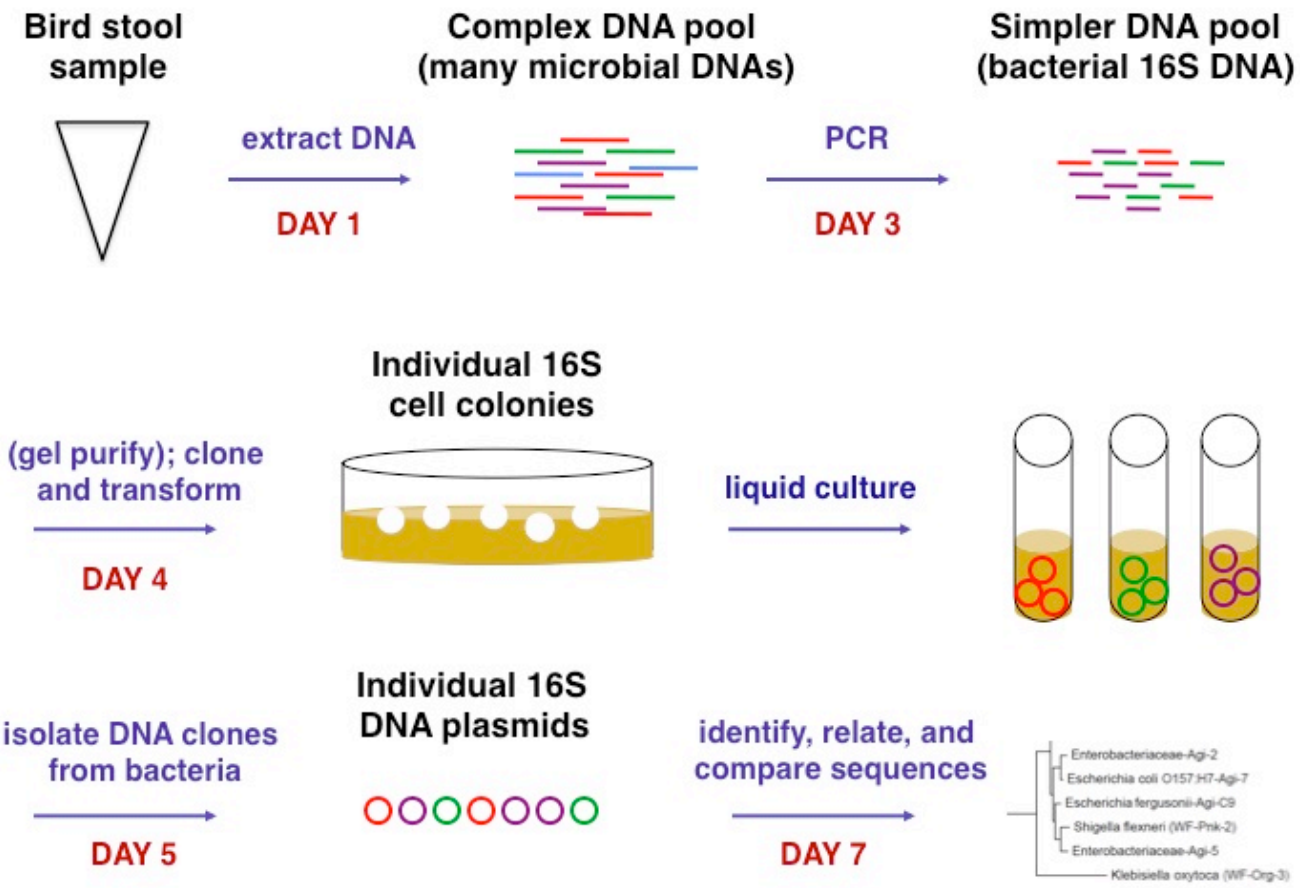
Lab business

1. Lab treat next time…real treats today ☺
   - Feel free to grab a goodie during any downtime
   - Door code =
2. Homework due M1D4 (today)
   - Exp #1 schematic diagram and methods draft
   - Exp #2 primer design table with caption
   - Ligation calculation spreadsheet
3. Notebook entry will be collected M1D8
   - D4, D5, and D7 possible

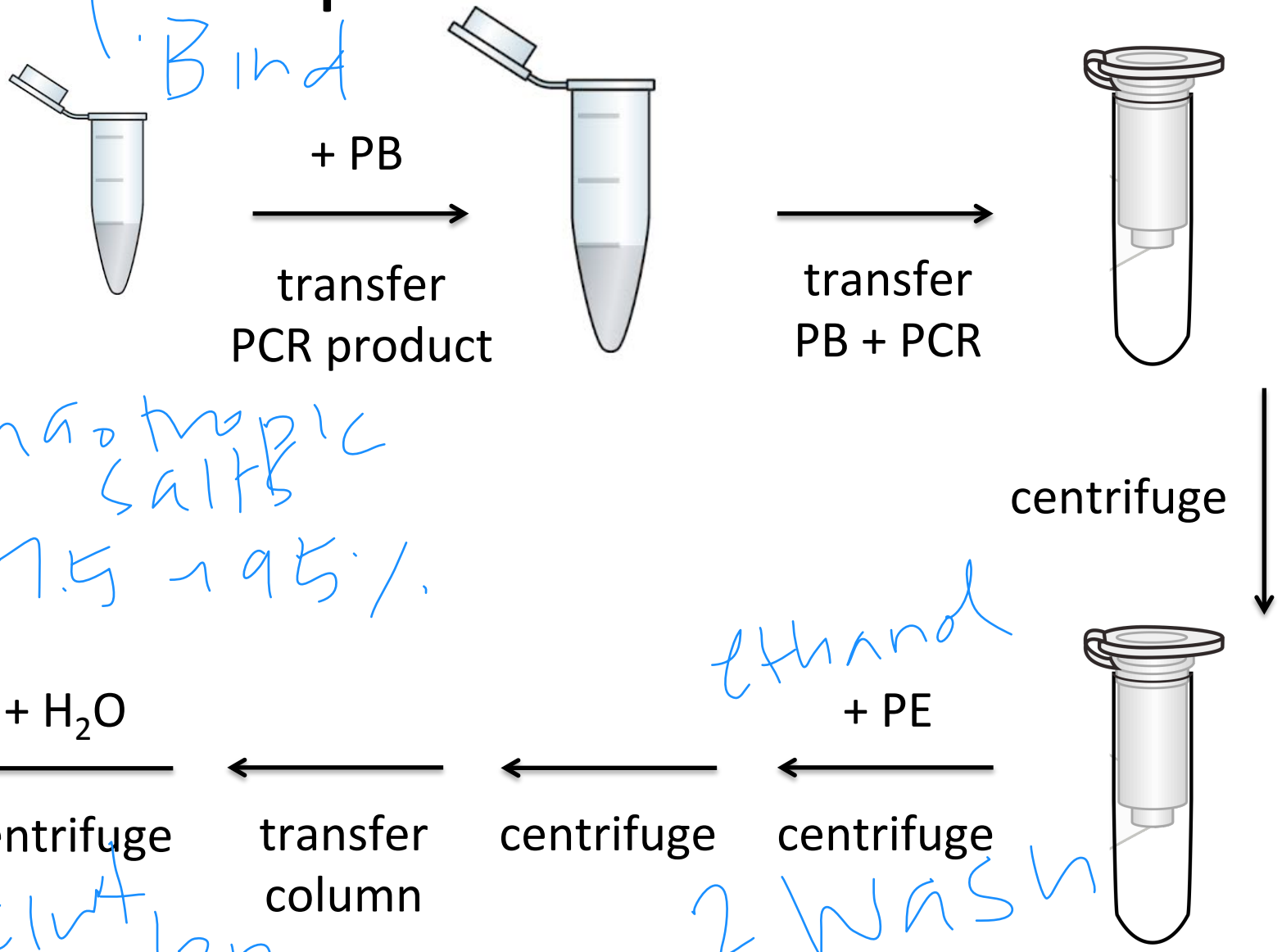
Module 1 conceptual overview



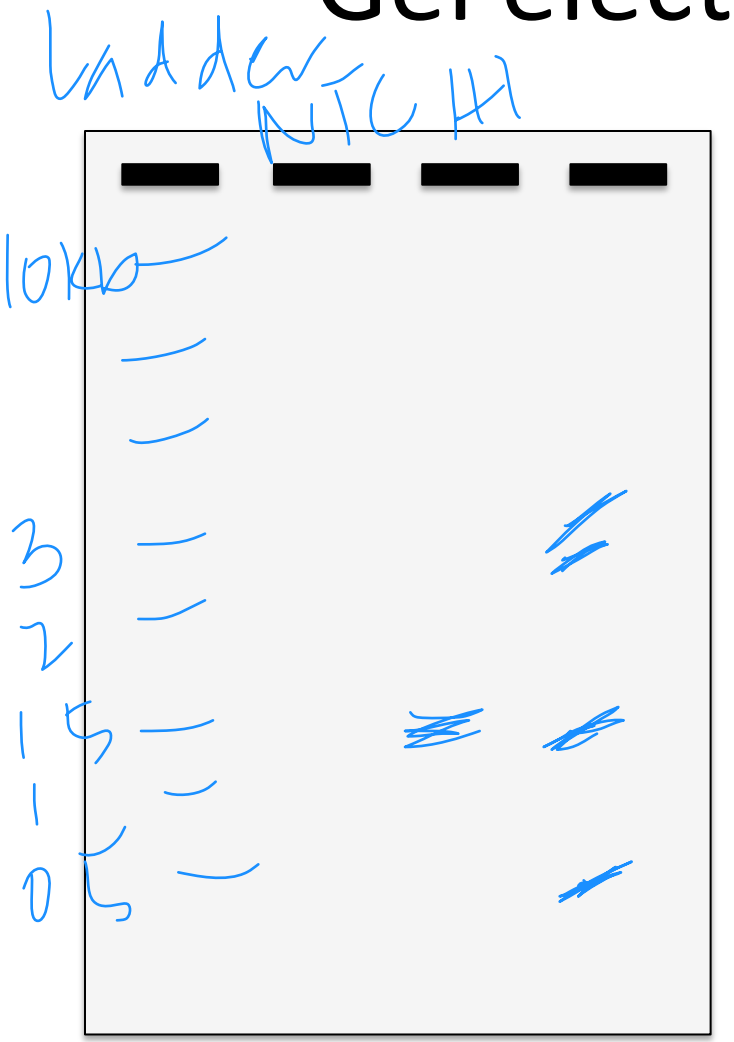
Experimental overview



PCR purification overview



Gel electrophoresis results



You have 16S sequences, now what?

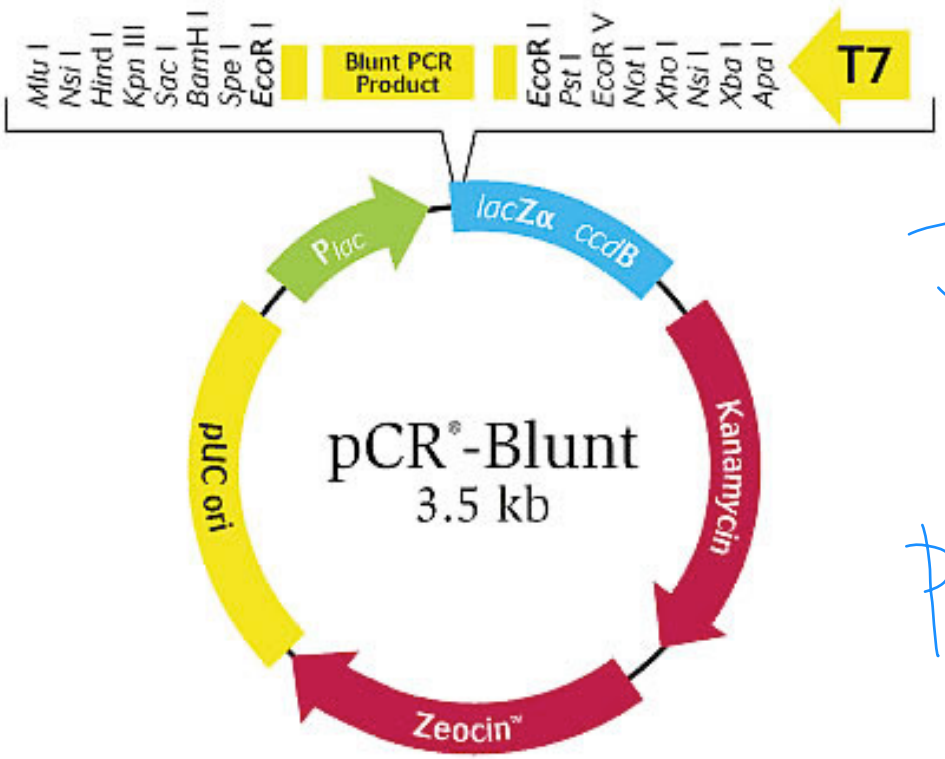
- Cloning with sticky ends

- Cloning with blunt ends

no overhang → T4 ligase
no control over orientation

cloning → separate PCR products

pCR-Blunt cloning system



T7 prom. / P_{lac}

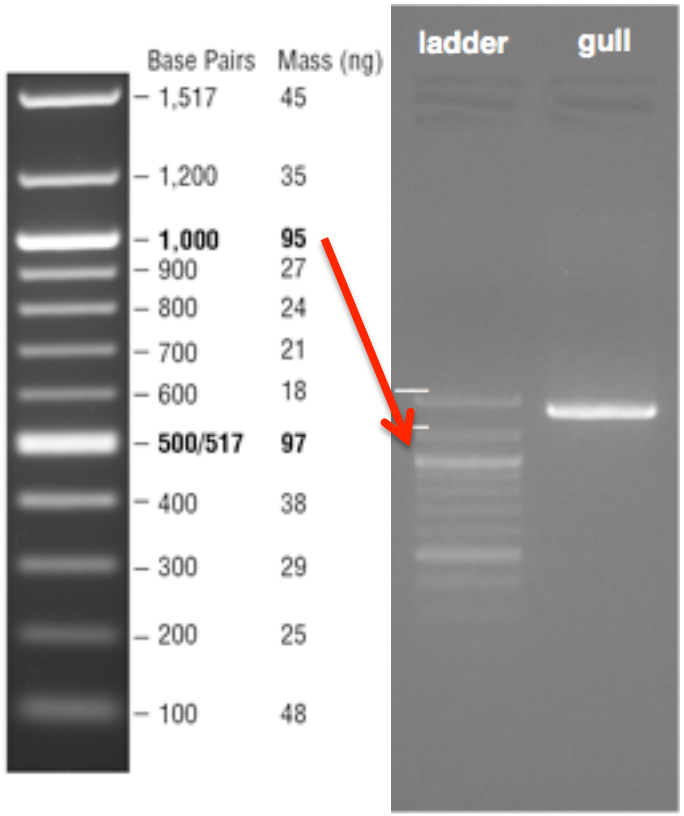
T7 M13R M13F priming

pUC ori

ccdB gene

Kan

Homework calculations

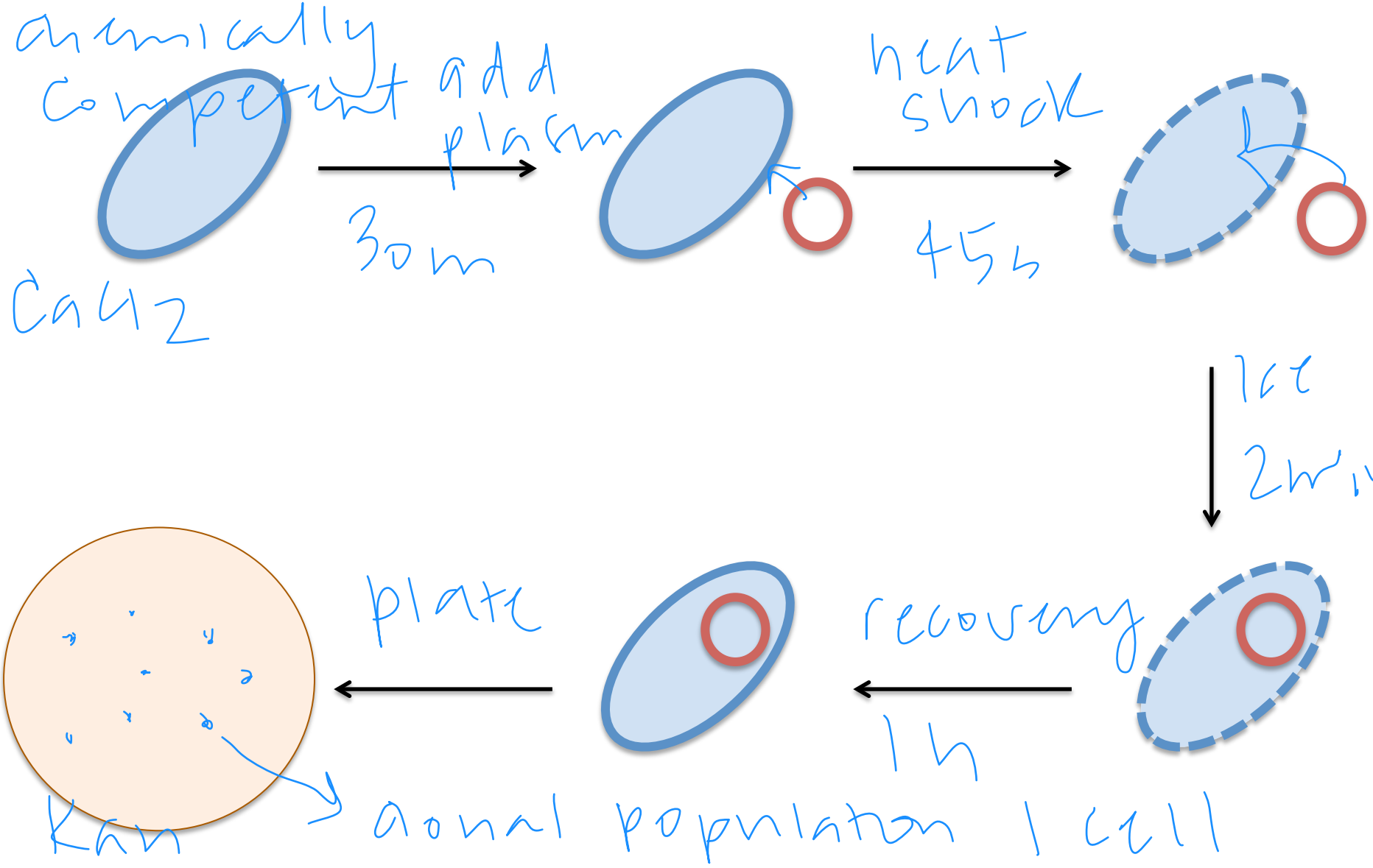


20uL ~~total~~
24uL total

19ng
16.7uL

25ng bks $\frac{1\,mol\ bp}{649\,ng}$ $\frac{10^9\,ng}{1\,mol}$ $\frac{1400\,bp}{325\,bp}$ $\frac{640}{16.7\,uL}$

You cloned your sequences, now what?



Why do we transform cloning products?

Seperate clones

Amplifying cloning product
→ sequencing

Important procedural notes

- Pair up with another group for centrifuge spins
- Use nitrile gloves when handling DNA gel electrophoresis supplies
- Keep the ligase enzyme on ice (only one tube for class)
- Be very careful with competent cells
- Wash your hands before you grab a snack

Today

1. PCR product clean up
2. Gel electrophoresis
3. (Pre)lab discussion
4. Cloning and transformation
5. Finish paper discussion
6. Complete transformation
7. Homework
   - Prepare figure with caption using PCR gel image
   - Choose article for journal club presentation