- Announcements
- Pre-lab Lecture
  - Module 1 review
  - Ligation and cloning
  - Bacterial transformation
  - Today in Lab: M1D4
  - ~~LATER~~: Writing a figure caption

Announcements

- Quiz next time
- Another long(ish) FNT:
  - background/motivation slide(s)
  - first data slide: today's gel (Stellar)
- Notebook due M1D7
  - D4, D5, or D7

bind exp

Homework

add intro text

add topic – change topic order

view all submissions – find submission

General

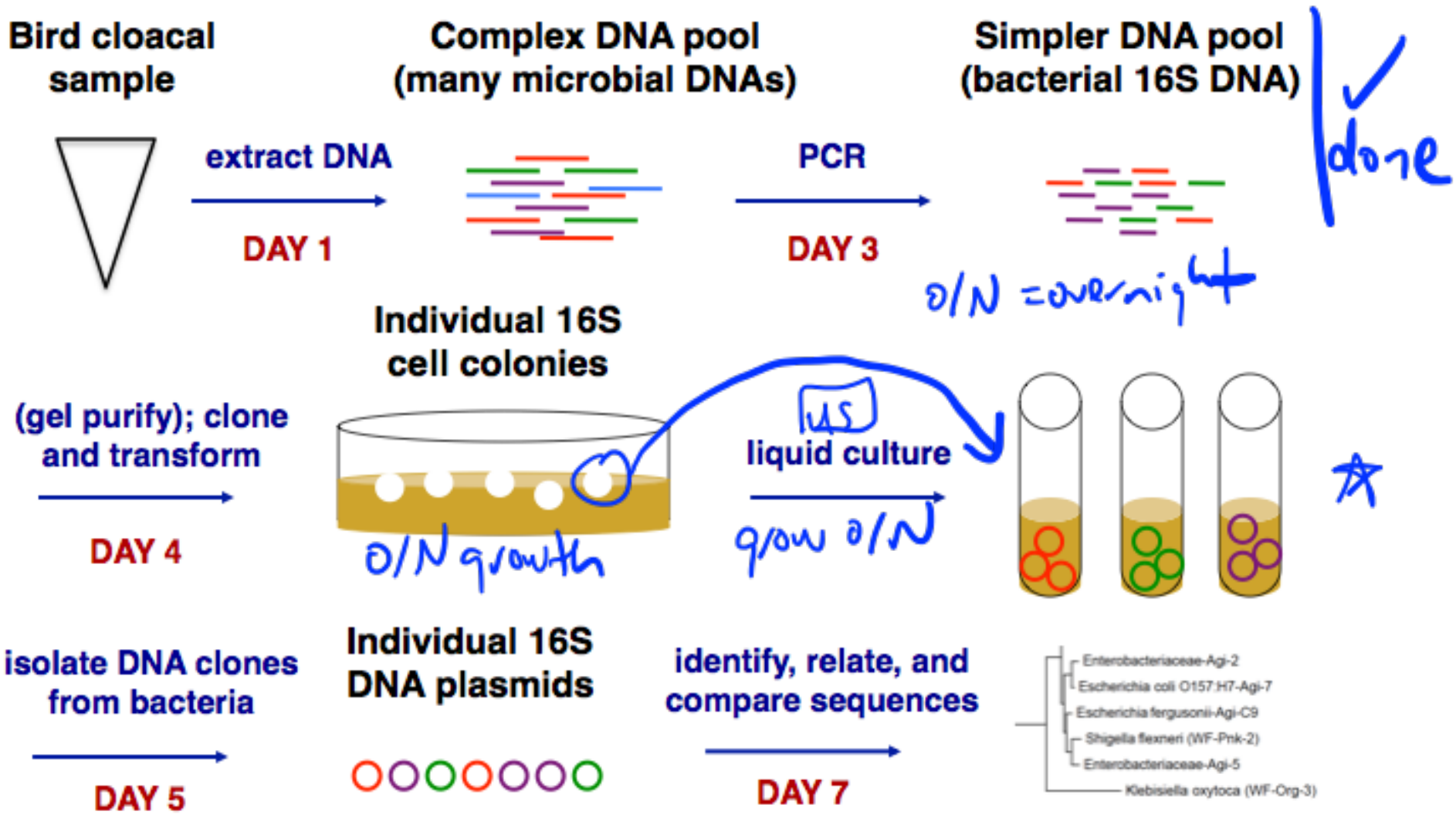
edit topic – delete topic – add assignment

M1D4 FNT TR -- Gel Figure edit – delete
Due 25 February 2014 1:00 p.m. Posted 20 Febr

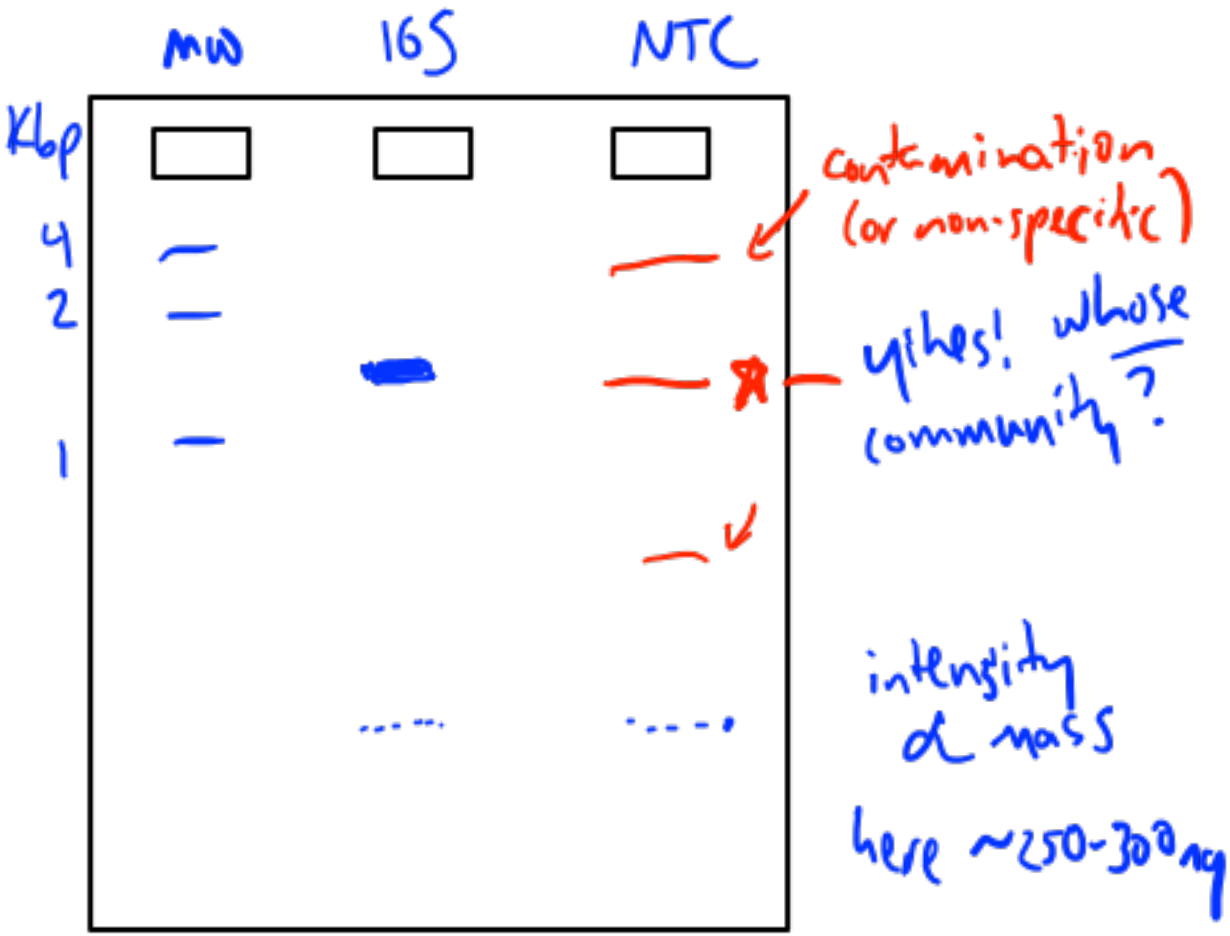
M1D4 FNT WF -- Gel Figure edit – delete

- Journal club coming up in 1 and 2 weeks!
  - sign up for a day (ASAP)
  - sign up for a paper (1 week before your presentation)
- Starting with Marilee presenting about figures/captions
- Then, prep gels – (re)-read M1D4P2 now! – t*hen* pre-lab

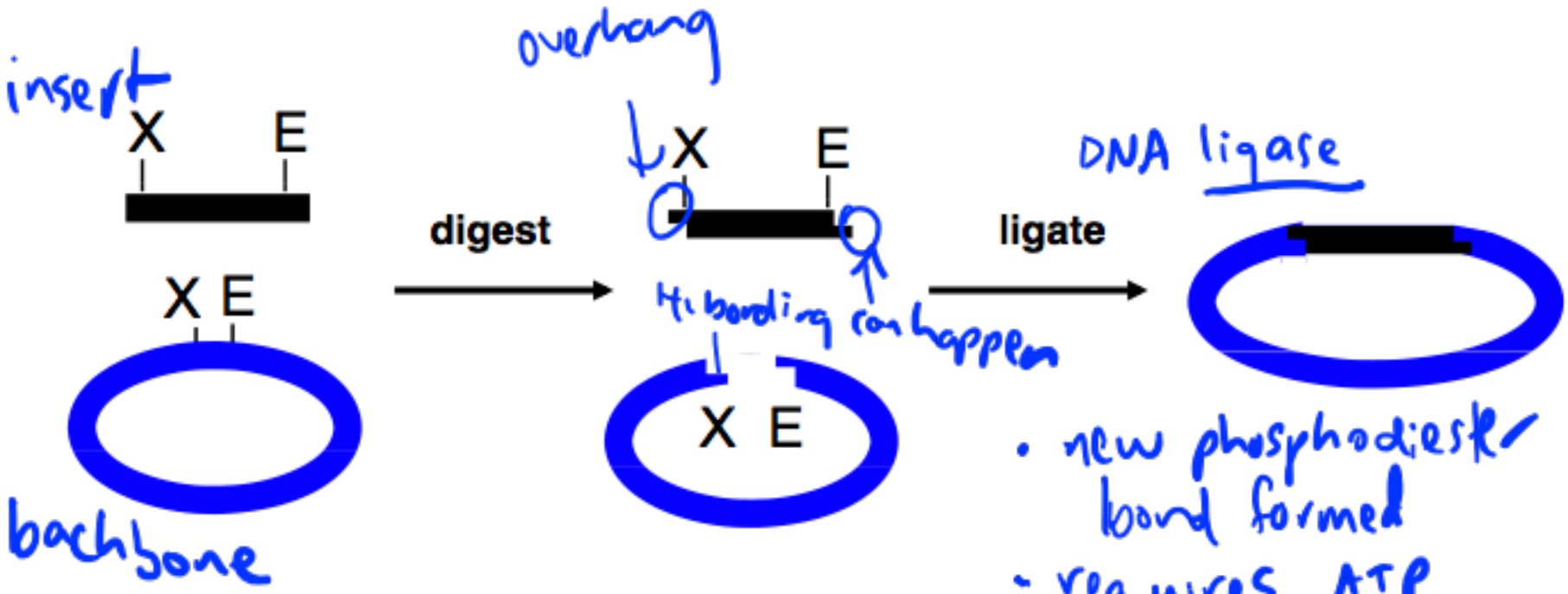
Bird microbial communities: review



Review: DNA EP

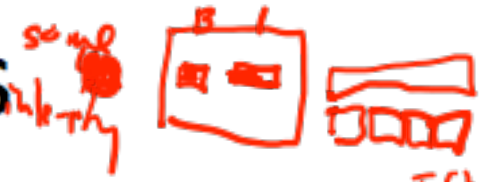


Review: cohesive end restriction cloning



X and E = restriction enzyme sites : recognized and cleaved by special enzymes

Ligation calculations



- insert volume in gel = $\frac{20\,\mu L\ DNA}{24\,\mu L\ total} = 16.7\,\mu L\ DNA$ — F(bp) = 1/4 B(bp)
- associated w/ 25 ng in FNT → 7.5 ng/μL insert
  → figure out today!

goal

- ★ go in your notebk

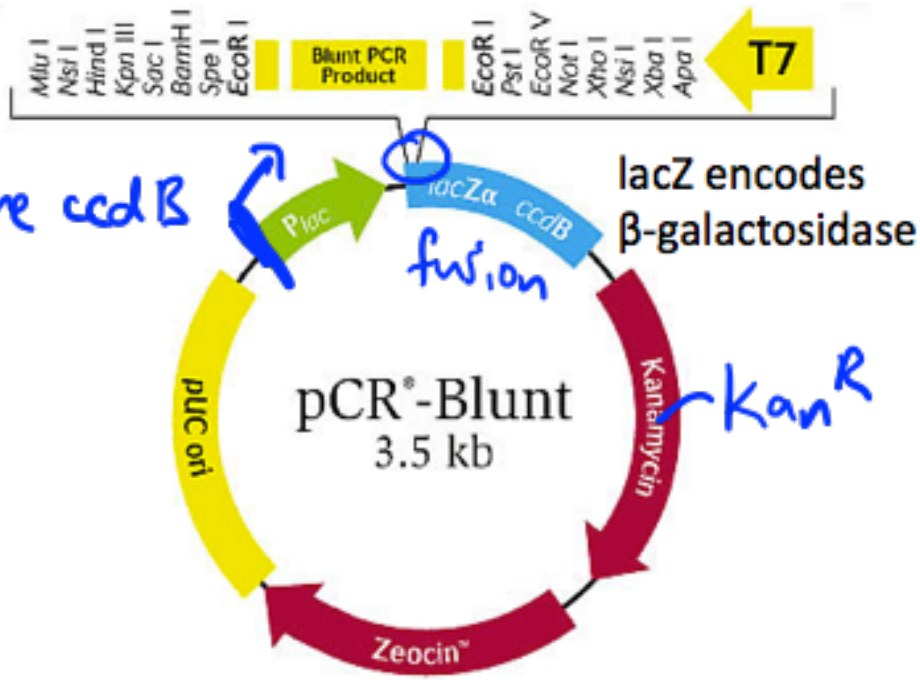
$$25\,ng\ bkb \times \frac{nmol \cdot bp \cdot bkb}{660\,ng} \times \frac{10\,nmol\ ins}{1\,nmol\ bkb} \times \frac{1400\,bp\ ins}{3500\,bp\ bkb} \times \frac{660\,ng}{nmol\ bp\ ins} \times \frac{\mu L\ ins}{7.5\,ng\ ins}$$

→ 13.3 μL insert if ↓ ins 5 μL → 3.75:1 ratio

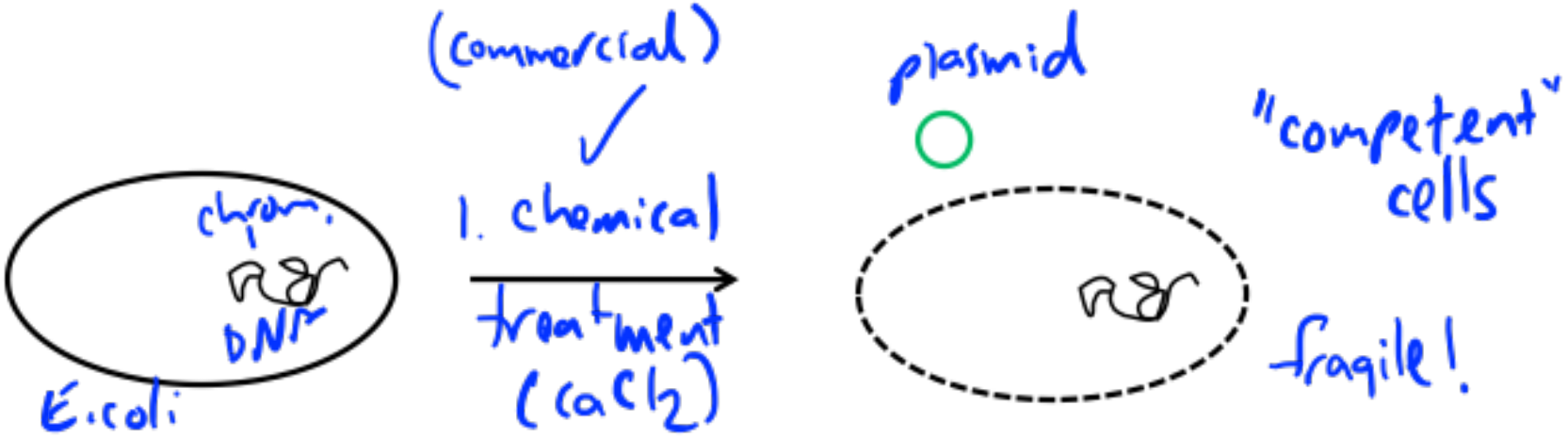
okay to do more steps such as fmoles DNA

Blunt-end ligation & cloning in our system

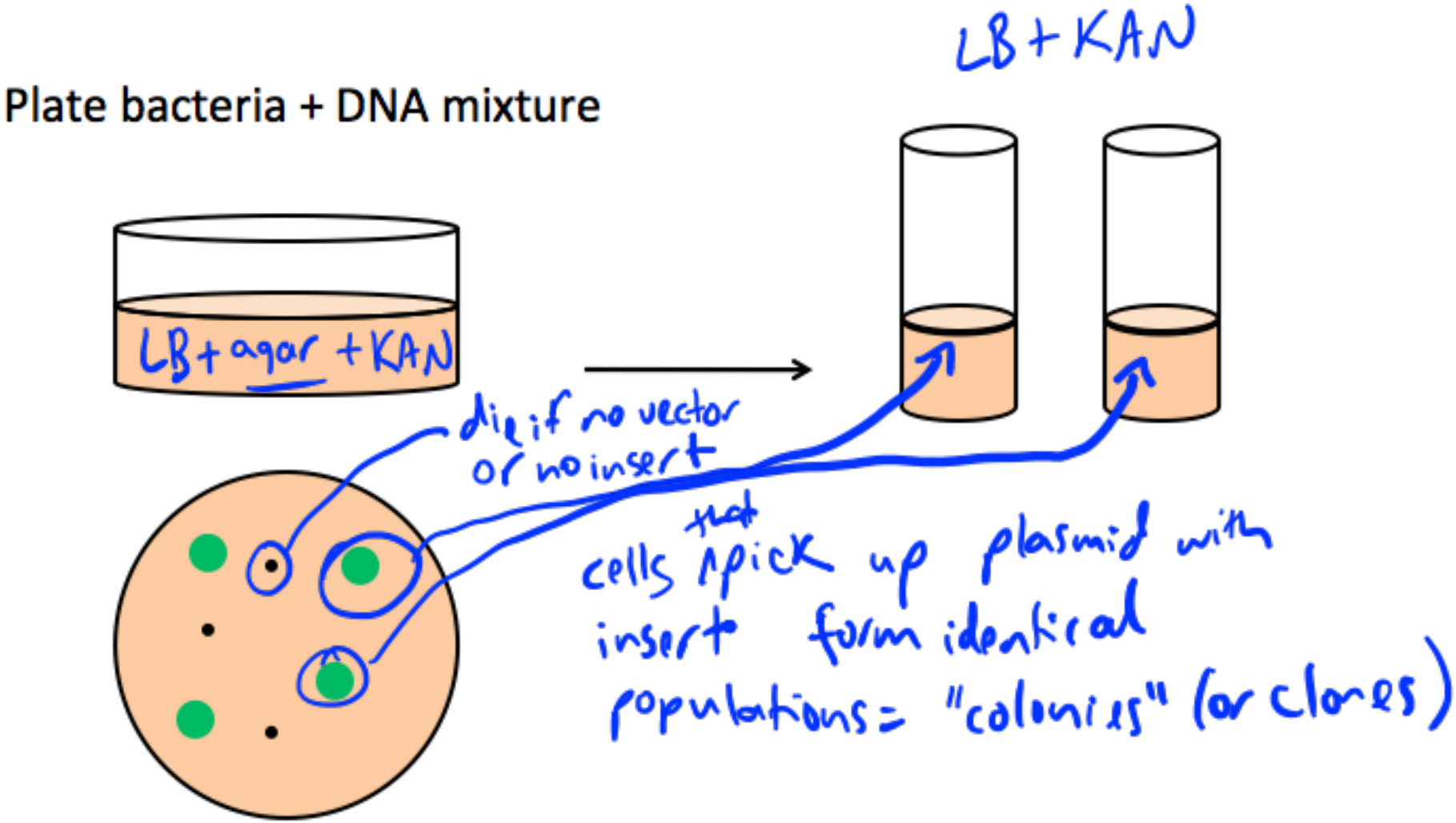
- "Non-directional" cloning
- Two-component selection
  - for intact vector: Kan^R
  - for insert inclusion:
    - disrupt toxin-producing gene ccdB
    - Plac promoter lacZ-ccdB
- Special cell strain:
  lacIq lac repressor lacI



Bacterial transformation



DNA amplification in bacteria



DNA EP: clean-up and safety

- Use nitrile gloves when handling DNA gels and all equipment used for gels.
- Gels and gel-contaminated papers are disposed of in solid chemical waste.
- Wear amber glasses (blue light) or face shields (UV) when cutting DNA bands out of a gel.

Today in Lab (M1D4)

- Part 2B:
  - gel purify IFF multiple products
  - **share** if no product
- Part 3:
  - use **filter tips** for preparing reaction
  - pay attention to **order** of addition
  - be **gentle** with competent cells! → Keep cold don't vortex
- During 1 hr incubation
  - transformation demo
  - *label* tubes for liquid O/N culture (we'll add LB/Kan later)

over for figure discussion

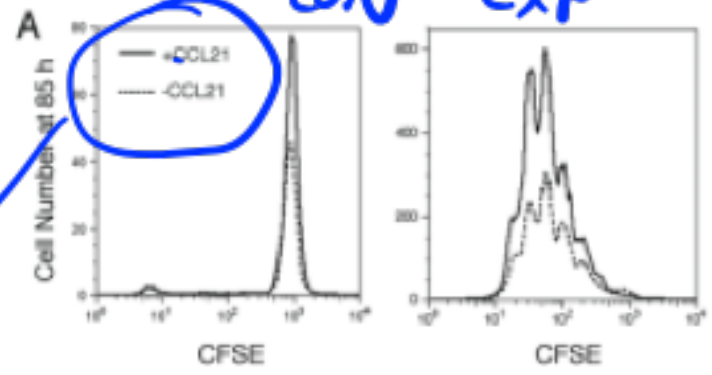
Figures: style and scope

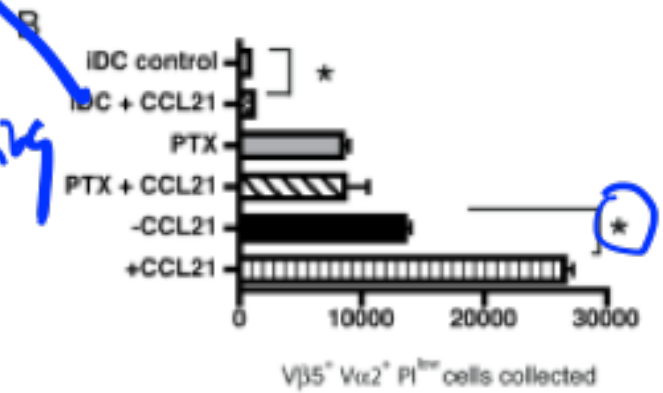
- Title: concise, informative, tells overall goal/result
- Caption: gives context for result from big → small
  - introduce what we are looking at
  - include just enough methods to understand result
  - define all elements (e.g., DNA ladder)
  - cover facts, not interpretation

    e.g., expected size
- Aesthetics: simplicity, clarity → at-a-glance

  labeling (e.g., some ladder band sizes)

Figures: example





con exp ← etc.

Co-culture labeling

title states result won't over-interpret

* defined in caption

Figure 3 **CCL21 impacts naïve T cell proliferation under conditions of rare Ag-specific T-DC encounters**. Co-cultures comprising 9% OVA-specific OT-II CD4+ T cells, 81% C57Bl/6 CD4+ T cells, 5% OVA-mDC and 5% iDC with/without CCL21 were analyzed by flow cytometry at 85 h. (A) Sample CFSE histograms are shown for control (left, iDC only) and experimental (right, with OVA-mDC) conditions. (B) OTII cell recovery for all conditions is shown. Ave ± std. dev. for 3 wells per condition. [* indicates bracketed conditions statistically different ($p \leq 0.05$)] (A-B) are from 1 representative of 5 experiments.

} expt'l overview

} figure walkthrough