M1D3:
PCR and paper discussion

2/13/15

Lab business

1. Lab treat…

2. Homework due M1D3 (today)
   - Sign-up for Journal Club presentation date
3. Homework assignment due M1D4 is long
   - Carryover due to snowstorm
   - Monday is a holiday (no class T or W)

Homework due M1D4

- Experiment #1 (gull microbiome)
  - Schematic diagram to be included in your Microbiome Abstract and Data Summary
  - Methods section draft of DNA purification and PCR
- Experiment #2 (AIV detection)
  - Primer sequences submitted on M1D2 Talk page
  - Table including your primer sequences and design details to be included in your Primer Design Memo
- Spreadsheet for M1D4 ligation calculation

Input [DNA] → output volume

Writing your methods section

Materials and Methods

The methods section should allow an independent investigator to repeat any of your experiments. Use sub-section headings to allow researchers to quickly identify experiment of interest to them (e.g. “Protein conjugation to hydrogels” or Cell culture and fluorescent labeling”). When commercially available kits were used, it is sufficient to cite the name of the kit and say that it was used according to the manufacturer’s protocol. The key to a good methods section is developing your judgment for what information is essential and what is extraneous.

Note that the methods section should be written in the past tense, since your experiments are already complete at the time you are writing your paper. This section should also be written in complete sentences and paragraphs, not in bullet point form.

subsection headers

* PCR → Amp. of bacterial 16S rRNA

** no lists → generalizable → no volumes → use Molar

use logical order

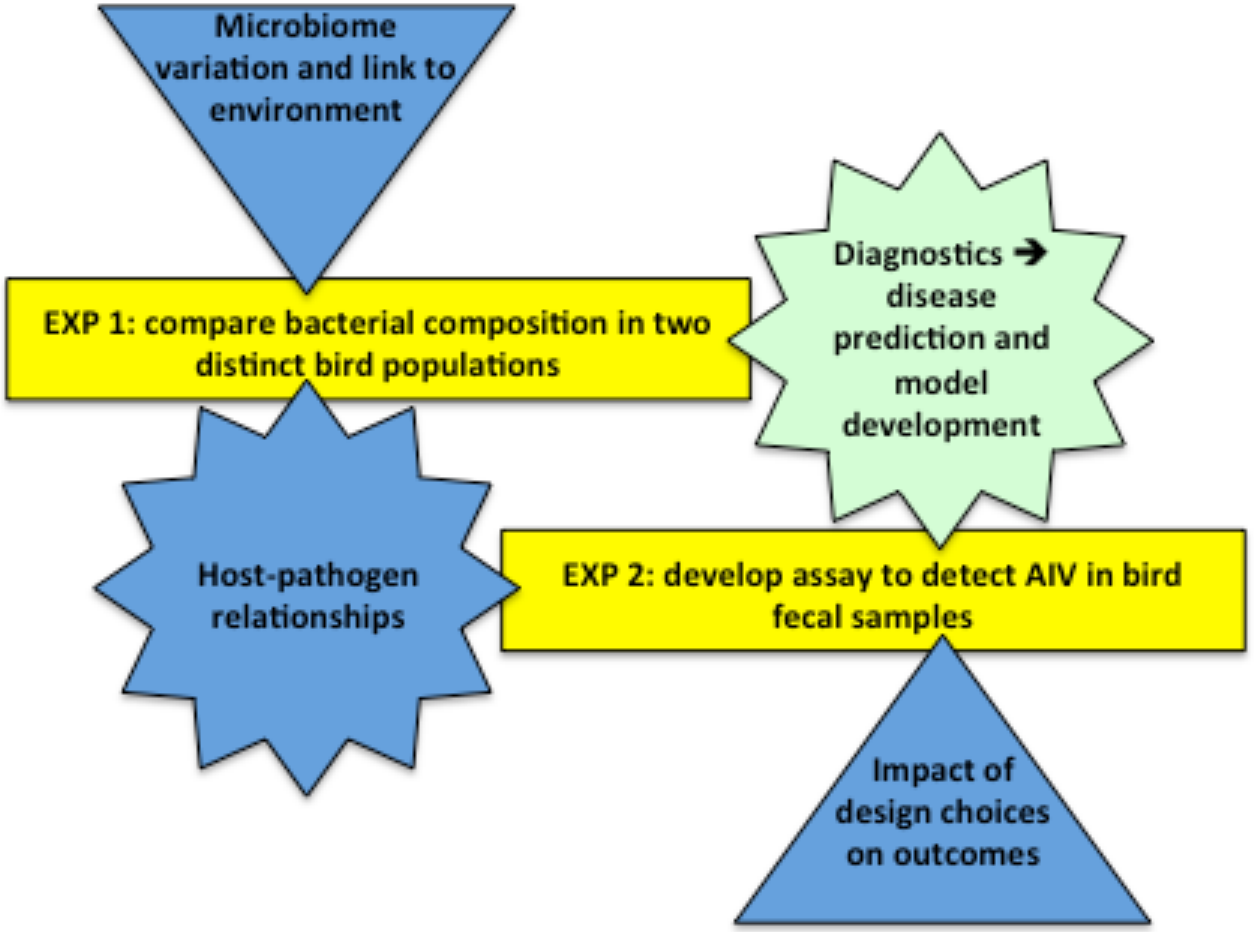
Who log Speak

Let's practice!

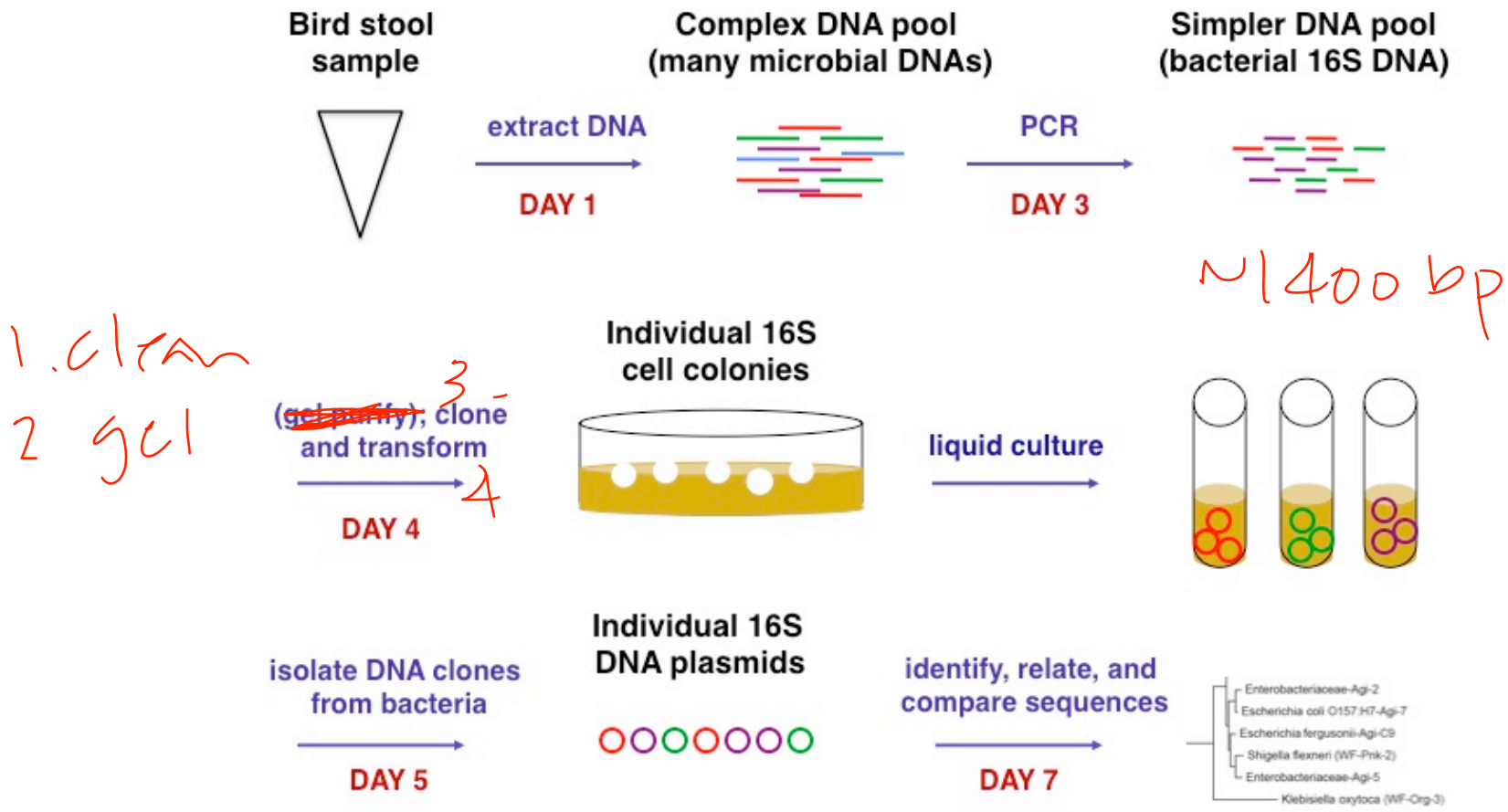
"Template DNA (5ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube.

Water was added to 50 uL. A tube without template was prepared and labeled control."

Module 1 conceptual overview



Experimental overview

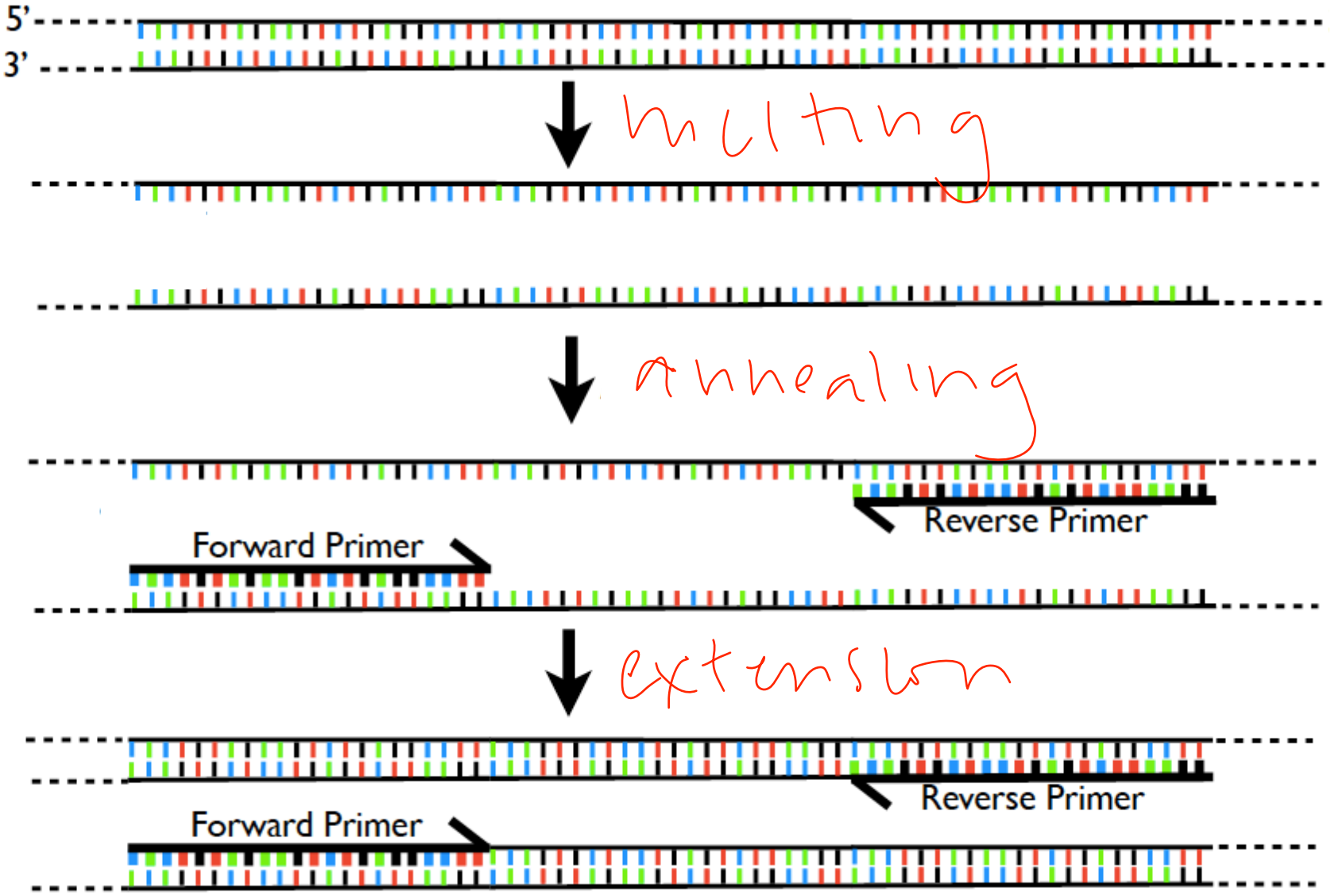


Why amplify the 16S sequence?

highly conserved in bacteria

PCR gives lots of product

Review of PCR

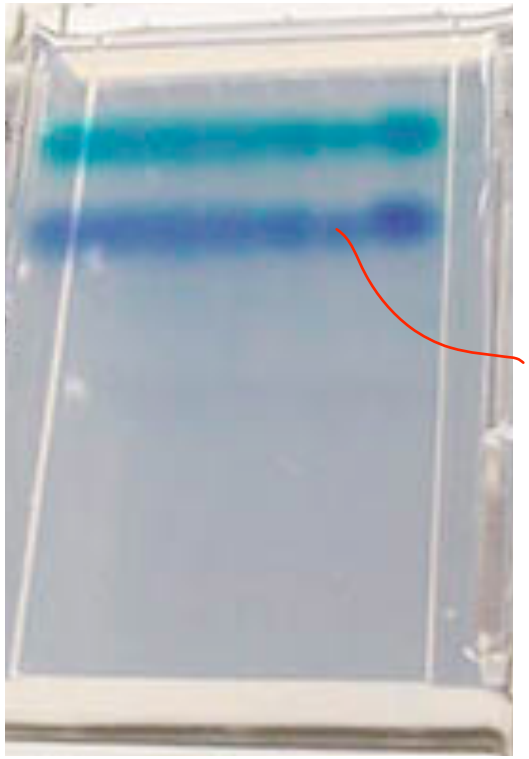


Temperature cycles for PCR

Reagents for PCR

Component	Purpose
dNTPs	bricks
template	original copy
polymerase Pfu high fidelity hotstart	catalyzes rxn
buffer Mg BSA	sets chemical environment
primers	initiates transcription

How do we visualize PCR products?



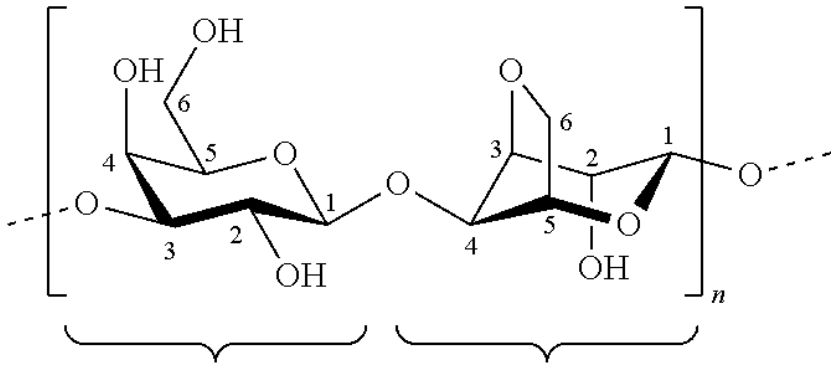
loading buffer
dye
glycerol

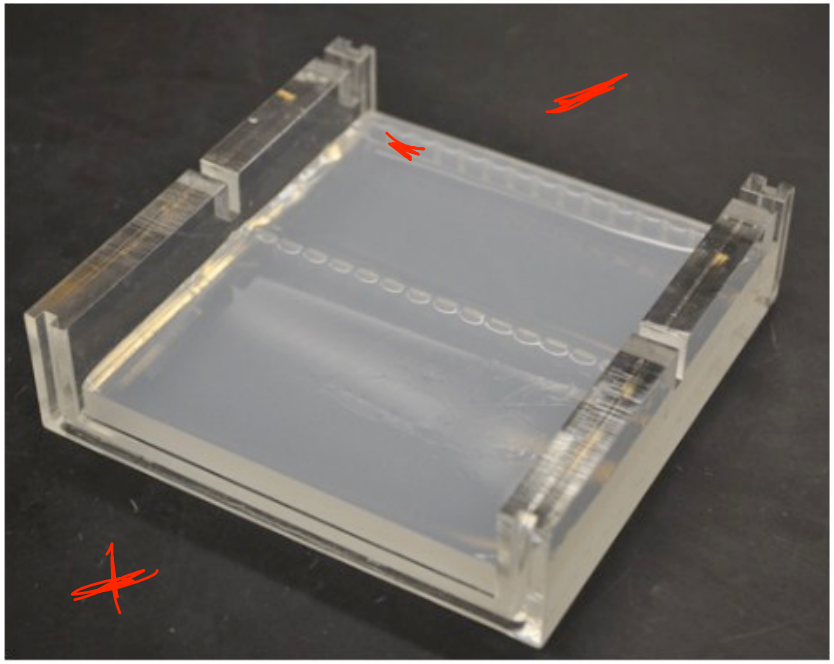
bromo phenol blue

stain

SybrSafe

Gel electrophoresis





- Driving force for separation is
  angle
- DNA moves − to + because of
  neg charge phos backbone
- Separation is based on
  size

Why do we visualize PCR products?

If we have product

tells it correct product is present

presence of secondary product

Important procedural notes

- Keep everything on ice
- Label your PCR tube (do not use a sticker)
- Use filtered pipet tips
- Be careful not to contaminate your reaction
  - Remember: our target sequence is found in all bacteria
- Aliquots are for two groups (6 reactions)
  - Spin down before use

Today

1. Prepare PCR (3 reactions per group)
   - 1 no template control per group
   - 1 reaction with template per person
2. Atissa will join us to discuss the art of giving great presentations
3. Figure presentations and paper discussion
4. Homework
   - For questions, email or come to office hours on R