M1D2:
Diagnostic primer design

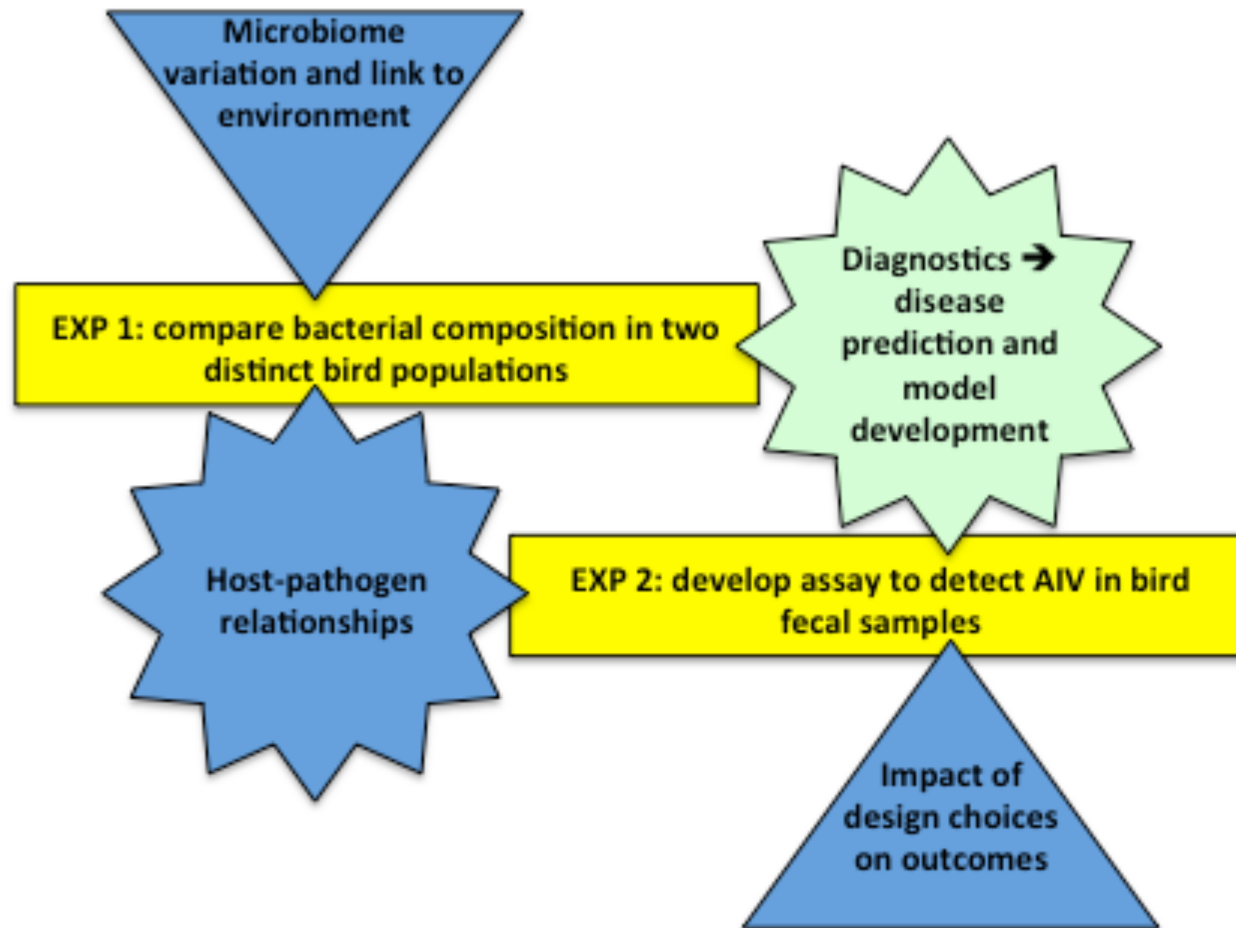
2/11/15

Lab business

- Lab treat next time (M1D3)
  - Content will be from lecture (M1D1) and pre-labs (M1D1 and M1D2)
  - All quiz dates posted on Schedule page of wiki
- Homework due M1D2 (today)
  - Leave wiki page printout on front bench
- Office hours begin tomorrow
  - Noreen: M 2-4p, R 2-4p, and by appointment
  - Leslie: M 1-2p, R 1-2p, and by appointment

14-429b

Module 1 conceptual overview



AIV screening project

- Primary research question?

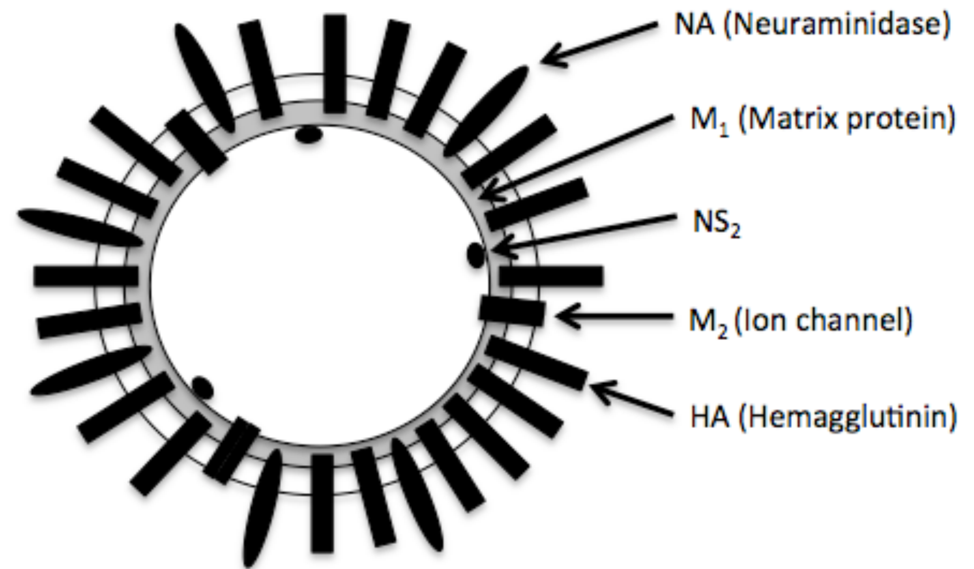
Can we improve the detection of AIV in gulls?

- Broader impact?

Improving the screen better predict disease model development

How will you develop your AIV screen?

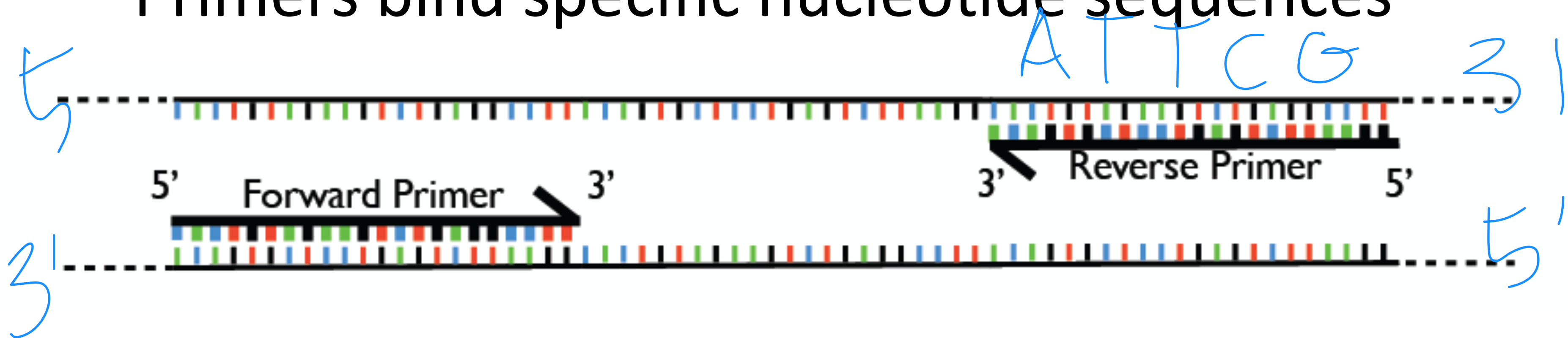
- NA and HA are variable and used to define AIV subtypes
- M1 (MA) is highly conserved



- Which is a better target for screening bird samples for AIV?

How will we target M1?

- Primers bind specific nucleotide sequences



- Forward primer binds the α-sense strand

- Reverse primer binds sense strand

make sure to use reverse complement

Primer sensitivity

- In diagnostic assays, sensitivity is important for determining presence/absence
  - Some false positives okay, but want as few as possible
- Specificity is important in identifying strain or subtype information
- Is your goal to increase sensitivity or specificity?

Primer design considerations

1. Length
   >17 bp
   10 bp $4^{10} = 10^4$ $3 \cdot 10^9$ bp
2. Melting temperature
   55-60C 58C ± 3
   Tm = 50% bound/unbound
3. GC content
   ↑ GC ↑ Tm GC clamp 3'
   40-50%

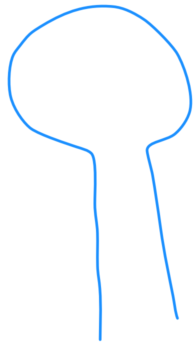
Primer design considerations

5. Long repeats

nonspecific binding

T stretches → polyA tails

6. Secondary structure



energetically favorable

Primer design considerations

6. Primer dimers

homodimers
heterodimers

OligoAnalyzer 3.1

Instructions | Definitions | Feedback

Sequence — # Bases 21
5'- CCTCTCCGGAACCAAACCCTG

Target Type DNA
Oligo Conc 0.25 µM
Na^+ Conc 50 mM
Mg^{++} Conc 0 mM

Analyze | Hairpin | Self-Dimer | Hetero-Dimer | NCBI Blast | TM Mismatch

Clear Sequence

Results | 5' mods | Internal Mods | 3'

HOMO-DIMER ANALYSIS ?

Dimer Sequence
5'- CCTCTCCGGAACCAAACCCTG -3'

Maximum Delta G -43.97 kcal/mole

Delta G -12.9 kcal/mole
Base Pairs 6

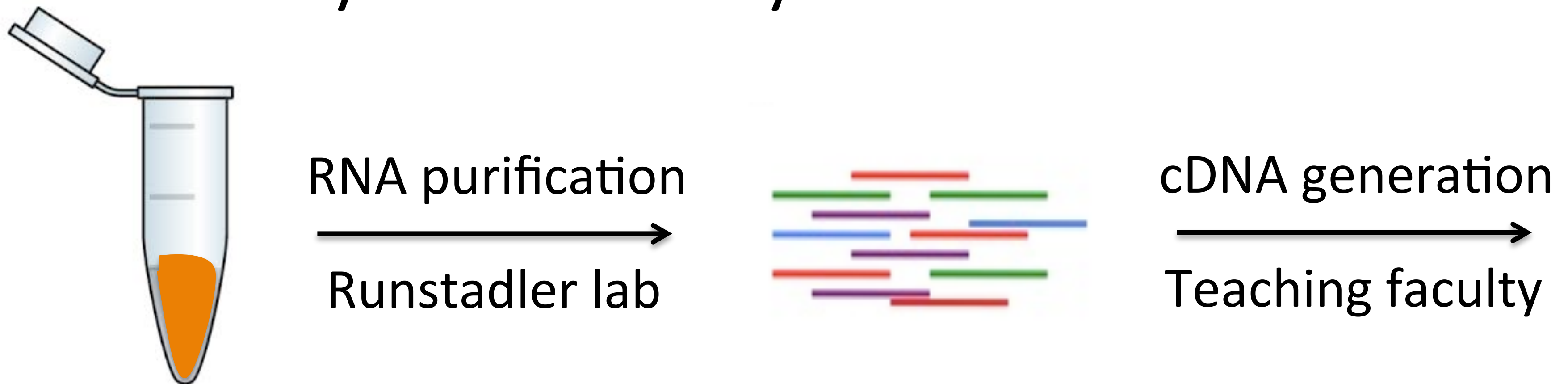
IDT Website: "You can also compare the value of the maximum delta g (the delta g for a perfect duplex) to that of each individual self dimer. If the values are within 10% of each other, you should redesign. Heterodimer analysis works the same way."

You have your primers, now what?

- You will screen fecal samples collected from ‘scallop pile’ in Nantucket

Experimental overview

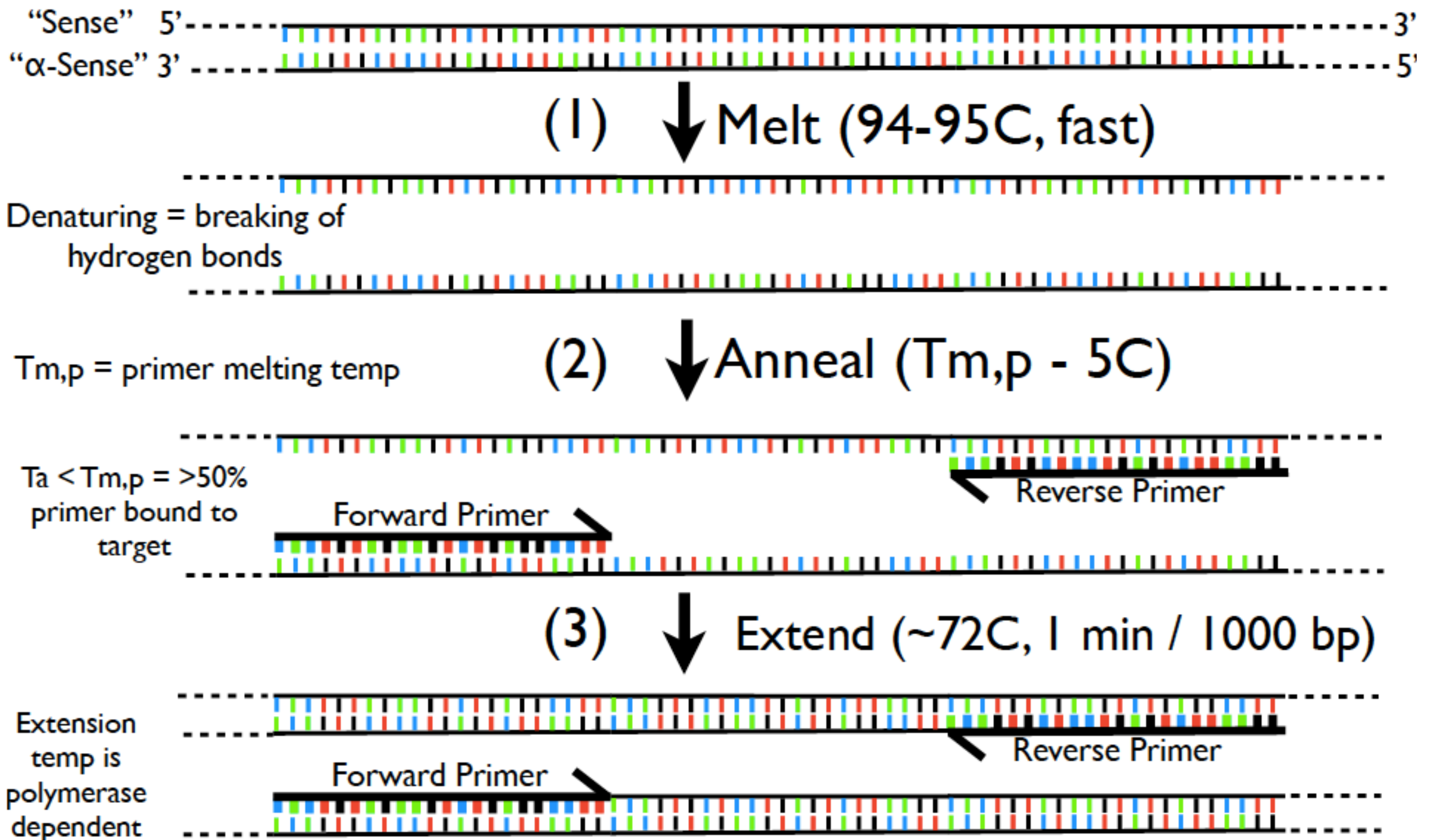
- While you were away...



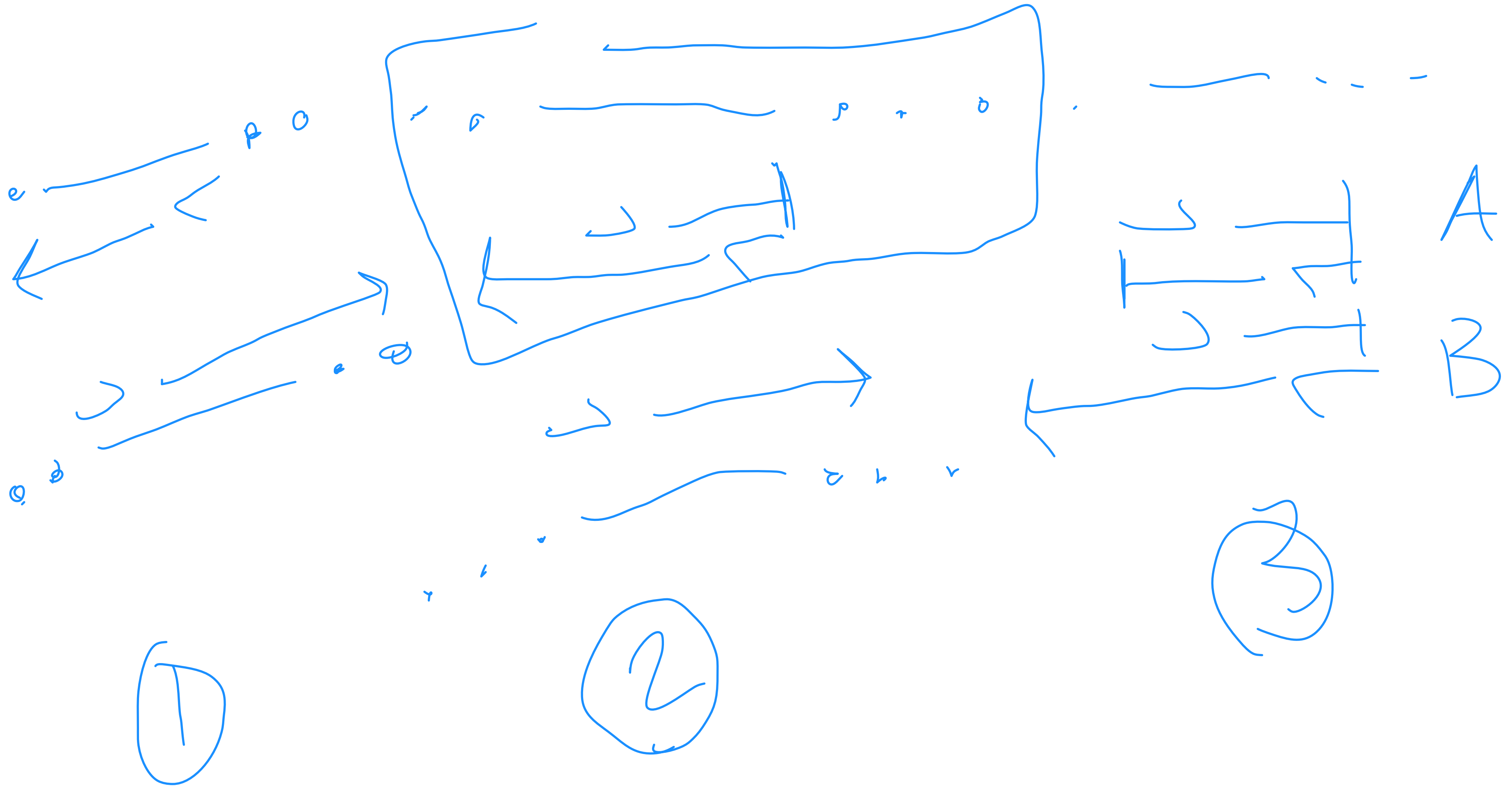
- Your part...

Screen cDNA pool using your primers.

Polymerase chain reaction (PCR)



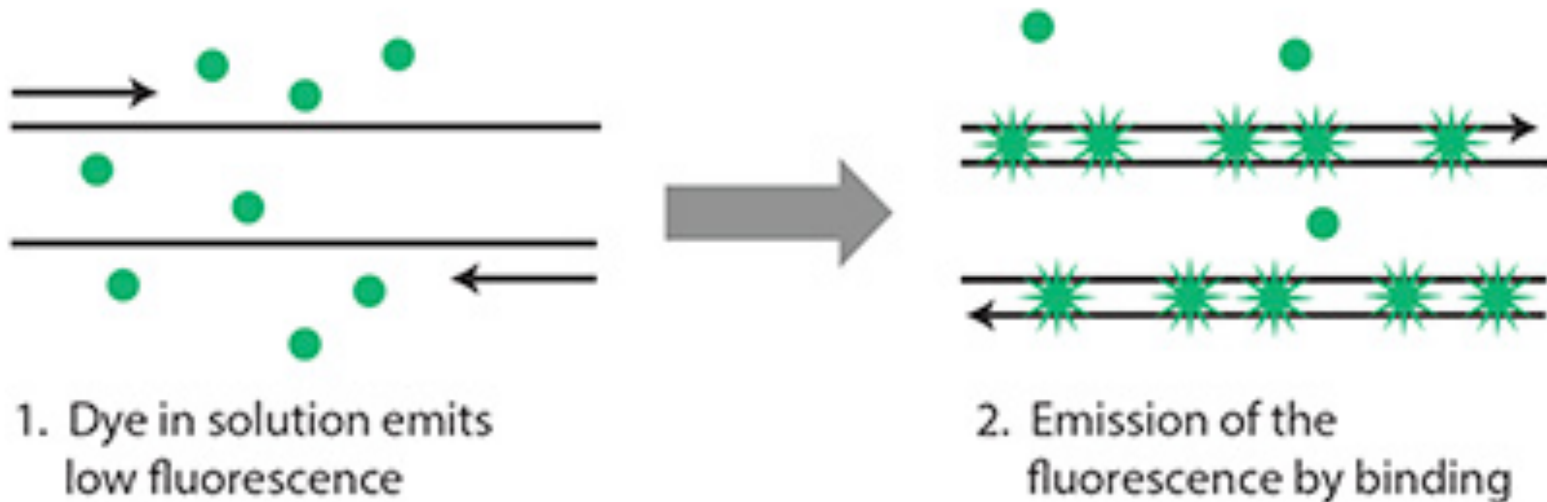
Draw the first three cycles



In which cycle do you first see your amplicon?

Quantitative PCR

- SYBR green dye binds double stranded DNA
- Amount of fluorescence is proportional to the amount of PCR product



PCR versus qPCR

- Utility of PCR?

cloning

- Utility of qPCR?

Today

- Explore current AIV primers
- Develop your AIV screening assay
  - Specifically, you will design your primers
  - Enter sequences on M1D2 Talk page
- Homework
  - Prepare for paper discussion
  - Discuss your primer design choices (by M1D4)
  - Post final primer design (by M1D4)
  - Choose journal article presentation day