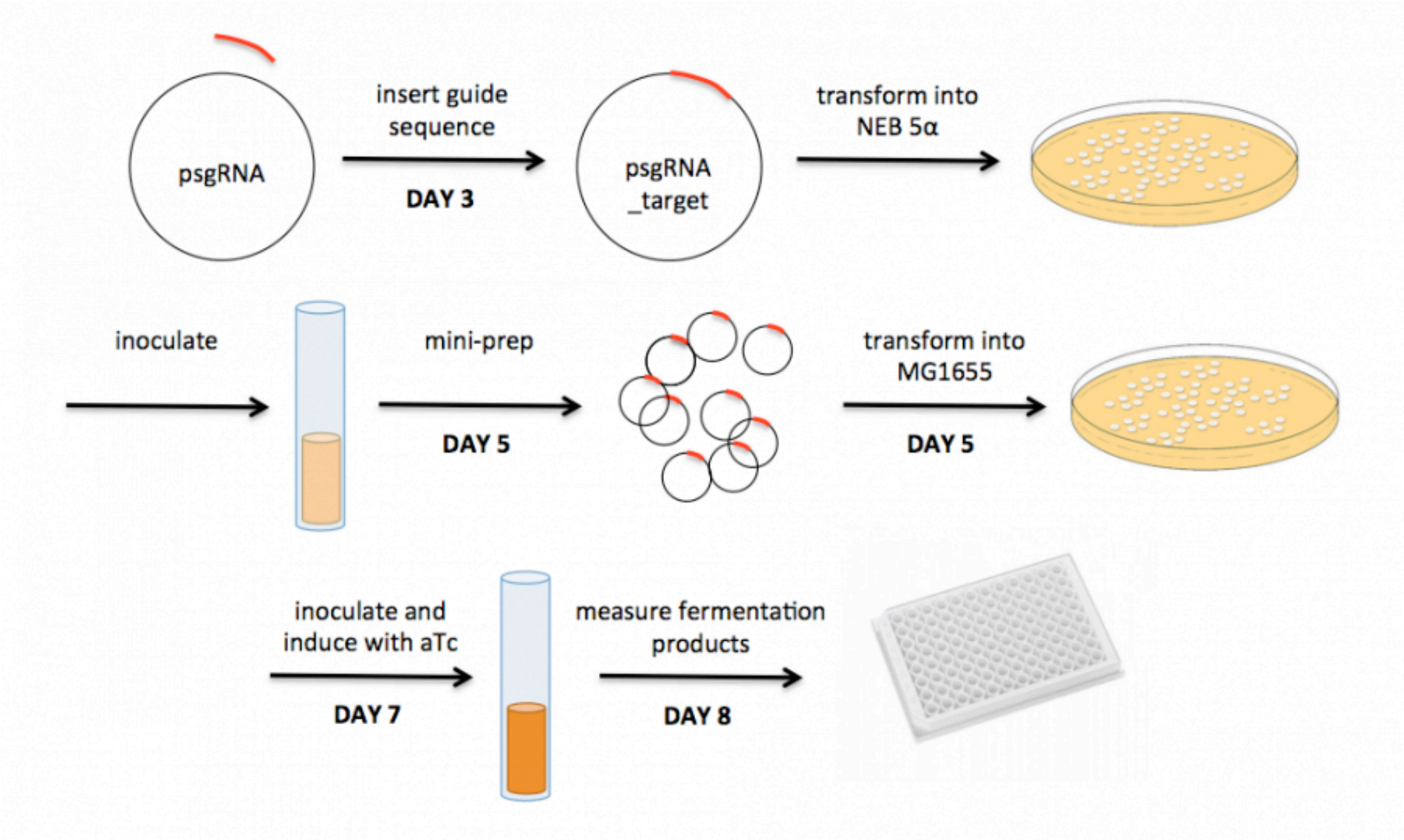
M2D3: Generate gRNA plasmid

10/20/16

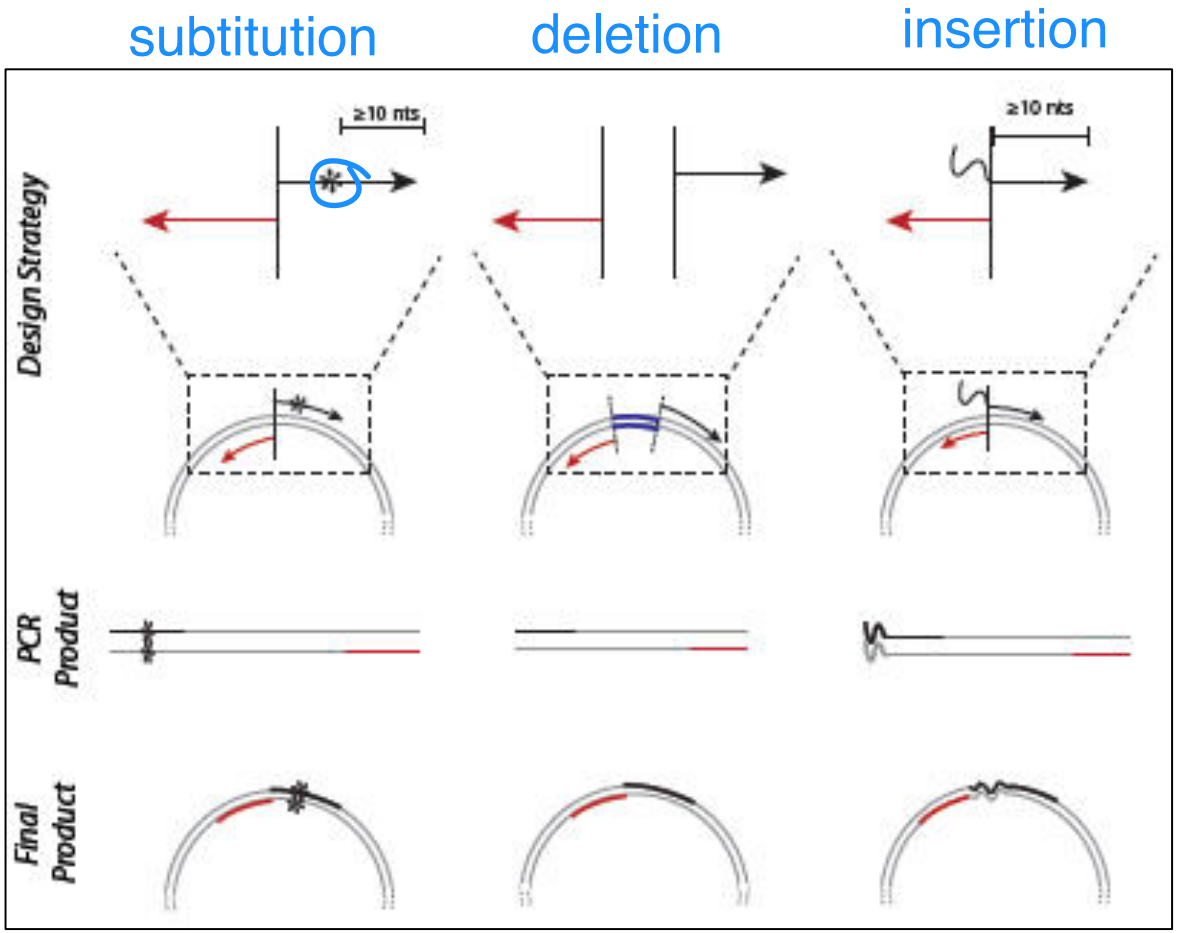
1. *Quick* Pre-lab Discussion
2. Set up PCR to generate gRNA plasmid
3. BE Communication workshop: Journal Club
   - 56-302
4. Discussion of Otoupal *et al.*

M2 experimental overview



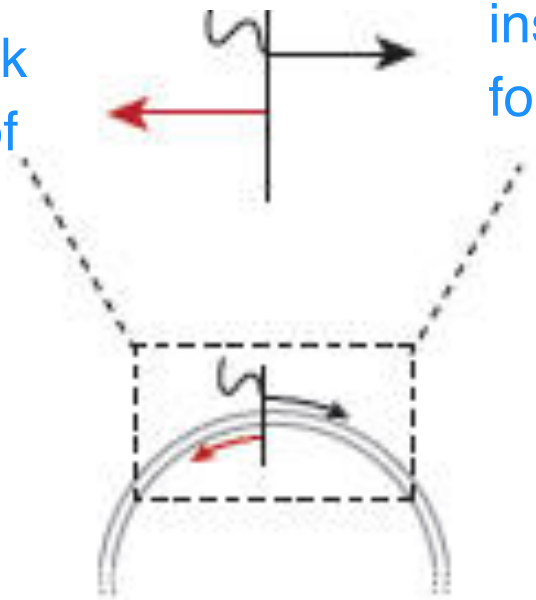
Use of Site-directed mutagenesis (SDM) to engineer DNA

- Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer contains the desired mutation
- The final PCR product is processed and annealed back-to-back
- NEB Q5 SDM kit

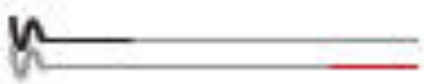


Insertion of DNA via SDM

reverse primer anneals back to back with comp. region of forward primer



insertion incorporated 5' of forward primer

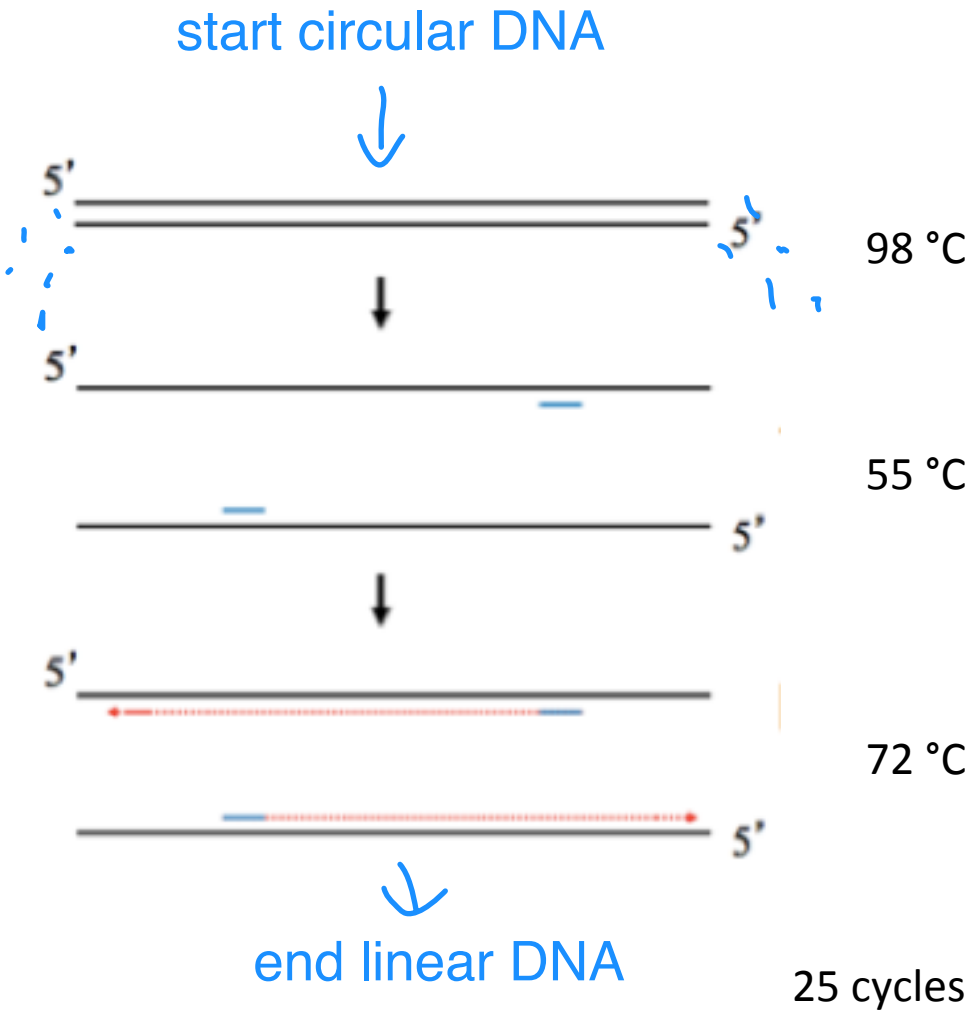


major product

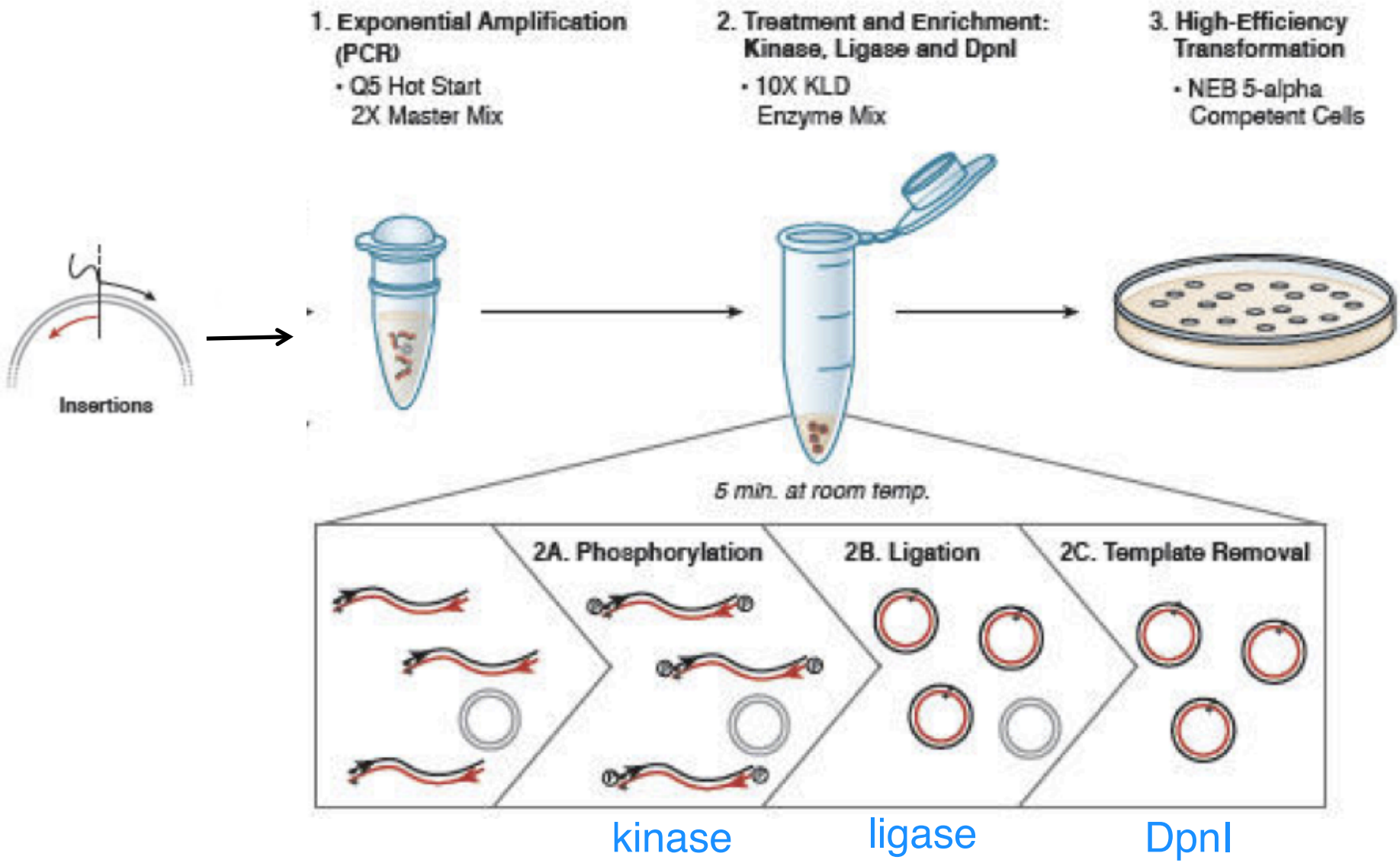
circular after blunt ligation

SDM ingredients and cycling conditions

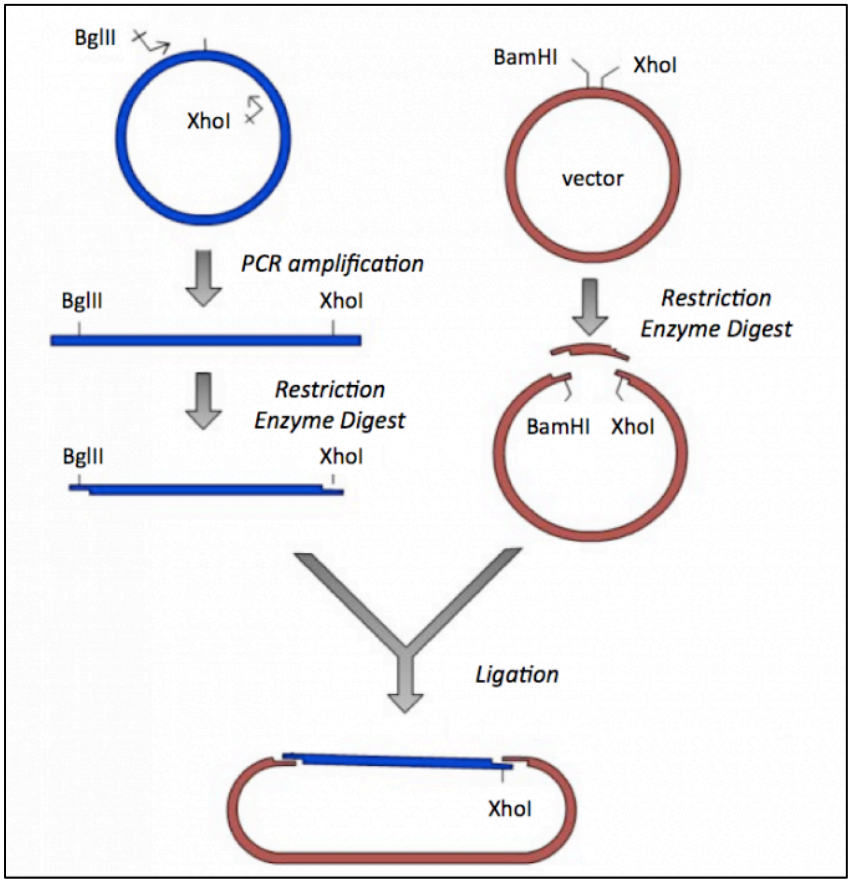
SDM ingredients
DNA polymerase
primers
dNTPs
template DNA
buffer, cofactors Mg2+
H2O

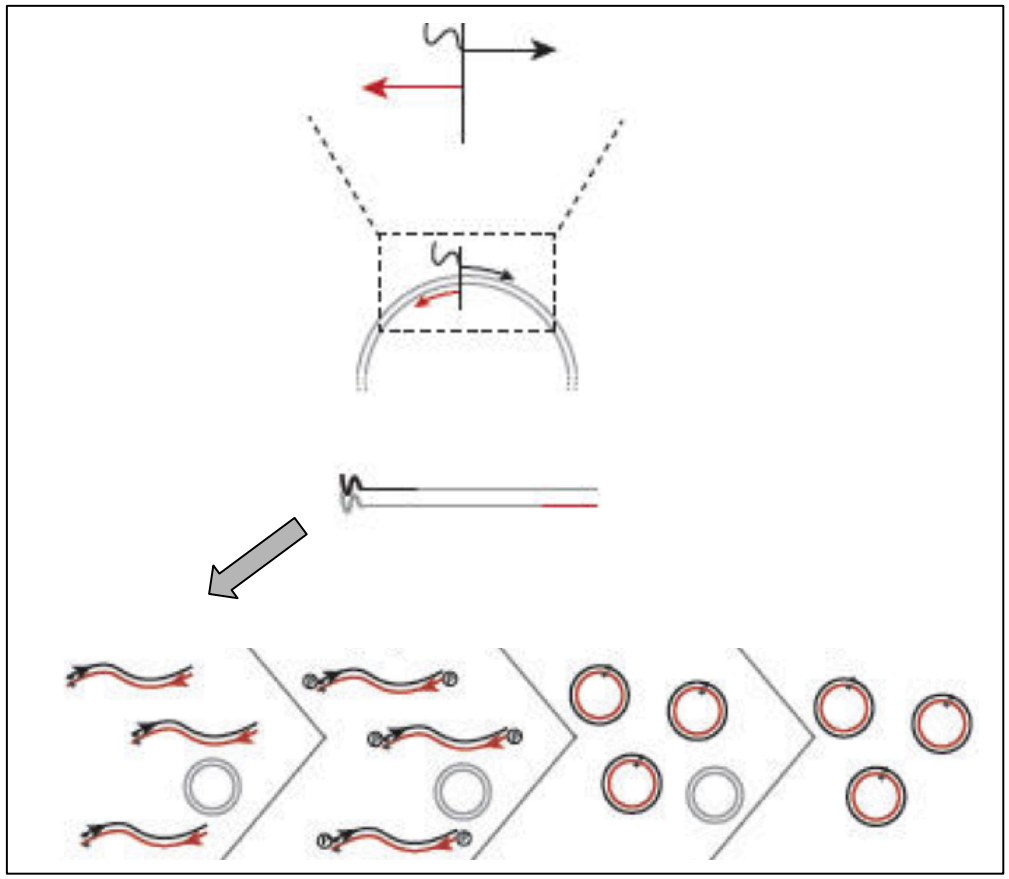


Additional steps necessary to recover circular plasmid



Traditional cloning vs. Site Directed Mutagensis





Today in lab…

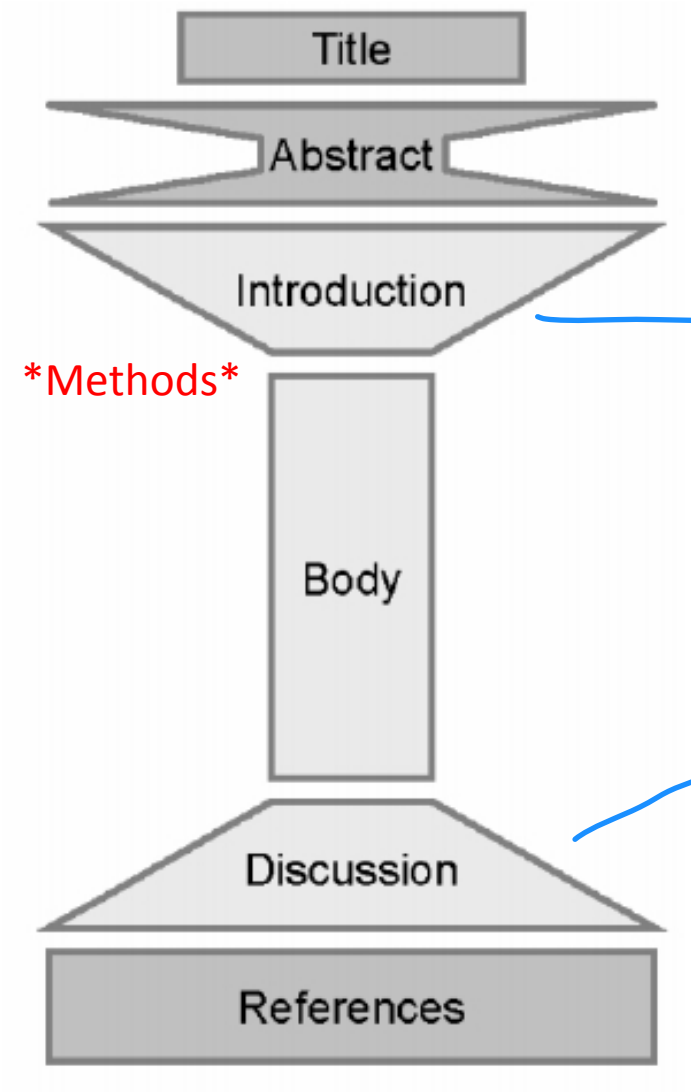
- Pick up your gRNA oligo and reconstitute in H2O
- Set up your gRNA insertion and amplification reaction
- Workshop then paper discussion

No homework M2D4, although there is a Quiz!

M2D5 HW: Intro, Schematic, Discussion

- Draft Introduction
  - "Big Picture" paragraph
  - overview/ topic sentence (first sentence) of each additional paragraph
  - references in text and brief summary of each reference
- Schematic of Mod2 experimental approach
  - no referencing other images for Mod2 report
  - Include a figure title and caption
- Draft Discussion for confirmation agarose gel figure

In the king / hourglass models, the Discussion mirrors the Introduction



Intro: *general background/motivation
*specific background
*knowledge gap/central question
*hypothesis
*preview results
*Implications

Discussion: *Recap Results
*Interpret Results
*discuss expectations
*propose next steps