M2D3:
Generate and amplify pgRNA

10/21/2016

Today in lab

- quick pre-lab
- set up PCR to generate gRNA plasmid
- BE Communication workshop: Journal Club in 16-275
- class discussion of Otoupal *et al.*
- (evening) finish gRNA plasmid generation

ACS SyntheticBiology

Research Article

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CRISPR Perturbation of Gene Expression Alters Bacterial Fitness under Stress and Reveals Underlying Epistatic Constraints

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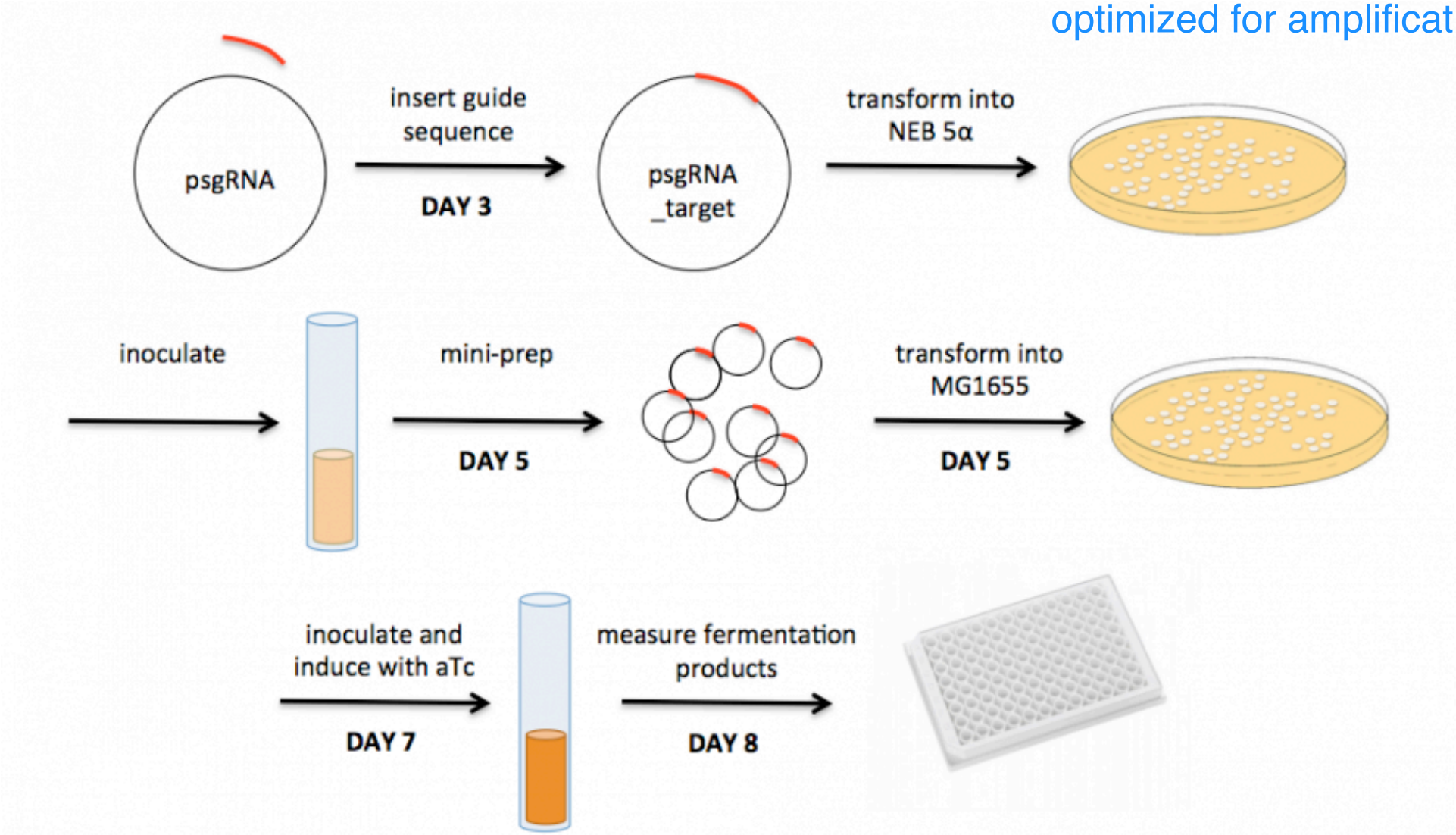
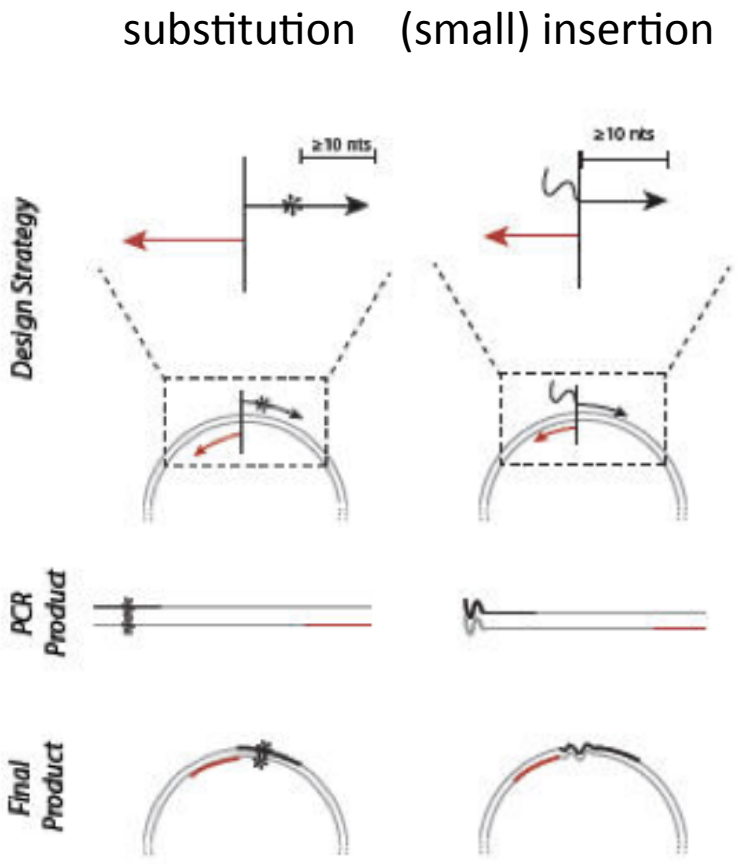
M2 experimental overview

optimized for amplification

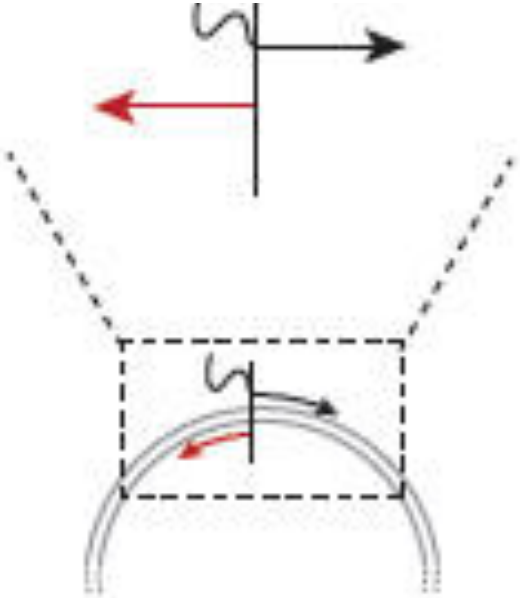


Site-directed mutagenesis (SDM) for insertion: another DNA engineering tool

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



Insertion of DNA using SDM



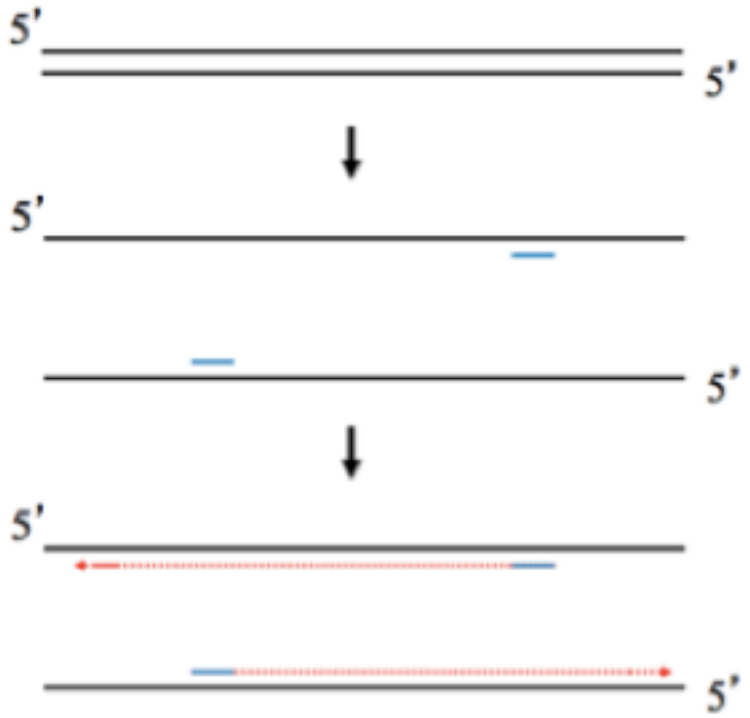
F-primer contains
the gRNA_target
(Cas9 recognition flap)

PCR product linear

re-circularize
to create
pgRNA plasmid

SDM ingredients and cycling conditions

SDM ingredients
dNTPs
polymerase (HF > Pfu > Taq)
primers
template
Mg2+ as a cofactor
buffer
water



98 °C

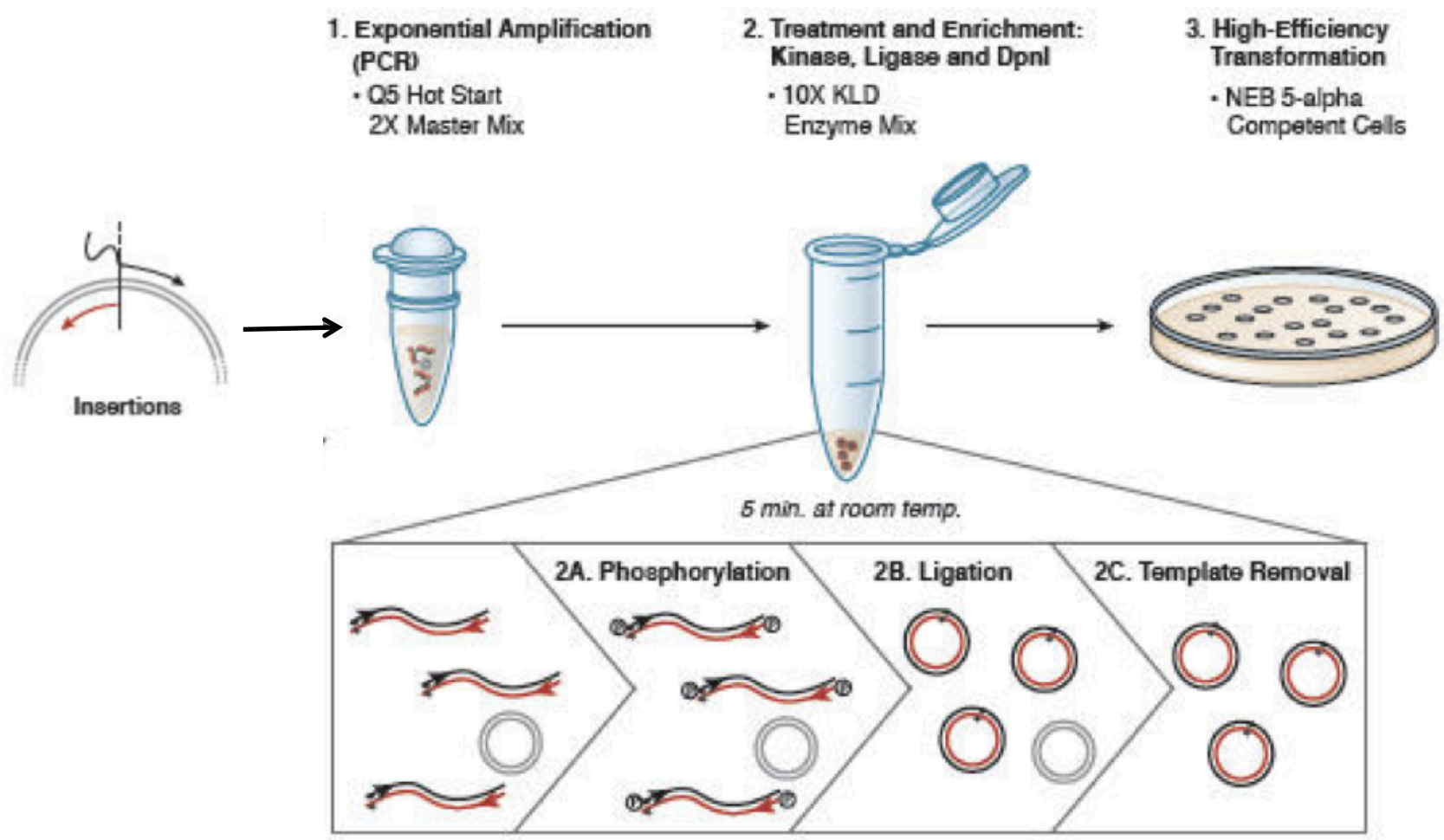
55 °C

72 °C

25 cycles

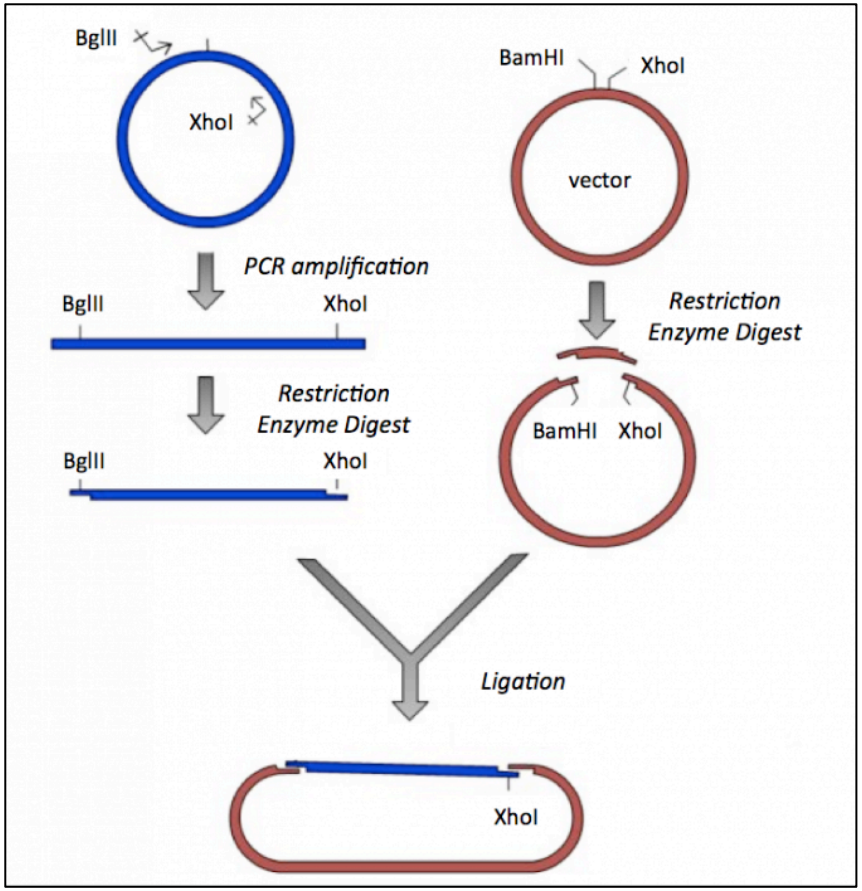
SDM steps with NEB Q5 kit to recover circular plasmid

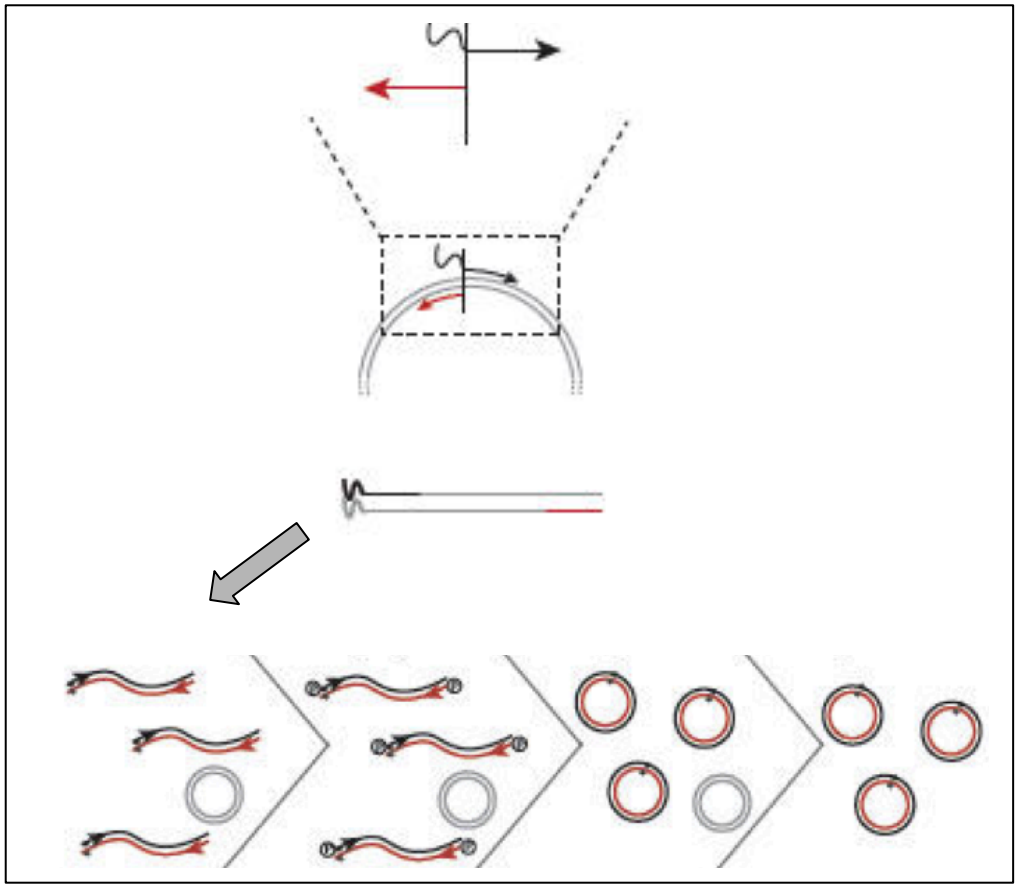
KLD



https://www.neb.com/applications/dna-modification/dna-end-modification/dna-phosphorylation

We have learned two ways to engineer DNA





traditional cloning *vs.* site-directed mutagenesis

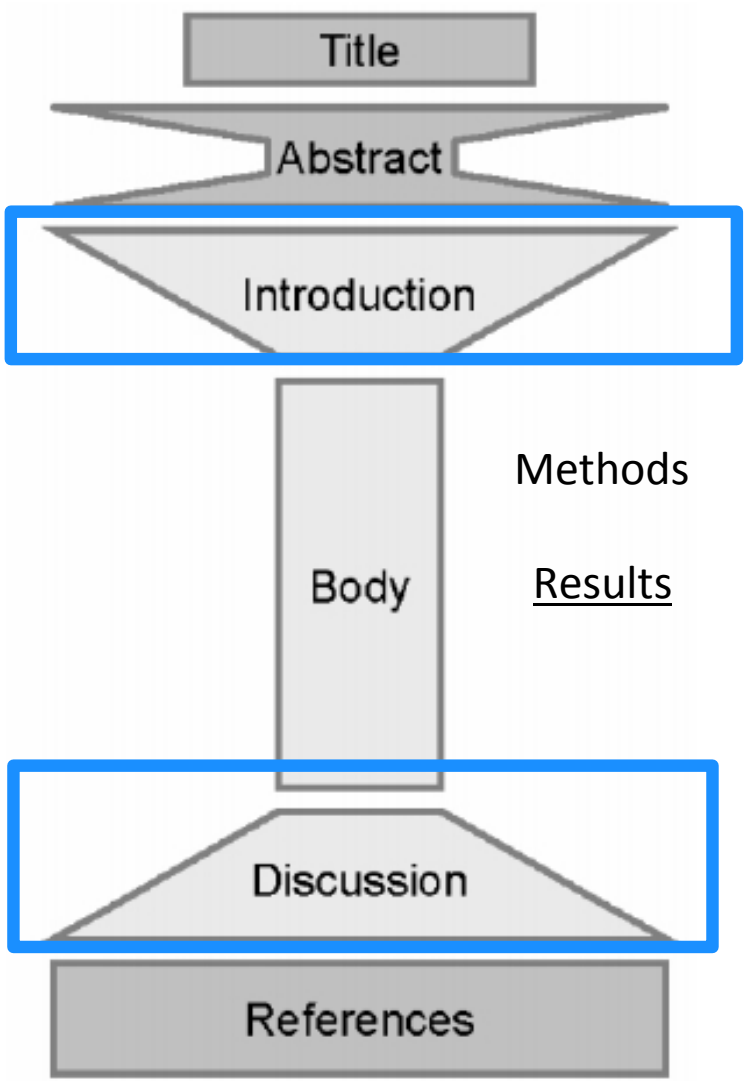
For M2D4 and M2D5

- Journal club presenters
  - Read and re-read and re-read your paper
  - What story do you want to tell?
  - Outline your slides (titles) to tell this story
  - Make beautiful slides!

- Quiz on M2D4:
  - before or after presentations?

- Long homework for M2D5
  - Introduction (of M2 research article)
  - Schematic (of M2 experimental approach)
  - Discussion (of confirmation digest)

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



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