- Announcements
- Lab Quiz
- Ku80 paper discussion
- Pre-lab Lecture
  - Restriction enzymes intro and restriction sites in M2 context
  - Rest of today in lab (M2D3)

Announcements

- Prof. Samson is joining us for the paper discussion today; after the quiz, take 10' to review guiding Q
- M1 microbiota summary drafts to be returned this Friday from Jon; Leslie is already done (see Stellar)
- Short FNT – plan digest conditions/calculations
- Just a few days till spring break!

Module 2: basic goal and approach

Question: How well does NHEJ repair different-looking damage?

Step 1 damage DNA

Step 2 measure repair with blue fluorescence

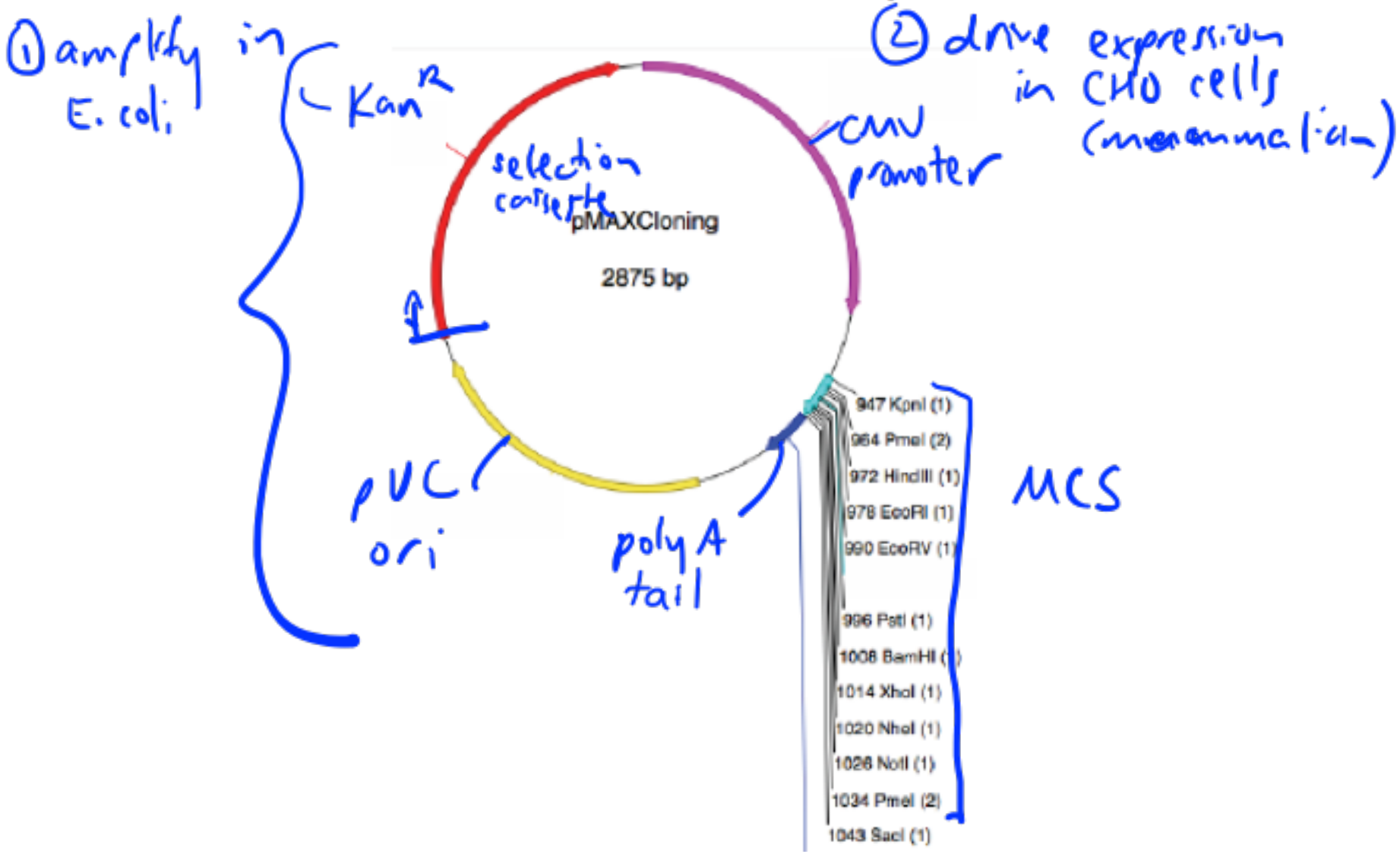
Specific methods used in our approach

Step 1 cut with restriction enzymes

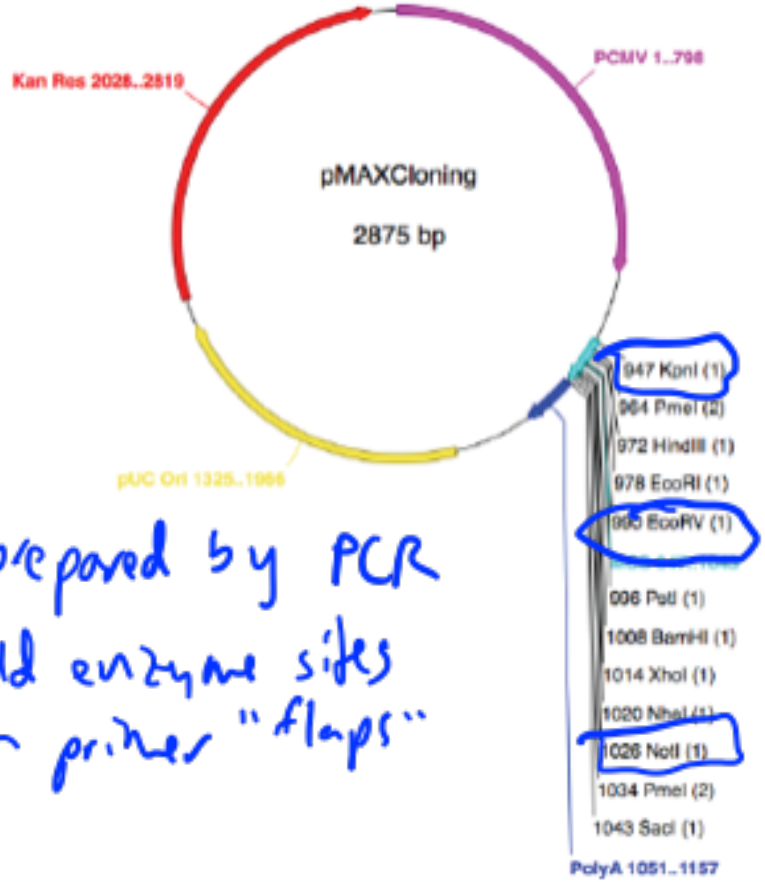
Step 2 flow cytometry

Side note: RE defend against viruses
← bacteria protected by self-methylation
mammalian cells don't have those specific Gallis-transferases

Plasmid redux: mammalian expression vectors



Restriction site choices for cloning: topology



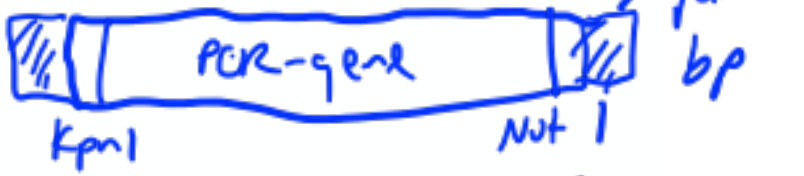
insert prepared by PCR
→ add enzyme sites on primer "flaps"

Where do you introduce a gene?

in MCS

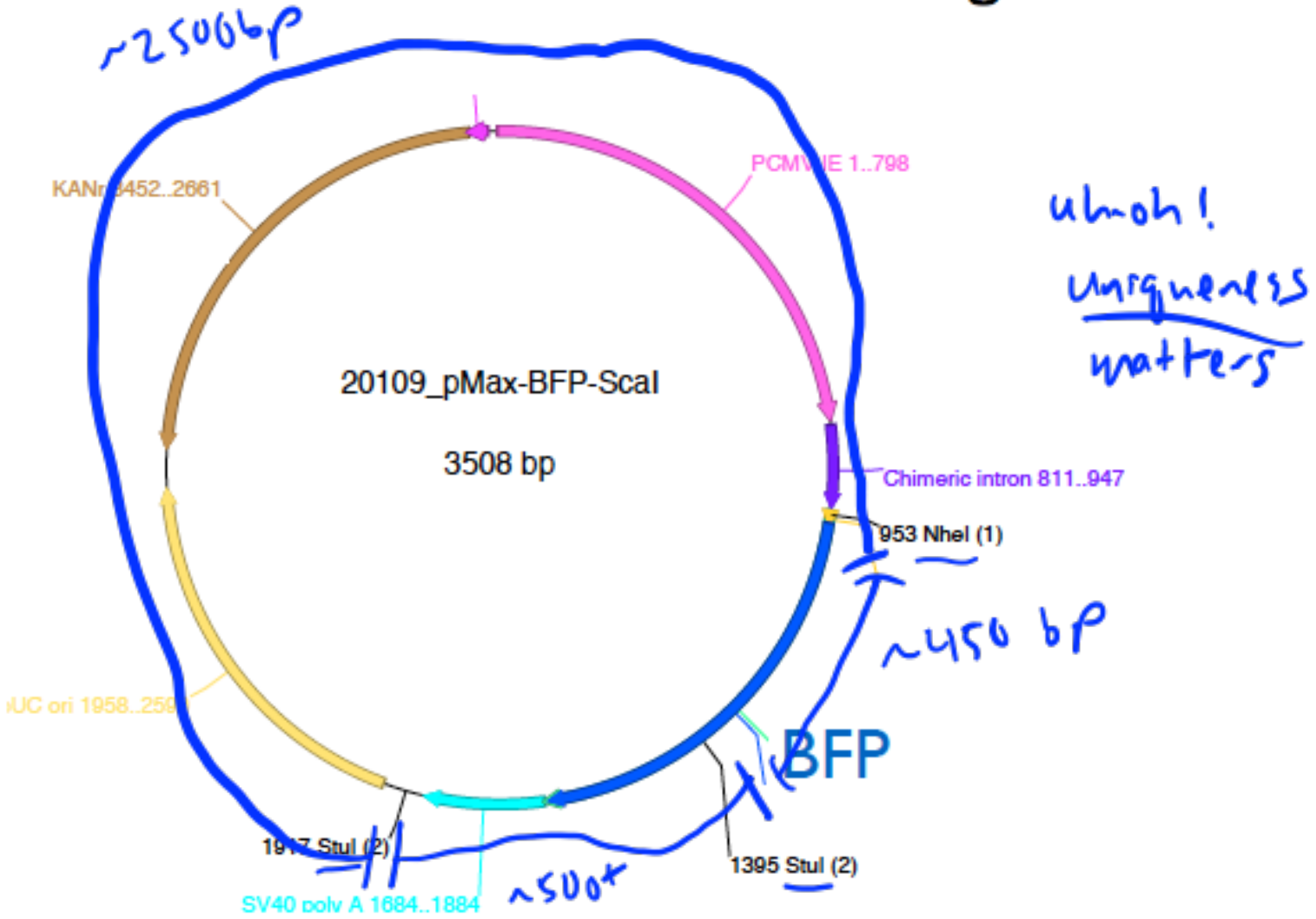
A) blunt e.g., EcoRV
* don't have to purify

B) sticky e.g., KpnI + NotI
* leaves ~70 bp fragment
⇒ gel purify

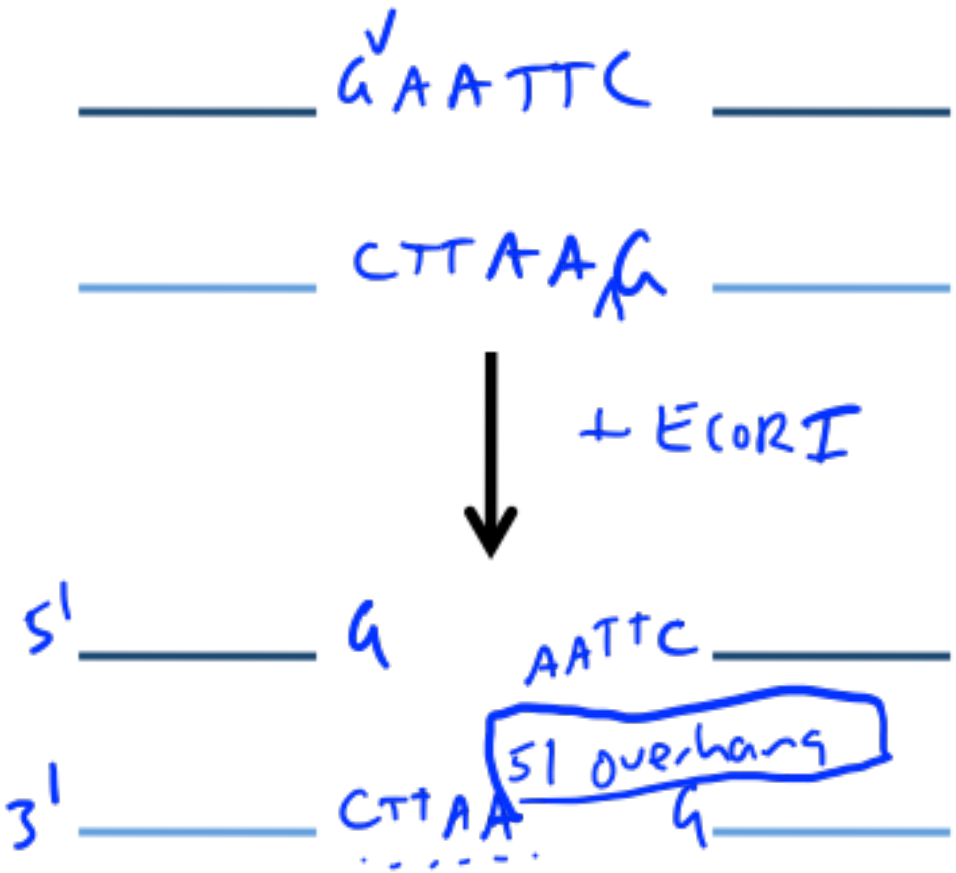


junk - ↑ binding + ∴ cutting efficiency of the enzyme

Restriction site choices for cloning: careful!



Base-pair view of restriction enzymes



cf EcoRV

GAT | ATC

CTA | TAG

no overhangs / blunt

cf PstI 3' overhang

CTGCA G

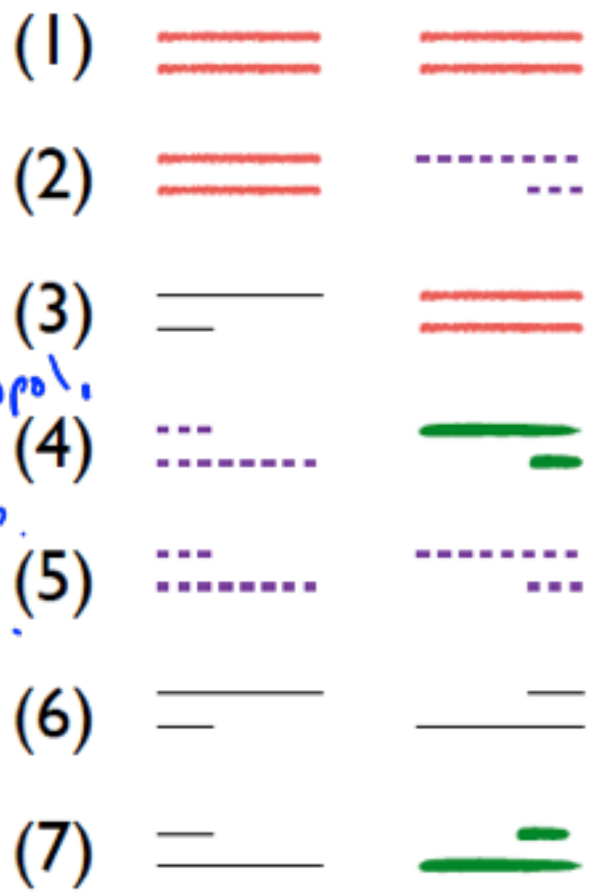
G ACGTC

CTGCA G

G CGTA

Topologies that we will test: let's predict!

Possible cut topologies:



Same topol. diff. SEQ

same TOP. same SEQ

neither same

NHEJ Hypothesis: (error-prone pathway)

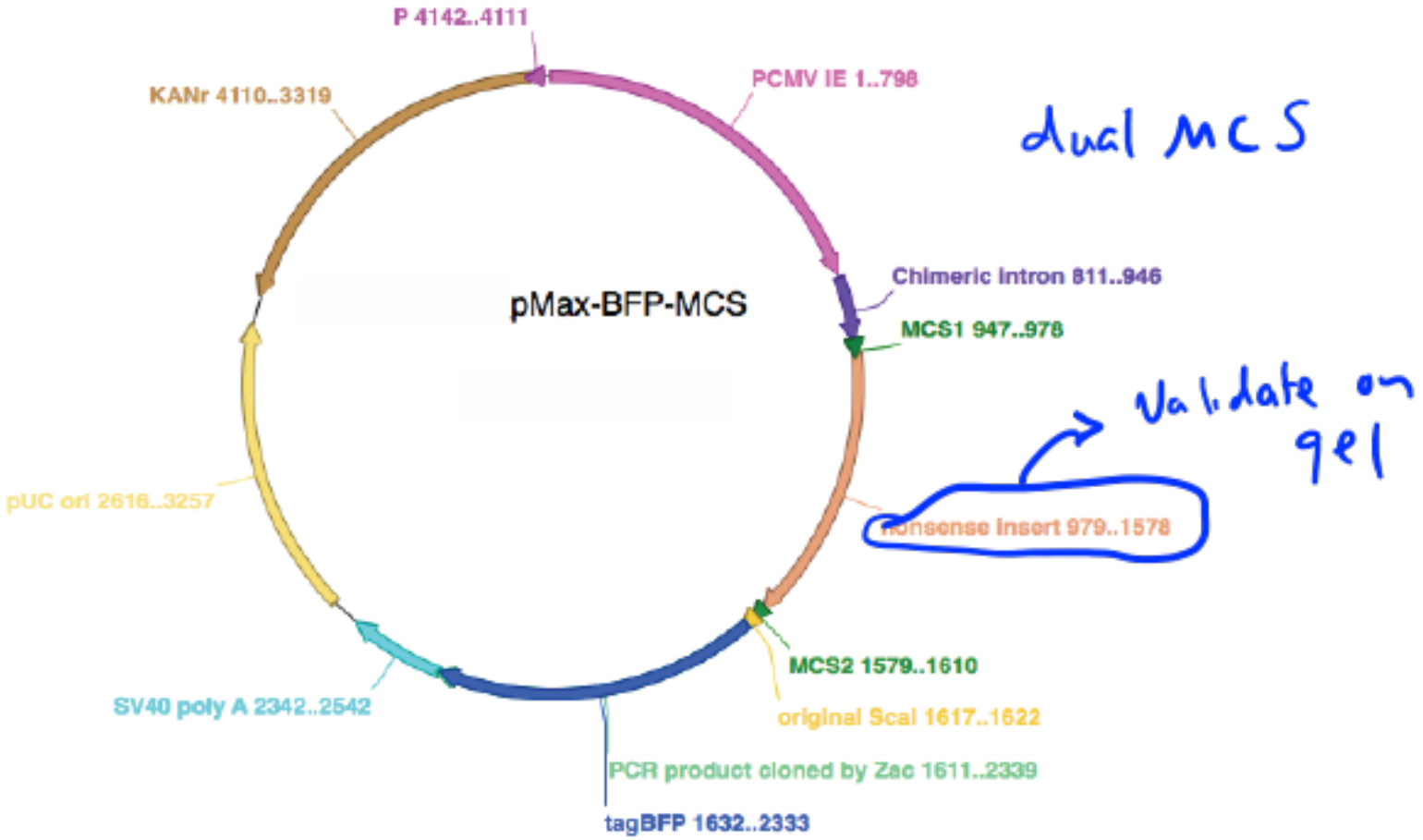
1 best?

2+3 better than 4?

} best?

worst

Today you will build our system (virtually!)



Today in Lab: M2D3

- Investigate system
  - parent vector
  - original NHEJ assay iteration (Zac/Samson)
  - our NHEJ assay variant (Agi/Zac/Samson)
- Don't leave without signing up for a cut topology to test! (One per group.)
- Note: today's notebook can NOT be collected. Develop your understanding; don't craft your prose.

- Thanks to Shannon for most of the images in this pre-lab!