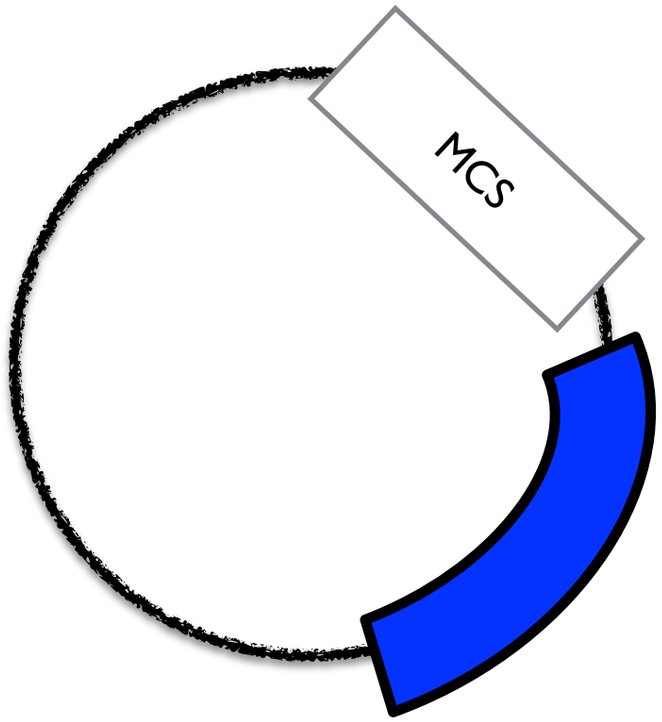
M2D3: System Conditions + Paper

3/18/14

1. Lab treat!
2. Review the paper guiding questions to prep for discussion.
3. Ku80 in NSCLC
4. Pre-lab lecture — Restriction Enyzmes
5. Investigate plasmid system for studying NHEJ
6. Choose design parameters (type of cut topology)

Mod2 Goal: Quantify NHEJ efficiency given different DNA damage topologies.



Non-technical explanation:

How good is NHEJ @ repairing damage?

* Introduce DNA damage

⇓

* Measure repair through Blue Fluorescence

Mod2 Goal: Quantify NHEJ efficiency given different DNA damage topologies.

Why do they work in mammalian cells?

No specific methyltransferases

Ok - how do we do it?

* Cut w/ Restriction Enzymes

↓

* flow cytometry

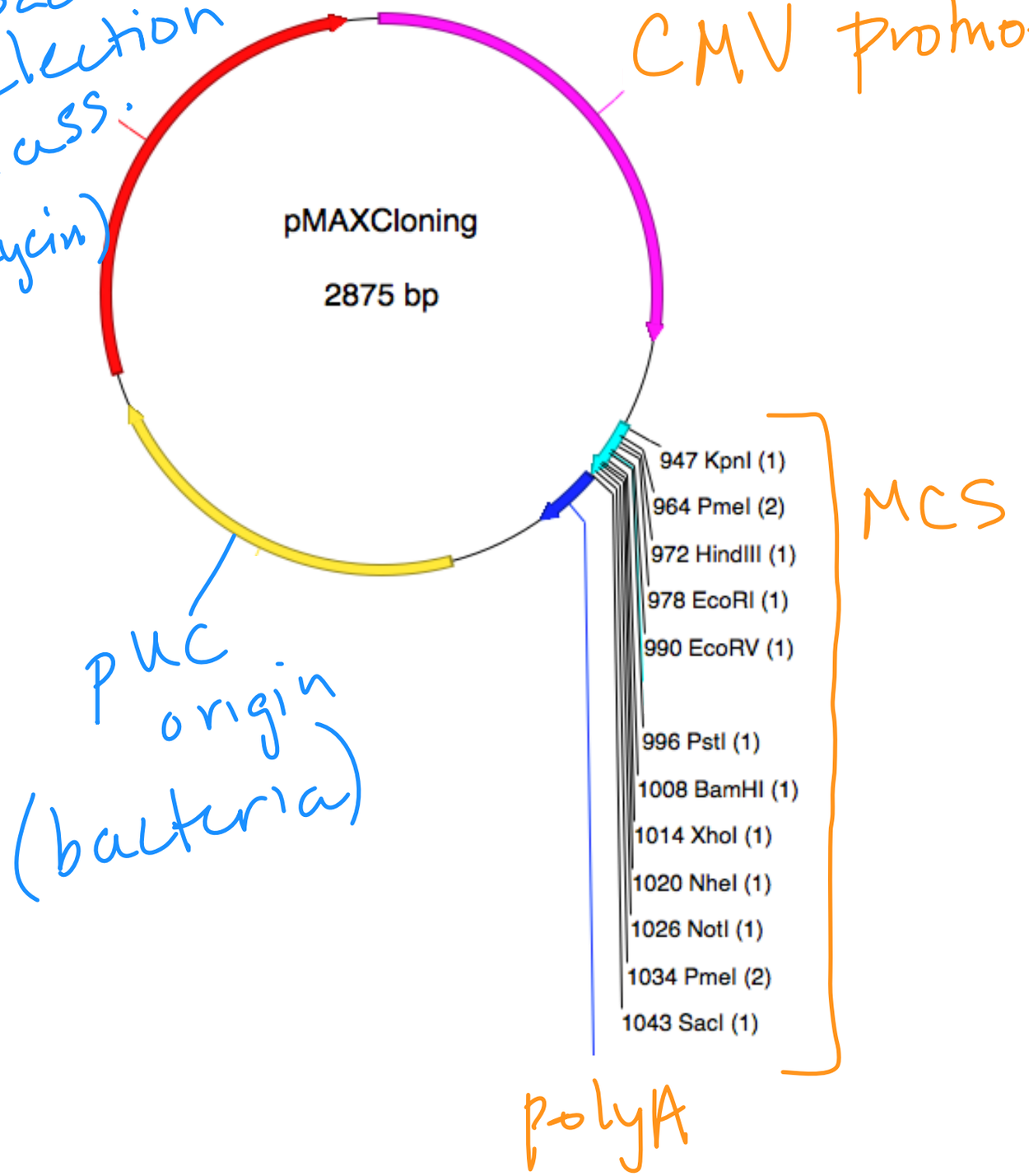
Mammalian expression vectors:

1) Amplify our plasmid e.coli (bacteria)

Selection cass. (Kanamycin)

- drive expression in CHO cells

CMV promoter



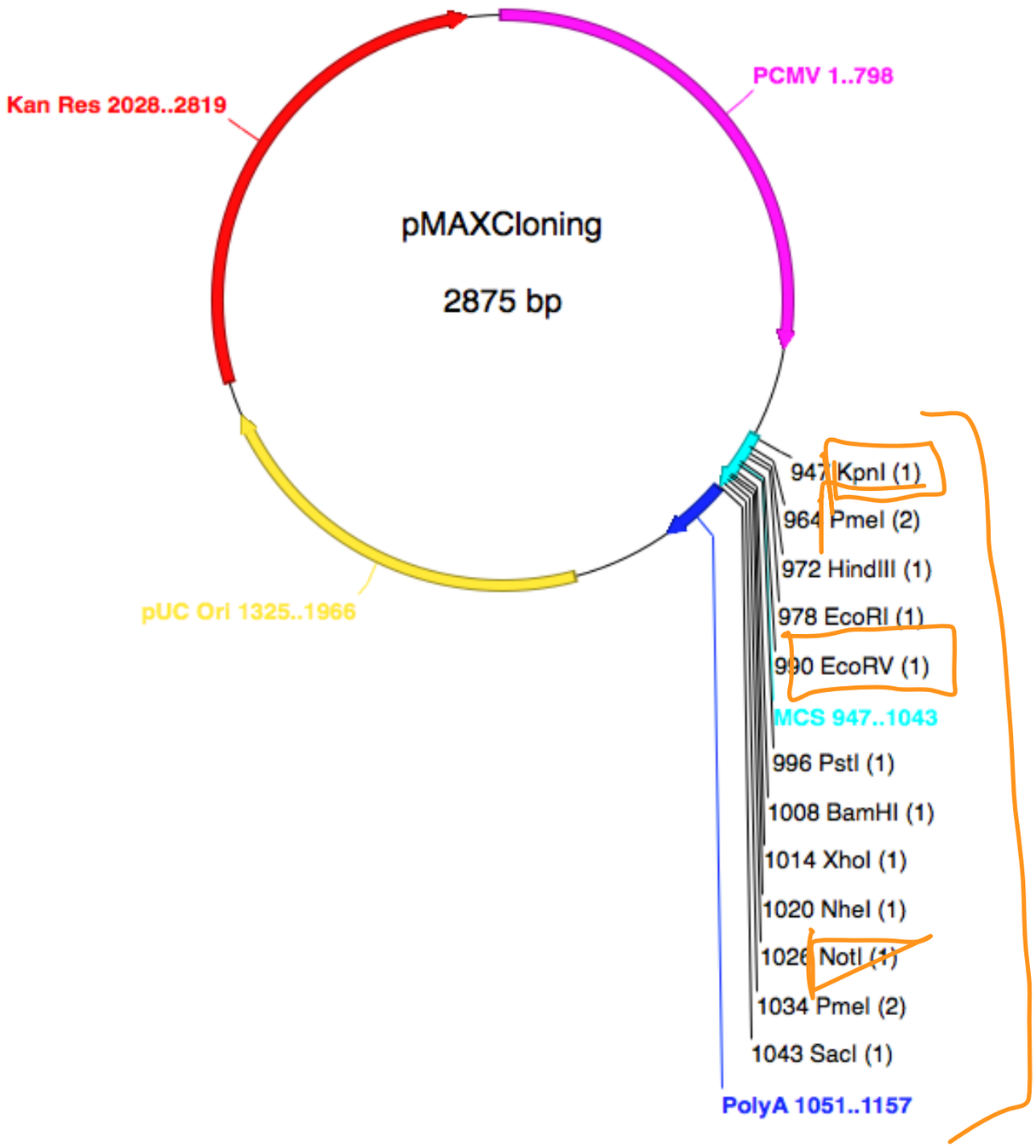
pUC origin (bacteria)

MCS

polyA

Introduction to Restriction Enzymes

Lonza



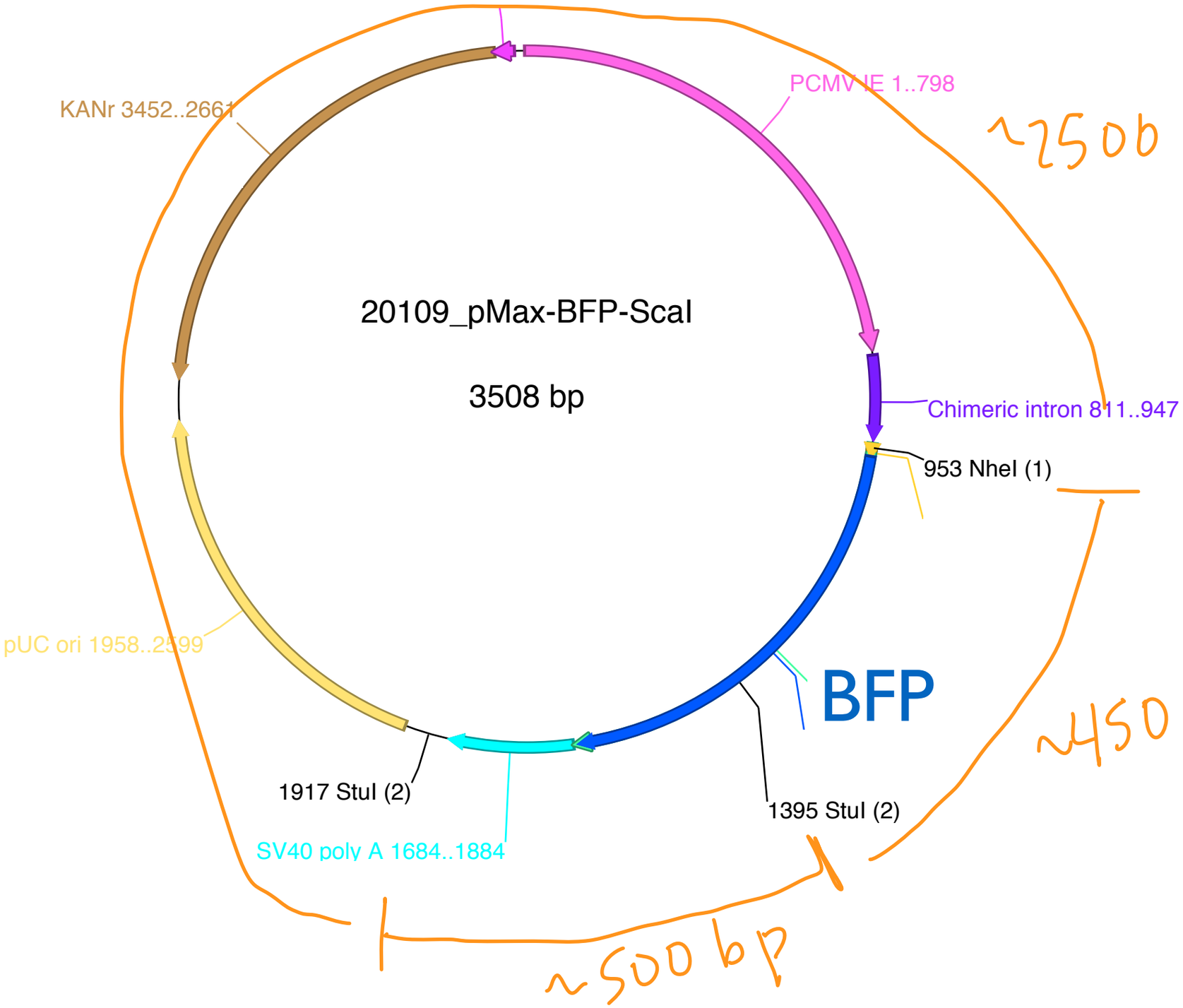
Where do you introduce a gene?

1) Blunt → don't have to purify

2) sticky
KpnI
NotI
> ~70 bp] gel purity

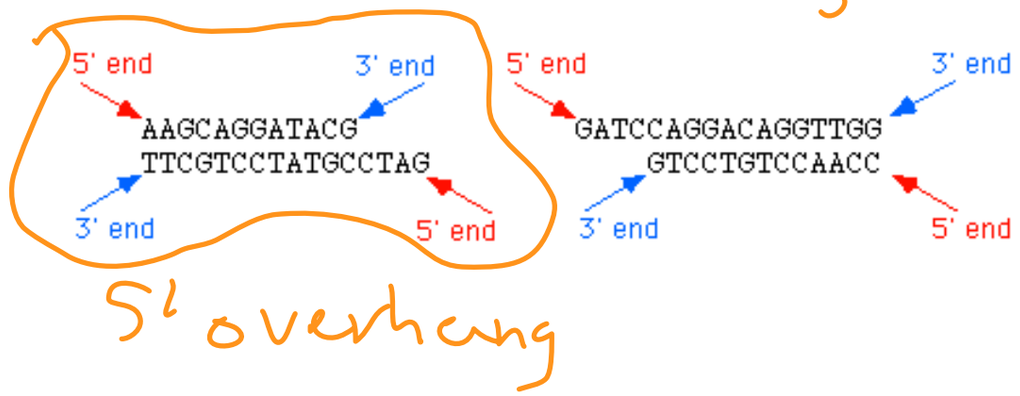
increase RE binding & cut efficiency

Introduction to Restriction Enzymes



Introduction to Restriction Enzymes

Possible cut topologies:



3’ overhang
ex. PstI

5’ overhang
ex. EcoR1

Blunt
ex. EcoRV

★NHEJ is very error prone

Our System:

1 - highest repair
7 - worst repair

Possible cut topologies:

NHEJ Hypothesis:

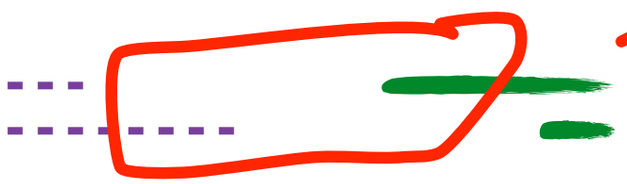
(1) 

2

(2) 

(4-6)

(3) 

(4) 

- Same topology - different seq.
- different topology - same topology - comp. sequences

3

(5) 

1-A

(6) 

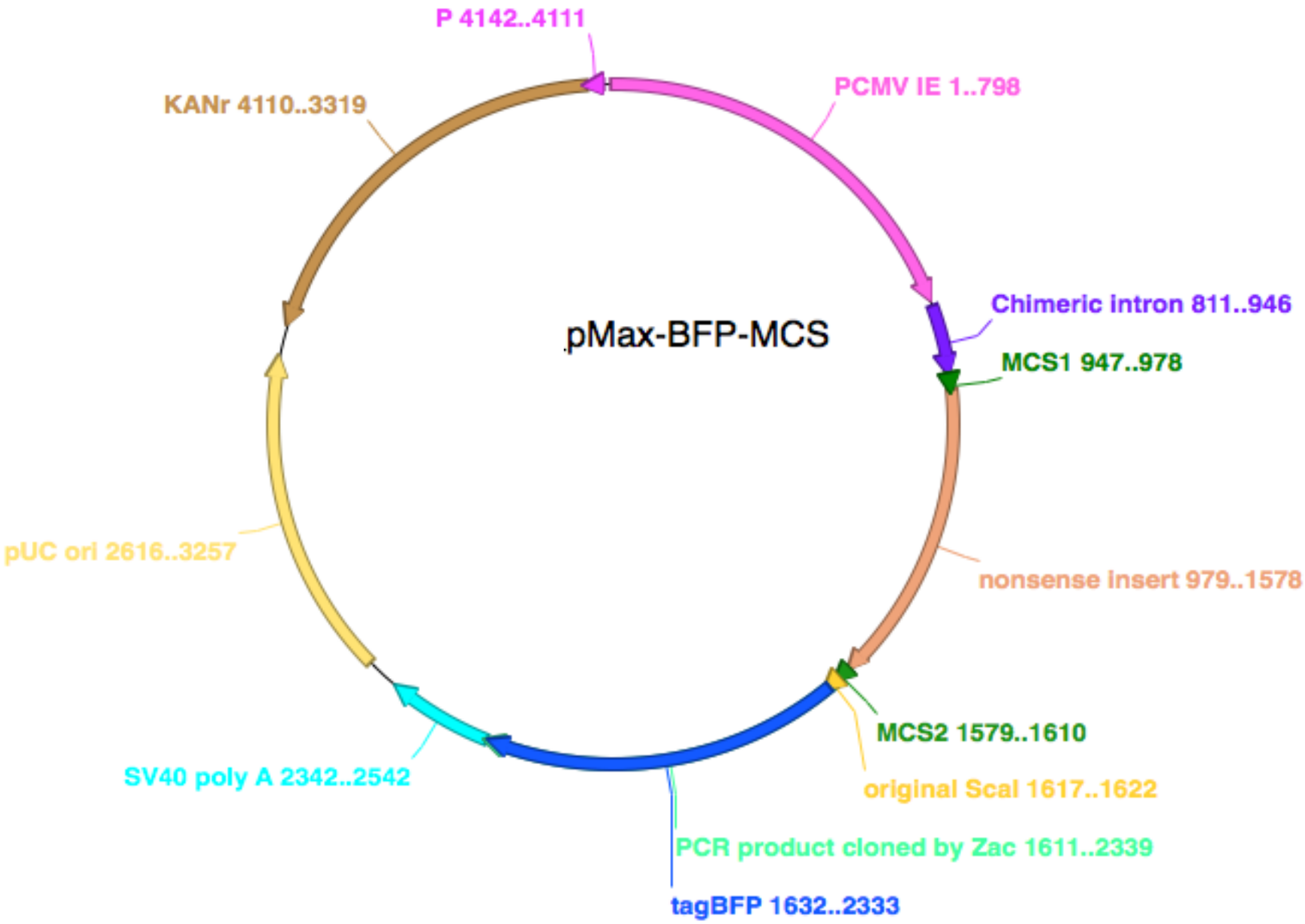
1-B

(7) 

- different topology & different sequence

7

Today you will build our system (virtually!)



Today in Lab:

1. Follow protocol on wiki to learn about system.
2. Sign up for DNA damage topology to study using our BFP plasmid system.

Due on M2D4:

1. Plan your restriction enzyme digest

Next time:

1. Second part of WB to evaluate Ku80 expression.
2. Prepare your cut DNA for the NHEJ repair assay.