DNA Languages: Reading and Editing

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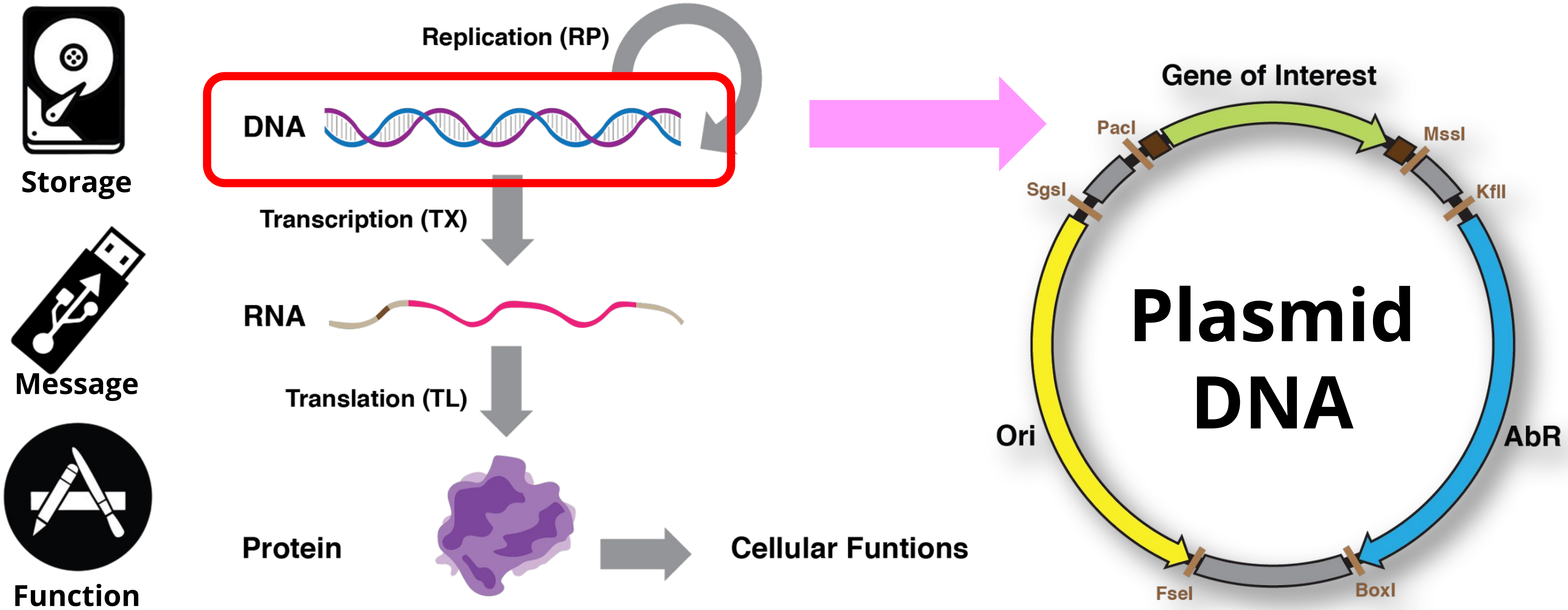
HTGAA Bootcamp
January 23rd, 2026

Overview of the session

- Why do we need specific molecular biology tools? Can't we just use **Notepad, Microsoft Word** or **Google Docs**?
- Show how to use simple **Benchling** Operation
  - Attaching Primers --- find existing primers and pair primers
  - In silico PCR Fragments
- Navigate Simple Assembly Wizard in Benchling
  - Restriction-Ligation (Cut-Connect) of colored proteins
- Discuss some Scenarios while cloning a new plasmid along the way

Most biological information were stored in the form of DNA



Storage

Message

Function

Replication (RP)

DNA

Transcription (TX)

RNA

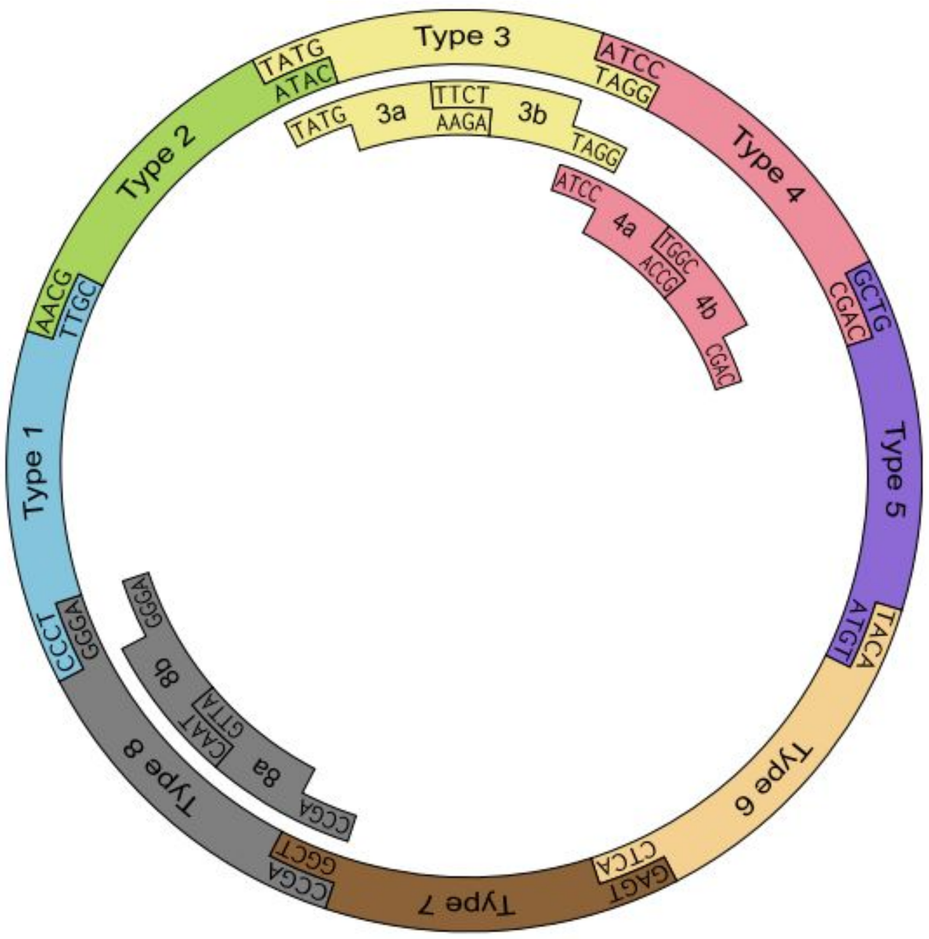
Translation (TL)

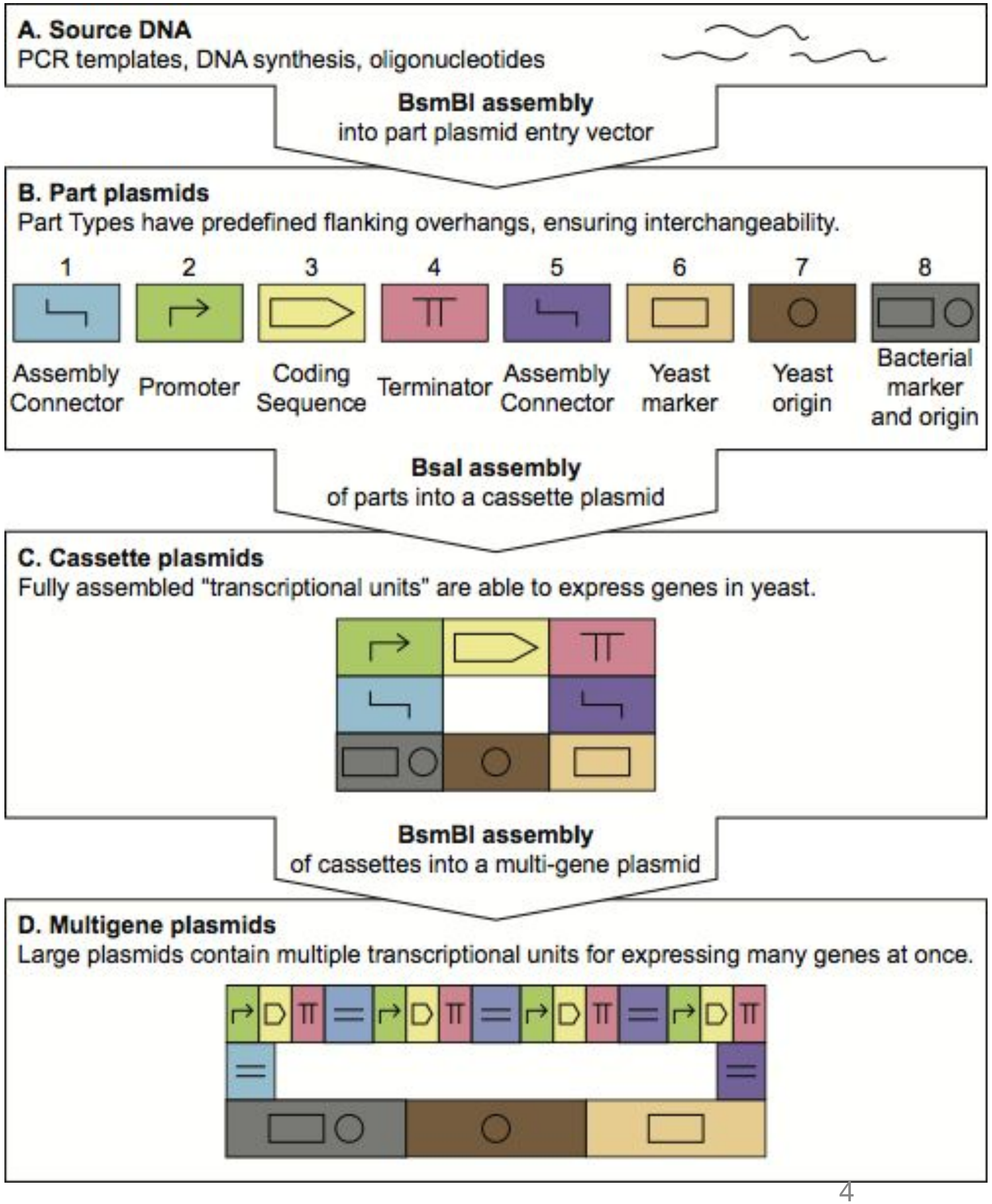
Protein

Cellular Funtions

Plasmid DNA

DNA as LEGO pieces





A. Source DNA
PCR templates, DNA synthesis, oligonucleotides

BsmBI assembly
into part plasmid entry vector

B. Part plasmids
Part Types have predefined flanking overhangs, ensuring interchangeability.

BsaI assembly
of parts into a cassette plasmid

C. Cassette plasmids
Fully assembled "transcriptional units" are able to express genes in yeast.

BsmBI assembly
of cassettes into a multi-gene plasmid

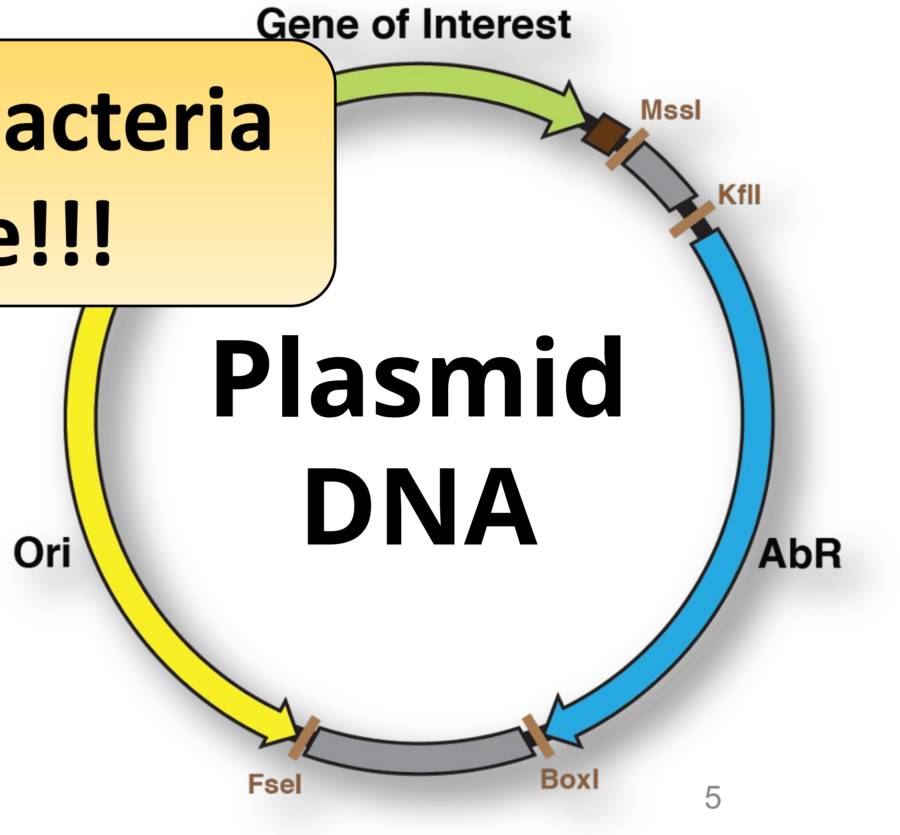
D. Multigene plasmids
Large plasmids contain multiple transcriptional units for expressing many genes at once.

Lee et al. *ACS Synth. Biol.* 2015, 4, 9, 975–986

General Plasmid Architectures

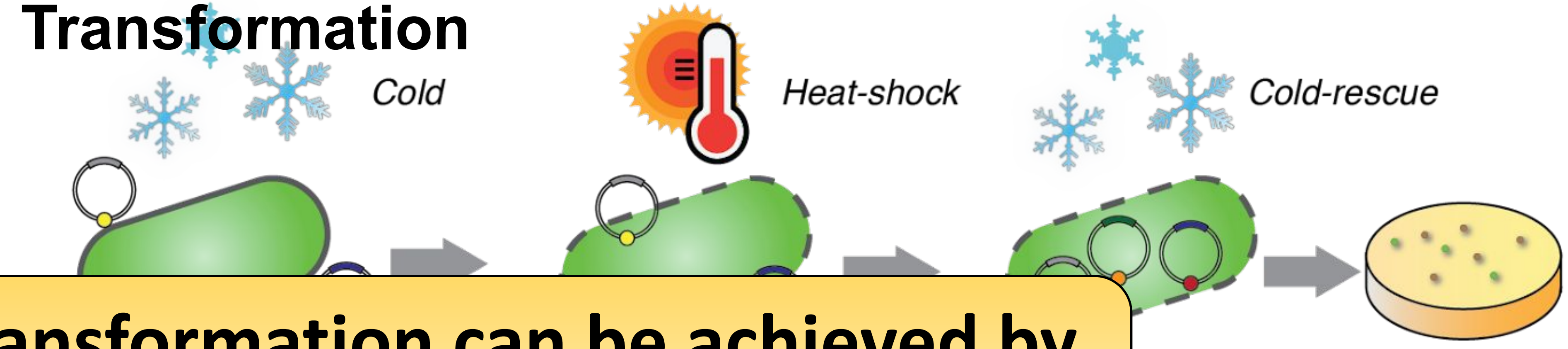
- At their most basic level, plasmids are small circular pieces of DNA that replicate independently from the host's chromosomal DNA
- General eleme
  - Origin of repli
    - And its replic
  - Homologous region (integrative)
  - Selectable marker
  - Gene of interests
    - Multiple Cloning Sites (MCS)

Putting plasmid into bacteria is relatively simple!!!



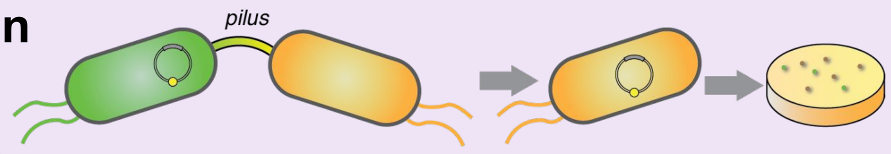
Putting DNA into bacteria (or yeast)

Chemical Transformation



Transformation can be achieved by heat-shock or electric-shock

Conjugation



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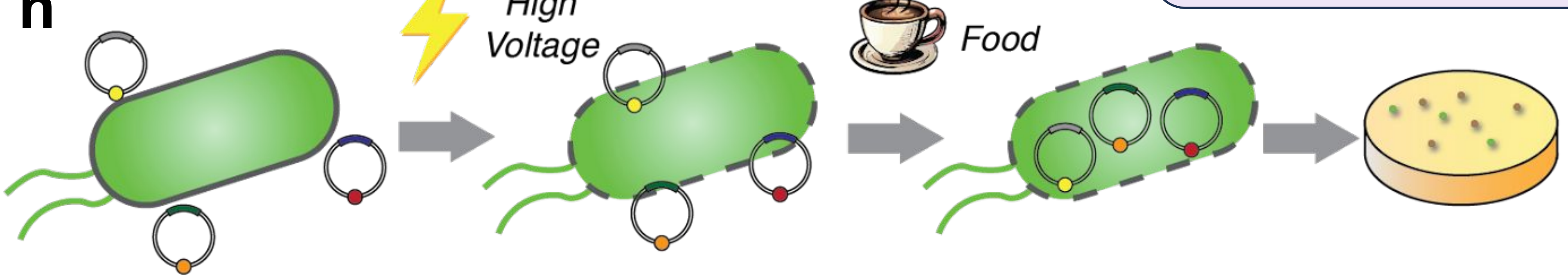
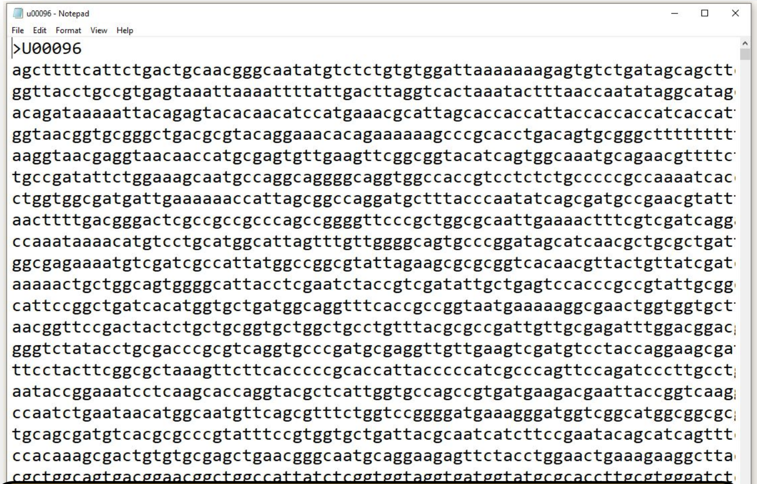


Visualizing DNA Features

- DNA data can be stored in various formats e.g.
    - .fasta
    - .genbank or .gb or .gff
    - .dna (Snapgene)



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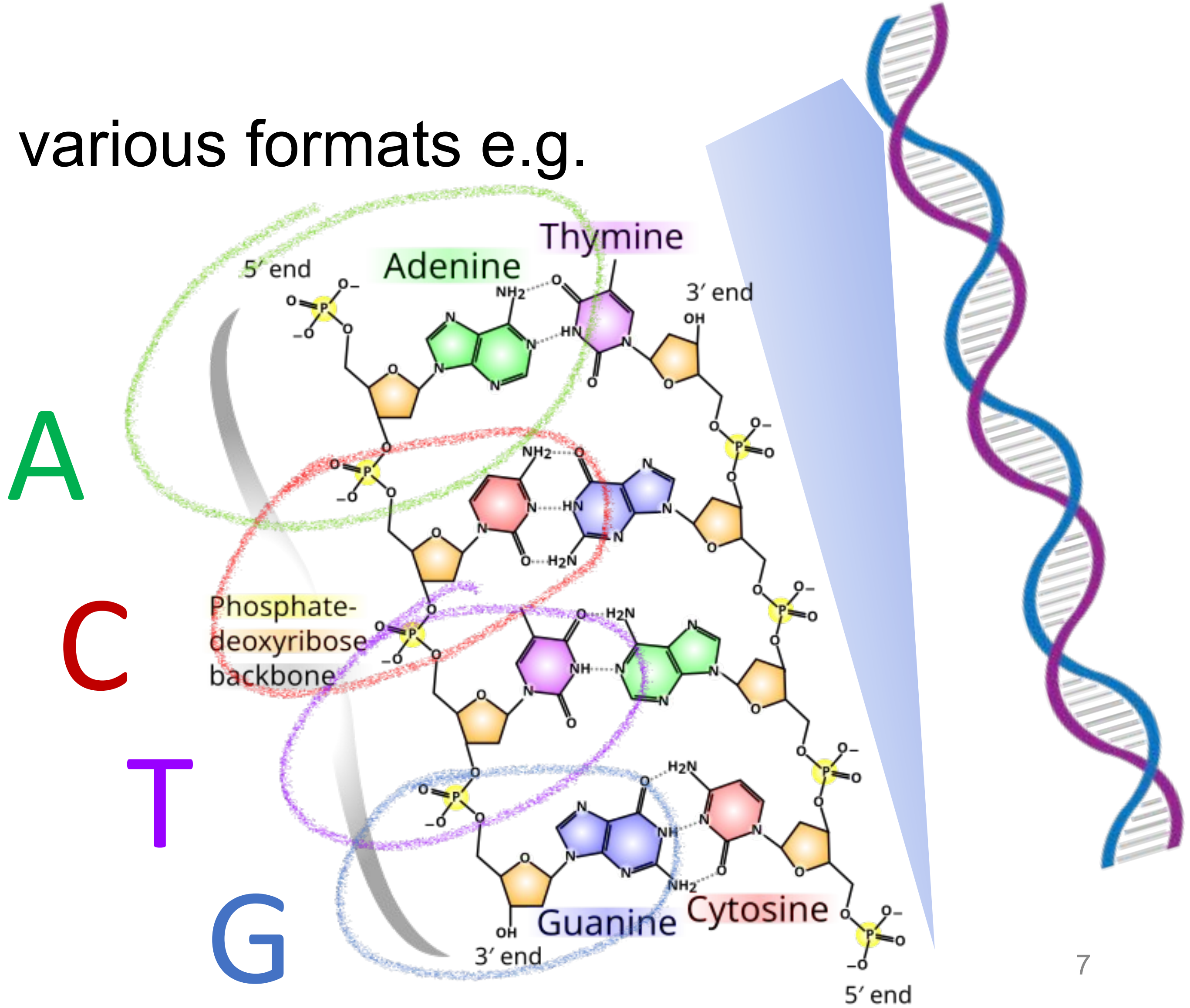

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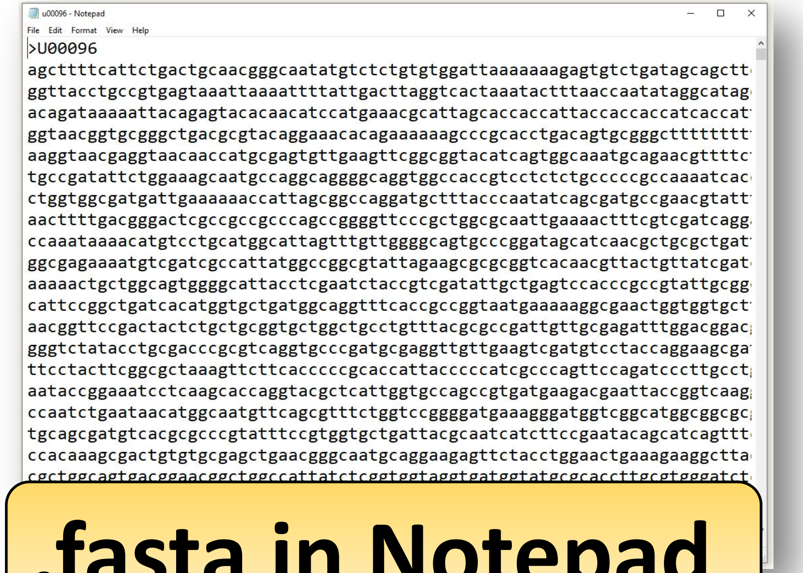

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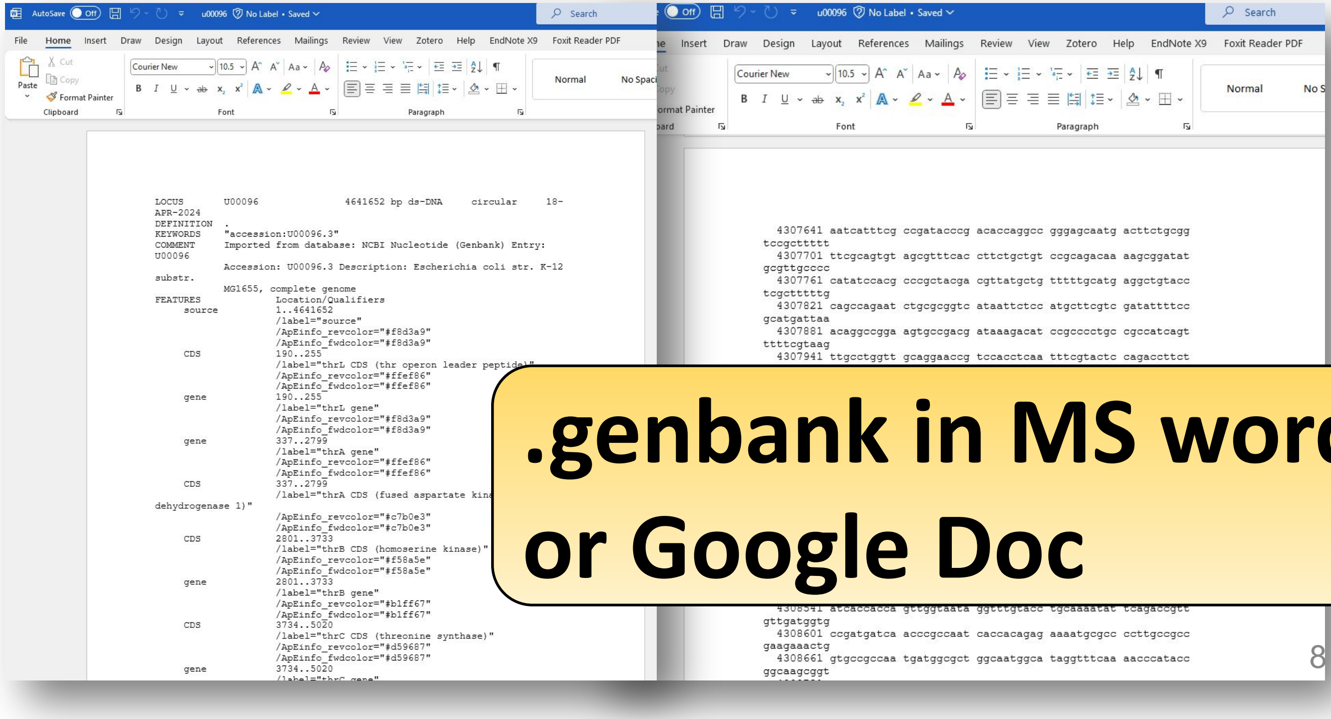
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.genbank in MS word or Google Doc

Visualizing DNA Features

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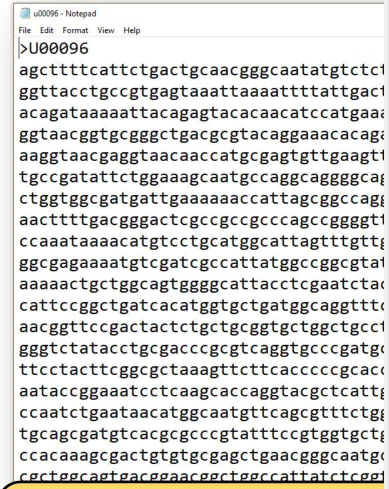
  - ▯ .genba
  - ▯ .dna (S



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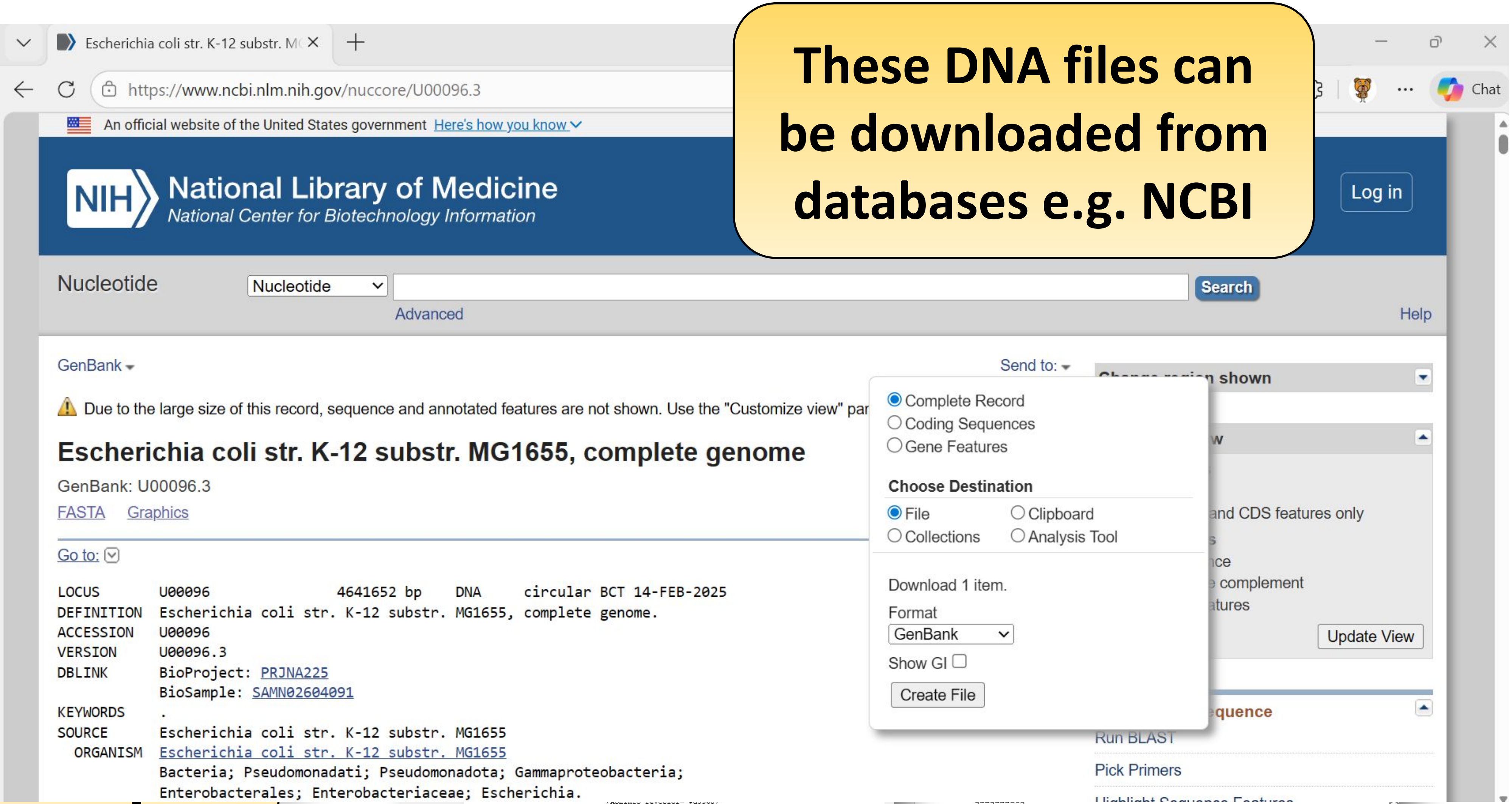
Escherichia coli str. K-12 substr. M
https://www.ncbi.nlm.nih.gov/nuccore/U00096.3
Chat
An official website of the United States government Here's how you know
NIH National Library of Medicine
National Center for Biotechnology Information
Log in
Nucleotide
Nucleotide
Search
Advanced
Help
GenBank
Send to:
Change region shown
w
Update View
equence
Run BLAST
Pick Primers

⚠ Due to the large size of this record, sequence and annotated features are not shown. Use the "Customize view" par

Escherichia coli str. K-12 substr. MG1655, complete genome

GenBank: U00096.3

FASTA Graphics

Go to:

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● Complete Record
○ Coding Sequences
○ Gene Features

Choose Destination

● File ○ Clipboard
○ Collections ○ Analysis Tool

Download 1 item.

Format: GenBank

Show GI □

Create File

and CDS features only
s
nce
complement
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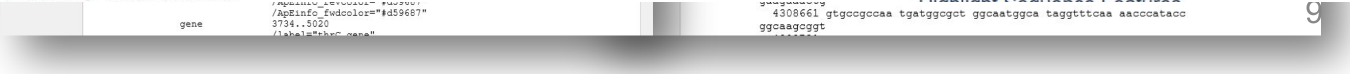
These DNA files can be downloaded from databases e.g. NCBI



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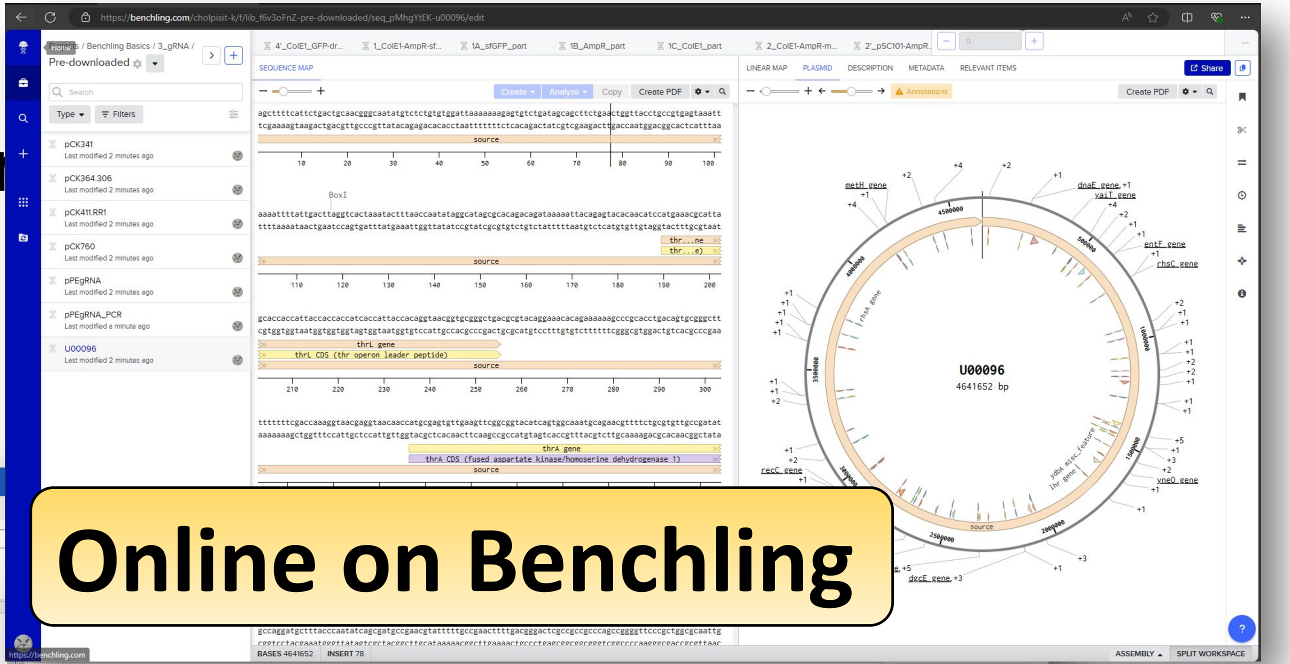
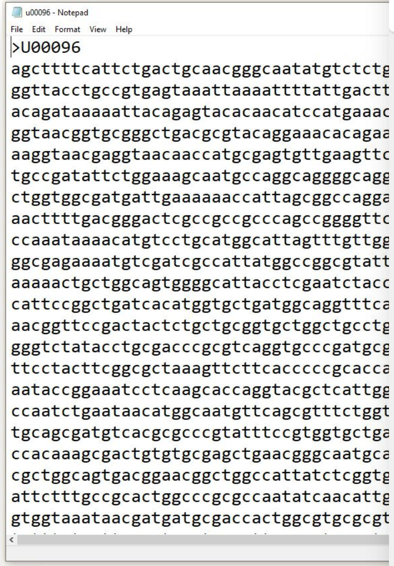
Visualizing DNA Features

- DNA data can be stored in var
  - .fasta
  - .genbank or .gb or .gff
  - .dna (Snapgene)



Online on Benchling



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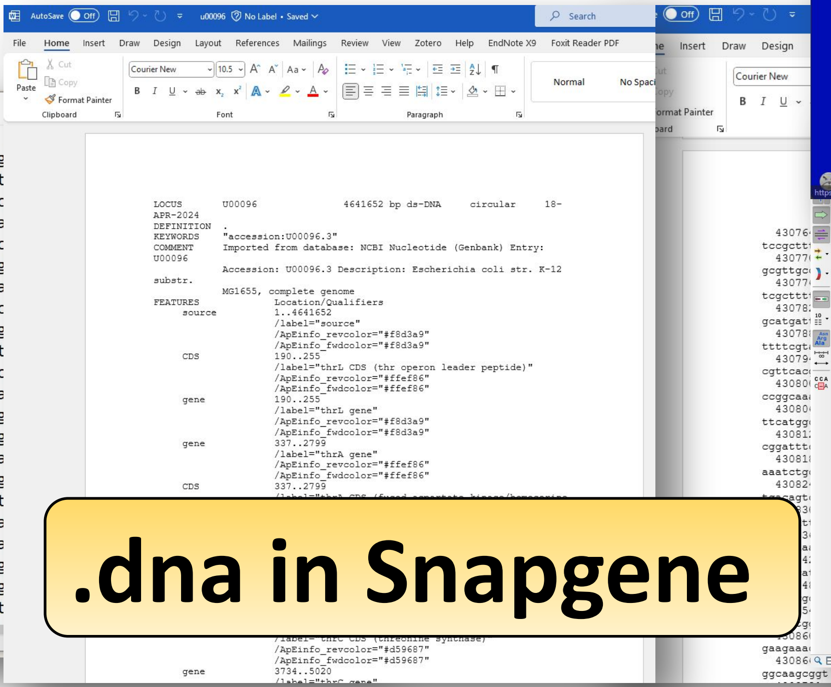

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            MG1655, complete genome
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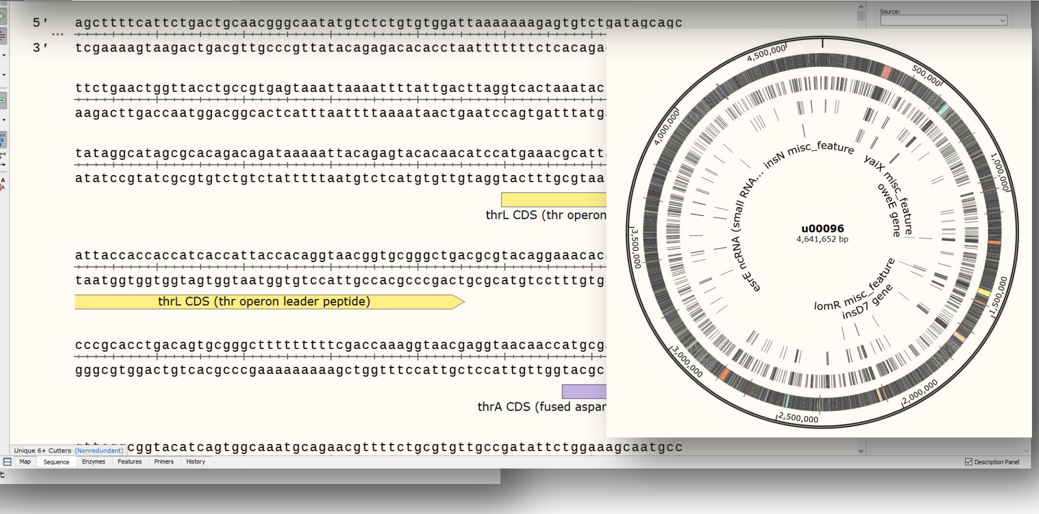

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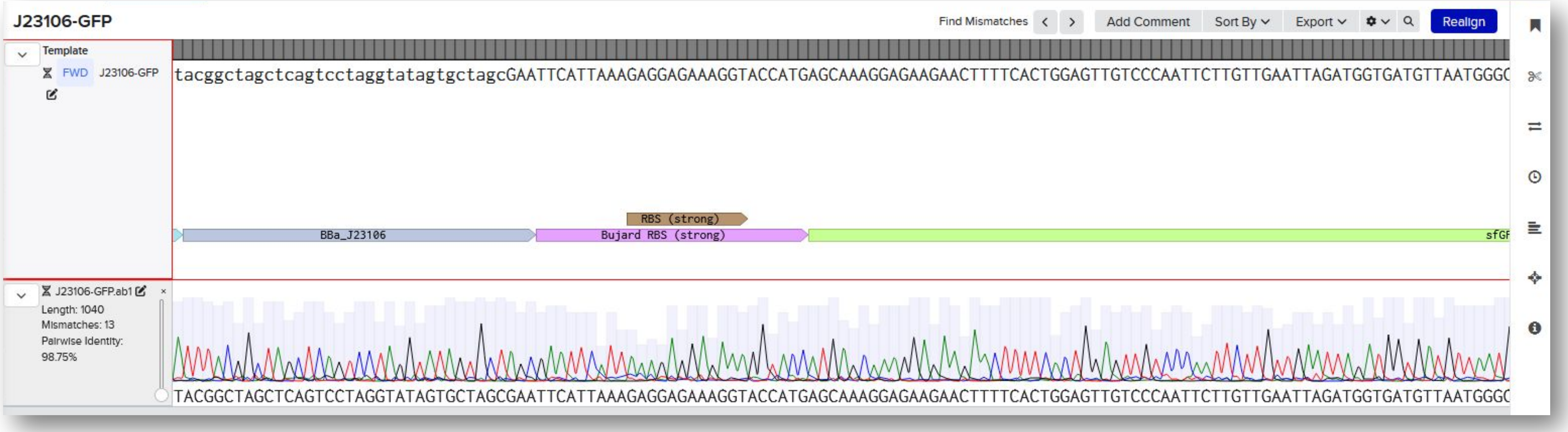
Validation of new DNA: Sequencing

DNA sequencing could be performed by different technologies including

1. Sanger sequencing (cheap),
2. Next-gen sequencing (massively parallel)
3. 3rd-gen sequencing (long read)



J23106-GFP

Template

FWD J23106-GFP

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J23106-GFP.ab1

Length: 1040
Mismatches: 13
Pairwise Identity: 98.75%

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Each nucleotide yield different signal (color). Then, they can be aligned to that of template or expected sequence for validation

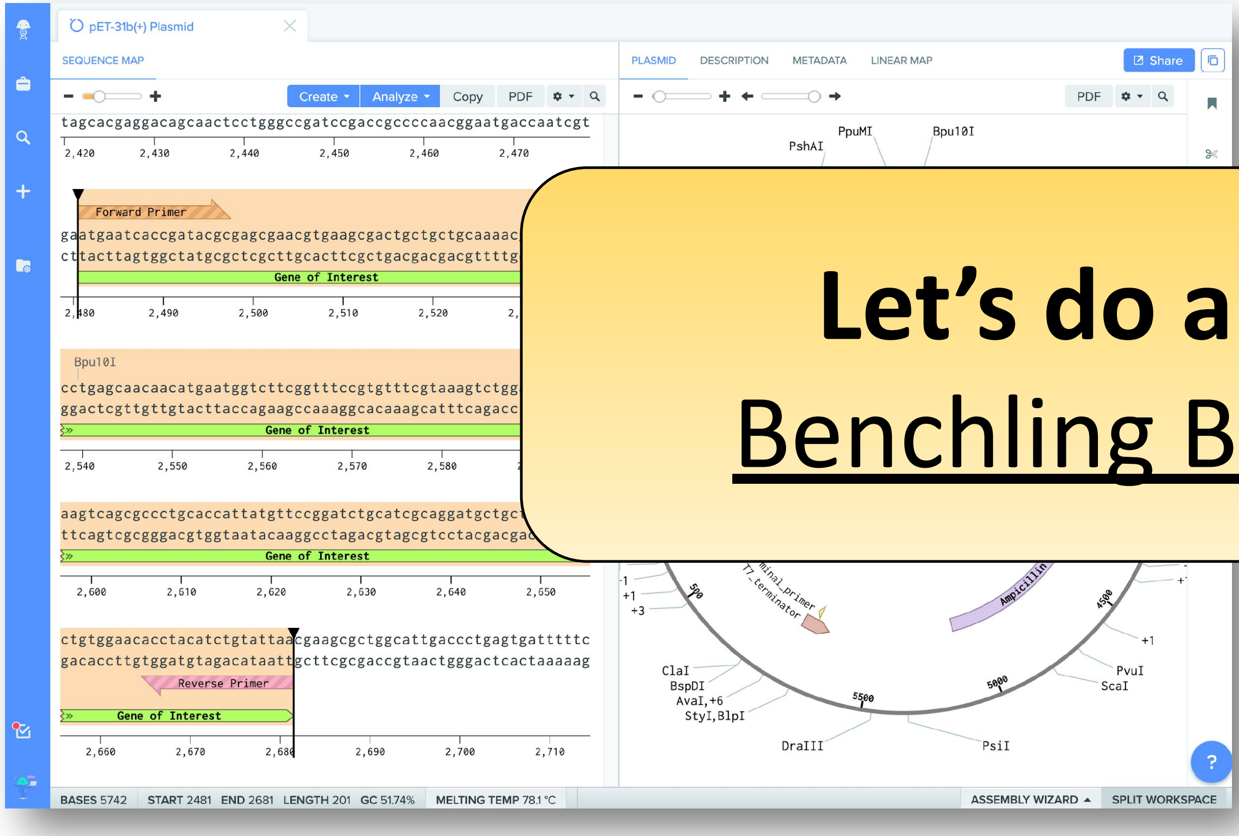
In silico analysis of DNA sequence

Benchling®: Free for academic users

A more powerful version available for **Enterprise user**

SnapGene Viewer: Free

A more powerful version available as **SnapGene®**



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 2,420     2,430     2,440     2,450     2,460     2,470

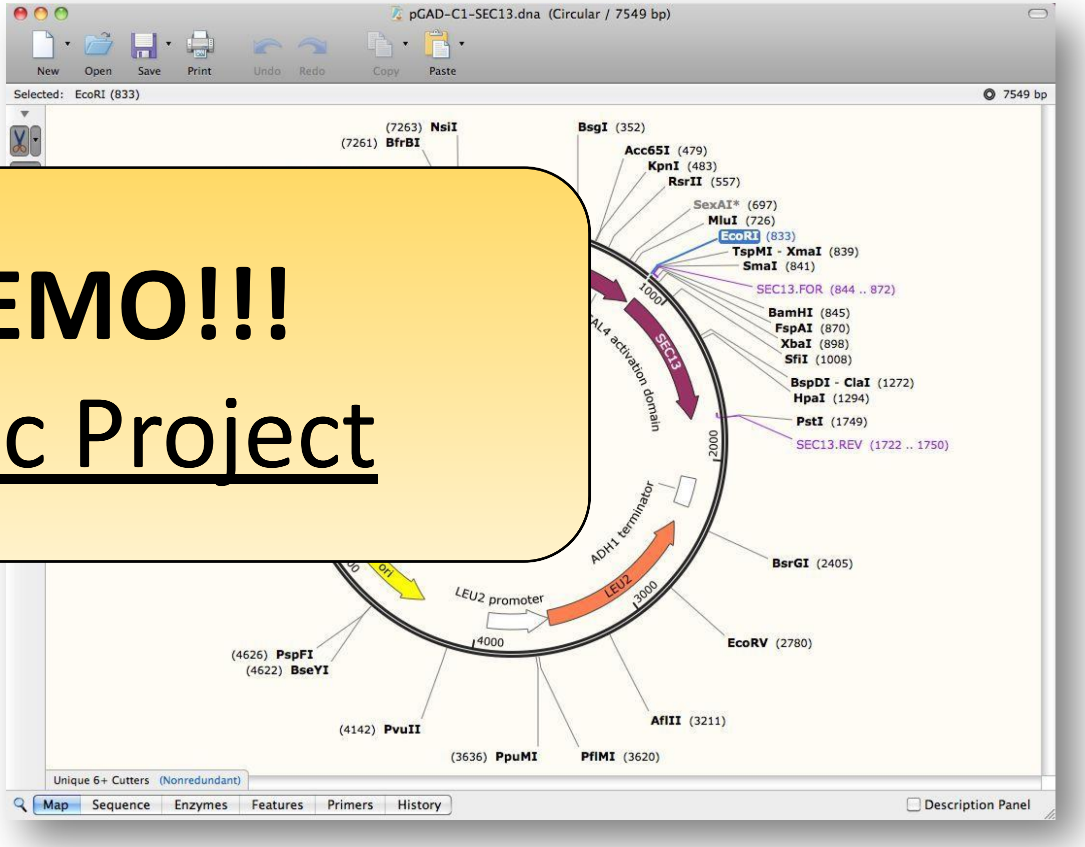
gaatgaatcaccgatacgcgagcgaacgtgaagcgactgctgctgcaaaac
cttacttagtggctatgcgctcgcttgcacttcgctgacgacgacgttttg
 2,480     2,490     2,500     2,510     2,520

cctgagcaacaacatgaatggtcttcggtttccgtgtttcgtaaagtctgg
ggactcgttgttgtacttaccagaagccaaaggcacaaagcatttcagacc
 2,540     2,550     2,560     2,570     2,580

aagtcagcgccctgcaccattatgttccggatctgcatcgcaggatgctgc
ttcagtcgcgggacgtggtaatacaaggcctagacgtagcgtcctacgacga
 2,600     2,610     2,620     2,630     2,640     2,650

ctgtggaacacctacatctgtattaacgaagcgctggcattgaccctgagtgattttc
gacaccttgtggatgtagacataattgcttcgcgaccgtaactgggactcactaaaaag
 2,660     2,670     2,680     2,690     2,700     2,710
```

BASES 5742 START 2481 END 2681 LENGTH 201 GC 51.74% MELTING TEMP 78.1 °C



pGAD-C1-SEC13.dna (Circular / 7549 bp)

Selected: EcoRI (833) 7549 bp

(7263) NsiI
(7261) BfrBI
BsgI (352)
Acc65I (479)
KpnI (483)
RsrII (557)
SexAI* (697)
MluI (726)
EcoRI (833)
TspMI - XmaI (839)
SmaI (841)
SEC13.FOR (844 .. 872)
BamHI (845)
FspAI (870)
XbaI (898)
SfiI (1008)
BspDI - ClaI (1272)
HpaI (1294)
PstI (1749)
SEC13.REV (1722 .. 1750)
BsrGI (2405)
EcoRV (2780)
AflII (3211)
PflMI (3620)
(3636) PpuMI
(4142) PvuII
(4626) PspFI
(4622) BseYI

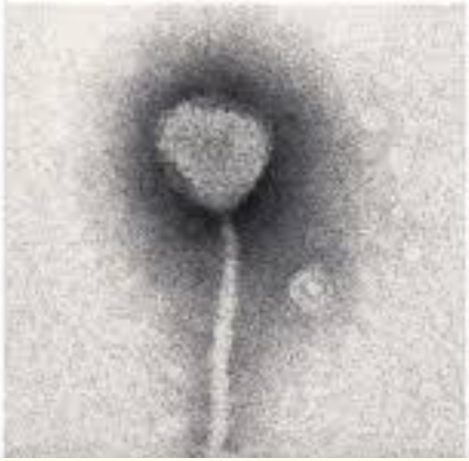
Let's do a DEMO!!!

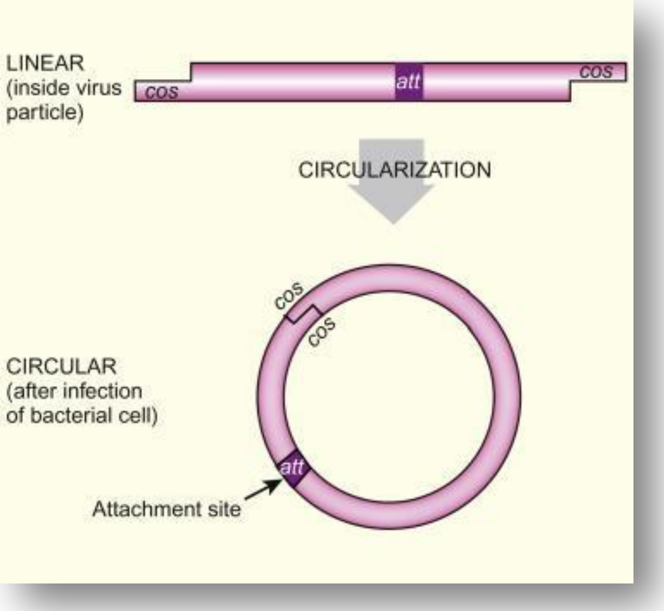
Benchling Basic Project

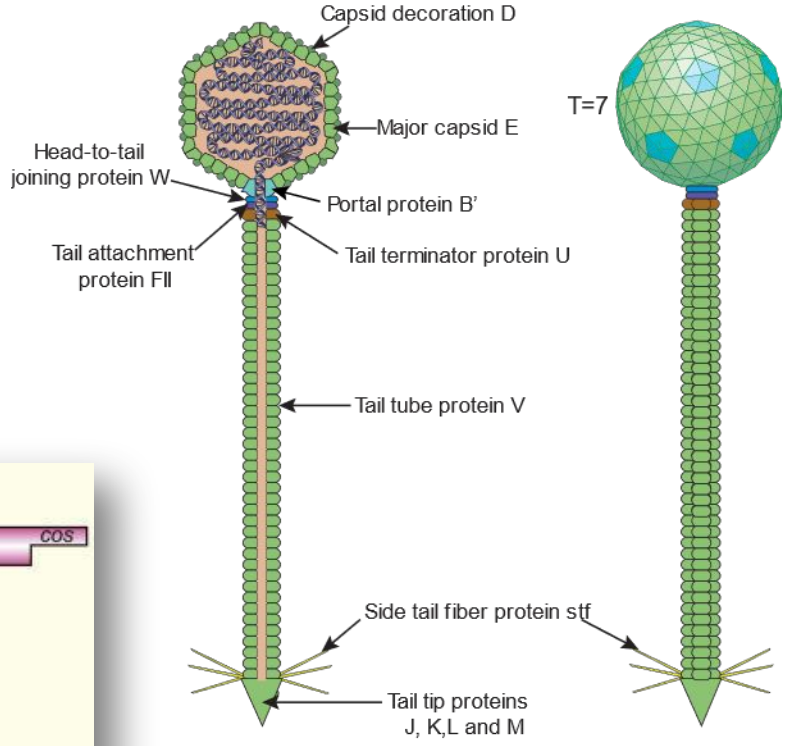
https://benchling.com/

https://www.snapgene.com/snapgene-viewer

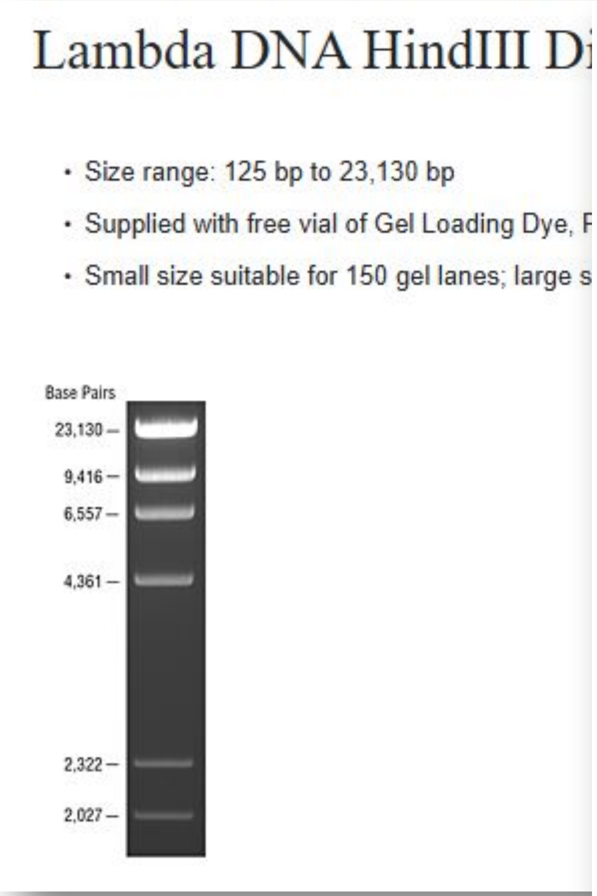
Let’s do DNA Separation: *in silico* gel





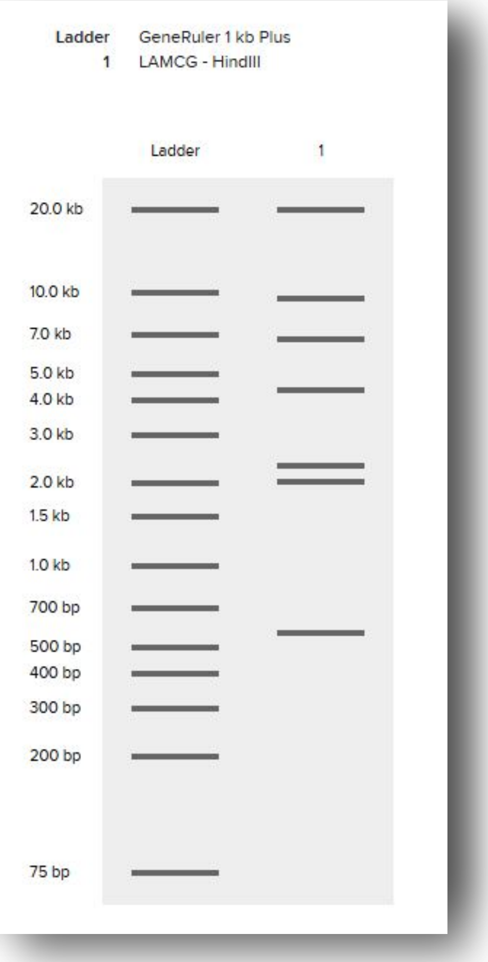


Phage Lambda Genome ~45kb



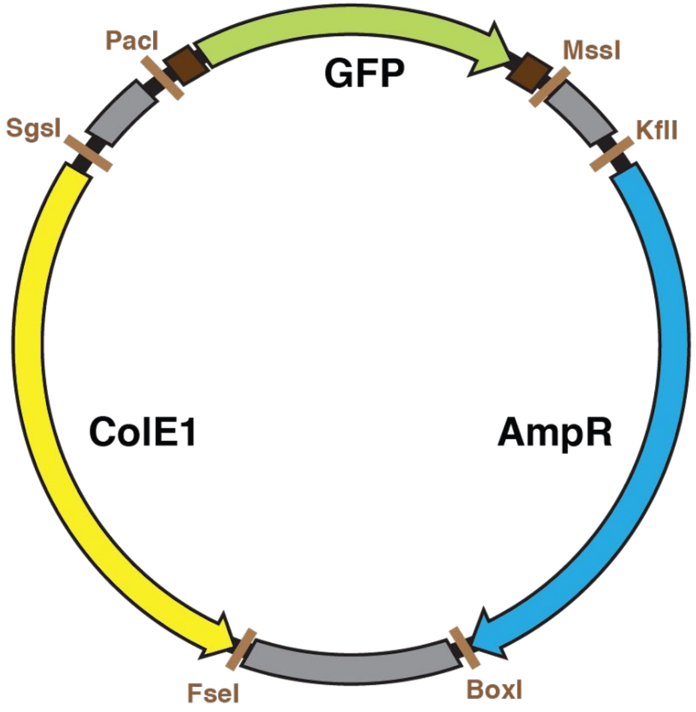
Lambda DNA HindIII Di

- Size range: 125 bp to 23,130 bp
- Supplied with free vial of Gel Loading Dye, P
- Small size suitable for 150 gel lanes; large s



Ladder GeneRuler 1 kb Plus
1 LAMCG - HindIII

Assembly of 3 Fragments into a plasmid – ColE1-AmpR-GFP

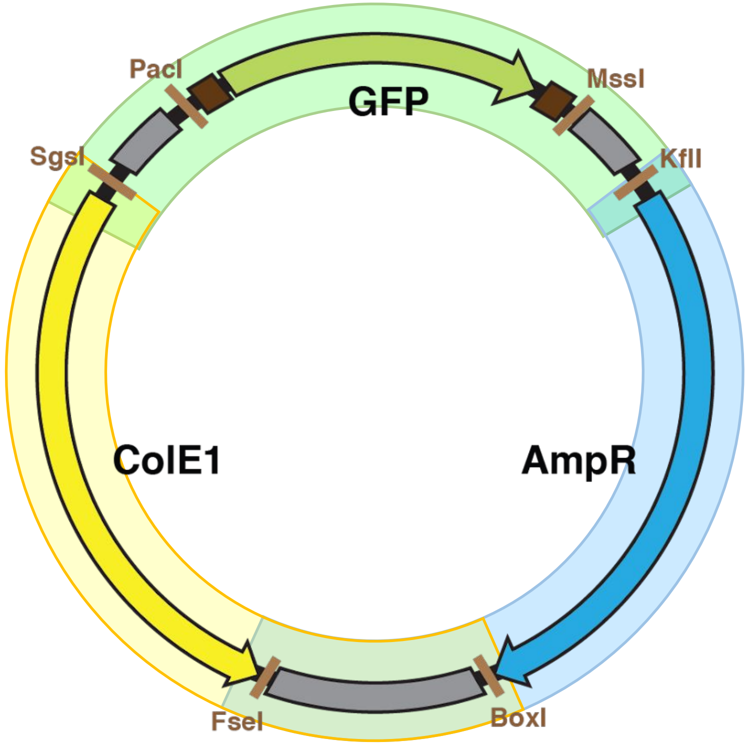


Make different DNA fragments by PCR

DNA fragments could also be made by
1) restriction digest reaction or
2) ordered for chemical synthesis

Links to DNA sequences:
Desired sequence: ColE1-AmpR-sfGFP plasmid
1) sfGFP_part, 2) AmpR_part, 3) ColE1_part

Assembly of 3 Fragments into a plasmid – ColE1-AmpR-GFP



Make different DNA fragments by PCR

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1) restriction digest reaction or
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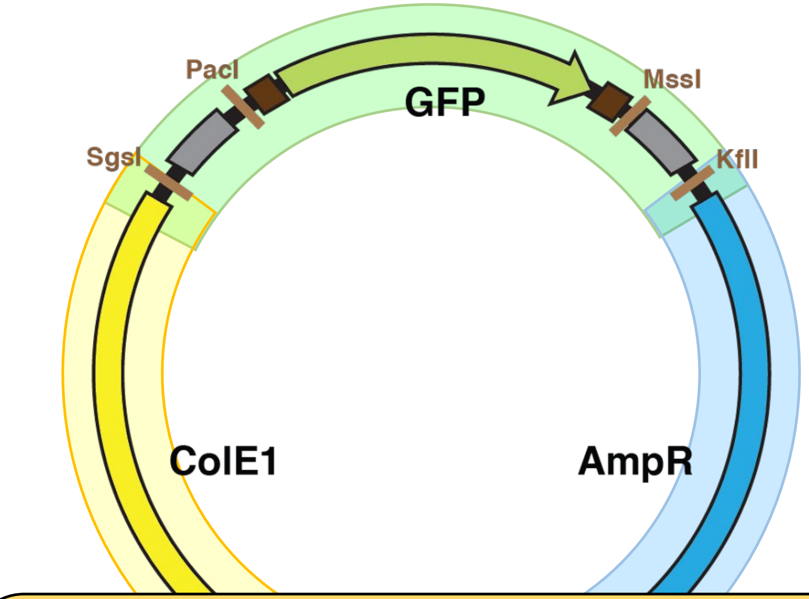
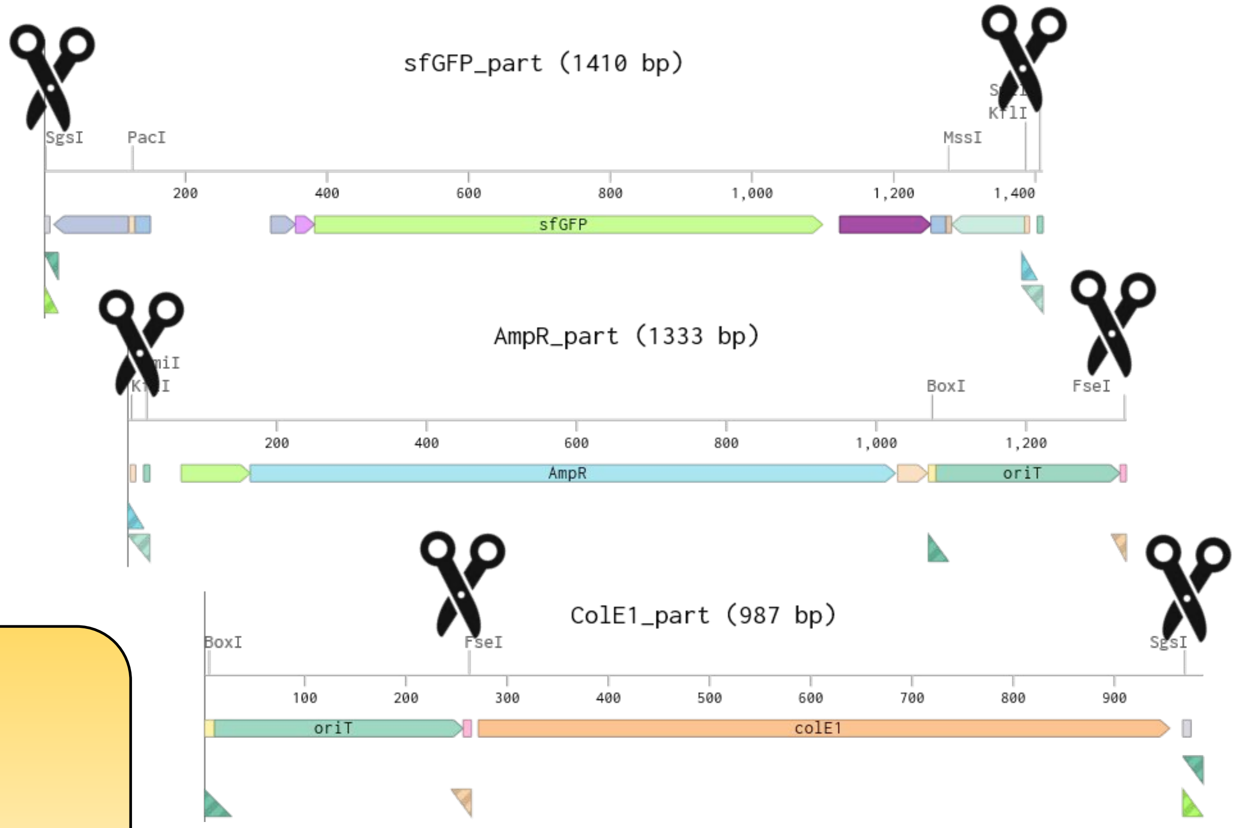
Links to DNA sequences:
Desired sequence: ColE1-AmpR-sfGFP plasmid
1) sfGFP_part, 2) AmpR_part, 3) ColE1_part

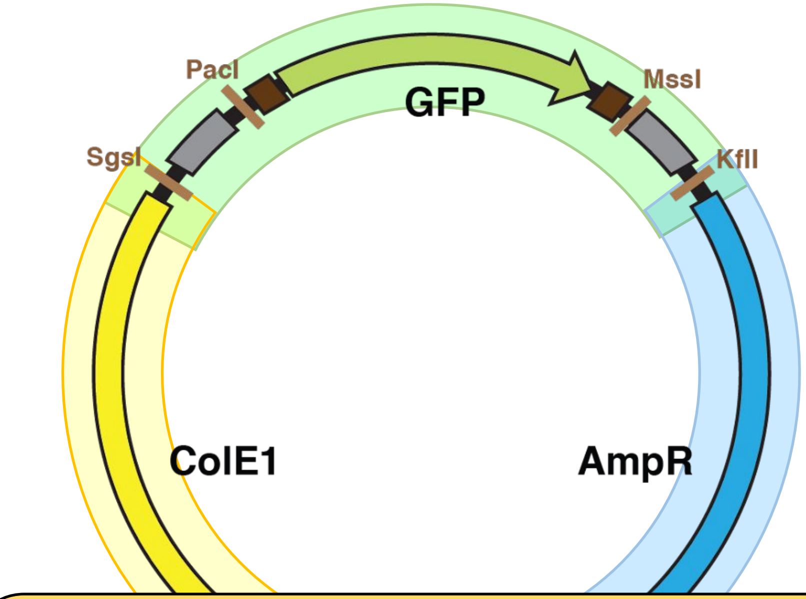
Assembly of 3 Fragments into a plasmid – ColE1-AmpR-GFP

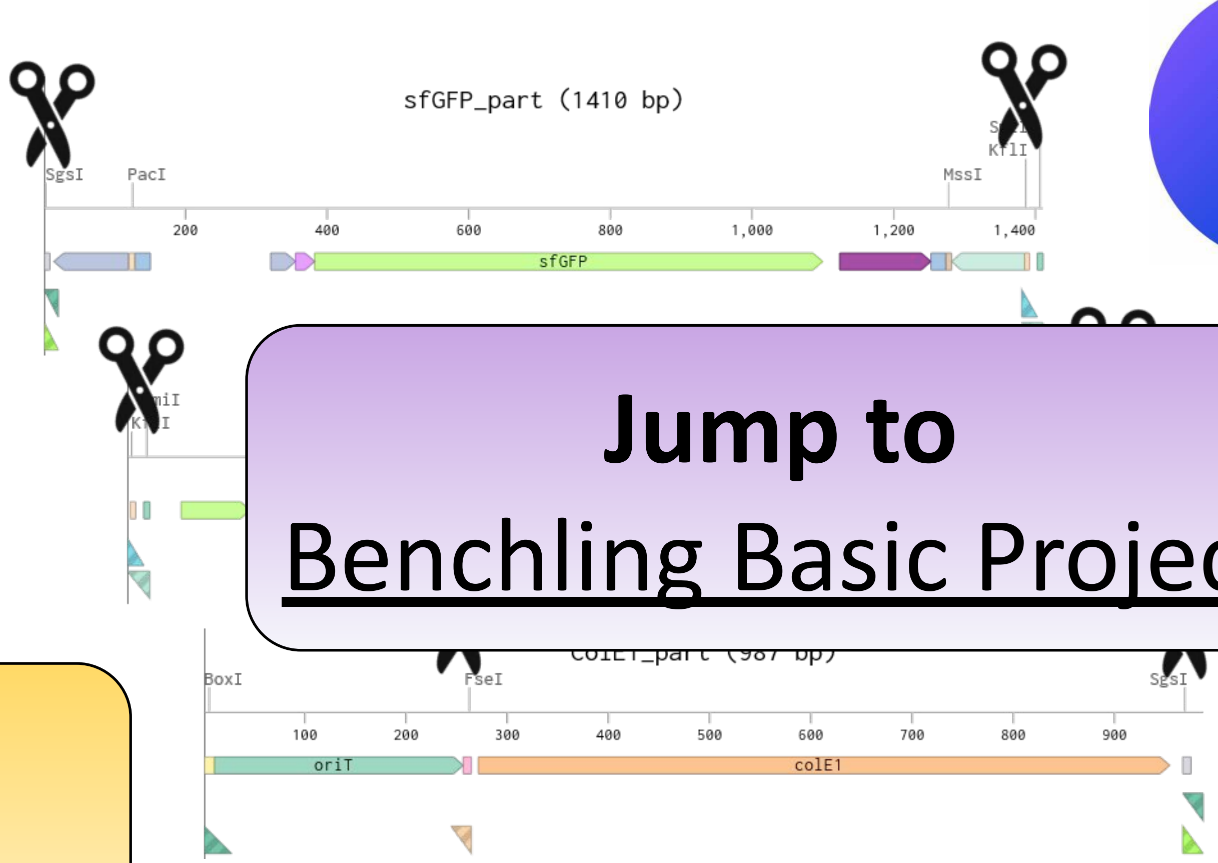




Make appropriate sticky-ends for ligation

Assembly of 3 Fragments into a plasmid – ColE1-AmpR-GFP



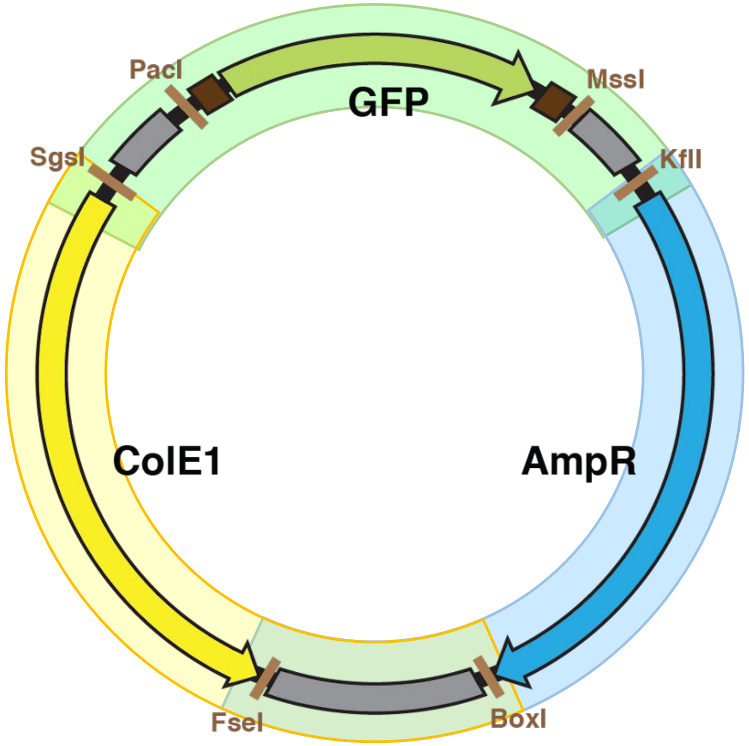


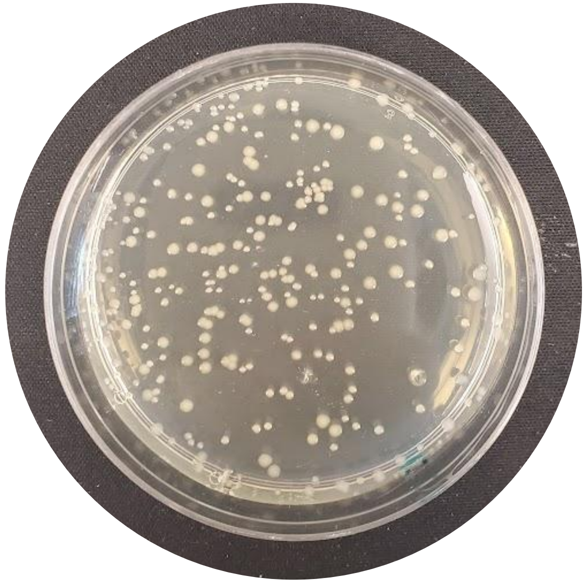
Jump to

Benchling Basic Project

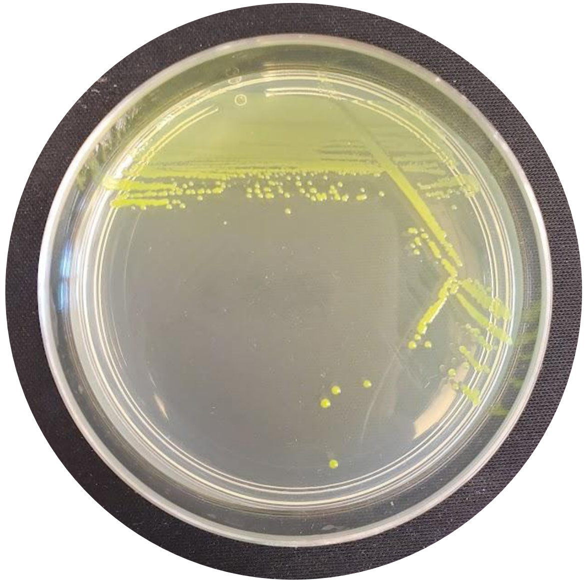
Make appropriate sticky-ends for ligation

Assembly of 3 Fragments into a plasmid – ColE1-AmpR-GFP



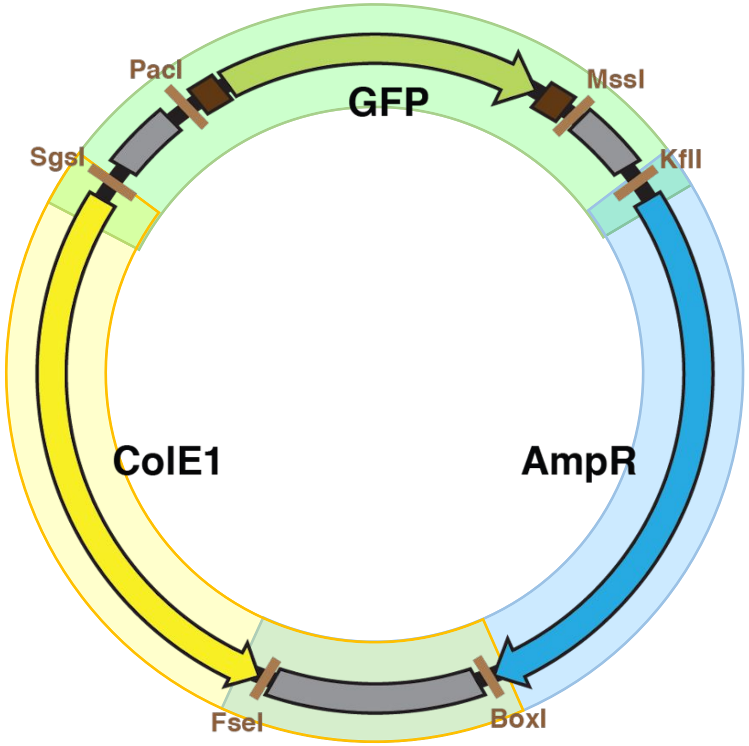


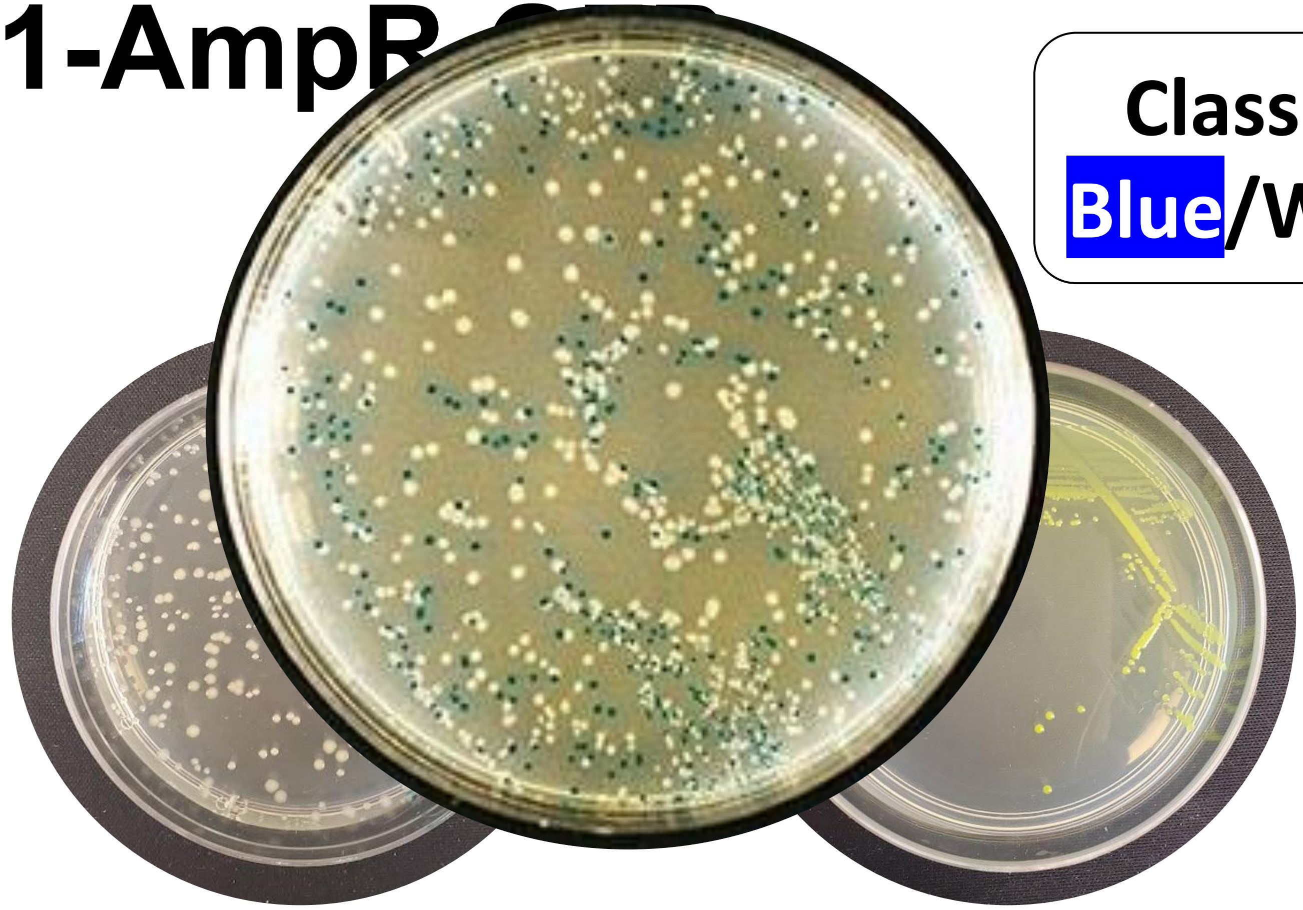
White



Green

Assembly of 3 Fragments into a plasmid – ColE1-AmpR



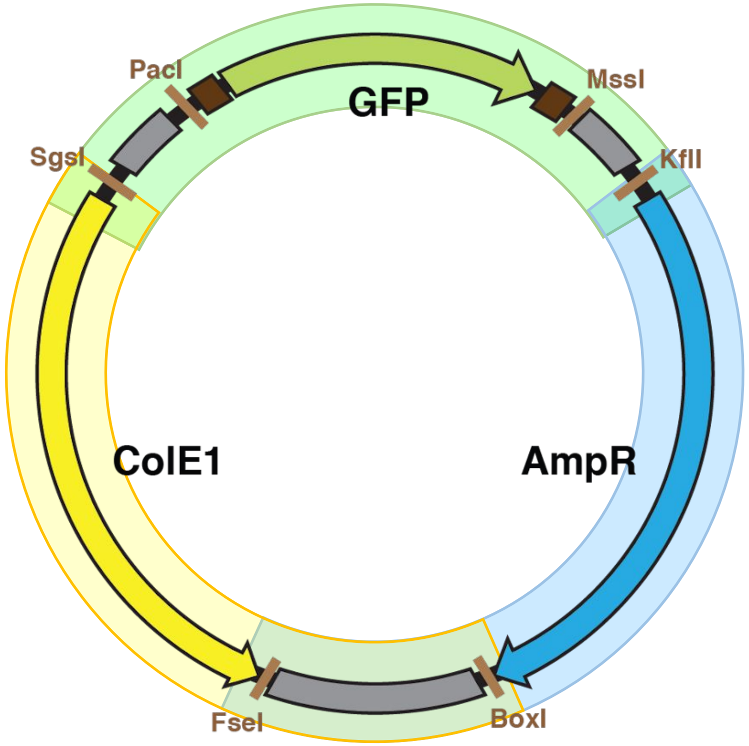


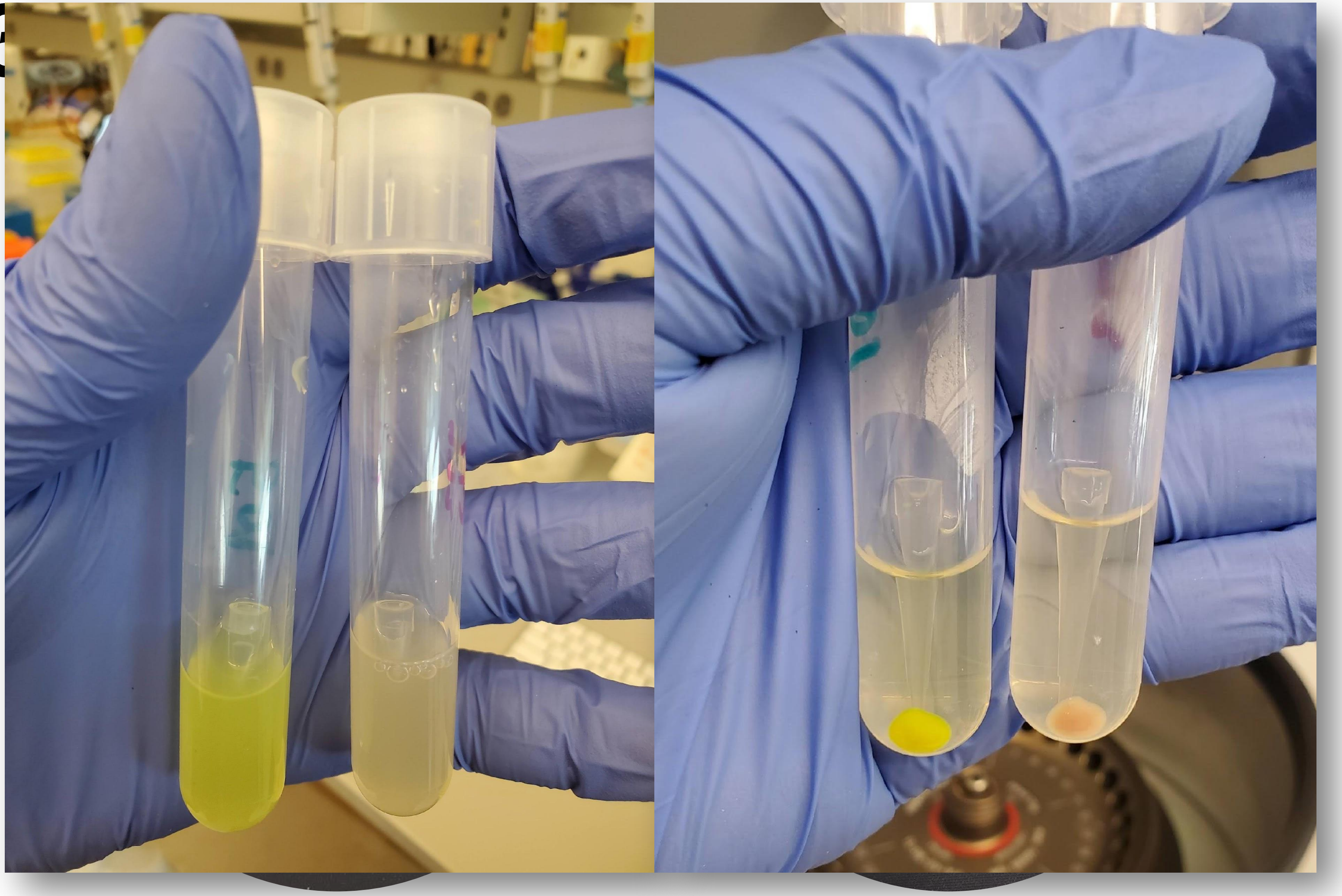
Classical Blue/White

White

Green

Assembly of 3
plasmid – Col





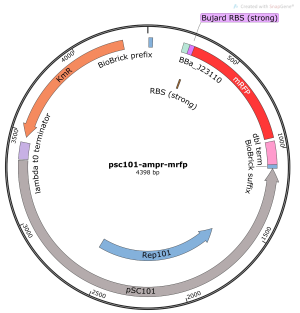
White

Green

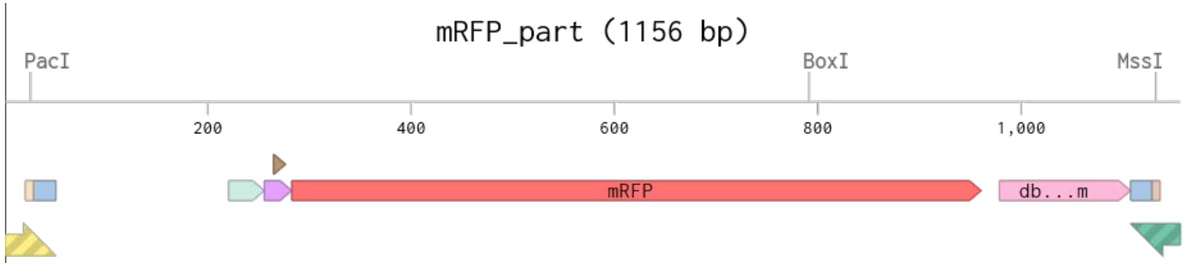
Converting GFP into RFP

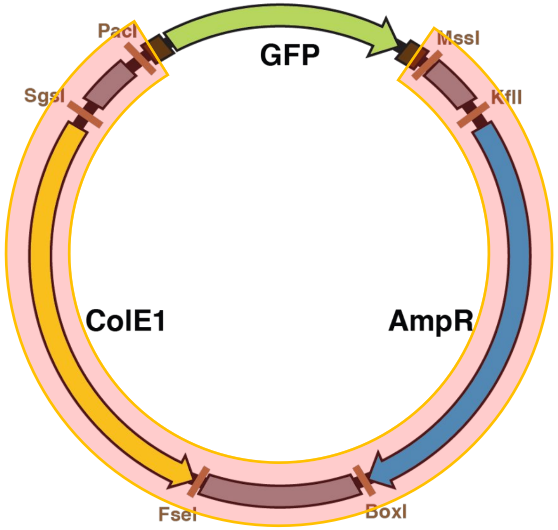
Or DNA Synthesis

Links to DNA sequences:
Starting ColE1-AmpR-sfGFP plasmid
□Digested ColE1-AmpR
mRFP template
□PCR mRFP insert

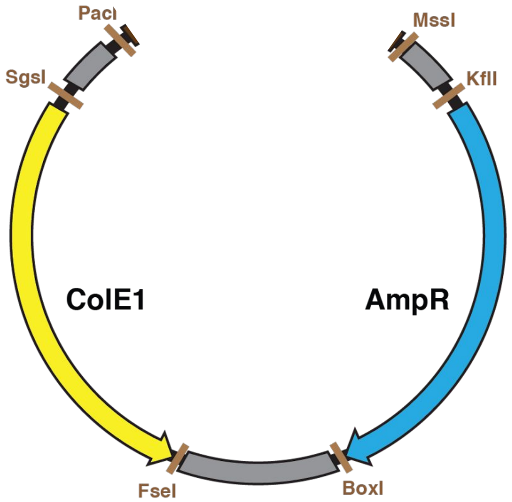


PCR

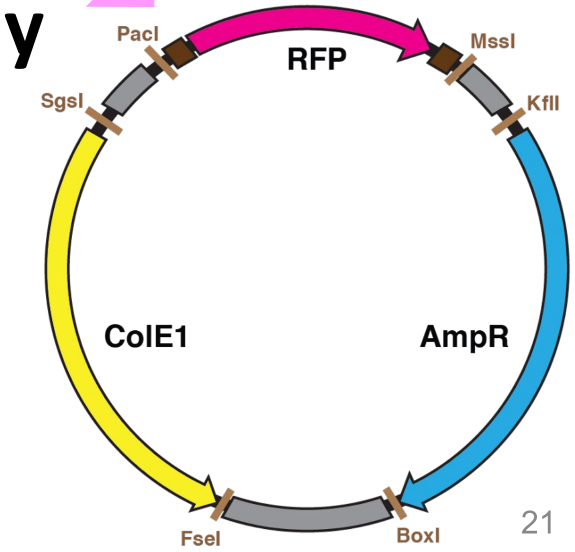




Digest



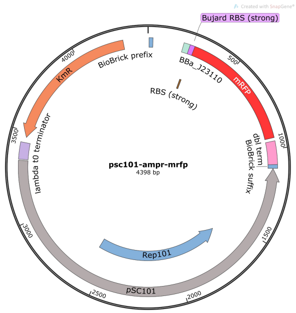
Assembly



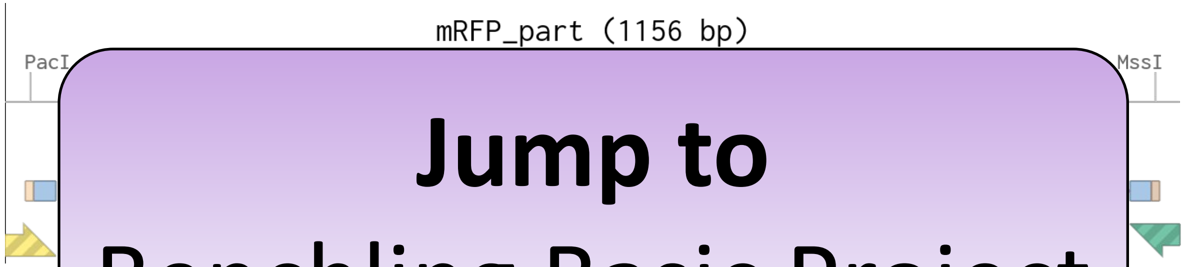
Converting GFP into RFP

Links to DNA sequences:
Starting ColE1-AmpR-sfGFP plasmid
□Digested ColE1-AmpR
mRFP template
□PCR mRFP insert

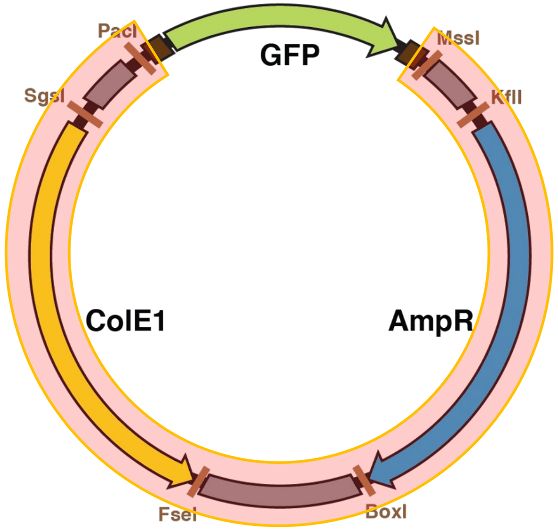
Or DNA Synthesis



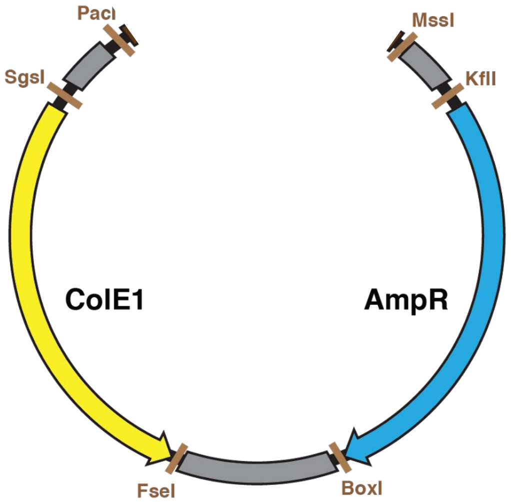
PCR



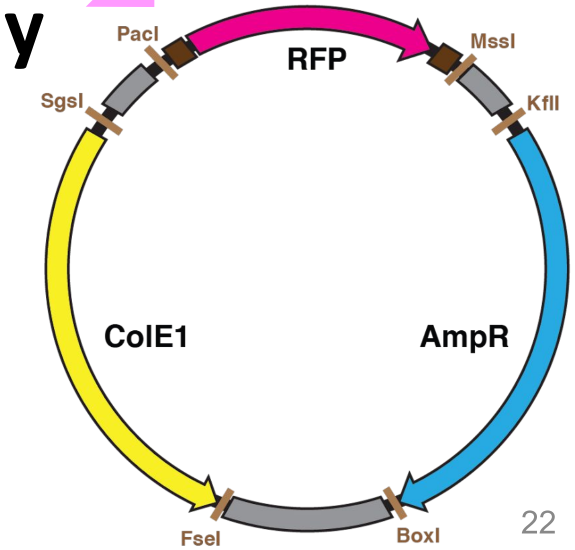
Jump to
Benchling Basic Project



Digest



Assembly

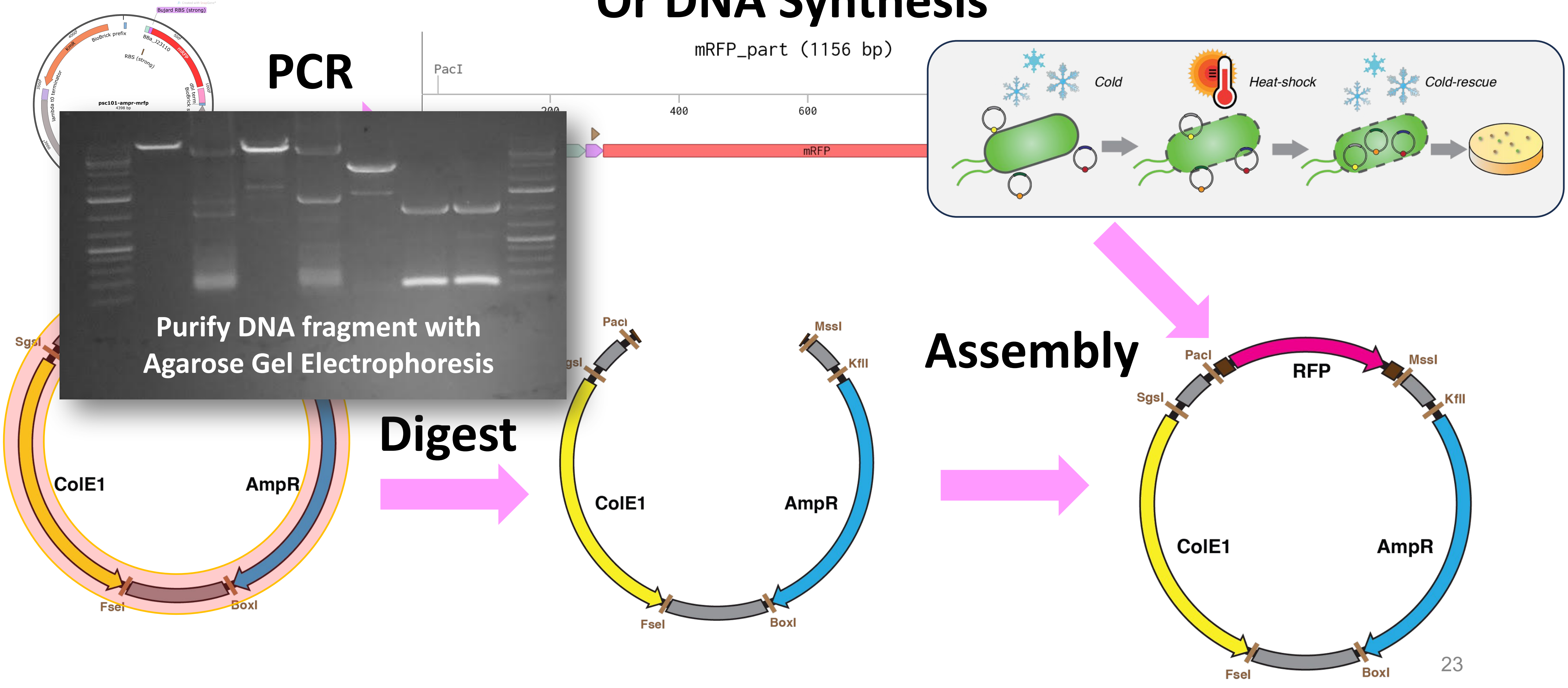


Converting GFP into RFP

Or DNA Synthesis

Links to DNA sequences:

Starting ColE1-AmpR-sfGFP plasmid
□Digested ColE1-AmpR
mRFP template
□PCR mRFP insert



PCR

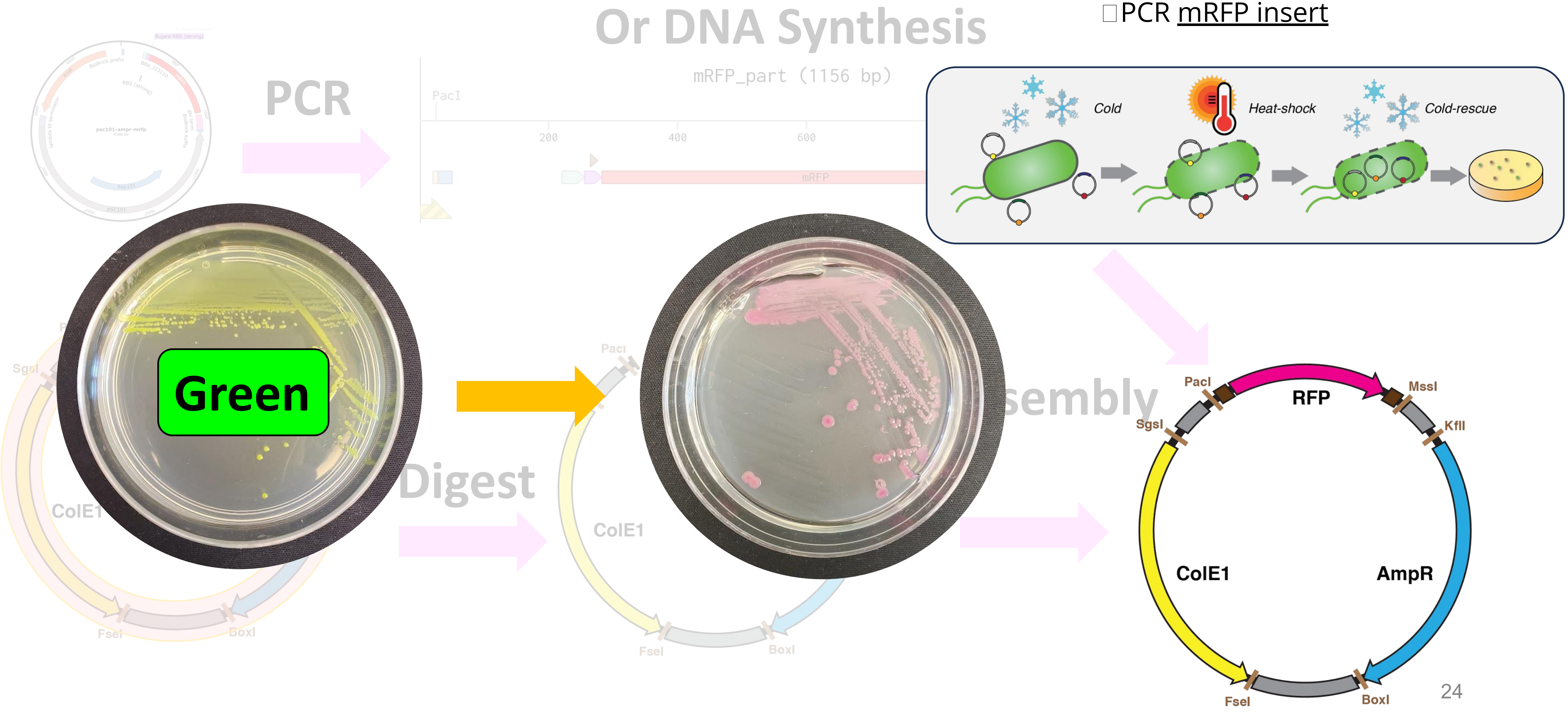
Purify DNA fragment with Agarose Gel Electrophoresis

Digest

Assembly

Converting GFP into RFP

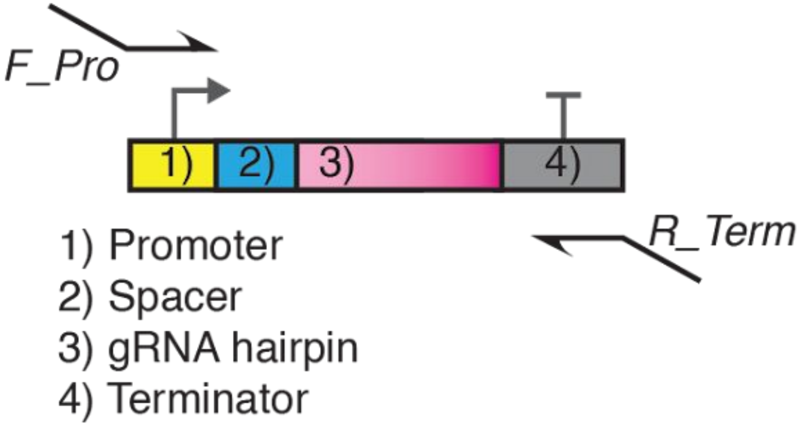
Links to DNA sequences:
Starting ColE1-AmpR-sfGFP plasmid
□Digested ColE1-AmpR
mRFP template
□PCR mRFP insert



Skipped during the talk

See 2025 recording if interested in this demo

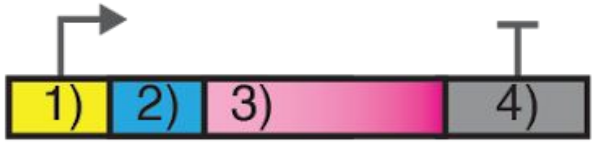
sgRNA expression cassette in bacteria



Spacer
sgRNA
Hairpin

CRISPRi - pCK411.RR1

Ones can also install cut-site between 1) and 2) so that spacer can be changed by PCR with only one oligonucleotide order

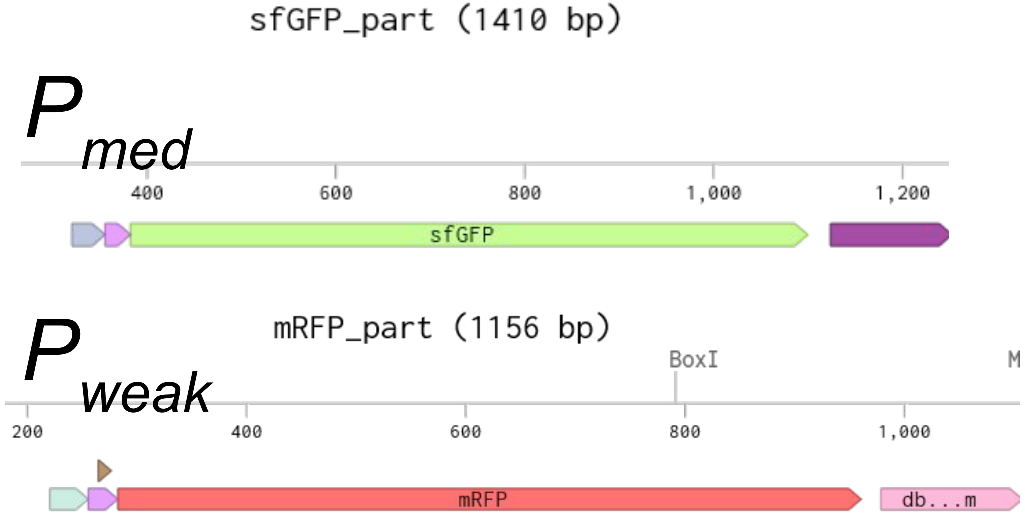


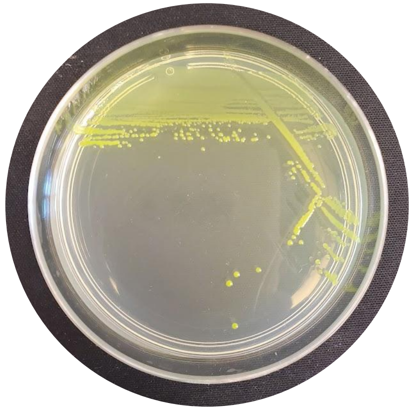
Oligo order is ~$10-20 per 60 bases
20 nt is a regular spacer size
40 nt can be attributed to priming and overhang site

Skipped during the talk

sgRNAs can be used to program phenotypes

Reporter genes Addgene: pCK760





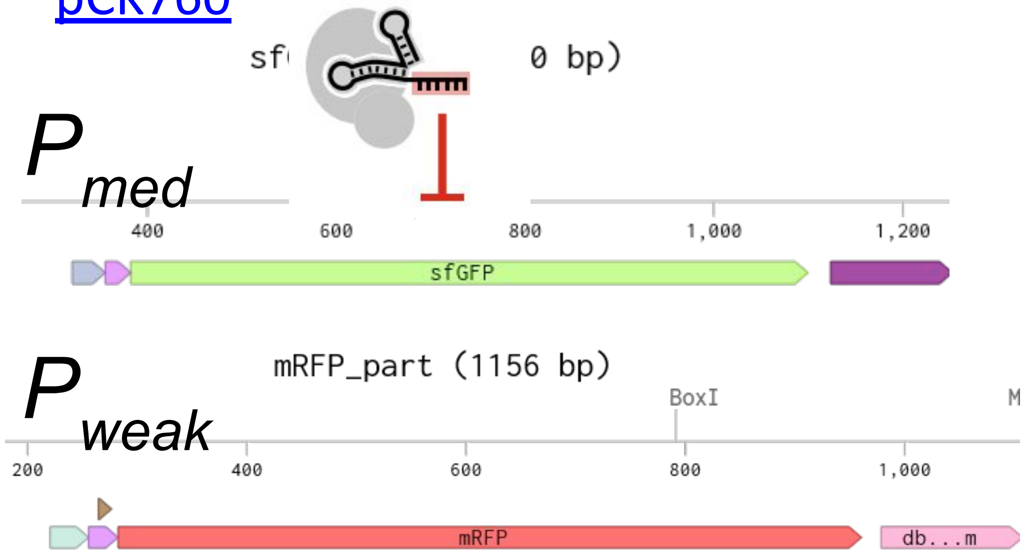
Green

Skipped during the talk

sgRNAs can be used to program phenotypes

CRISPR interference

Reporter genes [Addgene: pCK760]



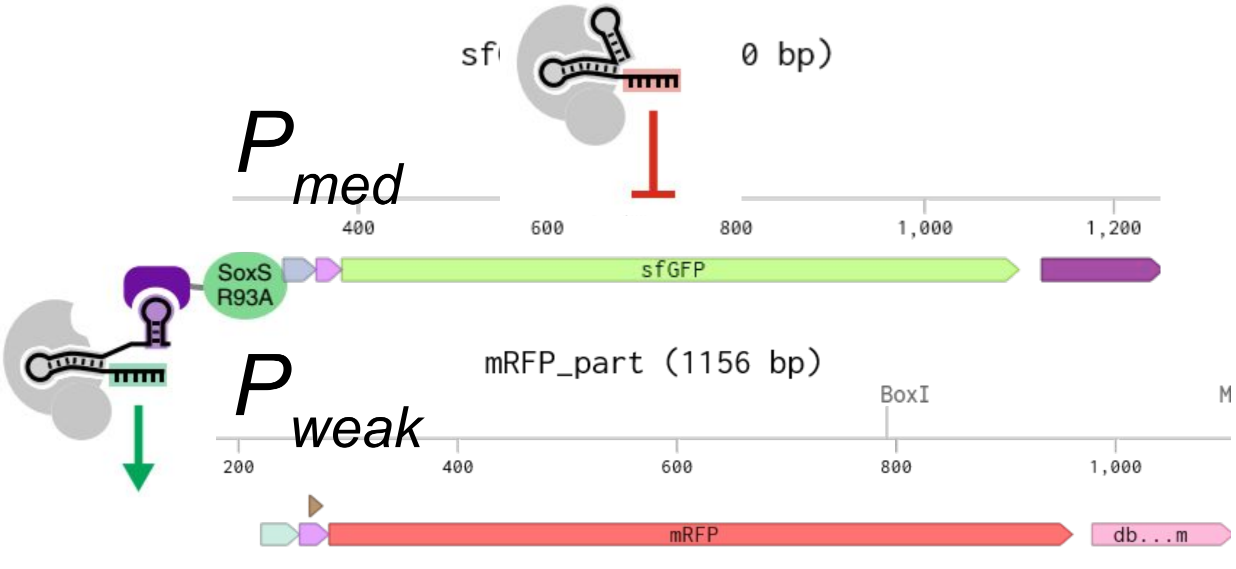
Colorless

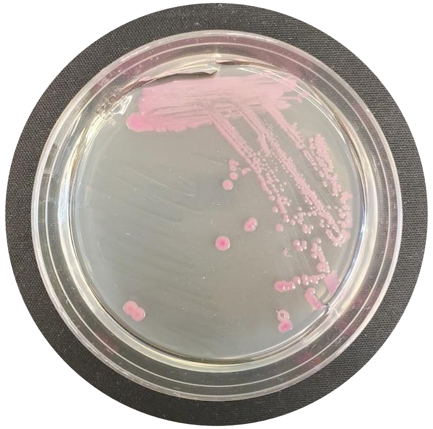
Skipped during the talk

sgRNAs can be used to program phenotypes

CRISPR interference and activation

Reporter genes Addgene: pCK760

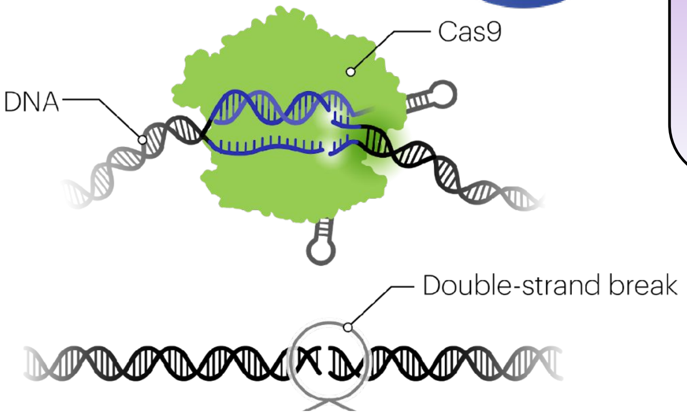




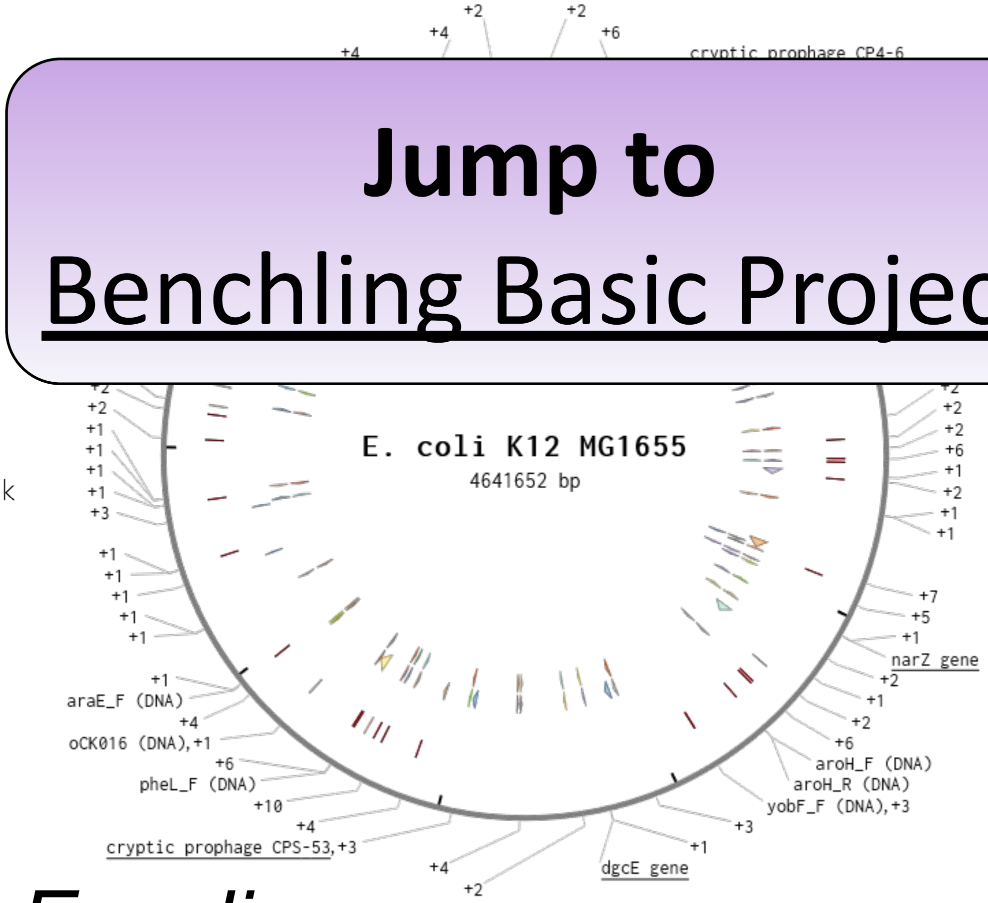
Red

Skipped during the talk

sgRNAs can be used to program phenotypes



Try knocking out ampC gene ---ampicillin resistance



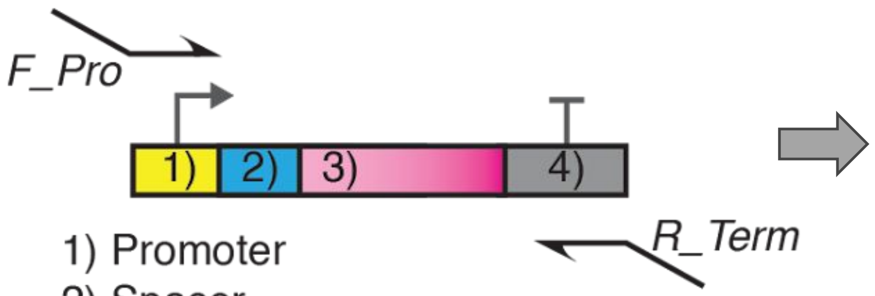
Jump to

Benchling Basic Project

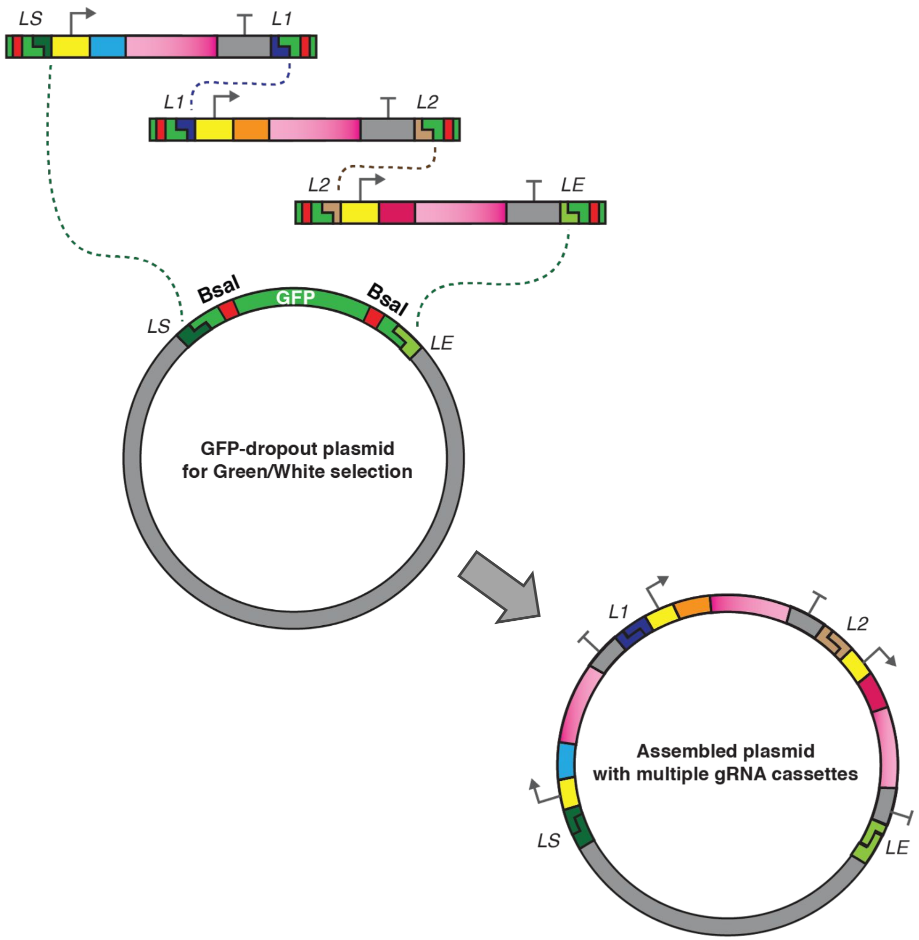
E. coli genome: GenBank: U00096.3

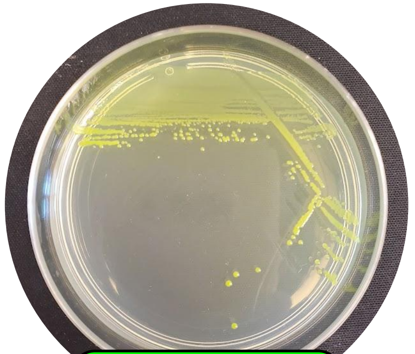
Skipped during the talk

Multiple gRNA cassettes by Golden-Gate



1) Promoter
2) Spacer
3) gRNA hairpin
4) Terminator





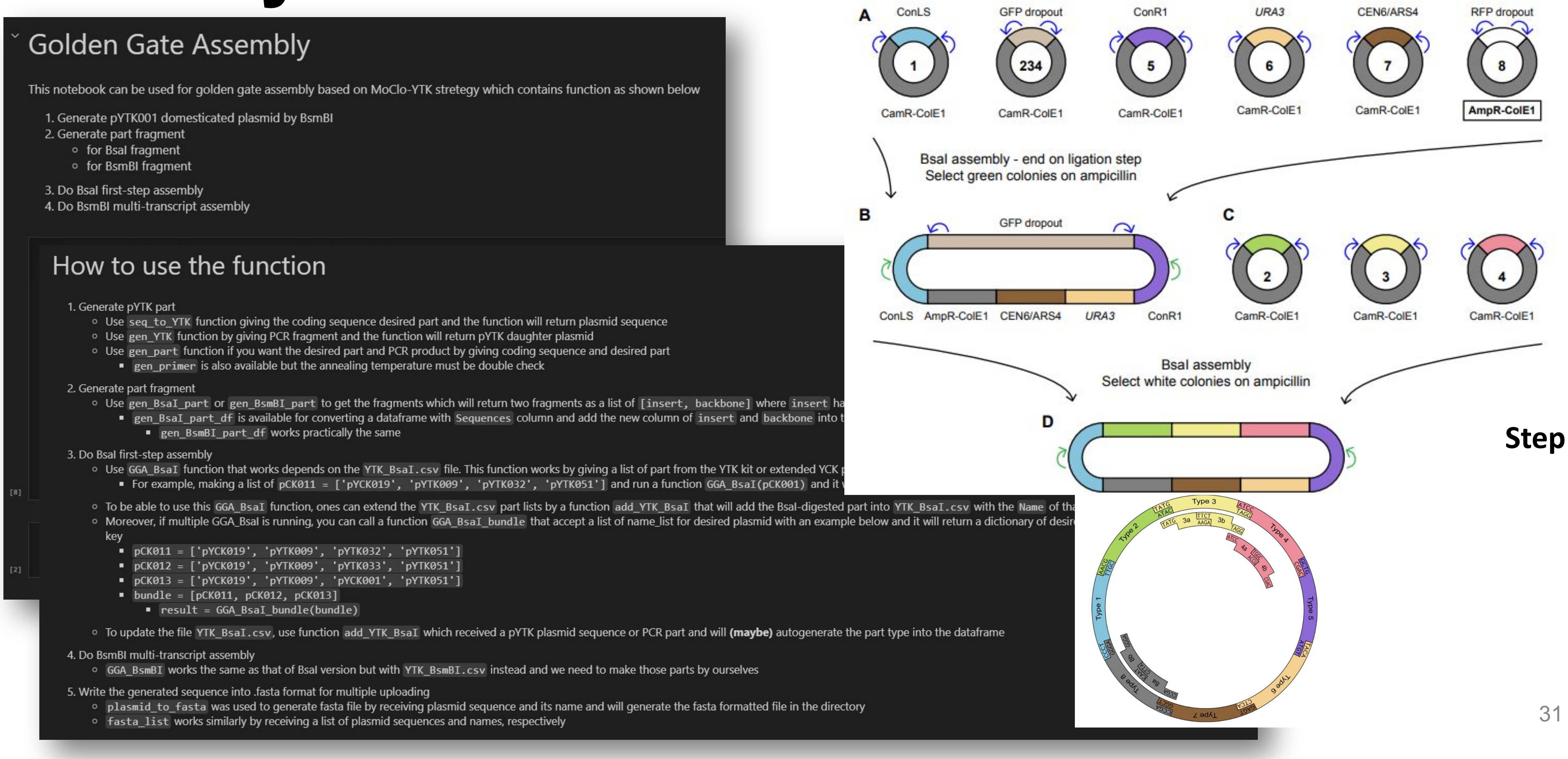
Green

Colorless

3 gRNAs in one cloning reaction

Skipped during the talk

ate Assembly with BioPython



Golden Gate Assembly

This notebook can be used for golden gate assembly based on MoClo-YTK stretegy which contains function as shown below

1. Generate pYTK001 domesticated plasmid by BsmBI
2. Generate part fragment
   - for BsaI fragment
   - for BsmBI fragment
3. Do BsaI first-step assembly
4. Do BsmBI multi-transcript assembly

How to use the function

1. Generate pYTK part
   - Use `seq_to_YTK` function giving the coding sequence desired part and the function will return plasmid sequence
   - Use `gen_YTK` function by giving PCR fragment and the function will return pYTK daughter plasmid
   - Use `gen_part` function if you want the desired part and PCR product by giving coding sequence and desired part
     - `gen_primer` is also available but the annealing temperature must be double check
2. Generate part fragment
   - Use `gen_BsaI_part` or `gen_BsmBI_part` to get the fragments which will return two fragments as a list of `[insert, backbone]` where `insert` ha
     - `gen_BsaI_part_df` is available for converting a dataframe with `Sequences` column and add the new column of `insert` and `backbone` into t
       - `gen_BsmBI_part_df` works practically the same
3. Do BsaI first-step assembly
   - Use `GGA_BsaI` function that works depends on the `YTK_BsaI.csv` file. This function works by giving a list of part from the YTK kit or extended YCK p
     - For example, making a list of `pCK011 = ['pYCK019', 'pYTK009', 'pYTK032', 'pYTK051']` and run a function `GGA_BsaI(pCK001)` and it w
   - To be able to use this `GGA_BsaI` function, ones can extend the `YTK_BsaI.csv` part lists by a function `add_YTK_BsaI` that will add the BsaI-digested part into `YTK_BsaI.csv` with the `Name` of tha
   - Moreover, if multiple GGA_BsaI is running, you can call a function `GGA_BsaI_bundle` that accept a list of name_list for desired plasmid with an example below and it will return a dictionary of desir key
     - `pCK011 = ['pYCK019', 'pYTK009', 'pYTK032', 'pYTK051']`
     - `pCK012 = ['pYCK019', 'pYTK009', 'pYTK033', 'pYTK051']`
     - `pCK013 = ['pYCK019', 'pYTK009', 'pYCK001', 'pYTK051']`
     - `bundle = [pCK011, pCK012, pCK013]`
       - `result = GGA_BsaI_bundle(bundle)`
   - To update the file `YTK_BsaI.csv`, use function `add_YTK_BsaI` which received a pYTK plasmid sequence or PCR part and will **(maybe)** autogenerate the part type into the dataframe
4. Do BsmBI multi-transcript assembly
   - `GGA_BsmBI` works the same as that of BsaI version but with `YTK_BsmBI.csv` instead and we need to make those parts by ourselves
5. Write the generated sequence into .fasta format for multiple uploading
   - `plasmid_to_fasta` was used to generate fasta file by receiving plasmid sequence and its name and will generate the fasta formatted file in the directory
   - `fasta_list` works similarly by receiving a list of plasmid sequences and names, respectively

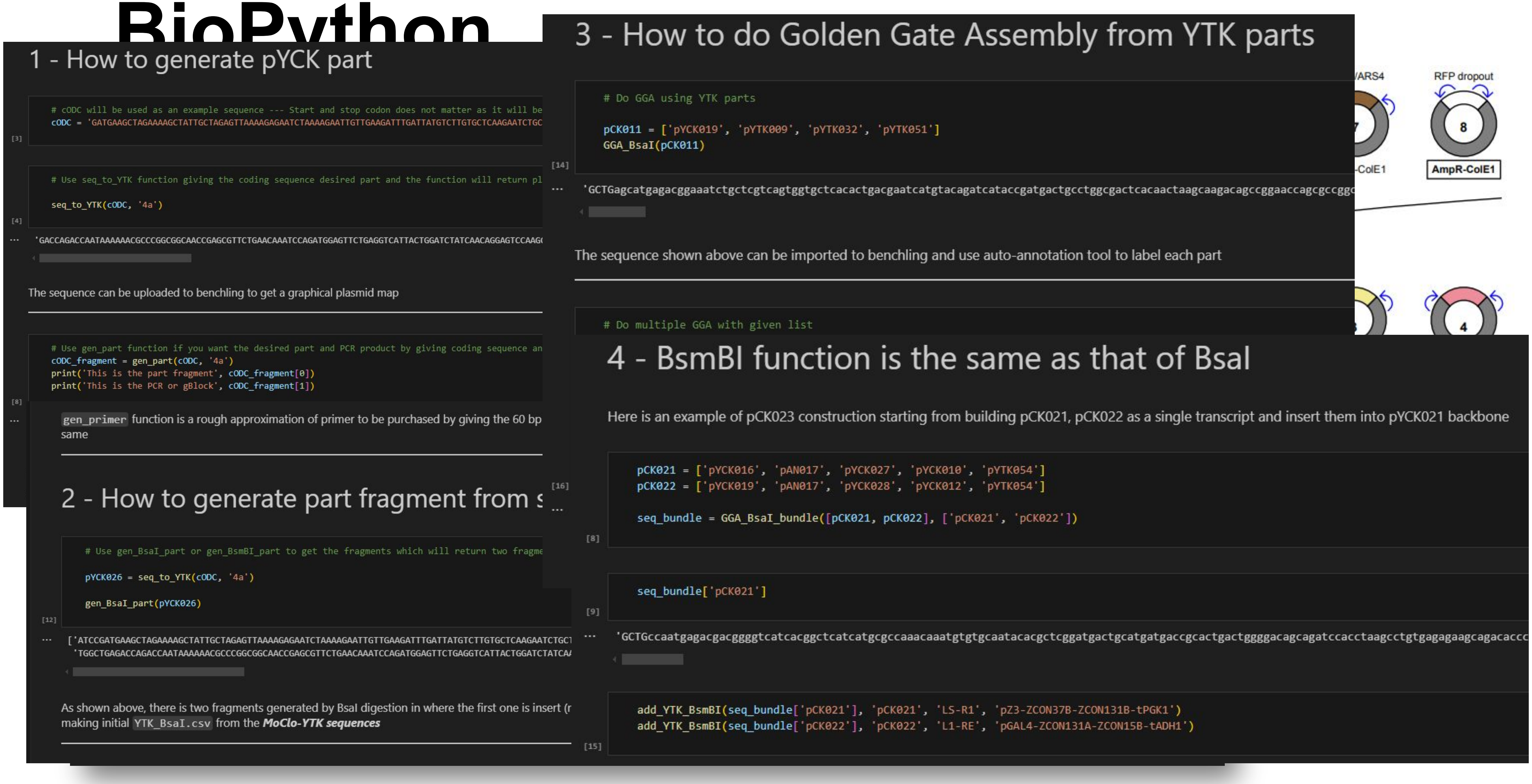
Step 1&2

Step 3

Step 4

Skipped during the talk

ate Assembly with BioPython



1 - How to generate pYCK part

```
# cODC will be used as an example sequence --- Start and stop codon does not matter as it will be
cODC = 'GATGAAGCTAGAAAAGCTATTGCTAGAGTTAAAAGAGAATCTAAAAGAATTGTTGAAGATTTGATTATGTCTTGTGCTCAAGAATCTGC
```
[3]

```
# Use seq_to_YTK function giving the coding sequence desired part and the function will return pl

seq_to_YTK(cODC, '4a')
```
[4]

```
'GACCAGACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAACAGGAGTCCAAG
```

The sequence can be uploaded to benchling to get a graphical plasmid map

```
# Use gen_part function if you want the desired part and PCR product by giving coding sequence an
cODC_fragment = gen_part(cODC, '4a')
print('This is the part fragment', cODC_fragment[0])
print('This is the PCR or gBlock', cODC_fragment[1])
```
[8]

`gen_primer` function is a rough approximation of primer to be purchased by giving the 60 bp same

2 - How to generate part fragment from s

```
# Use gen_BsaI_part or gen_BsmBI_part to get the fragments which will return two fragme

pYCK026 = seq_to_YTK(cODC, '4a')

gen_BsaI_part(pYCK026)
```
[12]

```
['ATCCGATGAAGCTAGAAAAGCTATTGCTAGAGTTAAAAGAGAATCTAAAAGAATTGTTGAAGATTTGATTATGTCTTGTGCTCAAGAATCTGCT
 'TGGCTGAGACCAGACCAATAAAAAACGCCCGGCGGCAACCGAGCGTTCTGAACAAATCCAGATGGAGTTCTGAGGTCATTACTGGATCTATCAA
```

As shown above, there is two fragments generated by BsaI digestion in where the first one is insert (r making initial `YTK_BsaI.csv` from the ***MoClo-YTK sequences***

3 - How to do Golden Gate Assembly from YTK parts

```
# Do GGA using YTK parts

pCK011 = ['pYCK019', 'pYTK009', 'pYTK032', 'pYTK051']
GGA_BsaI(pCK011)
```
[14]

```
'GCTGagcatgagacggaaatctgctcgtcagtggtgctcacactgacgaatcatgtacagatcataccgatgactgcctggcgactcacaactaagcaagacagccggaaccagcgccggc
```

The sequence shown above can be imported to benchling and use auto-annotation tool to label each part

```
# Do multiple GGA with given list
```
[16]

4 - BsmBI function is the same as that of BsaI

Here is an example of pCK023 construction starting from building pCK021, pCK022 as a single transcript and insert them into pYCK021 backbone

```
pCK021 = ['pYCK016', 'pAN017', 'pYCK027', 'pYCK010', 'pYTK054']
pCK022 = ['pYCK019', 'pAN017', 'pYCK028', 'pYCK012', 'pYTK054']

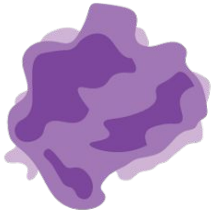
seq_bundle = GGA_BsaI_bundle([pCK021, pCK022], ['pCK021', 'pCK022'])
```
[8]

```
seq_bundle['pCK021']
```
[9]

```
'GCTGccaatgagacgacggggtcatcacggctcatcatgcgccaaacaaatgtgtgcaatacacgctcggatgactgcatgatgaccgcactgactggggacagcagatccacctaagcctgtgagagaagcagacaccc
```

```
add_YTK_BsmBI(seq_bundle['pCK021'], 'pCK021', 'LS-R1', 'pZ3-ZCON37B-ZCON131B-tPGK1')
add_YTK_BsmBI(seq_bundle['pCK022'], 'pCK022', 'L1-RE', 'pGAL4-ZCON131A-ZCON15B-tADH1')
```
[15]

Key Takeaways



- Use Molecular Biology tools like Benchling or Snapgene for DNA/RNA/Protein works: Read, Compare, Edit, or Design

- Benchling is a simple *in silico* molecular biology tools and is free for academic users

- *In silico* operations can save a lot of experimental time, especially when the procedure didn’t go as planned

Resources

- https://benchling.com/
- https://www.snapgene.com/snapgene-viewer
- https://www.geneious.com/guides/molecular-cloning-methods
- ApE, A Plasmid Editor: A Freely Available DNA Manipulation and Visualization Program
- https://www.addgene.org/mol-bio-reference/cloning/
- https://biopython.org/
- http://plannotate.barricklab.org/
    - For plasmid annotation