ß1 Extra Loop of Proteasome in *P. falciparum*

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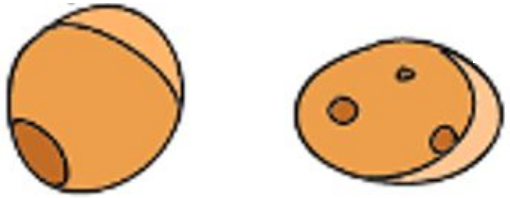
Introduction : Plasmodium

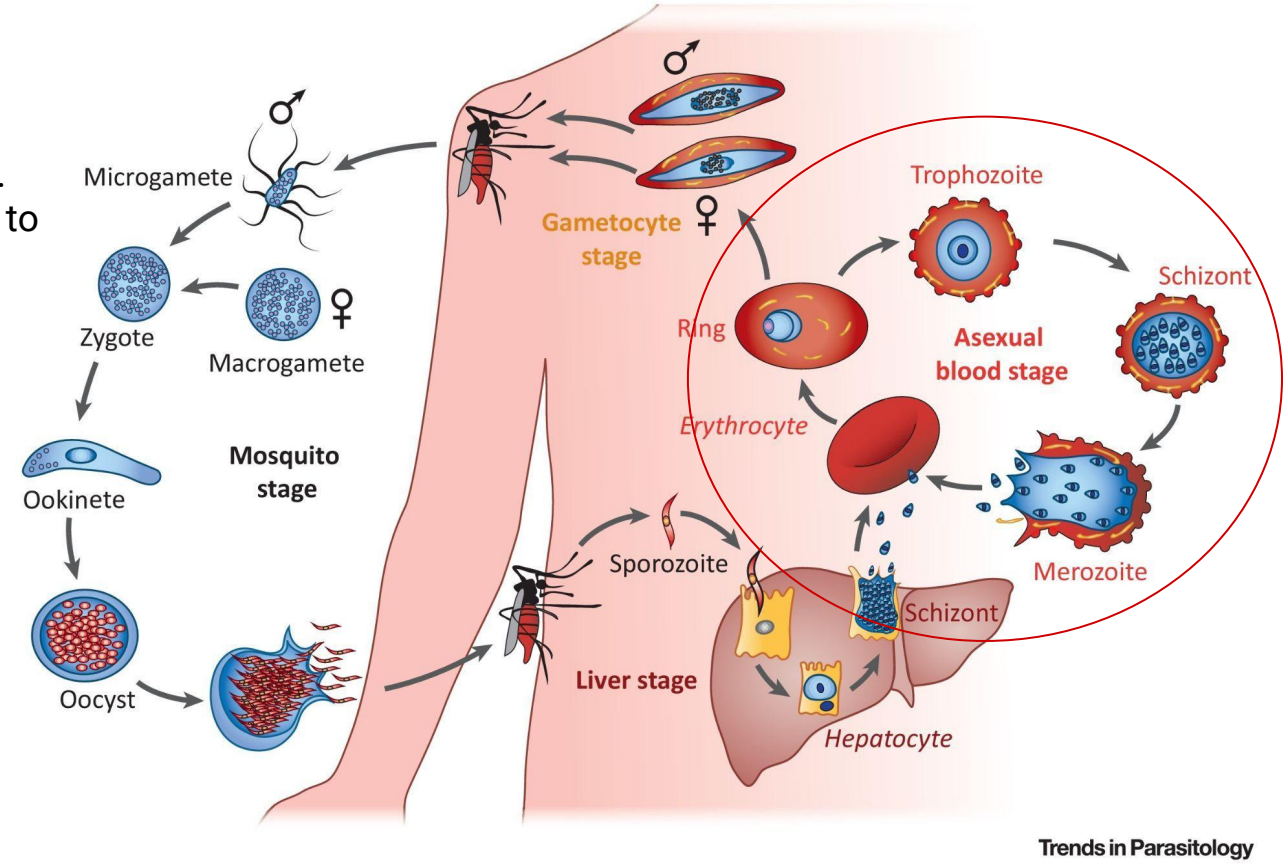
I'm P. Vivax! I'm the most common species that causes infectious in humans. I'm so popular that even George Washington and Abraham Lincoln were infected with me!



I'm P. ovale! I'm pretty rare and less severe than my fellow species. I guess you could say I'm the nice one?

I'm P. Malariae! I cause infectious around the world. I'm pretty benign compared to other Plasmodium species.

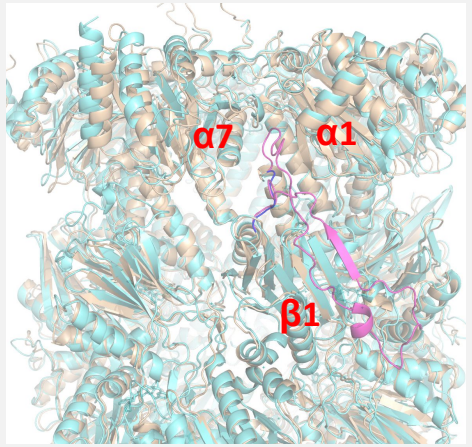
I'm P. falciparum! I may look funny because I'm shaped like a banana, but I'm the most dangerous of the bunch! I'm mostly found in sub-saharan Africa.



Red blood cell developmental cycle

Human vs Malaria Proteasome

In comparison to the human, the *P. falciparum* proteasome has extra loops.

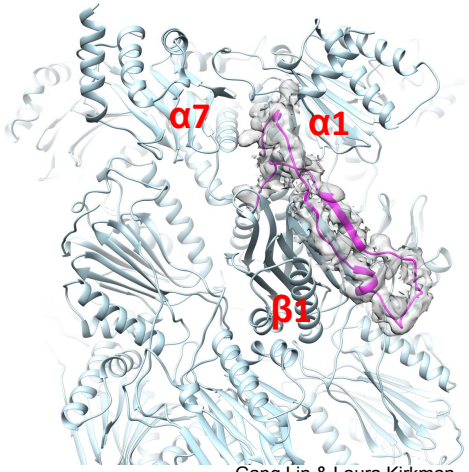


Structural alignment of Human and Pf proteasome

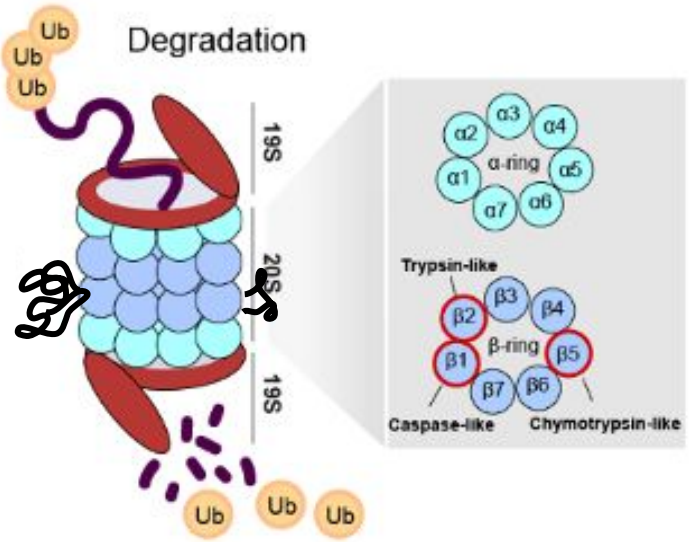
```
Hsβ1  70 LNEP-------------------------------------------------  73
Pfβ1  70 NRKKGRFHEGETIYDETTYDEEIDIDSINYLDYNNNNDNNLVTKNKYFYEDKFNDYN 126

Hsβ7 115 YADGES---------------------------------- 120
Pfβ7 118 INSQKYDNNDDNVLLYTNKNNDDEQNEYKNNEEYKEIHKDDL 159
```

Gang Lin & Laura Kirkman

Pf proteasome



Gang Lin & Laura Kirkman

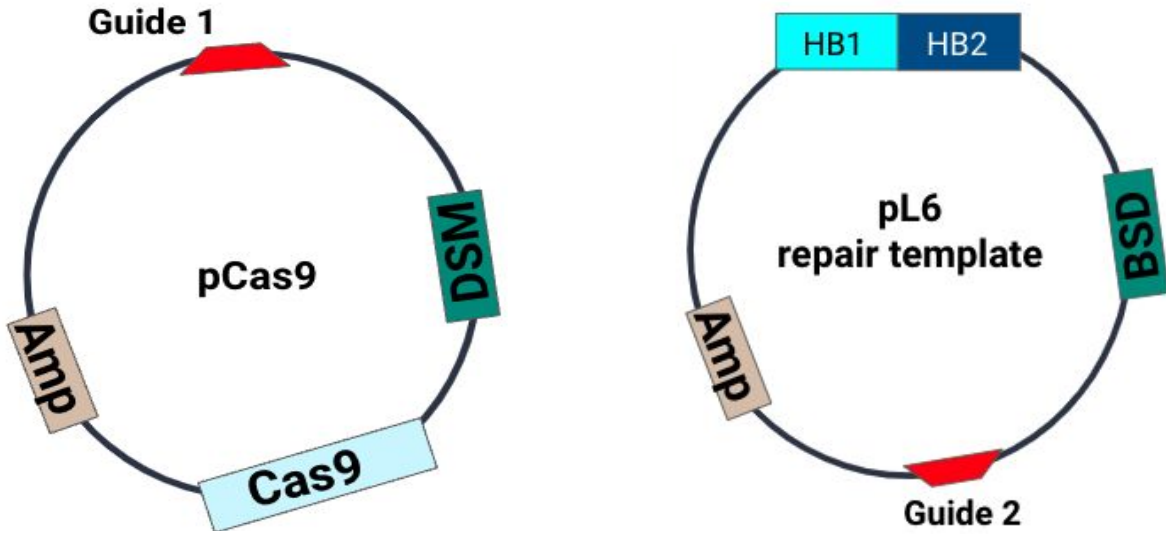


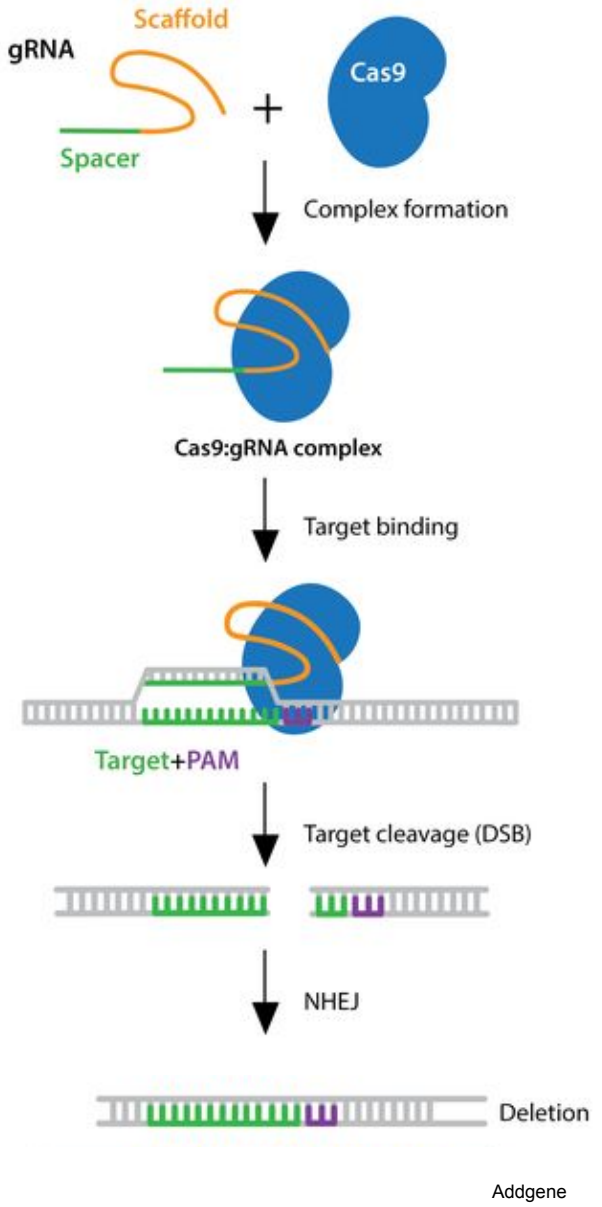
Goals:

1. To identify if the extra loop in ß1 subunit is **essential for parasite survival**
2. To better understand the **unique** parts of parasite proteasome function
3. To possibly use proteasome inhibitors for the treatment of malaria.

Approach:

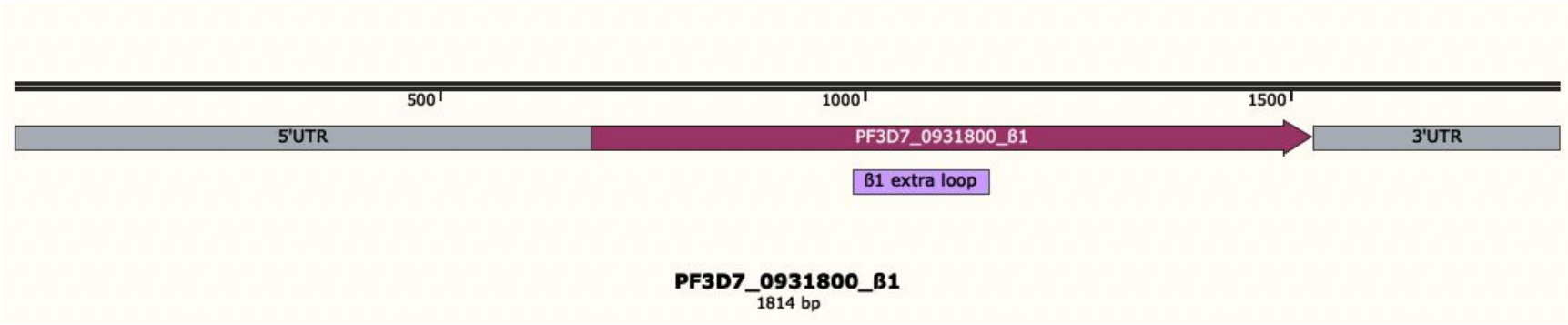
CRISPR/Cas9- mediated deletion of extra loop in *Plasmodium falciparum*



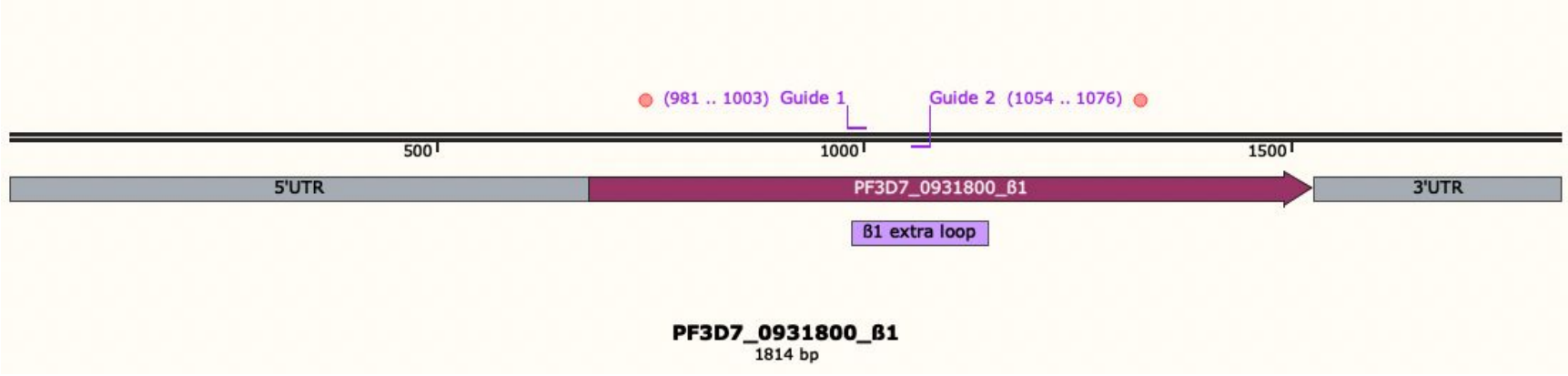


Step 1: Guide Selection

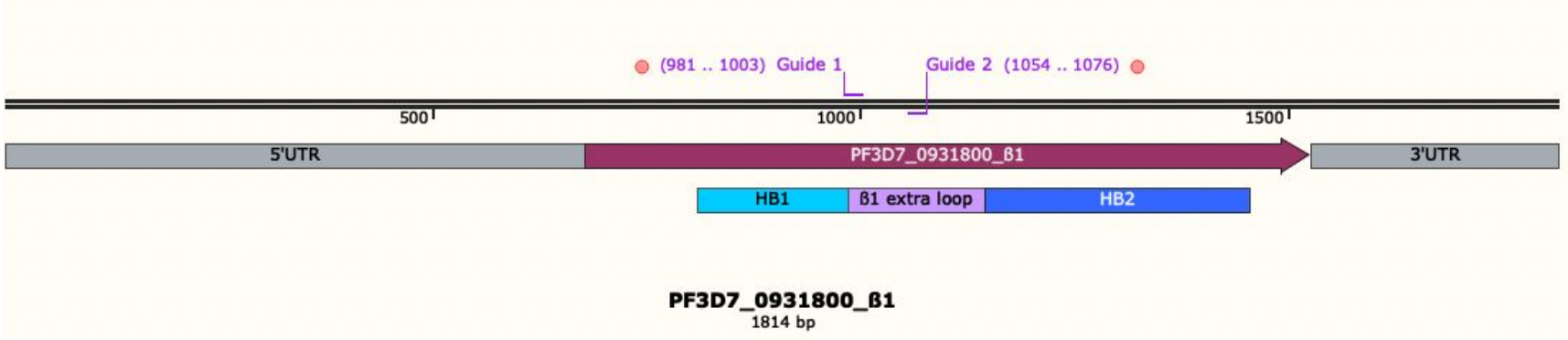
ß1 gene



Guide selection (targeting a cut in the extra loop)



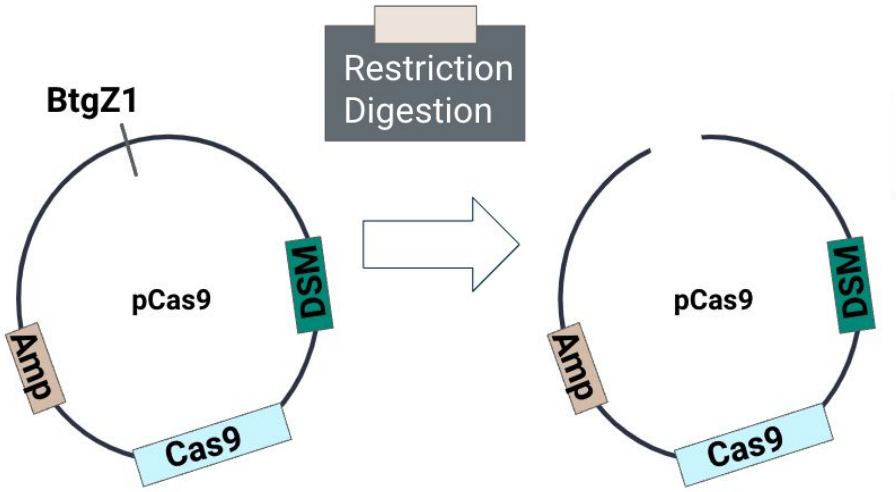
Homology block for repair template



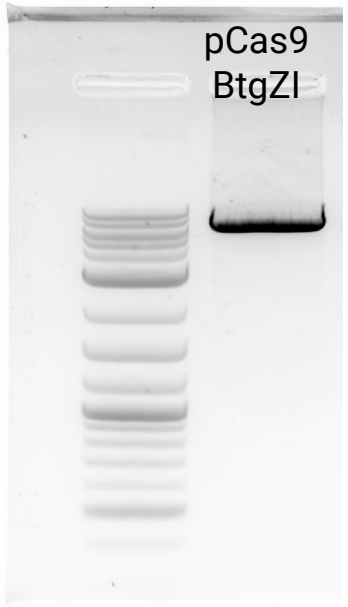
Step 2: Preparation of pCas9 plasmid

Plasmid 1:
pCas9 + Guide1

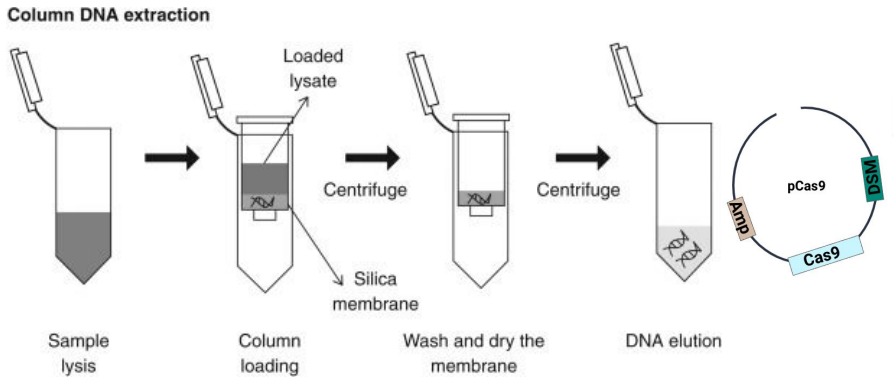
a) Vector preparation



Agarose gel run



DNA extraction from gel



Step 3: Preparation of pCas9 plasmid

Plasmid 1:
pCas9 + Guide1

b) Insert preparation

1. Order guides as single stranded DNA

Guide 1

Top strand

Bottom strand

Guide 2

Top strand

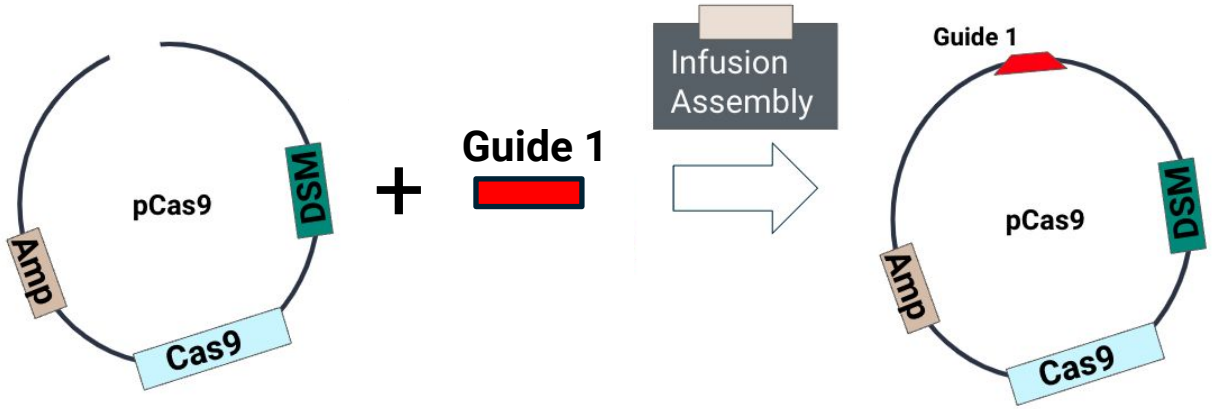
Bottom strand

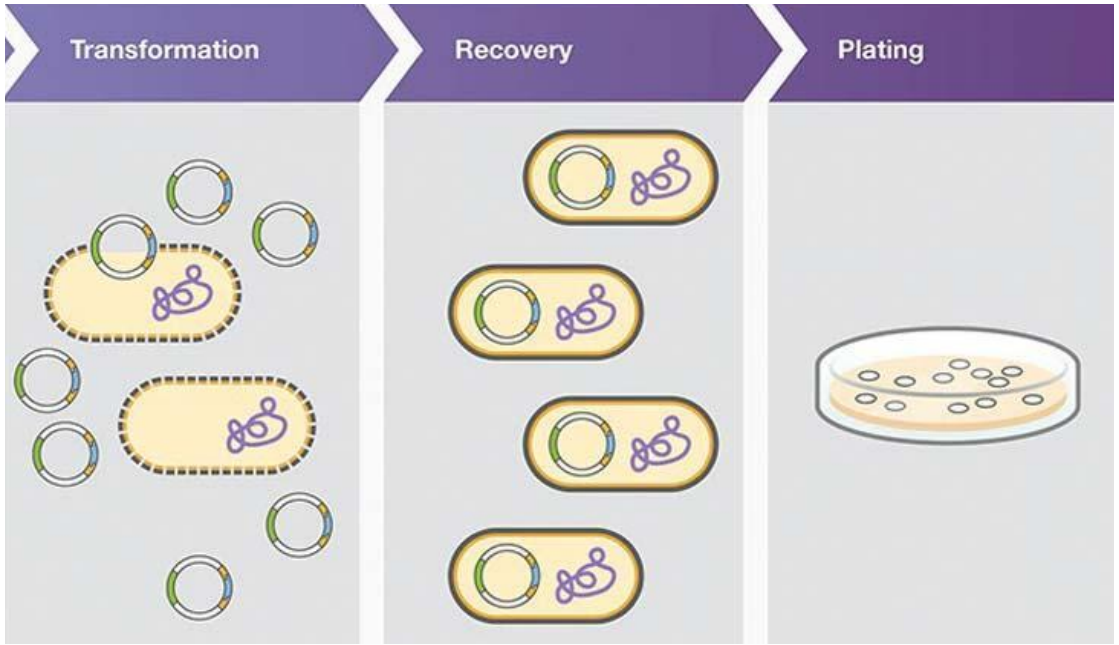
2. Anneal the top and bottom strand

Guide 1

Guide 2

c) Plasmid preparation

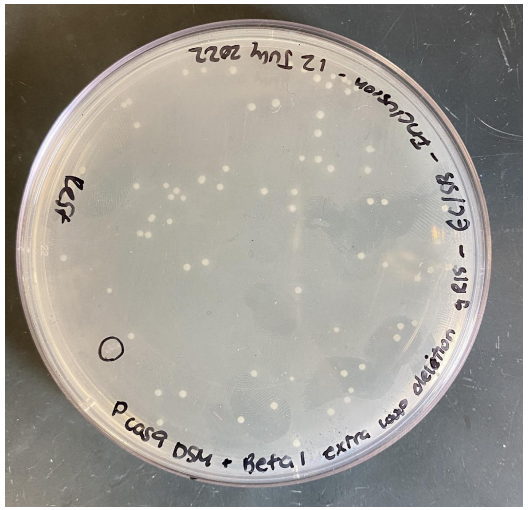




Step 4: Preparation of pCas9 plasmid

Plasmid 1:
pCas9 + Guide1

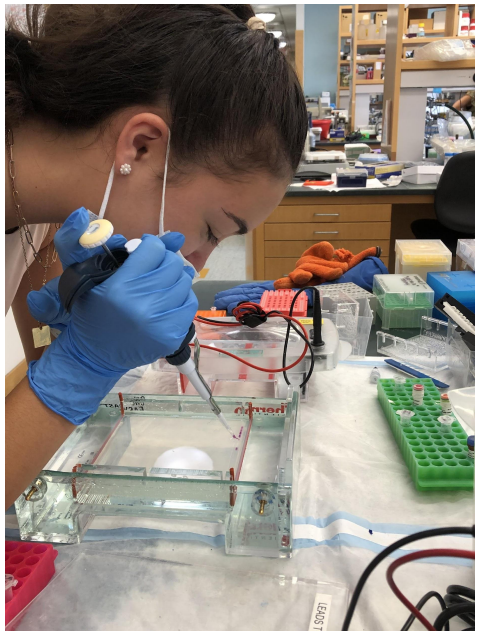
d) Steps to See if Guide Inserted into Plasmid

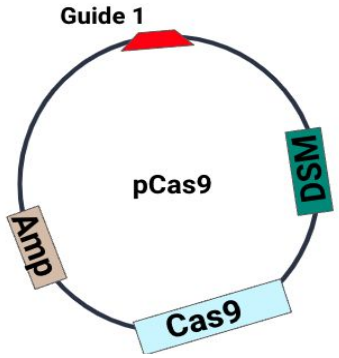


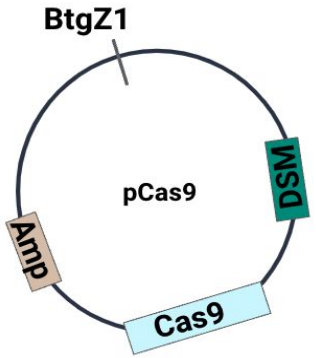
Pick bacterial colonies

Grow bacteria

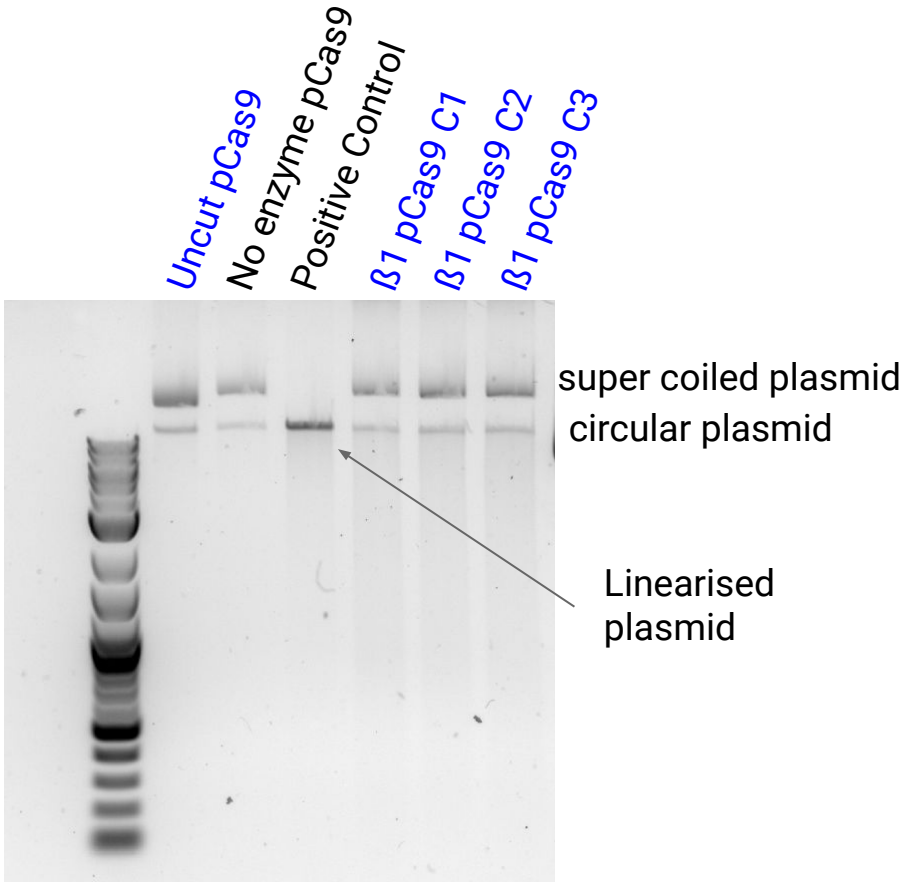
Plasmid isolation

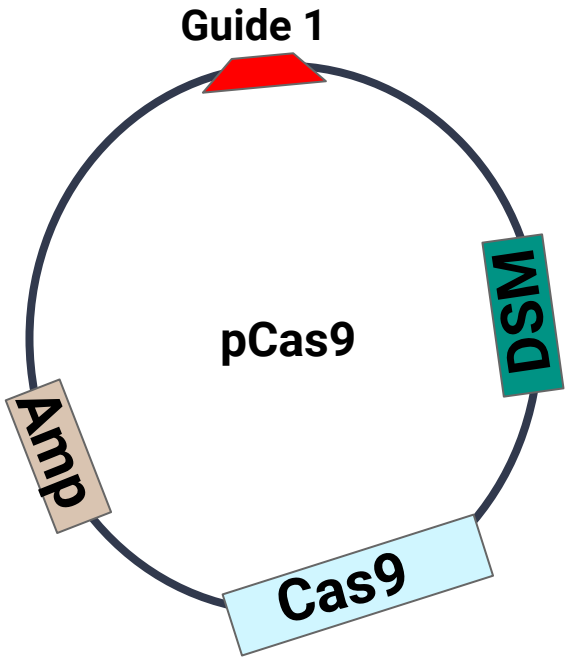


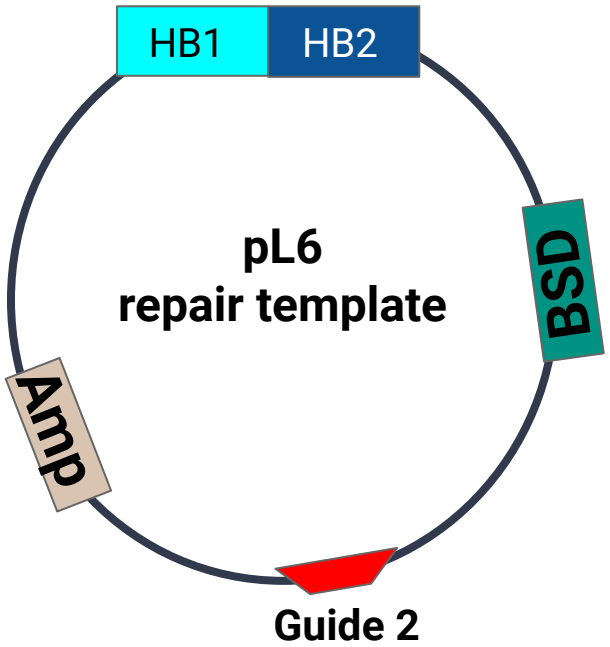


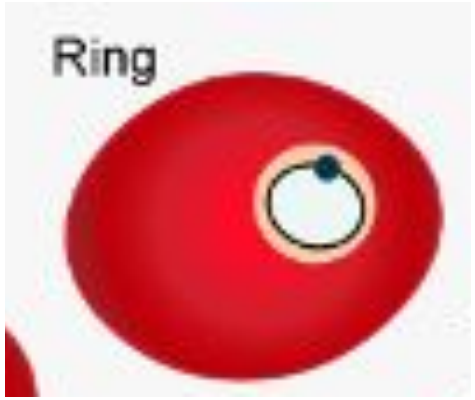


Diagnostic digest
using BtgZI







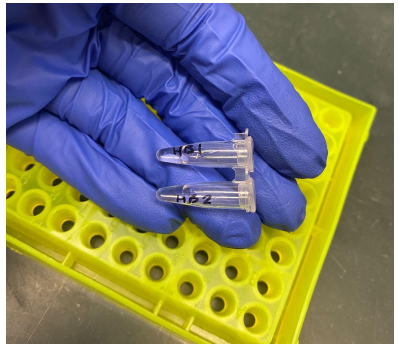


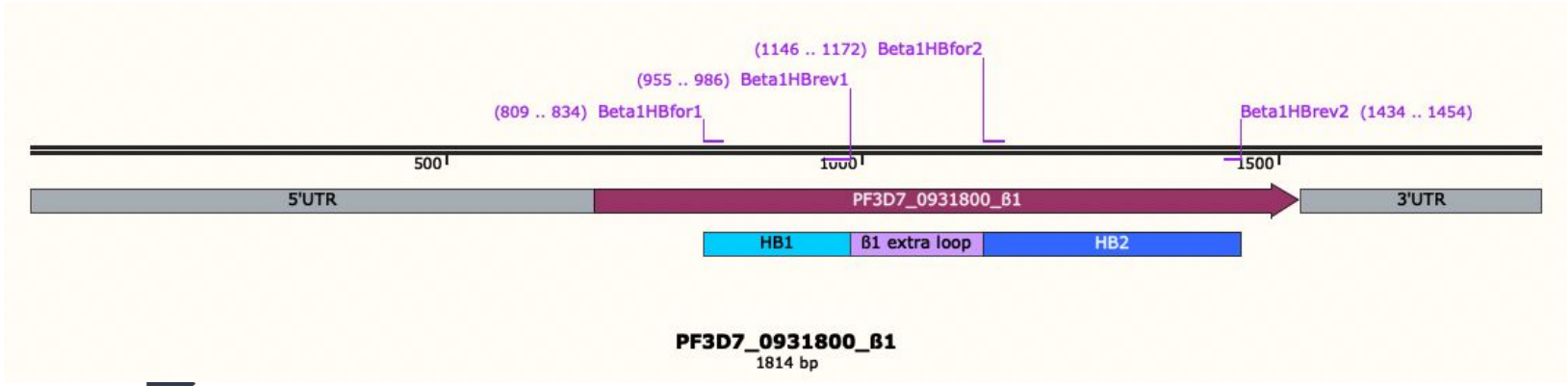
Transfection into parasites

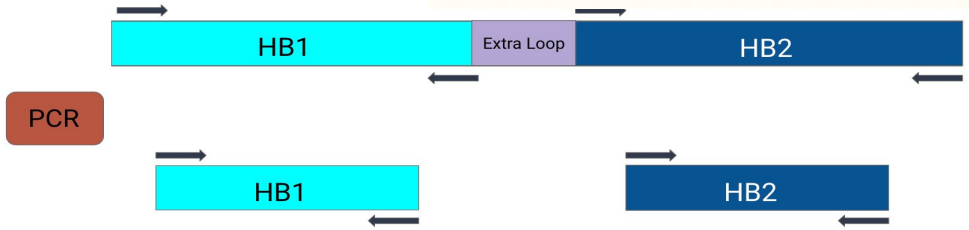
Preparation of pL6 repair plasmid

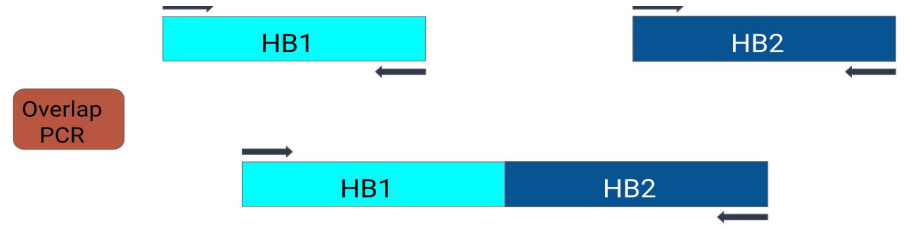
Plasmid 2:
pL6 + HB

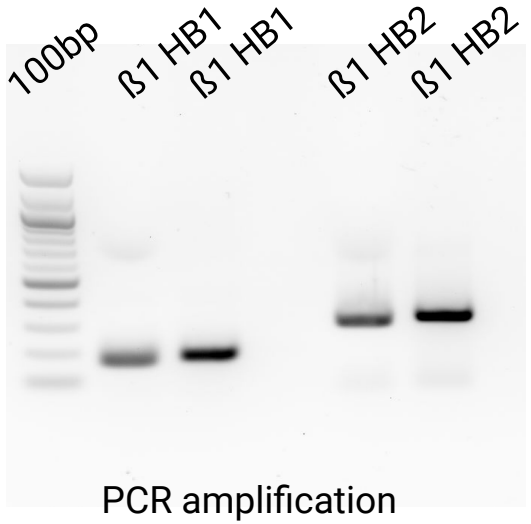
Insert (repair template) preparation



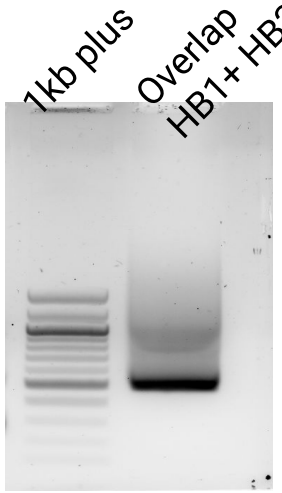




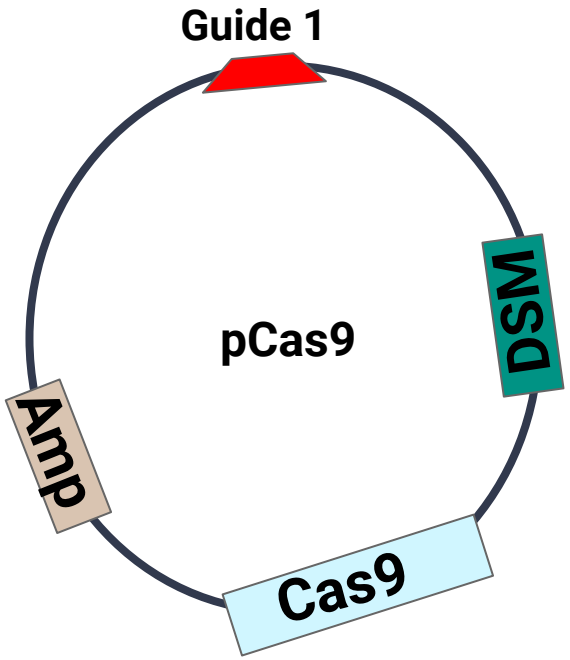


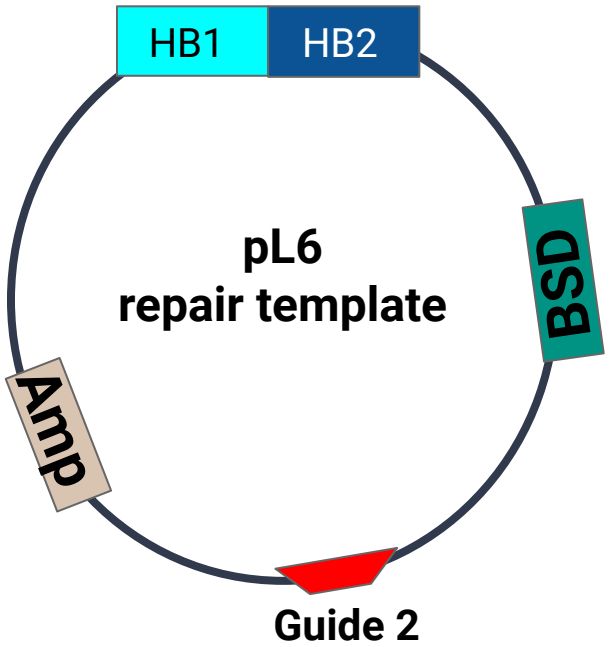


PCR amplification

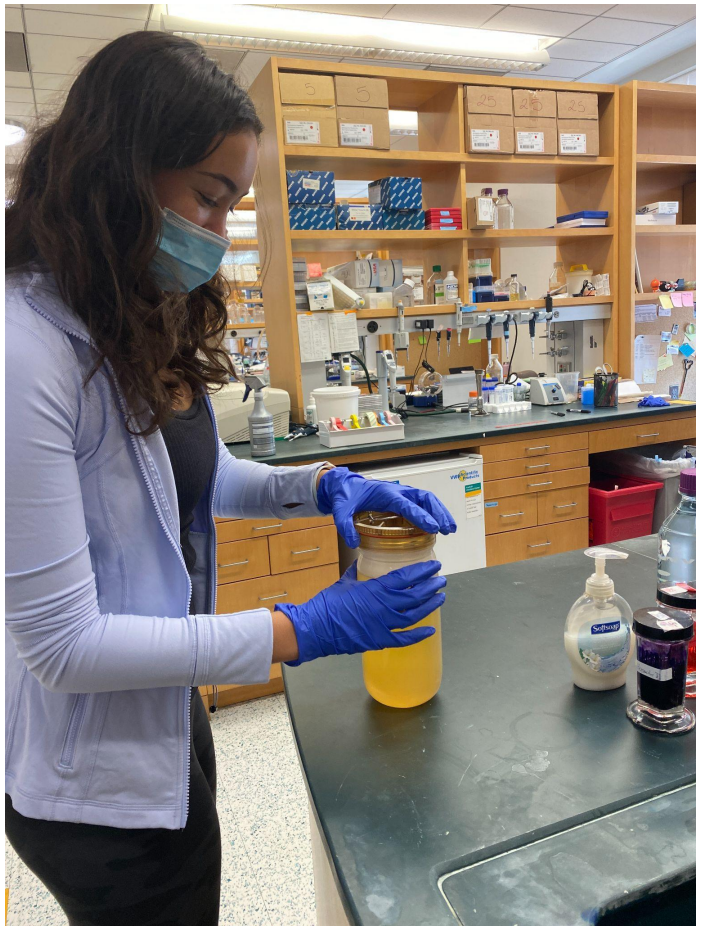


Overlap PCR





Confirmed the sequence of inserts by sanger sequencing



Mega prep for isolating large amount of plasmid DNA

Takeaways

New Skills:

- Working with complex scientific equipment (eg: centrifuge, pipettes, and a gel electrophoresis apparatus)
- Presenting scientific material
- Understanding the complex dynamics between a combined hospital and laboratory setting

Reflections:

- Patience is key
- Step out of your comfort zone
- Say yes to new opportunities
- Always bring your headphones and a book on the subway

Thank You & Questions

Dr. Laura Kirkman, Dr. Shubha Subramanyaswamy, and the Weill Cornell Infectious Disease Laboratory for hosting me this summer.

Thank you to Dr. Krug and the Holton-Arms Summer Science Research Program for giving me this opportunity and supporting me throughout the process.

Any Questions?