Primer Design and Purchase

Participants: Rori Hoover, Patrick Jiang

Date: Monday, June 12, 2023

Protocol:

1. Designed primers in Benchling for the genes G4.1, G4.2, and G4.3 with the following configuration:

   Insert sketch from lab notebook

2. Ordered G4.1, G4.2, and G4.3 from IDT

Results: N/A

Conclusion: N/A

pRE Digestion

Participants: Rori Hoover, Patrick Jiang

Date: Monday, June 12, 2023

Protocol:

1. Prepared a 28.7 µL digestion reaction with the following components:

Component	Volume
4189.02 ng pRE	24.2 µL
HindIII-HF	1 µL
NdeI	1 µL
10X rCutSmart buffer	2.5 µL
Total	**28.7 µL**

Results: N/A

Conclusion: N/A

We didn't sequence our backbone before ordering the primers, and we used an online sequence. Something had already been cloned in so it was not the same. HiFi uses high fidelity enzymes that can fix bp mismatches

pRE has a total length of ~6 kb because there's an insert, but we cut it out, and there's only 1 kb left

The original plan of ligating them individually and MEGAWHOP as only one insert wouldn't work, so do HiFi assembly – just do one step of adding everything together at the same time. Too many steps originally, so just one

Our insert is 6 times larger than your backbone

We will be digesting pRSET-gluSnFr with NDEI and HindIII. Due to a minor design error, we have to perform Hifi due to 2-6 bp mismatches. We will digest pRSET-E, which has total ng value of 4189.02 ng. It has a volume of 24.2 uL. We will digest it all

We thought that because the enzyme wasn't

We ran a gel after, and you didn't see the expected band – which means digest DNA – speculated because enzyme was faulty – so troubleshooting

pRE Gel Purification

Participants: Rori Hoover, Patrick Jiang

Date: Monday, June 12, 2023

Protocol:

1. Prepared a 1% agarose running gel:
    a. Added 0.25 g of agarose to 25 mL 1X TBE in an Erlenmeyer flask
    b. Heated flask in a microwave in 10 second increments until fully dissolved
    c. Cooled flask until it was manageable to touch
    d. Added 3 µL SYBR Safe and swirled the flask to mix
    e. Poured solution into a gel mold with a comb and left to solidify for 20 minutes
    f. Removed comb and covered gel cast completely with 1X TBE
2. Loaded gel:
    a. Loaded 12 µL of 1 kb DNA ladder into the first well
    b. Added 6 µL gel loading dye to the digested pRE
    c. Loaded 20 µL of pRE DNA into the second and third well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:

Insert photo from scan (page 22)

Conclusion:

We were unsuccessful. We expected bands at 2752 bp for the backbone and 1677 bp for the section between the cut sites.

There was only one band on the gel, so we suspected human error, faulty data collection on Addgene, or the enzymes are dead

The backbone is pRSET right?

If HindIII-HF works, we expect a band at 413 bp, and another band at approximately 6000 bp

If NDEI works, we expect one band at 350 bp and a second at approximately 6000 bp

If there's uncut DNA, it will run oddly, but because there was one band, at least one of the enzymes cut something

Troubleshoot

Our initial PCR (what PcR) suggests that at least one of the enzymes cut at their recognition site, so if both our pDuskk digests only have one band then something is wrong

Tomorro we will run a gel

We have generated the template DNA that G4.1, G4.2, and G4.3 will be inserted to. Our next step is to construct a megaprimer that contains all three genes.