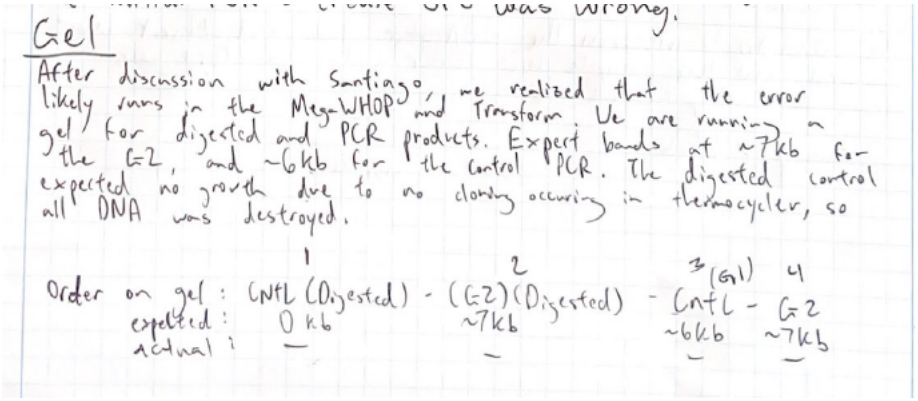

was wrong.

Gel

After discussion with Santiago, we realized that the error likely runs in the MegaWHOP and Transform. We are running a gel for digested and PCR products. Expect bands at ~7kb for the G-2, and ~6kb for the control PCR. The digested control expected no growth due to no cloning occurring in thermocycler, so all DNA was destroyed.

	1	2	3 (G1)	4
Order on gel:	CNTL (Digested)	(G-2) (Digested)	CntL -	G-2
expected:	0 kb	~7kb	~6kb	~7kb
actual:	—	—	—	—

What is this?

mScarlet Megaprimer Generation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, May 16, 2023

Protocol:

1. Prepared a 50 µL reaction in a PCR tube with the following components:

Component	Volume
10 µM forward primer	2.5 µL
10 µM reverse primer	2.5 µL
10 ng template DNA (R8)	0.625 µL
Q5 High-Fidelity 2X Master Mix	25 µL
Nuclease-free water	19.4 µL
Total	**50 µL**

2. Amplified the DNA in the 25 µL reaction with a thermocycler, using the following conditions:

Step	Temperature	Time	Number of cycles
Initial denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	30
Annealing	61 °C	30 sec	30
Extension	72 °C	30 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

3. Prepared a 1% agarose running gel:
    a. Added 0.25 g of agarose to 25 mL 1X TBE in an Erlenmeyer flask
    b. Heated the flask in a microwave in 10 second increments until fully dissolved
    c. Cooled the flask until it was manageable to touch
    d. Added 2.5 µL SYBR Safe and swirled the flask to mix
    e. Poured the solution into a gel mold with a comb and left it to solidify for 20 minutes
    f. Removed the comb and covered the gel cast completely with 1X TBE
4. Loaded gel:
    a. Loaded 5 µL of 1 kb DNA ladder into the first well
    b. Added 3 µL gel loading dye to the PCR product
    c. Loaded 5 µL of PCR sample into the second well
5. Placed the gel cast in a blueGel electrophoresis system and left the sample to run for 30 minutes

Results:

Conclusion: We were successful in generating the mScarlet Megaprimer because a thin band appeared at 1 kb. At this point, we had still not realized that we had forgotten to dilute the forward and reverse primer from 100 µM to 10 µM, so the larger bands appearing at approximately 200 bp are again the result of self-primerization.

Gel Extraction of mScarlet Megaprimer

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, May 16, 2023

Protocol:

1. Excised mScarlet Megaprimer DNA from the agarose gel and placed it in a microfuge tube.
2. Added 584.8 µL ethanol to the agarose gel piece
3. Incubated the agarose piece at 50 °C while shaking at 400 rpm until the gel completely dissolved
4. Inserted a spin column into a collection tube and loaded the sample into the column
5. Centrifuged the tube at 13,000 rpm for 1 minute and discarded the flow-through
6. Added 200 µL DNA Wash Buffer 1 to the column and centrifuged the column at 13,000 rpm for 1 minute
7. Added another 200 µL DNA Wash Buffer 1 to the column and centrifuged the column at 13,000 rpm for 1 minute
8. Transferred the spin column to a clean microfuge tube
9. Added 30 µL DNA Elution Buffer
10. Waited 1 minute before centrifuging the tube at 13,000 rpm for 1 minute

Results: N/A

Conclusion: N/A

Measurement of mScarlet Megaprimer DNA Concentration

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, May 16, 2023

Protocol:

1. Cleaned NanoDrop spectrophotometer with DI water
2. Blanked NanoDrop with 2 µL Elution Buffer
3. Cleaned NanoDrop before loading 2 µL extracted mScarlet megaprimer

Results: The DNA concentration of the extracted mScarlet megaprimer sample was found to be 69.1 ng/µL.

Conclusion: N/A

G1 with mScarlet Megaprimer Generation

Participants: Brianna Branson, Rori Hoover, Patrick Jiang

Date: Tuesday, May 16, 2023

Protocol:

1. Prepared a 50 µL MEGAWHOP reaction in a PCR tube with the following components:

Component	Volume
13.82 ng G1 plasmid	2 µL
138.2 ng mScarlet megaprimer	1.56 µL
Q5 High-Fidelity 2X Master Mix	25 µL
Nuclease-free water	21.44 µL
Total	**50 µL**

2. Amplified the DNA with a thermocycler using the following conditions:

Step	Temperature	Time	Number of cycles
Initial denaturation	98 °C	30 sec	1
Denaturation	98 °C	10 sec	30
Annealing	62 °C	30 sec	30
Extension	72 °C	3 min 35 sec	30
Final extension	72 °C	2 min	1
Hold	10 °C	Forever	-

Results: N/A

Conclusion: N/A