pDusk Megaprimer Generation

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Protocol:

1. Prepared a 25 µL reaction in a PCR tube with the following components:

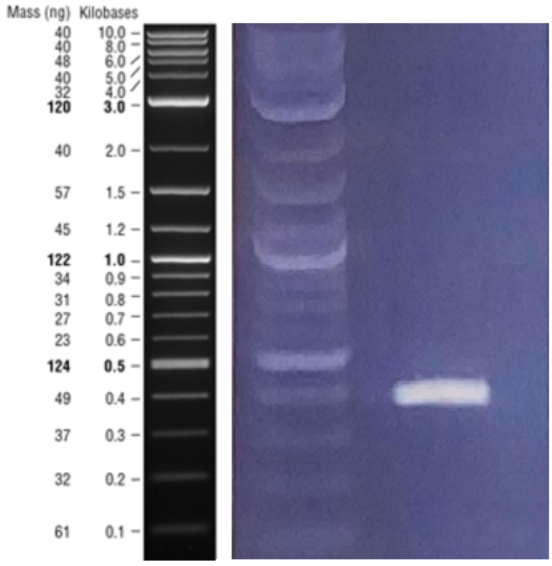
Component	Volume
10 µM forward primer	1.25 µL
10 µM reverse primer	1.25 µL
10 ng template DNA (pDusk)	1.4 µL
Q5 High-Fidelity 2X Master Mix	12.5 µL
Nuclease-free water	8.6 µL
Total	**25 µL**

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	60°C	30 sec	30
Extension	72°C	12 sec	30
Final extension	72°C	2 min	1
Hold	10°C	Forever	-

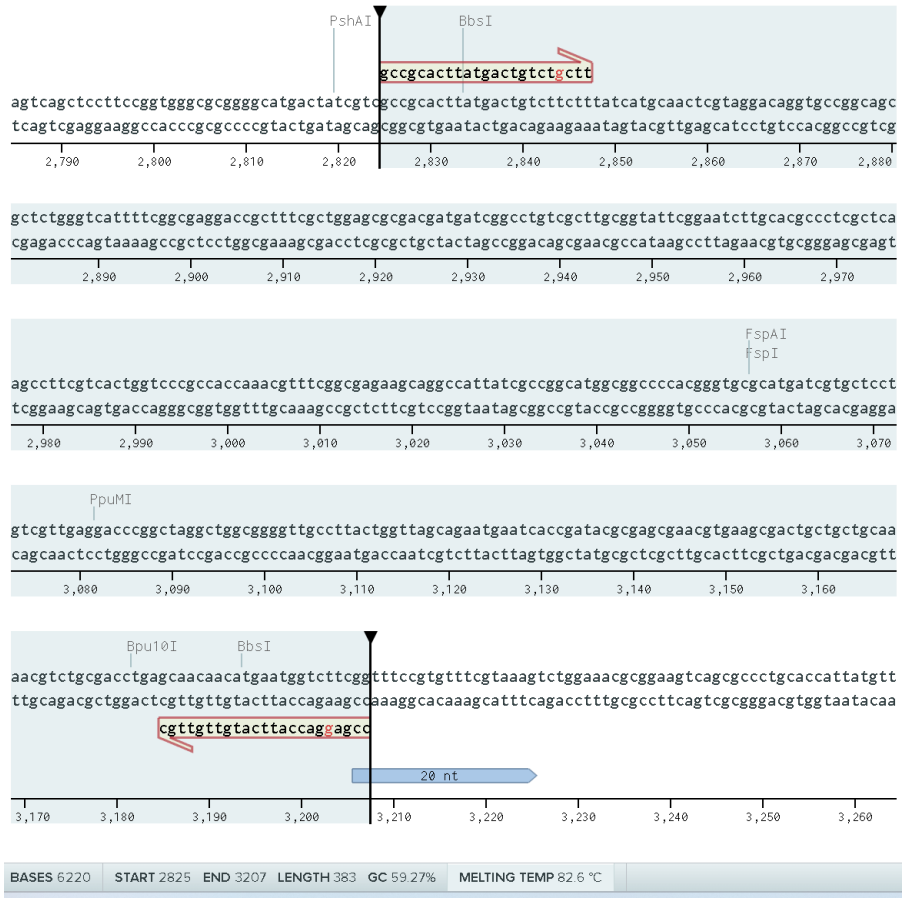
3. Prepared a 1% agarose running gel:
    a. Added 0.25 g agarose to 25 mL 1X TBE in an Erlenmeyer flask
    b. Heated the flask in a microwave in 10 second increments until fully dissolved
    c. Cooled the flask until it was manageable to touch
    d. Added 2.5 µL SYBR Safe and swirled the flask to 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 1 kb DNA ladder into the first well
    b. Added 3 µL gel loading dye to the PCR product
    c. Loaded 5 µL PCR product 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:

According to our plasmid map in Benchling, the pDusk megaprimer should be 383 bp long.



After performing gel electrophoresis and comparing the migration distance of our sample to the 1 kb DNA ladder, we determined that our sample was approximately 400 bp. Thus, it is highly likely that we successfully created the pDusk megaprimer.

Our next steps are to purify the pDusk megaprimer and then insert it into pDusk plasmid via a MEGAWHOP reaction. For simplicity, we will refer to this new plasmid as “G1.”