Re-Digest

- **We're trying to see ~50 bp so we need ~1 µg DNA**

Checklist

- **G3.1: 13 µL**
- **G3.2: 15 µL**
- **G3.3: 15 µL**
- **G3.4: 13 µL**
- **G3.5: 13 µL**
- **G3.6: 15 µL**
- **G3.7: 18 µL**
- **G3.8: 24.5 µL**

G3 Digestion

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Date: Friday, July 28, 2023

Protocol:

1. Prepared 56 µL digestion master mix with the following components:

Component	Volume
10X rCutSmart buffer	40 µL
XbaI concentration?	8 µL
BamHI-HF	8 µL
Total	**56 µL**

2. Added 7 µL digestion master mix to each miniprepped G3 sample
3. Prepared a 2% agarose running gel:
    a. Added 0.4 g of agarose to 20 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 3 µL 100 bp DNA ladder into the first well
    b. Added 7 µL gel loading dye to each sample
    c. Loaded **??** µL of each sample in ascending order from left to right starting with the second well
3. Placed gel cast in a blueGel electrophoresis system and left the sample to run for 1 hour

Results:

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Conclusion: the PCR to remove His-tags did not work because