


# Fall 2022

MONDAY, 8/22/2022

## P. Destructans

- Inoculate a culture
  - Follow Up-

- Purify

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## P. destructans

- Cleaned and Concentrated PD seq 3a and 3b PCR product
- Ran a Bsmbl GGA with the PCR product to ligate both sequences together.
- 1 uL 3a seq
- 2 uL of 3b seq
- 2 ul of Bsmbl mix
- 2 uL of T4 Ligase buffer
- 13 uL dH2O
- Follow Up-
  - Run a PCR of this assembly to amplify the entire sequence

## ABR Detection Threshold

- Grew up a culture of 013 strain for detection threshold
  - Follow up-
    - Perform a transformation with the decreasing DNA concentrations of NPTII
    - Perform a transformation with the decreasing DNA concentrations of Tem-1

## Co-Culture Experiment

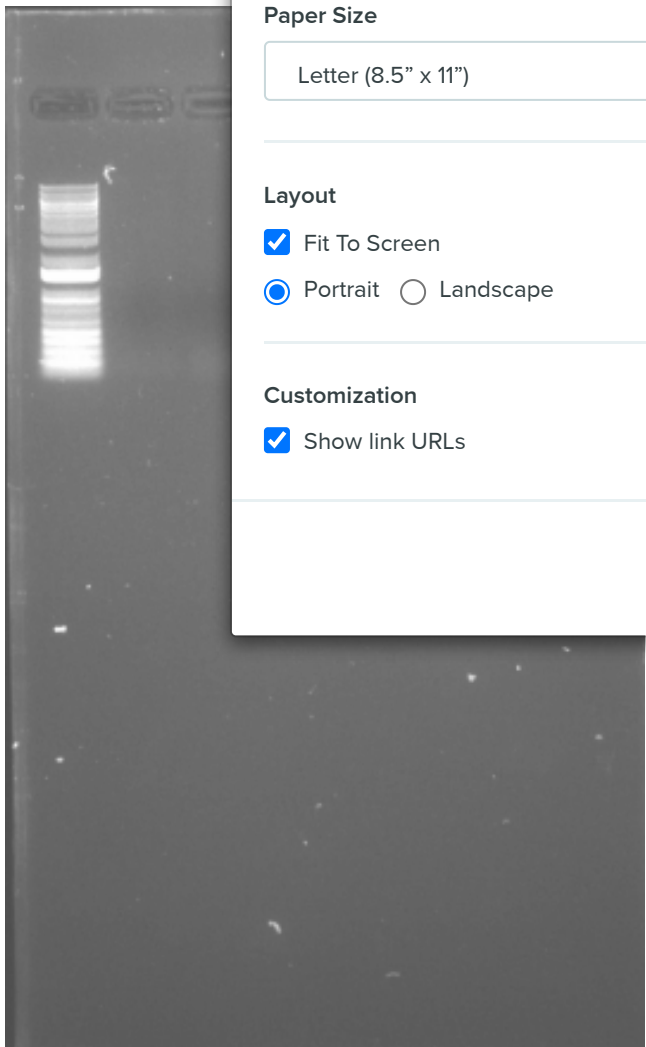
- Grew up a culture of 019 for TDK Transformation into 2049
  - Follow Up-
    - Add 35 uL of the overnight culture to 500 uL LB + 2049 tdk/kan sequence
- Ran a PCR of the 2049 Tdk/kan gene
  - 001 AND 004
  - Run at 59° with an extension time of 2:15

TUESDAY, 8/23/2022

## Co-Culture Experiment-

- Ran the gel for the 2049 PCR I ran yesterday
- Gel Order: Ladder - Control - tdk - tdk - GGA

- Got no bands for the TDK gene so I think the annealing temp was too high



Pd seq Gel.png

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## P. Destructans Detector Test-

- Run a PCR pf yesterday's Golden Gate Assembly of the two PCR's
  - This PCR will ligate the two PD sequences to make the entire target sequence
- Primers = 063 + 066
  - Run at 64°
  - Run at 66°
  - 124 uL of water
  - 40 uL of HF buffer
  - 10 uL of 063
  - 10 uL of 066
  - 6 uL DMSO
  - 4 uL dntp
  - 2 uL polymerase


2049 Up + PD 3a (1200+500) 30 sec /kb  
001 +064

2049 Repressor + PD 3p + 2049 down (1000+500+1200  
004 + 061

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