

# Leia Jiang 4/16-4/18

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## SATURDAY, 4/16/2022

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- Resuspended ampD primers and made 10x diluted stock solutions

- Phusion buffer
- ddH2O
- dNTPs
- Primer forward
- Primer reverse
- Phusion poly (ADD LAST TO MASTER MIX)
- Template (ADD TO EACH PCR TUBE)

### Annealing Temperatures: (NEB Tm calculator, 250 nM primer concentration, Phusion)

- ampD (Leia):
  - Upstream: 64 C
  - Downstream: 64 C
- acrB (Sai):
  - Upstream: 64 C
  - Downstream: 66 C
- pbpG (Jeffrey):
  - Upstream: 65 C
  - Downstream: 64 C
- recJ (Vivek):
  - Upstream: 61 C
  - Downstream: 61 C
- ACIAD2257 (Keaton):
  - Upstream: 63 C
  - Downstream: 62 C
- Inoculated 2 of each frozen stock from last week in 5 mL LB and 5 uL Kan, and an uninoculated control

## SUNDAY, 4/17/2022

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- Loaded yesterday's PCR products into gel
- Whole-cell PCR with 1000x diluted samples of overnight culture
  - ACIAD2049 upstream forward and downstream reverse
  - 45 uL mastermix, 5 uL 1000x dilution as template
  - - Control with untransformed ADP1-ISx
  - PCR didn't turn out well, no bars
- Casted gel for PCR tomorrow

## MONDAY, 4/18/2022

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- Didn't make it to lab, Marisa loaded PCR