

Jeffrey Chuong 4/16 - 4/17

SATURDAY, 4/16/2022

Rehydrated primers to create a 10x stock solution

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

- pbpG (Jeffrey):
 - Upstream: 65 C
 - Downstream: 64 C
- recJ (Vivek):
 - Upstream: 61 C
 - Downstream: 61 C

Set up PCR reactions and a negative control for each downstream and upstream flanks

Inoculated 2 of each -80C stock from last week in 5 mL LB and 5 uL Kan

Inoculated a negative control

SUNDAY, 4/17/2022

Created gels to visualize PCR products

Whole Cell PCR to confirm *tdk/kan* cassette insertion (<https://barricklab.org/twiki/bin/view/Lab/ProtocolsStandardPCR>)

Make 1000-fold dilutions of overnight culture, 1 ul in 1 mL ddH₂O in an Eppendorf tube

Combine 5 ul of the 1000-fold dilution with mastermix for 50 ul PCR reaction

Set up 135 ul mastermix on ice with ACIAD2049 Upstream Forward and Downstream Reverse primers

Gel 3 wells

Ladder

Jeffrey

Upstream

Upstream

Upstream Negative Control

Downstream

Downstream

Downstream Negative Control

Vivek

Downstream

Downstream

Downstream Negative Control

Upstream


Upstream

Upstream Negative Control

Most gels didn't show very strong bands, need to troubleshoot PCR

Is gel imager working properly? Imaged gel after 20 minutes, Imaged gel after 30 minutes

 IMG_0466.heic

 IMG_0467.heic