Lab Notebook Entry 38

Date Performed

29-May

Procedure

PCR of pTlpA36-wasabi and of previous gel extracted pTlpA

Details

Purpose: We are going to try this part again. This time we will just do PCR cleanup instead of gel extraction since the gel extraction had really bad yeild. The product will have a mix of the two bands, but the "bad" bands hopefully wont be compatible with the other peice when we do gibson and will be filtered out by that step. Also trying to PCR the gel extracted product from last time since that is theoretically 'pure' pTlpA even if it has a bad concentration.

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)