Lab Notebook Entry 14

Date Performed

5/5/17

Procedure

2nd attempt PCR of pTlpA36 with way less template

Details

Purpose: Our second attempt to make lots of our starter plasmid in a linear form. Eliminates LacI junk and adds Gibson assembly compatible ends. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid, primers, PCR tubes, Q5 2x master mix, nuclease free water

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: 98C for 30secs, 30 cycles of [98C for 10secs, 72C for 3mins30secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)