7/13/17

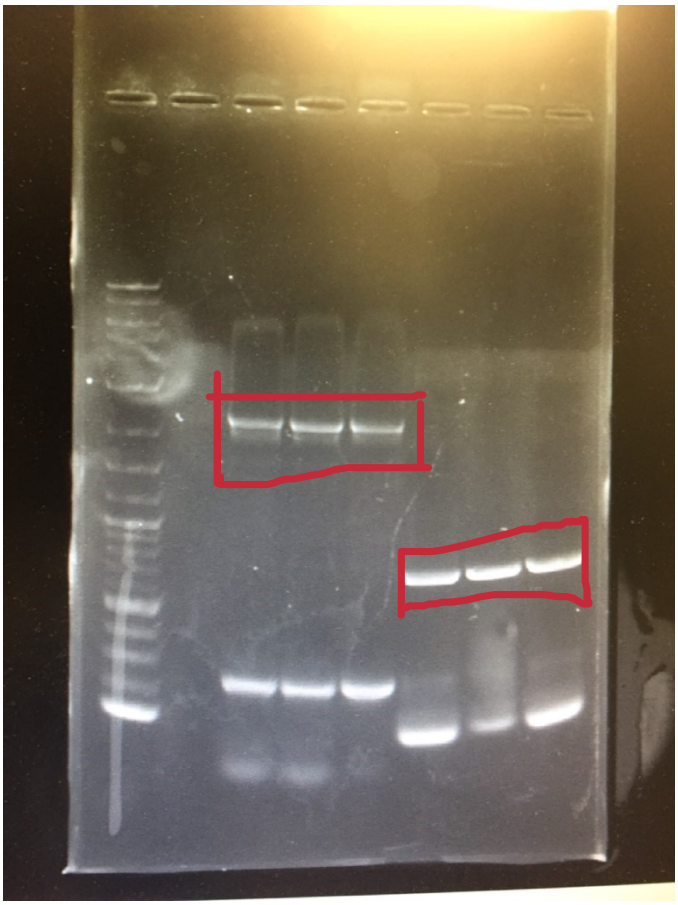
Friday, July 14, 2017 1:00 PM

Who's in Lab today: Martin, Ayesha, Jeff, Ana, Nathan, Qingxi

GIBSON ASSEMBLY POSITIVE CONTROL:

A 40 mL agarose gel was made with the same gradient PCR products from 7/12 as the gel was run too long and the YCP product ran off the gel .

JOE's annealing temp: 54.1 C **(lane 3)**, 55.0 C **(lane 4)**, 57.2 C **(lane 5)**
YCP's annealing temp: 59.0 C **(lane 6)**, 60.0 C **(lane 7)**, 62.0 C **(lane 8)**
Lane 1 contains 2-log DNA ladder
Lane 2 was left empty.



The red boxed areas are the major product that we wanted.
JOE (LEFT) has about a band size of 2.3 kb as expected and YCP (RIGHT) has a band size of about 0.7 kb as expected.

The bands shown underneath however in the gel are not as expected and they are either primer dimers or smaller sections of the DNA plasmid that was amplified.

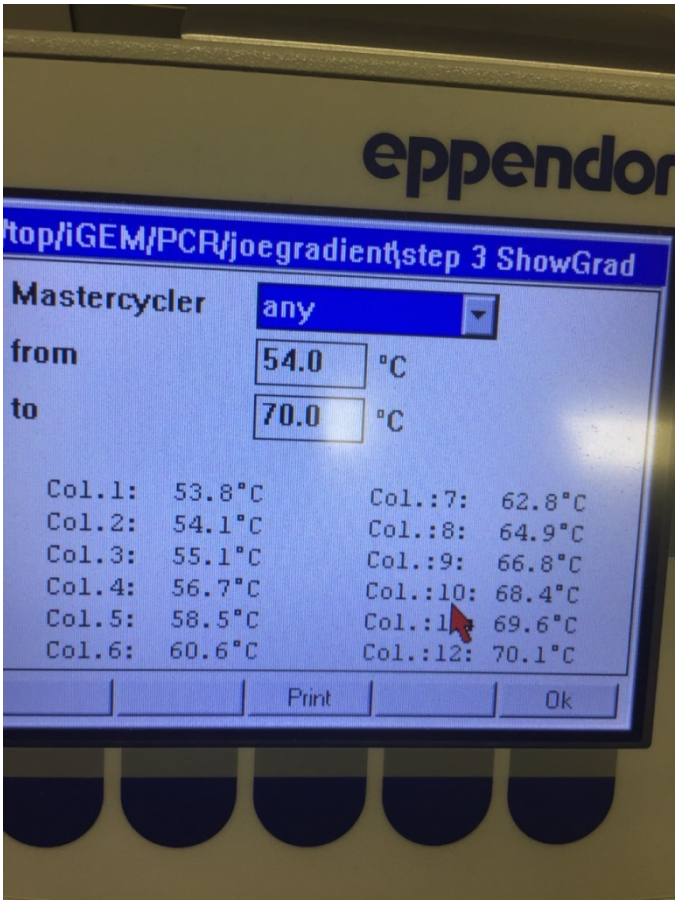
The bands of the minor product are also three times as bright as the major product indicating that that we had too much plasmid DNA for the DNA polymerase amplify that expected longer segments.

Hence, the stocks of both JOE and YCP plasmid was diluted to 1/10th. [so 90ul water with 10uL plasmid].

And **ANOTHER** PCR reaction gradient was set up using the diluted DNA.

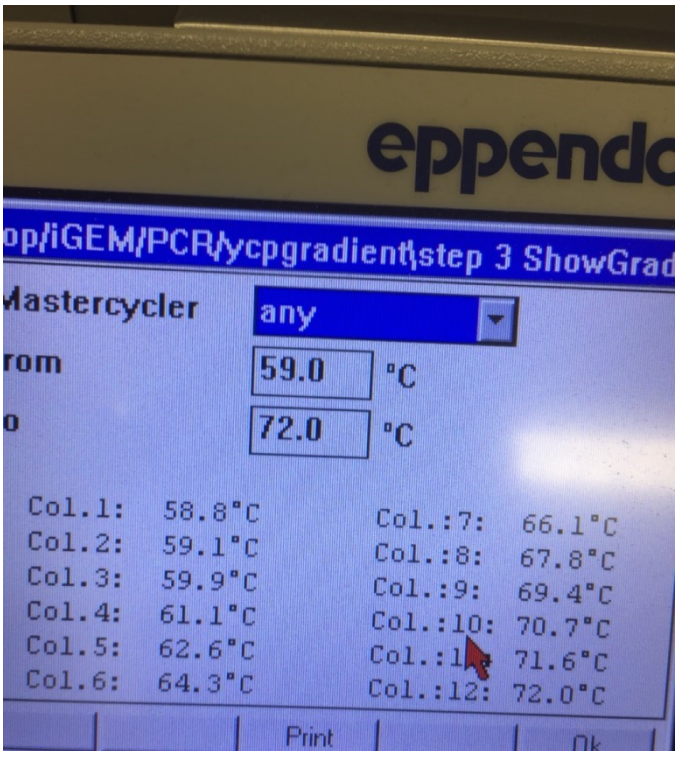
This second PCR gradient was set up with the following temperatures:

JOE (High Expression Vector):



A total of SIX PCR tubes were set up for JOE with even numbered temperatures (Col. 2 , Col. 4, Col. 6 etc.) listed above as the temperatures at which the PCR reactions took place.

YCP (Yellow Chromoprotein):



A total of SIX PCR tubes were set up for YCP with even numbered temperatures (Col. 2 , Col. 4, Col. 6 etc.) listed above as the temperatures at which the PCR reactions took place.

INTERLAB STUDY:

The plates transformed using Kit Plate 7 for the PCR study showed no colony growth. Measurement committee was emailed and we were advised to perform a cell competency test.