7/14/17

Friday, July 14, 2017 1:00 PM

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

GIBSON ASSEMBLY POSITIVE CONTROL

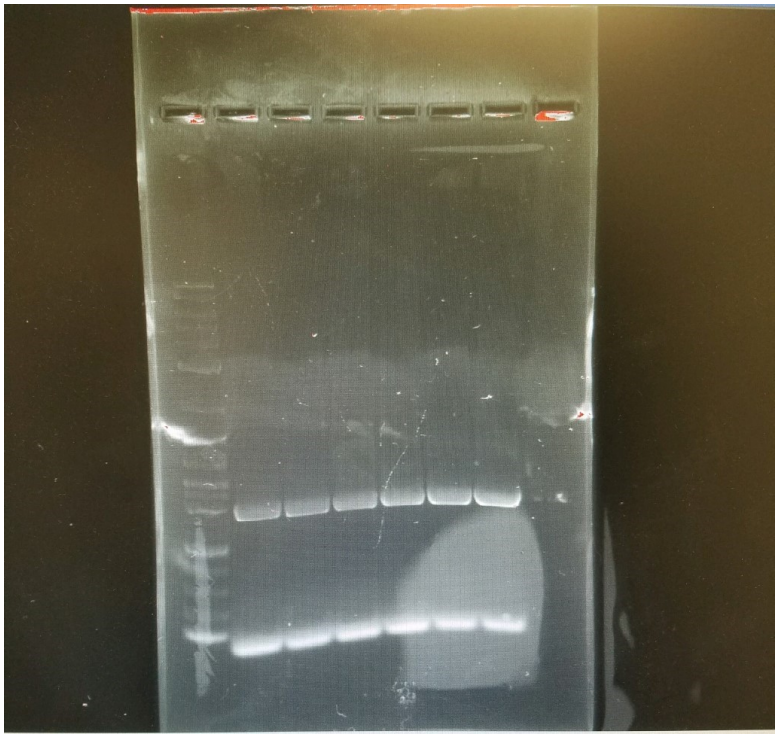
We made two eight lane (WIDE COMB) 40 mL agarose gels. Both were 1 % agarose gels.

The gradient PCR products from 7/13/17 were loaded into the gels.

One gel was for the 6 YCP gradient PCR products.

-	Approx. 30 uL of PCR product with 6uL loading dye was loaded into each well.
-	3 uL of DNA ladder was used

(Left to Right)
Lane 1 contains 2-log DNA ladder
Lane 2 was left empty.
Lane 3 59.1 deg C
Lane 4 61.1 deg C
Lane 5 64.3 deg C
Lane 6 67.8 deg C
Lane 7 70.7 deg C
Lane 8 72.0 deg C



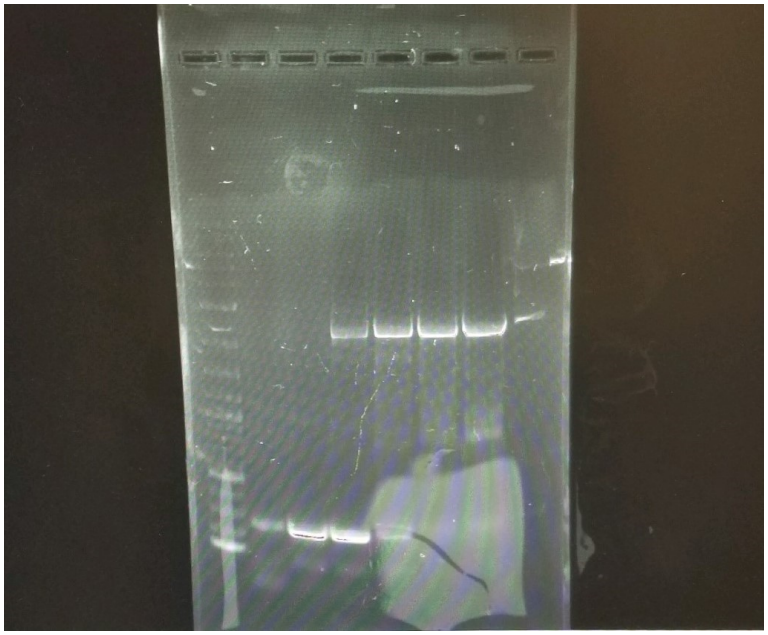
One gel was for the 6 JOE gradient PCR products.

-	Approx. 30 uL of PCR product with 6uL loading dye was loaded into each well.
•	3 uL of DNA ladder was used.

(Left to Right)
Lane 1 contains 2-log DNA ladder

Lane 2 was left empty.
Lane 3 54.1 deg C
Lane 4 56.7 deg C
Lane 5 60.6 deg C
Lane 6 64.9 deg C
Lane 7 68.4 deg C.
Lane 8 70.1 deg C

As the temperature increased, the PCR product majority seem to be the amplified JOE (brighter bands 2.3 kb) rather than primer dimers as seen in the lanes with lower temperatures (lanes 3 and 4). Lane 7 yielded best results.



After gel visualization, the products were cut up under Dr. Jensen's guidance and a gel purification was performed.

Results yielded enough concentration to use for Gibson Assembly.

H6

	A	B	C	D	E	F	G
1	dsDNA_1		260	280	Conc ng/µ	Ratio	Sample ID
2		A1	0.008	0.0037	8	2.16	YCP
3		A2	0.0032	0.0008	3.2	4	JOE
4							
5							
6							
7							

The JOE and YCP gel extraction products were placed in the 4 degrees fridge (the big one).