Amplification of hht_genes

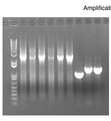


Table 1

Lane	Primers	Gene
1	KC430/1	hht1
2	KC432/3	hht2
3	KC434/5	hht3

Plasmid Digest for Overlab cloning

Plasmid digest

	Reagent	amount	volume
1	Plasmid	1ug	
2	10X Cutsmart	5	
3	AccI	2.5	
4	PacI	2.5	
5	H20	to 50 uls	

Overlab cloning

Table 2

	vector	vector volume	insert	insert volume	H2O	Colonies?	Forward Primer	Reverse Primer	
1	1276	1	-	0	4	NO	-	-	
2	1275	1	-	0	4	OK	-	KC260	
3	428	1	-	0	4	OK	-	KC260	
4	1276	1	430	0.5	3.5	NO	-	-	
5	1275	1	430	0.5	3.5	OK	KC430	KC260	
6	428	1	430	0.5	3.5	OK	KC430	KC260	
7	1276	1	432	0.5	3.5	NO	-	-	
8	1275	1	432	0.5	3.5	OK	KC432	KC260	
9	428	1	432	0.5	3.5	OK	KC432	KC260	
10	1276	1	434	0.5	3.5	NO	-	-	
11	1275	1	434	0.5	3.5	OK	KC434	KC260	
12	428	1	434	0.5	3.5	OK	KC434	KC260	

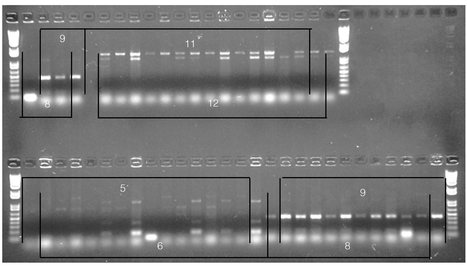
colony PCR for hht genes

P2: Table 1: PCR Mix

	Reagent	Volume (uls)	Master Mix	
1	10X Rxn Buffer	2.5	50	
2	2 mM NTP set	2.5	50	
3	Template (pFA6a plasmid)	0.2	4	
4	H2O	16.3	326	
5	Forward Primer (10 uM)	1.25	25	
6	Reverse Primer (10 uM)	1.25	25	
7	Taq	1	20	
Total		25	500	25

cPCR checking

MM #	Primer (forward)	Primer 2 (reverse)	Plate #
1	KC430	KC260	210 - 5 and 6
2	KC432	KC260	210 - 8 and 9
3	KC434	KC260	210 - 11 and 12



Tuesday, July 1, 2014

- These seems to be some trouble with the 1276 plasmid. None of plates have more than the control. I'll try reisolating plasmid 1276 and try the OL cloning once more. I still have the PCR product so this should be easy.
- The rest look nice with many more colonies than control
- I'll set up a PCR to check with oligos that amplify the gene and pTEF promotor

Wednesday, July 2, 2014: This looks pretty bad. I think there were some problems with the annealing temperature that made the PCR reaction to promiscuous. I'll try this again with a higher annealing temperature.

- Rerun this colony PCR using an annealing temperature of 60C to check for some good colonies

P2: Table 1: PCR Mix

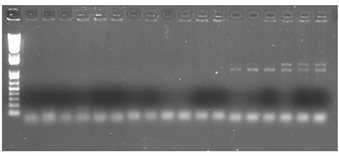
	Reagent	Volume (uls)	Master Mix	
1	10X Rxn Buffer	2.5	25	
2	2 mM NTP set	2.5	25	
3	Template (pFA6a plasmid)	0.2	2	
4	H2O	16.3	163	
5	Forward Primer (10 uM)	1.25	12.5	
6	Reverse Primer (10 uM)	1.25	12.5	
7	Taq	1	10	
Total		25	250	12.5

cPCR checking-1

MM #	Primer (forward)	Primer 2 (reverse)	Plate #	
1	KC430	KC260	210 - 5 and 6	6 RXNS
2	KC432	KC260	210 - 8 and 9	6 RXNS
3	KC434	KC260	210 - 11 and 12	6 RXNS

cPCR number 2

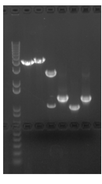
Sample #	Plate Number	colony number
1	5	2,6,8
2	6	1,2,5
3	8	4,5,8
4	9	1,2,4
5	11	2,3,7
6	12	2,3,6



This still does not look good. I'm going to try using the internal hph plasmid for the next set to check for the successful plasmids

Monday, July 7, 2014

- Restart cloning of hht1,2,3



Plasmid and Insert volumes

Lane		ng/ul	volume for 50 ngs vector	volume for 25 ngs insert
1	Plasmid 428	160	0.3	-
2	Plasmid 1275	115	0.4	-
3	hht1	191	-	0.13
4	hht2	115	-	0.2
5	hht3	161	-	0.15

Reactions

Reaction	Vector	Vector volume	Insert	Insert Volume	H2O
1	Plasmid 428	0.3	hht1	0.13	4
2	Plasmid 428	0.3	hht2	0.2	4
3	Plasmid 428	0.3	hht3	0.15	4
4	Plasmid 1275	0.4	hht1	0.13	4
5	Plasmid 1275	0.4	hht2	0.2	4
6	Plasmid 1275	0.4	hht3	0.15	4
7	Plasmid 428	0.3	-	-	4
8	Plasmid 1275	0.4	-	-	4

Tuesday, July 8, 2014

- Yesterday, each of these reactions was cut in half. I removed 2.5 uls from each tube and added 7.5 uls of the isothermal, Single-Reaction Enzyme Mix
- It looks like we have some nice successful plates. The negative controls look very nice. Today I'll do a colony PCR to check on the cells

P2: Table 1: PCR Mix

	Reagent	Volume (uls)	Master Mix	
1	10X Rxn Buffer	2.5	15	
2	2 mM NTP set	2.5	15	
3	Template (pFA6a plasmid)	0.2	1.2	
4	H2O	16.3	97.8	
5	Forward Primer (10 uM)	1.25	7.5	
6	Reverse Primer (10 uM)	1.25	7.5	
7	Taq	1	6	
Total		25	150	7.5

cPCR number 2-1

Reaction #	Plate #	colony number	forward Primer	Reverse Primer
1	1	1,2,3,4	KC430	KC331
2	2	1,2,3,4	KC432	KC331
3	3	1,2,3,4	KC434	KC331
4	4	1,2,3,4	KC430	KC333
5	5	1,2,3,4	KC432	KC333
6	6	1,2,3,4	KC434	KC333