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P24 – Site directed mutagenesis

Primer 1(forward)	**Primer 2 (reverse)**	**Template**	**hht gene**
KC438	KC439	2–1	hht2
KC440	KC441	3–1	hht3
KC436	KC437	4–1	hht1
KC436	KC437	4–2	hht1
KC438	KC439	5–2	hht2
KC440	KC441	6–1	hht3

PCR Master Mixes (6 mixes, 50μL each, 12.5μL for each PCR set)

Reagent	**Concentration**	**Volume (μL)**	**Master Mix (μL)**
Forward Primer	5μM	1	4
Reverse Primer	5μM	1	4
dNTP Mix	2mM each dNTP	0.25	2
10X KOD Buffer	–	2.5	10
25mM $MgSO_4$	25mM	1	4
Template DNA	1ng/μL	0.25	1
KOD enzyme	–	0.25	1
ddH_2O	–	7.5	30
DMSO	2% of final volume	0.255	–
Total Volume (μL)		12.5	50

Aliquot 12.5μ from master mix into PCR reaction tube For the 2 DMSO sample sets (6 samples per set), add 0.255μL to each PCR reaction tube

PCR Programs

PCR Program 1 – To be run on 1 DMSO set, 1 no-DMSO set

Step	**1– Heating**	**2 – Denature**	**3 – Anneal**	**4 – Extend**	**5 – End**
Temp	94C	94C	60C	72C	4C
Time	02m00s	00m15s	00m30s	01m40s	Forever
Directions	Go to next			Go to step 2 40X	Hold

PCR Program 2 – To be run on 1 DMSO set, 1 no-DMSO set

Step	**1– Heating**	**2 – Denature**	**3 – Anneal**	**4 – Extend**	**5 – End**
Temp	94C	94C	T_m–5C	68C	4C
Time	02m00s	00m15s	00m30s	05m00s	Forever
Directions	Go to next			Go to step 2 25X	Hold