

Fbxo2-VHC Mouse Embryonic Stem Cell Genotyping Protocol:

Produces a band of 3198bp to detect the presence of the Fbxo2-VHC allele.

Primers: (5`-3`)

Fbxo2-VHC\_F AACCAGCTGGGGCTCGAATCAAGC

Fbxo2-VHC\_R AGTTCTGTAGTCCTAAACTGTGTTTCAGGGAGGAAG

Reagent	$\mu$ l Vol for 25 $\mu$ l Rxn
water	14.5
5X Phusion HF buffer	5
10mM each dNTPs	0.5
DMSO	0.75
phusion hot start II enzyme	0.25
F primer (10 $\mu$ M)	1.25
R primer (10 $\mu$ M)	1.25
template DNA (DirectPCR Tail Lysate)	1.5
total	25

Cycling :

1. 98°C for 30s seconds
2. 98°C for 10 seconds
3. 72°C for 140 sec
4. go to step 2 35x
5. 72°C for 5 minutes
6. 4°C hold

