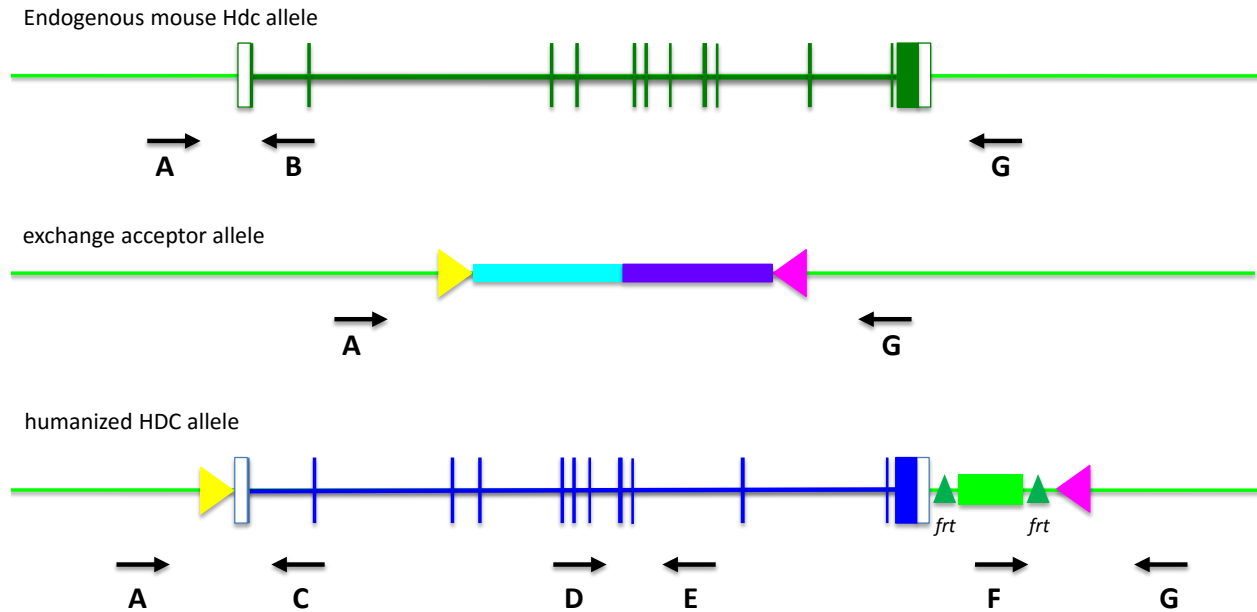


## PCR Protocols



PCR primers	Size of amplicon	comment
A/B	573 bp	Indicates presence of unmodified endogenous mouse Hdc locus
A/C	571 bp	Indicates correct 5' RMCE integration of human HDC
D/E	551 BP	Amplicon across intron 8 – exon 9; sequencing the amplicon will confirm wildtype (G) or mutant (G>A) HDC
F/G	439 bp	Indicates correct 3' RMCE integration of human HDC

Per PCR reaction:	Final concentrations	volumes
H2O		20.725 $\mu$ l
10 x Taq buffer incl. MgCl	1 x Taq buffer (0.5 mM MgCl)	2.5 $\mu$ l
10 mM dNTPs	0.1 mM	0.25 $\mu$ l
~10 $\mu$ M (100 ng/ $\mu$ l) forward primer	~0.1 $\mu$ M (1 ng/ $\mu$ l)	0.25 $\mu$ l
~10 $\mu$ M (100 ng/ $\mu$ l) reverse primer	~0.1 $\mu$ M (1 ng/ $\mu$ l)	0.25 $\mu$ l
Taq Polymerase 5U/ $\mu$ l	0.005 U	0.025 $\mu$ l
		24 $\mu$ l
Template DNA		1 $\mu$ l
		25 $\mu$ l

Cycling conditions:

94 C – 3 min

[94 C – 30 sec; 58 C – 30 sec; 72 C – 30 sec] x 30

72 C – 5 min

1% agarose gel

primer	direction	location	sequence
A	forward	Mouse genomic, 5' of Hdc start	5' - TATGGTCTGAATGCAGGGGCA
B	reverse	Mouse genomic, 3' of Hdc start	5' - AGAAGTAGGCACATGAGAGGC
C	reverse	Human genomic, 3' of HDC start	5' - AATGTTGCTGGTGCCAACTC
D	forward	Human genomic, 5' of HDC exon 9	5' - TCCACATCGATGCTGCTTATG
E	reverse	Human genomic, 3' of HDC exon 9	5' - GCCTCAAAGGCTGGTCTCATT
F	forward	Neo cassette	5' - CCTCGTGCTTTACGGTATCGCC
G	reverse	Mouse genomic, 3' of Hdc end	5' - TAGACCTCCTGACATGGGTAC