

Genotyping performed by Linh Trinh on 1_8_2019:

Conserved region 1

Forward primer	Sox17E1CEFwd: 5' GATAGATACTTTGGGAGGAGTGTGG
Reverse primer	Sox17E1CERev: 5' GTAGCCACCTAGAAATCCTGCACTG
Expected products	WT - 310bp; KO - 193bp
RNA Polymerase	Phusion

Component	Final concentration	25µl reaction	4
Nuclease-free water	To 25 µl	16.3	65.2
GC buffer	1X	5	20
10µM Forward Primer	0.5 µM	1	4
10µM Reverse Primer	0.5 µM	1	4
10mM dNTPs	200 µM	0.5	2
Phusion polymerase	1 Unit	0.2	0.8
cDNA		1	

Step		Temperature	Time
Initial Denaturation		98C	30s
40x	Denaturation	98C	10s
	Annealing	69C	10s
	Elongation	72C	10s
Final extension		72C	5min
Hold		4C	∞

CR1 mutation screening

