

Conserved region 2

Forward primer Sox17Δ128_For: CCAGAGGAACTCGTAAAGCTG
Reverse primer Sox17Δ128_Rev1: GAATTCAGCCTTCCTATTCC
Expected products WT - 341bp; KO - 215bp

RNA Polymerase Phusion

Component	Final concentration	25µl reaction	6
Nuclease-free water	To 25 µl	16.3	97.8
GC buffer	1X	5	30
10µM Forward Primer	0.5 µM	1	6
10µM Reverse Primer	0.5 µM	1	6
10mM dNTPs	200 µM	0.5	3
Phusion polymerase	1 Unit	0.2	1.2
cDNA		1	

Step		Temperature	Time
Initial Denaturation		98C	30s
40x	Denaturation	98C	10s
	Annealing	64C	10s
	Elongation	72C	10s
Final extension		72C	5min
Hold		4C	∞

CR2 mutation screening

