

Genotyping protocol for rCST3

Primer name	Sequence
rCST3 F1	5'-TGCTTGTGAATCCAGGCATA -3'
rCST3 R1	5'-CCCAATTCCCAGGATAAAT-3'

Master mix

reagent	volume
Dream Taq buffer 10x	2.5 µl
dNTPs (10mM)	0.5 µl
Primer forward F1 (10µM)	1.0 µl
Primer reverse R1 (10µM)	1.0 µl
Dream Taq polymerase	0.2 µl
DNA (DIRECT PCR)	3.0 µl
Tk H ₂ O	16.8 µl
Total volume	25.0 µl

DNA isolation: Direct PCR Lysis Reagent (Mouse Tail), ViagenBiotech, cat. number 102-T.

PCR (38 cycles):

Denaturation	95°C	5 min
Denaturation	95C	30 sec
Annealing	57°C	40 sec
Elongation	72°C	40 sec
Final Elongation	72°C	5 min
End	12°C	forever

Gel electrophoresis: 8ul on 3% agarose gel, 90V, 30min.. PCR product for wt is 460bp.

Digestion Maell (thermo) following company instruction

