



DNA173 - SEC203T1 PCR to Confirm Genotypes for Shipping

Reaction Components Vol (ul)

10X Sigma Buffer	5
25mM MgCl ₂	5
10mM dNTPs	1.25
LTR2 Primer	1.25
SEC203T1 3' Primer	1.25
5 U/ul Taq polymerase	0.5
Water	30.75
Total mix volume	45
Tail lysate (1:20 dilution)	5
Total reaction volume	50

Cycling Conditions: Touchdown

Step	Temp	Time	Note
1	94C	10'	
2	94C	15"	
3	65C	30"	Decrease 1C/cycle
4	72C	30"	Go to 2, 10 cycles
5	94C	15"	
6	55C	30"	
7	72C	30"	Go to 5, 30 cycles
8	72C	5'	

Primer sequences (5' to 3'):

LTR2	AAATGGCGTTACTTAAGCTAGCTTGC
SEC203T1 3'	TCGAGTTTAGAGTCCTTCCGACACA

Well	Sample	Genotype
1	Tail 158	het
2	Tail 159	het
3	Tail 160	het
4	Tail 161	het
5	ES DNA	het
6	wt lysate	wt
7	water	no amp



Mutant band: 386 bp

