

Myo1A Genotyping

I. Primer sequence

Myo1aF1584	TCAGGTGGGCAGACAGAGGTAGT	23 mer
Myo1aR3330	CCCCTCCCACCGTTAACTATCAGA	24 mer
RNeoc	TCGCCTTCTATCGCCTTCTTGACG	24 mer

II. Reaction Components

(We use PLATINUM PCR SUPERMIX from Invitrogen, 11306016)

	concertration	
PCR Supermix		22.5ul
Myo1aF1584	10 uM	0.6 ul
Myo1aR3330	10 uM	0.6 ul
NeoF1a	10 uM	0.6 ul
DNA		0.5 ul

The total volume in each reaction is 24.8ul.

III. Cycling Conditions

Step	Temp	Time	Note
1	94 °C	2 min	
2	94 °C	30 sec	
3	55 °C	30 sec	
4	72 °C	2 min	35 cycles
5	72 °C	5 min	
6	4 °C	∞	

Separated by gel electrophoresis on a 0.8% agarose gel.

iV. Products

wildtype = 1800 bp

heterozygous = 1800 bp and 650 bp

Knock out = 650 bp