

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: 129S-Pitx2^{tm1Jfm}/Mmucd MMRRC # 000124-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	18.05
10x Buffer (contains 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 20μM) Pitx2-JM1	0.5
Primer 2 (stock concentration is 20μM) MHOX Neo-1	0.5
Taq Polymerase 5Units/μL	0.25
DNA extracted with ☐ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	4:00	1
2. Denaturation	94	1:00	} 30x
3. Annealing	62	1:00	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Pitx2-JM1	CGG ACA CTT GAG CGC AGC CC
2: MHOX Neo-1	GCC ACT CCC ACT GTC CTT TC

Electrophoresis Protocol:

Agarose: 2% V: 100 Estimated Running Time: 60 min

Expected Bands	Genotype
300 bp	Pitx2 null -/-