

GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

Protocol Name: B6J.Cg-Y(IsXPAR;Y)Ei/EiJArnoMmucd **MMRRC:** 043694-UCD

Protocol:

Reagent/Constituent	Volume (μL)
Cat No./ID 204541	
QuantiTect Multiplex PCR Master Mix	5.0
Water	3.4
Target Probe mix	0.3
-21 μM Forward primer	
-21 μM Reverse primer	
-7 μM Mutant probe	
Reference Probe mix	0.3
-21 μM Forward primer	
-21 μM Reverse primer	
-7 μM Mutant probe	
Sample	1.0
TOTAL VOLUME OF REACTION:	10.0 μL

Comments on protocol:

- Use one target probe with one reference probe for real-time PCR. Several references are given as an example, but only one is needed. Another autosomal Taqman probe would also work

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	95	15:00	1
2. Denaturation	95	0:30	40x
3. Annealing/Elongation	60	1:00	40x
4. To step 2 for 40 cycles			

Primers:

Target probes

Integrated DNA technologies

43694-Prdx4-F	TTTGAGACAGTGTATCTATCCCT
43694-Prdx4-R	CCACTGAAAGCTACTGGTAAAG
43694-Prdx4-P	/56-FAM/CCTCAAACCT/ZEN/CTCCCAACAGCTCCC/3IABkFQ/

Integrated DNA technologies

43694-Amelx-F	AAATCATCCCTGTGCTGTCTC
43694-Amelx-R	AGGAACTGGCATCATTGGTT
43694-Amelx-P	/56-FAM/CTTCAGCCT/ZEN/CATCACCACCTTCCC/3IABkFQ/

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Reference Probes (pick one)

Applied Biosystems

Tcrd fp	CAGACTGGTTATCTGCAAAGCAA
Tcrd rp	TCTATGCCAGTTCCAAAAACATC
TM-TCRD-MGBV	VIC-ATTATAACGTGCTCCTGG-MGB

Applied Biosystems

QPCR-uc329-F	GTCATCAAGTGAGAAAGACATCCT
QPCR-uc329-R	CATCATGAATTTTGATAAGCCCATT
TM-UC329-MGBV	VIC-CTCCTGGCTGCCTG-MGB

QPCR-uc329-F	GTCATCAAGTGAGAAAGACATCCT
QPCR-uc329-R	CATCATGAATTTTGATAAGCCCATT
TM-uc329-P:	VIC - CTCCTGGCTGCCTGGCTGGC - TAMRA

Tcrd fp	CAGACTGGTTATCTGCAAAGCAA
Tcrd rp	TCTATGCCAGTTCCAAAAACATC
Tcrd probe	VIC-ATTATAACGTGCTCCTGGGACACCC-TAMRA

Example data

Fam-Prdx4

Vic-TCRD-

MGB

Fam-Amelx

Vic-TCRD-

MGB

	ΔCt	$\Delta\Delta Ct$	copy#	Prdx4#		ΔCt	$\Delta\Delta Ct$	copy#	AmelX#	Gonadal sex	Genotype
NTC					NTC						
XY	-0.3			1	XY	0.6			1		
43694-1	0.1	0.2	0.9	1	43694-1	-0.2	-1.0	2.0	2	male	XY*
43694-2	-0.2	-0.1	1.1	1	43694-2	-0.3	-1.1	2.1	2	male	XY*
43694-3	-0.3	-0.1	1.1	1	43694-3	-0.6	-1.4	2.6	2	male	XY*
43694-4	0.0	0.1	0.9	1	43694-4	-0.4	-1.2	2.3	2	male	XY*

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Genotyping Key

	Genotypes	Gonadal Sex	Prdx4 #	Amelx #
	Controls			
	XY	M	1	1
	XY*	M	1	2
	XX	F	2	2
Breeding	A. XX x XY*			
	X0	F	1	1
	XY*x	F	1	2
	XX	F	2	2
	XY*	M	1	2
	XXY*	M	2	2
Breeding	C. XY*x x XY			
	X0	F	1	1
	XY*x	F	1	2
	XX	F	2	2
	XXY*x	F	2	3
Breeding	D. XXY*x x XY Sry			
	XY Sry	M	1	1
	XXSry	M	2	2
	XXY*x Sry	M	2	3
	XXY*x Sry	M	1	2
Breeding	B. XYY^{8x} Sry x XX			
	X0	M or F	1	1
	XY	M or F	1	1
	XY*x	M or F	1	2
	XX	M or F	2	2
	XXY*x	M or F	2	3
	XXY*x	M or F	1	2
	XXY	M or F	2	2