GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **C57BL/6N-Dpp4em1(DPP4)Mbp/Mmucd** Stock: 068166-UCD

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) IVF	0.5
Primer 2. (stock concentration is 20µM) IVR	0.5
Primer 3. (stock concentration is 20µM) kiF	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. C19-Dpp4-IVF	GCACTCTCATTTGGCATCAGACG
2. C19-Dpp4-IVR	CCAGTCTCTTCAGCCATAACCCAG
3. C19_Dpp4-kiF	TAGGTGCTACAAAAACACAGCAAGG

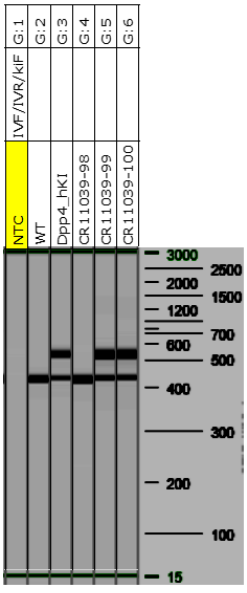
Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2	441	wildtype
1 & 3	542	mutant

Allele Description: CRISPR targeted humanized DPP4 KI locus and mouse Dpp4 (ENSMUSG00000035000.9) KO. Human CDS consisting of exons 2*-26 was inserted into mouse exon 2 (ENSMUSE00000662442) beginning at the 11th amino acid into zygotes. The mouse exon 2 is replaced with human exon 2 CDS/h3'UTR (human 3' untranslated region) in the HR template to prevent potential downstream splicing or RNP re-cleavage (4 engineered silent mutations in human CDS). This production targeted directly into zygotes to produce a founder and backcross to N1 to create germline Het animals. Long range PCR products are fully sequenced.



Het = #99-100; WT = #98

PCR protocol developed by MMRRC at University of California, Davis