GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **C57BL/6-*Ptpn6*^{m1Btlr}/Mmcd, (*spin*)** **MMRRC # 015198-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	12.675
10x Buffer (contains 15mM $MgCl_2$)	2.5
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 25mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) Spin PCR F1	0.5
Primer 2 (stock concentration is 20µM) Spin PCR R1	0.5
Taq Polymerase	0.5
DNA sample extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- PCR products are verified to contain the correct amplicon size by running ~10µl of the reaction on a gel and the remaining 15µl purified via column based PCR purification method for sequencing.
- The *spin* mutation introduces a *Hinc* II restriction enzyme site in the *Ptpn6* genomic DNA sequence. *Spin* genotyping can also be performed by amplifying the region containing the mutation using PCR, followed by *Hinc* II restriction enzyme digestion.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Spin PCR F1	ACG CTG GAG CTT CAG TGG AC
2: Spin PCR R1	TGA GGT GAG GGA GGA CGA
3: Spin Sequencing	Use Spin PCR F1 primer for sequencing

Electrophoresis Protocol:

Agarose: 2% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	300 bp	WT
SNP found at position ~ 150 of sequencing		
the novel *Hinc* II site is highlighted in gray		
Restriction Digest w/ *Hinc* II	192 bp, 108 bp	*spin*

Mutation site (red) and flanking sequence:

WT tttgtc **T**acctg
spin tttgtc **A**acctg