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SAPAP3 Genotyping Protocol

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kajsbr[1]

[1]University of California, San Francisco

Lisa Gunaydin Lab

kajsbr

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for SAPAP3 KO Mouse Model Genotyping from Dr. Lisa Gunaydin's Lab, University of California, San Francisco

1 **Cut the tails.**
To cut the tails and mark the animals, keep in mind to:
● cut as little amount of the tail as possible;
● always clean the scissors properly in between animals (EtOH and bleach);
● tattoo the animals at the same time as cutting the tails;
● use iso if needed.

2 **Digest the tails.**
Incubate the tails O/N at 55 C (water bath) in 200 ul 1x PBND buffer with Proteinase K at 0.25 mg/ml. Vortex before.
*200ul * (54+1) = 11000ul —> 11ml PBND*
*0.25mg/ml * 11ml = 2.75mg —> 0.00275g Proteinase K*
PBND (250 ml 1x, stored at 4 C)
2.5 ml 1M TrisHCl pH 8.3
1.125 ml IGEPAL
1.125 ml Tween 20
0.935 g KCl
0.125 g MgCl2.6H20
0.025 g Gelatin
Fill remainder with H2O
*Gelatin will dissolve only after heated

3 **Inactivate Proteinase K.**
After taking the tails out of the bath, see if they are digested. If so, vortex and boil them for 10 min. Vortex again and centrifuge at max speed for 5 min (table top centrifuge, something like 14,000 rpm).

4 **PCR reaction.**
We use the KAPABiosystems HotStart ReadyMix (2x) which contains DNA Polymerase, rxn buffer, dNTPs and MgCl2.
Pipette 24 ul of Master Mix into each PCR tube
Add 1 ul of DNA from each sample into each tube

Sap3 KO F1, Forward primer, 5' - 3': ATTGGTAGGCAATACCAACAGG
Sap3 KO R1, Reverse Primer 5' - 3': GCAAAGGCTCTTCATATTGTTGG
Extra Primer, TKF2: CTTTGTGGTTCTAAGTACTGTGG

	A	B	C	D	E
		1 rxn	MM SAPAP (__ rxns)	Primer name	Total Rxns
	Forward primer	1		Sap3 KO F1	
	Reverse primer	1		Sap3 KO R1	
	Extra primer	1		TKF2	
	Rxn mix	12.5			
	H20	8.5			
	DNA	1.0			
	PCR program		Sap3		

PCR Program for SAPAP3 Genotyping:
1. 95C for 4 minutes
2. 30 times:
   94C for 30 seconds
   62C for 30 seconds
   72C for 60 seconds
3. 72C for 5 minutes
4. 4C hold

Expected band lengths:
WT: one band at ~147 bp
Het: one band at ~147 bp and one band at ~222 bp
KO: one band at ~222 bp

5 **Run the 1% agarose gel**
200 ml TAE
2g agarose
2ul sybr safe gel stain
24ul DNA per well. 5ul DNA ladder before each new line