Ciphe Designing genes Profiling immunity		**Genotyping Mice Strategy Document** **Sept3$^{tm1a(KOMP)Wtsi}$** **Project**

PCR GENOTYPING SEPT3$^{TM1A(KOMP)WTSI}$ MICE STRATEGY

Validation date : 22/07/2014

Shipping date : …/…/….

Note: The PCR genotyping strategy and protocols are developed at the GEMTis facility core of CIPHE. The researcher will be able to implement and reproduce this protocol easily in his own laboratory.

Name of the researcher	Malissen/MDa
Engineer in charge of project	GEMTis
ES cell genetic background	C57Bl/6 N

For any question, please contact

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Quality approach Anxious to bring our customers a quality service and satisfy at best, we remain attentive to all your comments and suggestions on the development of the service. (Contact: Anne Marie MURA, Quality Manager, Email: anne-marie.mura@inserm.fr)

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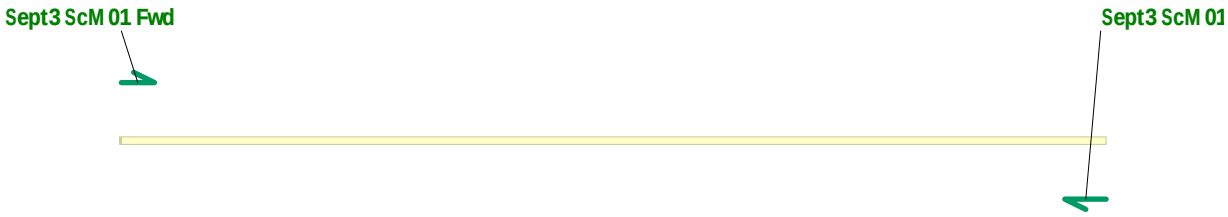
PCR GENOTYPING-SCREENING STRATEGY **F1** MICE: ZOOM VIEW

A specific set of primers was used to amplify genomic tail DNAs extracted from Wt and heterozygous littermates.

N°46187_Sept3_Wild_Type_Allele (Mice F1 WT): 591 pb

The map bellow represents **Wt allele**
The primers used to genotype mice and the amplicon size are depicted.



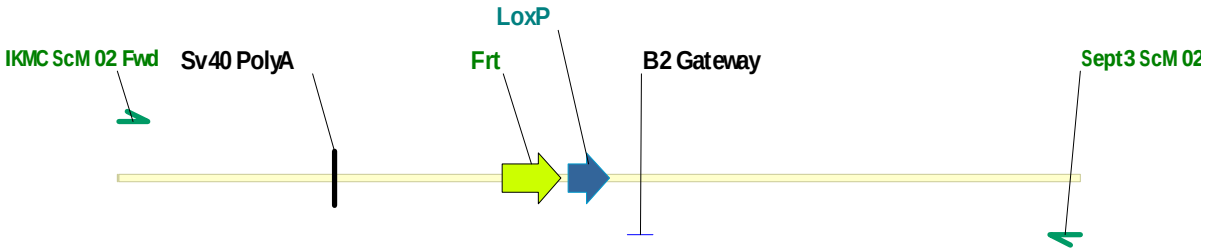
Wt Sequence

TGAAGCTGACAGTCATCGACACACCAGGCTTTGGAGACCAGATCAACAATGAAAACTGG
TATGTGCCTGCCTCTGAGATTTTCTTCCCCTTAAATACTCTGCTAGGAAGGCAAGGTGAA
GACTCATTCCCACTGAGAACTACCTGTATCCTCCCTTGACAAGGAGTTTGGTCCCATGG
GAGCTATTCTTGCTGGCCATCCATCGAAAGCCATTTGTTGGAGAGACTGAGGAAGAGCT
GGCCGTTGGGTTGAGAAAGGAGAGAGTGCGGAATTGTGCCCCGACTATGCCTCAGAGC
CACGCTGGAGTTTTGTCCTTGGATCTTTGGGTGGATCCCATCTGTCTTAGTTAGGGTTTT
ACTGCTGTGAACAGACACCATGACCAAGGCAACTCTTATAACGACAACATTTAATTGGG
ACTGGCTTACAGGACCAAAGGTTTAGTCCATTATCATCCAGGCAGGAGCATGGCCAGAC
GTGGAACTGAAGGAGCTGAGAGTTCCACATCTTGTTCCAAAGGCAGCTAGGAGAAGAC
TGACTTCCAGGCAGCTAGGATGAGGGTCTCCAAGCCCACGCCCACAGTGACACACCTA
CT

Size of wt amplicon (**591pb**).

N°46187_Sept3_Mutant_Allele

The map bellow represents mutant **allele**
The primers used to genotype mice and the amplicon size are depicted.



Mutant Sequence

GGGATCTCATGCTGGAGTTCTTCGCCCACCCCCCGGATCTAAGCTCTAGATAAGTAATG
ATCATAATCAGCCATATCACATCTGTAGAGGTTTTACTTGCTTTAAAAAACCTCCCACAC
CTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCA
GCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTT
CACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCC
GGGGGTACCGCGTCGAGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGA
ACTTCGTCGAGATAACTTCGTATAGCATACATTATACGAAGTTATGTCGAGATATCTAGA
CCCAGCTTTCTTGTACAAAGTGGTTGATATCTCTATAGTCGCAGTAGGCGGATTGTGCC
CCGACTATGCCTCAGAGCCACGCTGGAGTTTTGTCCTTGGATCTTTGGGTGGATCCCAT
CTGTCTTAGTTAGGGTTTTACTGCTGTGAACAGACACCATGACCAAGGCAACTCTTATAA
CGACAACATTTAATTGGGACTGGCTTACAGGACCAAAGGTTTAGTCCATTATCATCCAG
GCAGGAGCATGGCCAGACGTGGAACTGAAGGAGCTGAGAGTTCCACATCTTGTTCCAA
AGGCAGCTAGGAGAAGACTGACTTCCAGGCAGCTAGGATGAGGGTCTCCAAGCCCACG
CCCACAGTGACACACCTACT

Size of mutant amplicon (**792pb**).

CIPHE PROTOCOL

The protocol DNA extraction from mouse tail is located at the end of the document.

F1 MICE

1. PCR GENOTYPING PROTOCOL FOR F1 MICE OBTENTION

This section describes the composition of the mix and cycling conditions used for genotyping

Sequence of primers used for genotyping:

WT primers
Sept3_ScM_01_Fwd: TGAAGCTGACAGTCATCGAC
Sept3_ScM_01_Rev: TCCTAGCTGCCTTTGGAACA
Tm : 54,6°C

Mutant primers
IKMC ScM 02 Fwd : GGGATCTCATGCTGGAGTTCTTCG
Sept3_ScM_02_Rev: AGTAGGTGTGTCACTGTGGGCGTGG
Tm : 57°C

Amplicon WT size: 591pb
Amplicon mutant size: 792pb

Taq polymérase : *LongAmp Taq NEB*
PCR device: Eppendorf-Mastercycler-proS

PCR cycling parameters

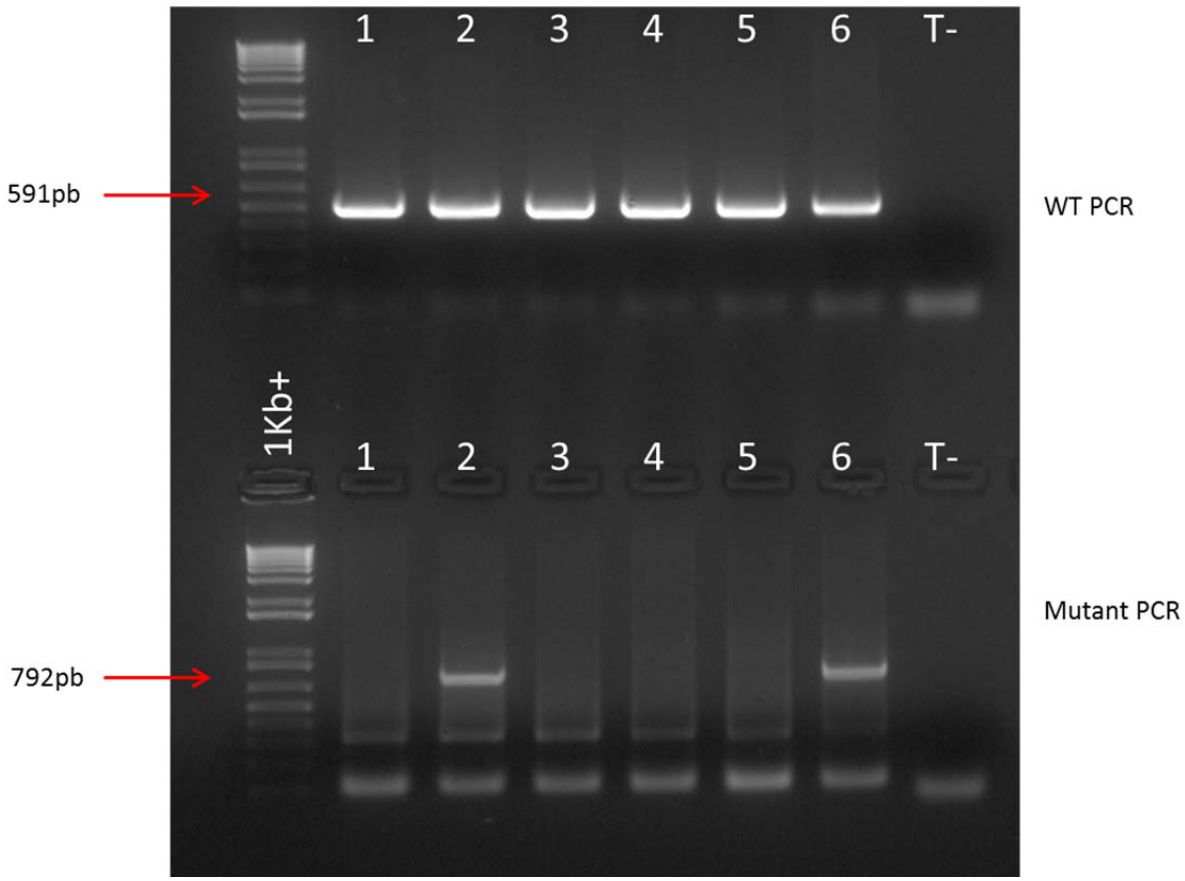
1- 94°C 5min
2- 10 cycles :
   a. 94°C 15s
   b. 65 to 55°C 15s (-1°C/cylces)
   c. 68°C
3- 30 cycles :
   a. 94°C 30 sec
   b. 55°C 45 sec
   c. 68°C 60sec
4- 68°C 5min

GEL: 1.5 % of agarose
Weight Marker: 1Kb+

PCR MIX

DNA	2µL
5X buffer	10µL
dNTP 10mM	1.5µL
100µM Primer Fwd	0.2µL
100µM Primer Rev	0.2µL
Taq	2µL
Nuclease free water	34.1µL
Final volume	50 µL

2. RESULTS OBTAINED FOR THE F1 MICE:



T-: Nuclease free water

Mice 2 and 6 have mutant allele.

PROTOCOL DNA EXTRACTION FROM MOUSE TAIL

Protocol applied:

1. Add 400µL of lysis buffer to biopsy and incubate over night at 55°C at 1200rpm
2. Inactivate Proteinase K at 95°C for 10min
3. Centrifuge at 14000 rpm for 10 min
4. Collect the supernatant and add 1.5 vol of isopropanol
5. Centrifuge at 14000 rpm for 10 min
6. Wash with 1 ml of ethanol 70%
7. Centrifuge at 14000 rpm for 5 min
8. Discard the supernatant
9. Dry the pellet at room temperature
10. Resuspend the pellet in 200 µL of distilled water

Lysis buffer :

Reagent	Final concentration
Tris-HCl pH=7,5	100mM
EDTA	5mM
SDS	200mM
H2O	0.2%
Proteinase K (PK)	0.5mg/mL
RNAse A	50µg/mL

Add PK and "RNAseA" extemporaneously