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EXCELLENCE IN MOUSE PHENOGENOMICS

M509T PM in the Lhcgr

Genotyping protocol

Kus6777 / IM00006777

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INVESTISSEMENTS D'AVENIR

Strategy proposal

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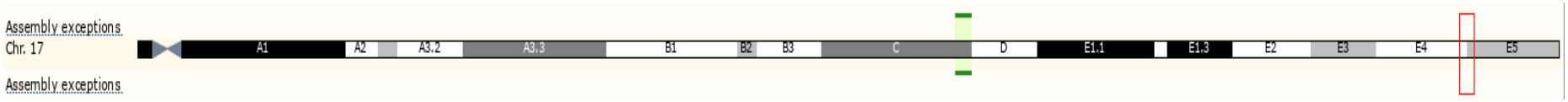
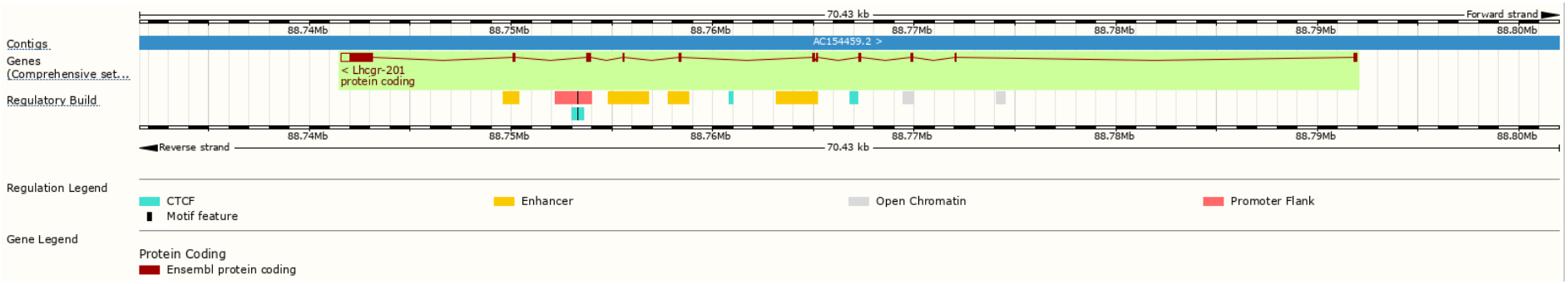
Lhcgr Mouse Genomic locus

Chromosome 17: 88,741,549-88,791,976



Gene: Lhcgr ENSMUSG00000024107



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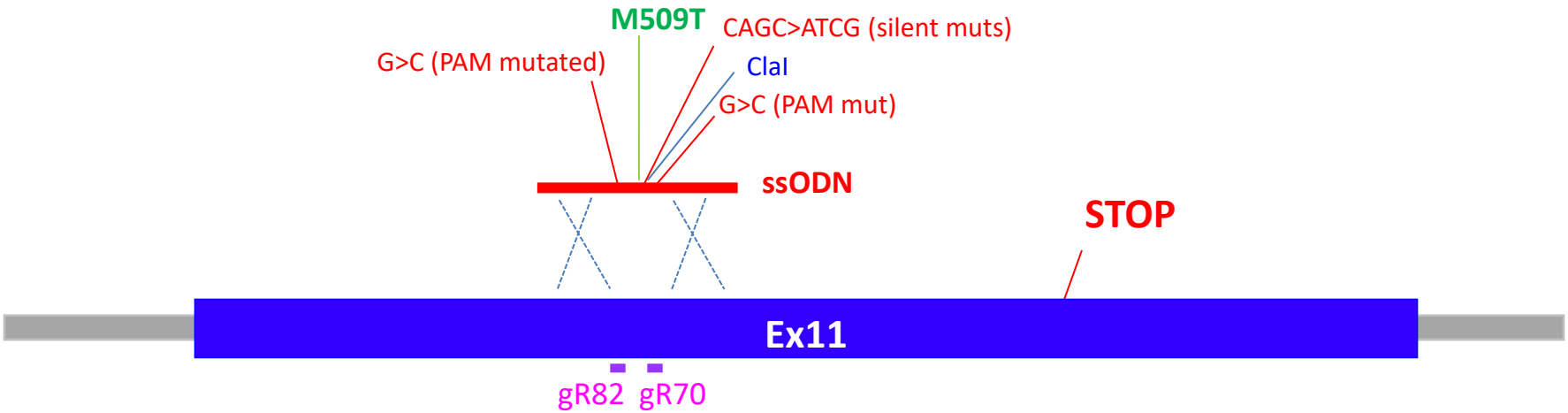
NOM mRNAs and proteins

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq
Lhcgr-201	ENSMUST00000024916.5	2553	700aa	Protein coding	CCDS29025	P30730	NM_013582 NP_038610

Lhcgr-201 ENSMUST00000024916.5

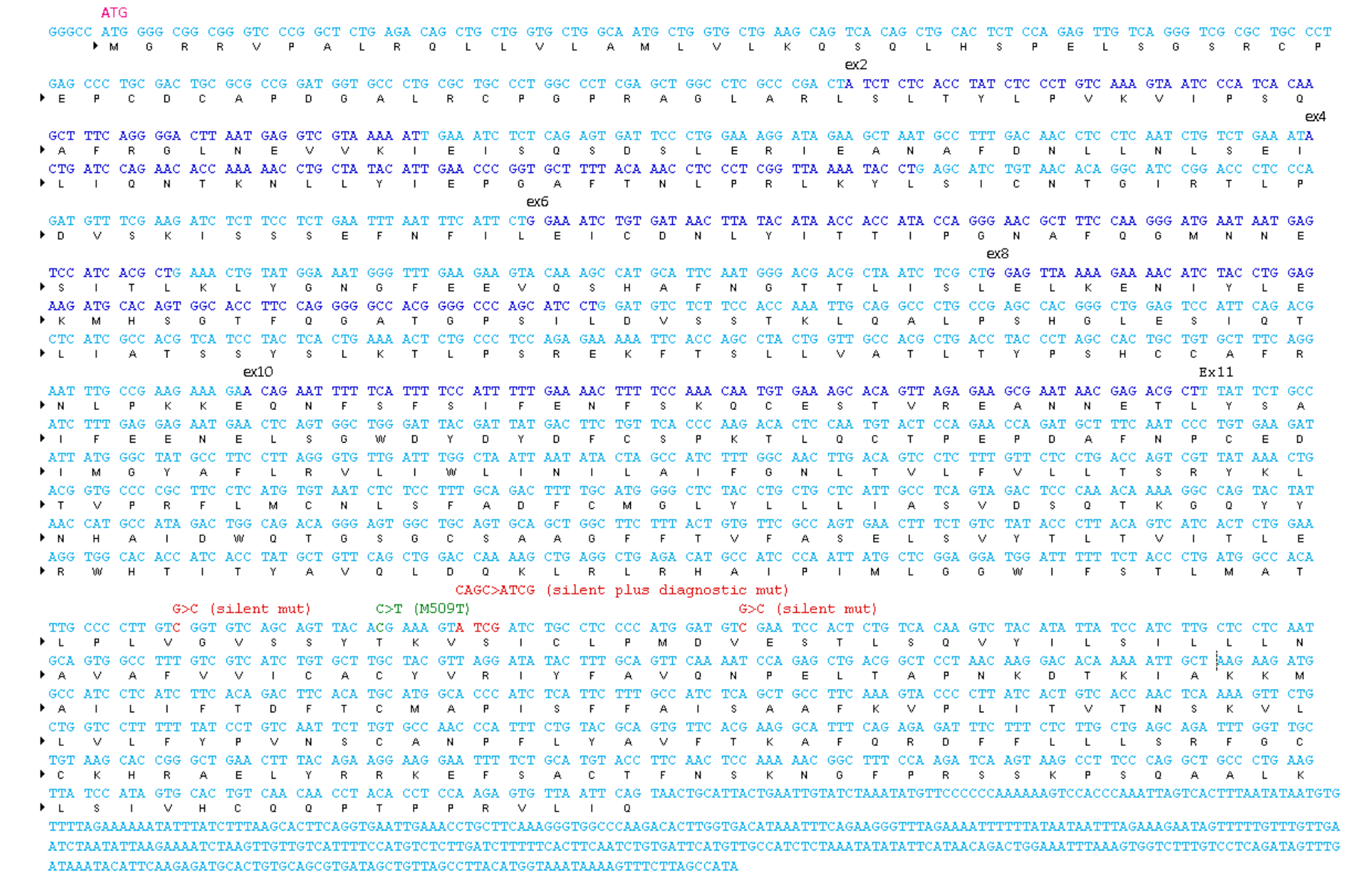
Strategy proposed: M509T point mutation

CRISPR/cas9 approach



Sequence of the homology directed repair (HDR) resulting allele

mRNA sequence after HDR



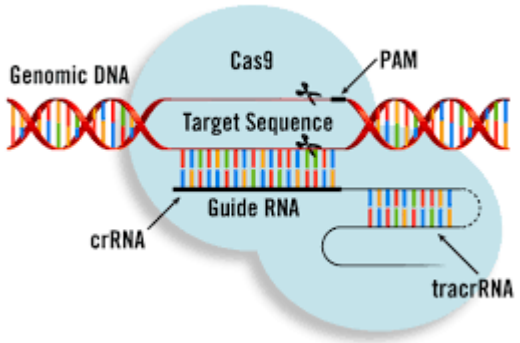
Strategy proposed : HDR by CRISPR/Cas9

Pros

- As asked
- Two sgRNAs will be used in order to increase the chance of insertion of the asked mutation (double strand breaks occur 3 bps 5' of the PAM sequences, respectively 22 and 26 bps from the asked mutation)
- A diagnostic ClaI site will be created with changes in the codon (but not the amino acid sequence
- Both Protospacer Adjacent Motif (PAM; NGG) will be mutated with silent mutation in order to avoid recuts after HDR.

Cons

- Silent mutations will be introduced and may change the genomic environment (ie splicing could be affected, other?)
- The sgRNA efficiency of double strand break cannot be anticipated (even if the capacity to generate DSB is tested *in vitro*)
- Efficacy is highly variable from one target sequence to another



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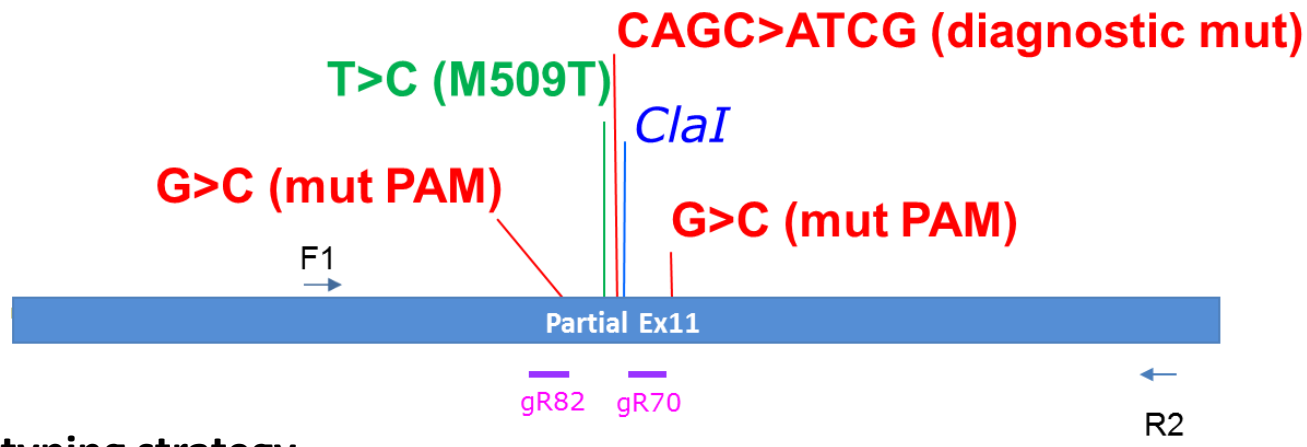
GENOTYPING INSTRUCTIONS



PCR genotyping strategy

Primer ref.	Sequence	Amplification product size WT	Sizes observed if ClaI restriction present (associated with asked PM)
F1	AAAGGTGGCACACCATCACCTATGC	436 bps	288 bps + 148 bps
R2	AGCAGAACTTTTGAGTTGGTGACAG		

Sequence of the PCR product

AAAGGTGGCACACCATCACCTATGCTGTTCAGCTGGACCAAAAGCTGAGGCTGAGACATGCCATCCCAATTATGCTCGGAGGATGGATTT
TTTCTACCCTGATGGCCACATTGCCCCTTGTCGGTGTCAGCAGTTACACGAAAGT*ATCG*ATCTGCCTCCCCATGGATGTCGAATCCACTCTG
TCACAAGTCTACATATTATCCATCTTGCTCCTCAATGCAGTGGCCTTTGTCGTCATCTGTGCTTGCTACGTTAGGATATACTTTGCAGTTCAAA
ATCCAGAGCTGACGGCTCCTAACAAGGACACAAAAATTGCTAAGAAGATGGCCATCCTCATCTTCACAGACTTCACATGCATGGCACCCAT
CTCATTCTTTGCCATCTCAGCTGCCTTCAAAGTACCCCTTATCACTGTCACCAACTCAAAAGTTCTGCT

Primers in orange

ClaI site in italic

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GENOTYPING INSTRUCTIONS

PCR Protocol

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

Reagents:	Volume (per sample):
- Phusion HS (Thermo Scientific) 5X Buffer	4 µl
- 10mM dNTP	0,4µl
- 5' primer (100 µM)	0.1µl
- 3' primer (100 µM)	0.1µl
- DNA (lysate 1/10)	2µl
- Phusion Hot Start II	0,2µl
- Sterile H2O	up to 20 µl

Cycling conditions

Temp	Time	#Cycles
96°C	5min	1
96°C	8s	
62°C	10s	30
68°C	45s	
68°C	5min	1
12°C	5min	1

Digestion protocol	**Volume / sample**
PCR product	10 µl
Buffer 10X	2 µl
ClaI Restriction enzyme	0,2 µl
H20	7,8 µl

This reaction is incubated 15 mins at 37°C then leaded on a 3% agarose. The 10 µl left over PCR reaction serves as negative control