phenomin

EXCELLENCE IN MOUSE PHENOGENOMICS

G424R KI in Pak3

$Pak3^{em1Ics}$

Genotyping protocol

Kus6767 / IM6767

by Marie-Christine Birling (PhD)

birlingm@igbmc.fr

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INVESTISSEMENTS D'AVENIR

Proposal

cnrs

centre européen de recherche en biologie et en médecine CERBMgie

Instituts thématiques Inserm

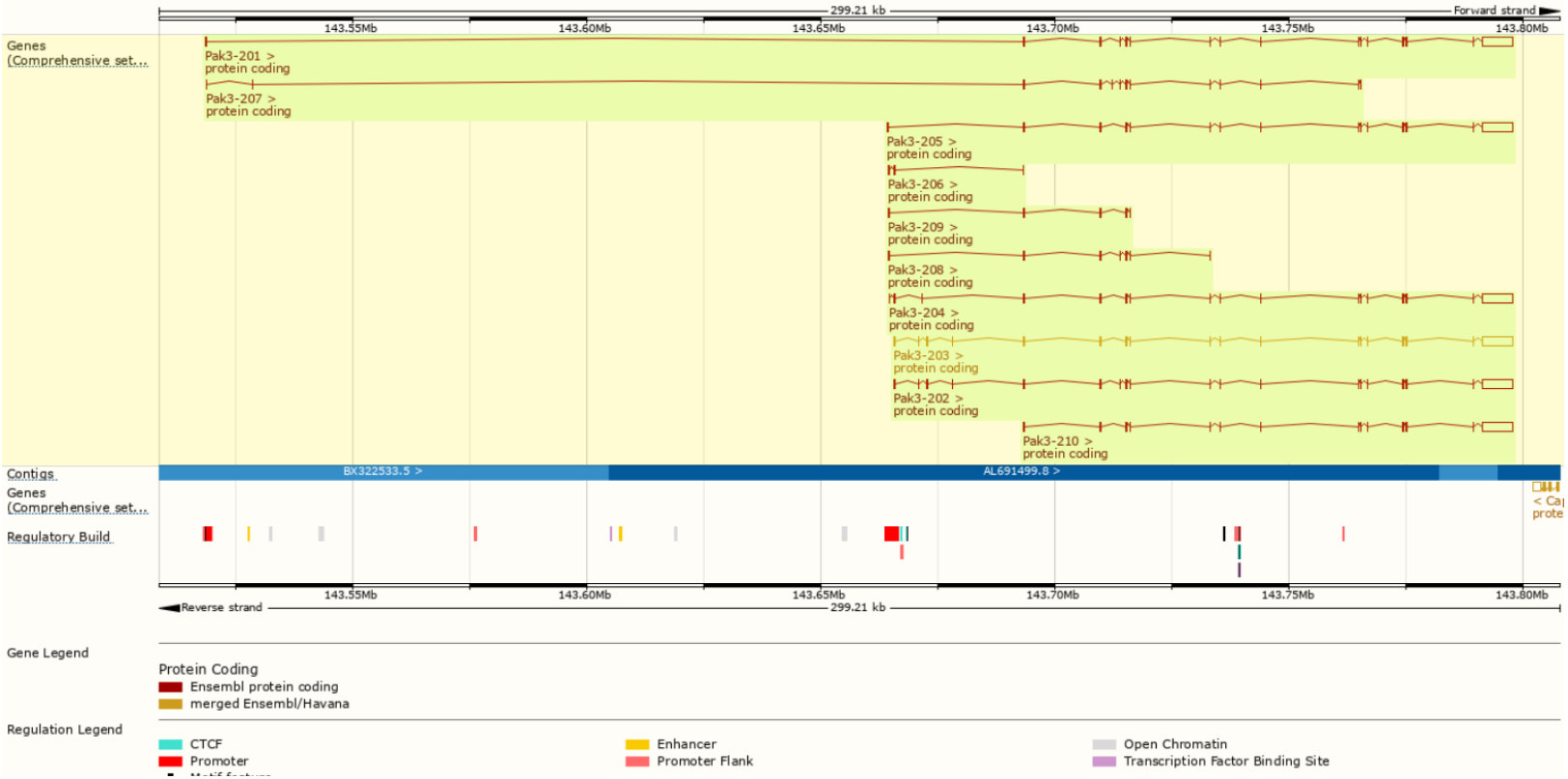
institut national de la santé et de la recherche médicale

UNIVERSITÉ DE STRASBOURG

Aix*Marseille université

Pak3 Mouse Genomic locus

Gene: Pak3 ENSMUSG00000031284



Chromosome X: 143,518,591-143,797,796

Pak3 mRNAs and proteins

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq	Flags
Pak3-201	ENSMUST00000033640.13	8348	559aa	Protein coding	CCDS57779	Q61036	NM_001195046 NM_001195048 NP_001181975 NP_001181977	TSL:1GENCODE basicAPPRIS ALT1
Pak3-202	ENSMUST00000112863.7	8657	559aa	Protein coding	CCDS57779	Q61036	-	TSL:5GENCODE basicAPPRIS ALT1
Pak3-203	ENSMUST00000112864.7	8612	544aa	Protein coding	CCDS30454	Q61036	NM_001195049 NP_001181978	TSL:1GENCODE basicAPPRIS P3
Pak3-204	ENSMUST00000112865.7	8484	544aa	Protein coding	CCDS30454	Q61036	-	TSL:5GENCODE basicAPPRIS P3
Pak3-205	ENSMUST00000112868.7	8163	544aa	Protein coding	CCDS30454	Q61036	-	TSL:5GENCODE basicAPPRIS P3
Pak3-206	ENSMUST00000126592.7	451	23aa	Protein coding	-	A3KGC2	-	CDS 3' incompleteTSL:2
Pak3-207	ENSMUST00000134402.7	1351	366aa	Protein coding	-	A3KGC5	-	CDS 3' incompleteTSL:5
Pak3-208	ENSMUST00000155215.7	701	195aa	Protein coding	-	A3KGC4	-	CDS 3' incompleteTSL:3
Pak3-209	ENSMUST00000156449.7	716	155aa	Protein coding	-	A3KGC3	-	CDS 3' incompleteTSL:5
Pak3-210	ENSMUST00000172330.2	8135	544aa	Protein coding	CCDS30454	Q61036	NM_001195047 NM_008778 NP_001181976 NP_032804	TSL:1GENCODE basicAPPRIS P3

Pak3-203 ENSMUST00000112864.7

sgRNA search

http://crispor.tefor.net/crispor.py?batchId=8XOGbo1bro9TfbCuhylQ

Guide Sequence + *PAM* + Restriction Enzymes + Variants Only G- Only GG- Only A-	Specificity Score	Predicted Efficiency Show all scores		Out-of-Frame score Click on score to show micro-homology	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score exons only chrX only
		Doench '16	Mor.-Mateos			
AGTAAACGAAGCACTATGGT *GGG* . . . . Enzymes: *OliI, SmiMI* **Cloning / PCR primers**	84	63	34	56	0 - 0 - 1 - 7 - 72 0 - 0 - 0 - 0 - 1 80 off-targets	4:intron:Ltbp1 3:intergenic:Gm6486-Gm3053 4:intergenic:Igf2bp1-Gip show all...

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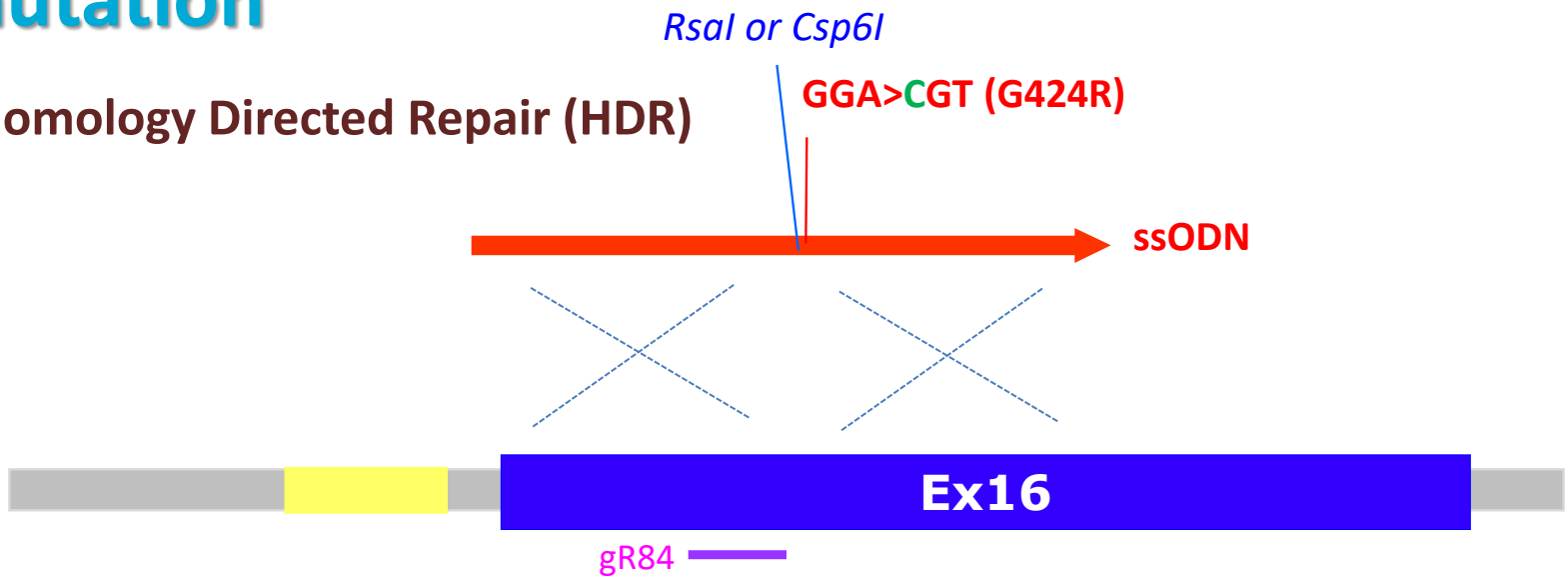
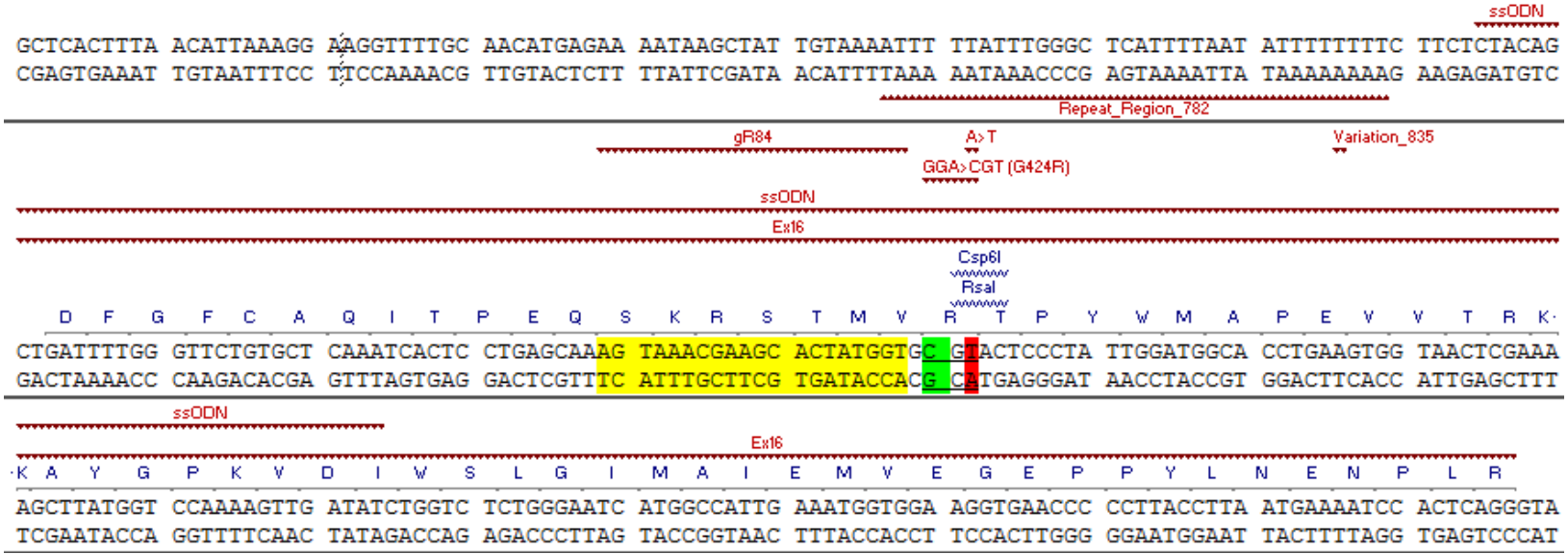
Strategy proposed: insertion of the G424R mutation



Sequence of the allele obtained after HDR



Strategy proposed: insertion of the G424R mutation

Pros

- Asked mutation introduced after genome editing
- The PAM will be mutated (introduction of a silent mutation) in the donor single strand oligonucleotide (ssODN). This mutation will avoid a new double strand break after HDR.
- A diagnostic silent mutation leading to RsaI (or Csp6I) restriction site will also be introduced in the donor ssODN (insertion of the A>T silent mutation)

Cons

- The introduction of the 1 silent mutations might alter the level of expression of the *Pak3* mRNA and protein (ie change in genomic environment)

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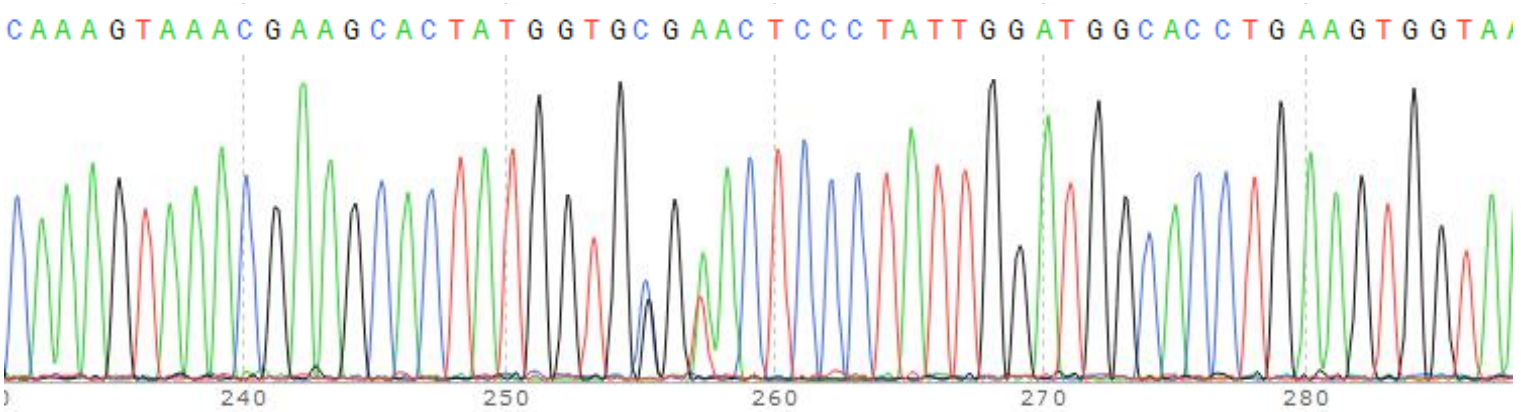
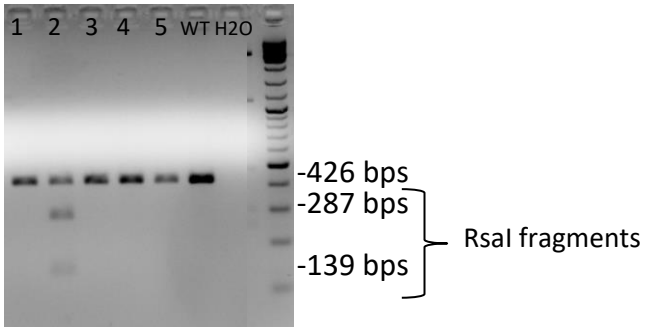
Genotyping of F1

Chromatogram on one F1 animal



GGA>CGT (G424R)

Example of F1 genotyping



Line Kus6767-6-PM established

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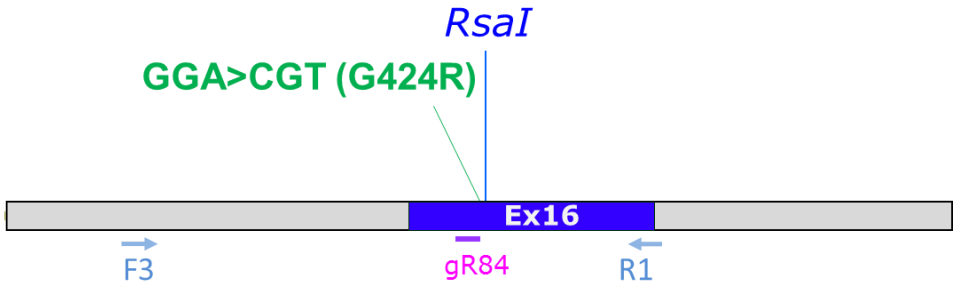
GENOTYPING INSTRUCTIONS

PCR genotyping strategy



Primer ref.	Sequence	Amplification product size WT
F3	AACTCTATCTATGCAAGTCTACTGA	426 bps (+ RsaI : 287 bp + 139 bp)
R1	CTTACCCTGAGTGGATTTTCATTAA	

Sequence of the PCR product (with mutations introduced)

AACTCTATCTATGCAAGTCTACTGAAAATGACAGCACTTGTATCAATTGTTTCAGATCCTTTTAAACTCAGGAGCAGATGAGAGATT
AGGAAAAGATCAAGATATGAGCTAGGCACTTGTGAGAGCTCACTTTAACATTAAAGGAAGGTTTTGCAACATGAGAAAATAAGCT
ATTGTAAAATTTTTATTTGGGCTCATTTTAATATTTTTTTTCTTCTCTACAGCTGATTTTGGGTTCTGTGCTCAAATCACTCCTGAGCA
AAGTAAACGAAGCACTATGGTG***CGT***ACTCCCTATTGGATGGCACCTGAAGTGGTAACTCGAAAAGCTTATGGTCCAAAAGTTGAT
ATCTGGTCTCTGGGAATCATGGCCATTGAAATGGTGGAAGGTGAACCCCCTTACCTTAATGAAAATCCACTCAGGGTAAG

	Forward and reverse primers
	Introduced mutation
Italic	Restriction enzyme

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GENOTYPING INSTRUCTIONS

PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping F0.

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
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.