phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

R187C in Grin2b
Grin2b^{em1Ics}

Genotyping protocol

Kus7403 / IM7403

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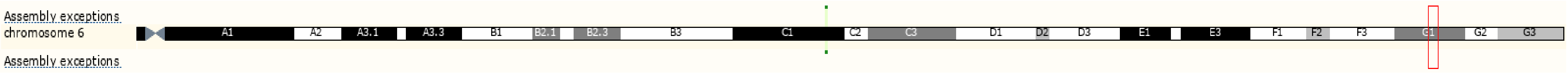
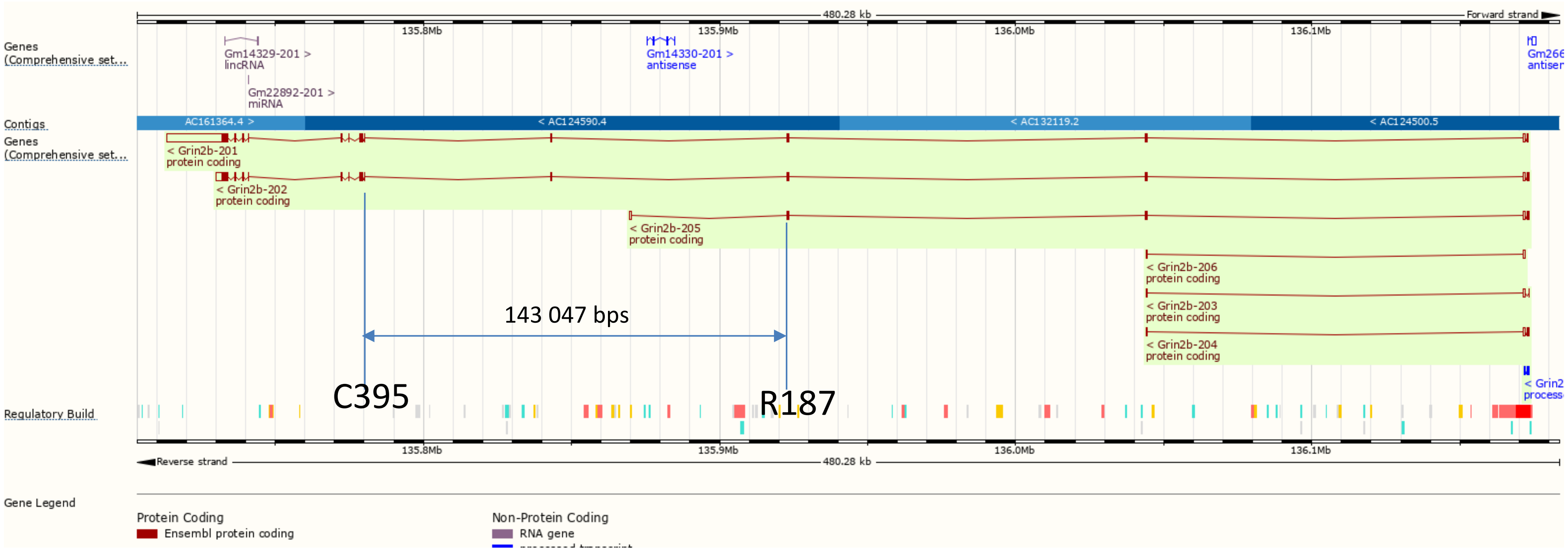
Validated Approach

Grin2b Mouse Genomic locus

Location: Chromosome 6: 135,713,233-136,173,511



Gene: Grin2b ENSMUSG00000030209



The distance between R187 and C395 is 143 047 bps in the mouse genome. It is not possible to knock-in both mutation at the same time.

Grin2b mRNAs and proteins

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grin2b-201	ENSMUST00000053880.12	24060	1482aa	Protein coding	CCDS20648	G3X9V4	TSL:5GENCODE basicAPPRIS P1
Grin2b-202	ENSMUST00000111905.7	7484	1482aa	Protein coding	CCDS20648	G3X9V4	TSL:5GENCODE basicAPPRIS P1
Grin2b-205	ENSMUST00000152012.7	2461	337aa	Protein coding	-	A0A0G2JEA7	TSL:1GENCODE basic
Grin2b-204	ENSMUST00000143943.7	703	35aa	Protein coding	-	Q8CG69	CDS 3' incompleteTSL:1
Grin2b-203	ENSMUST00000125905.1	630	35aa	Protein coding	-	Q8CG69	CDS 3' incompleteTSL:1
Grin2b-206	ENSMUST00000188999.2	601	36aa	Protein coding	-	A0A087WR33	CDS 3' incompleteTSL:1
Grin2b-207	ENSMUST00000198283.1	241	No protein	Processed transcript	-	-	TSL:5

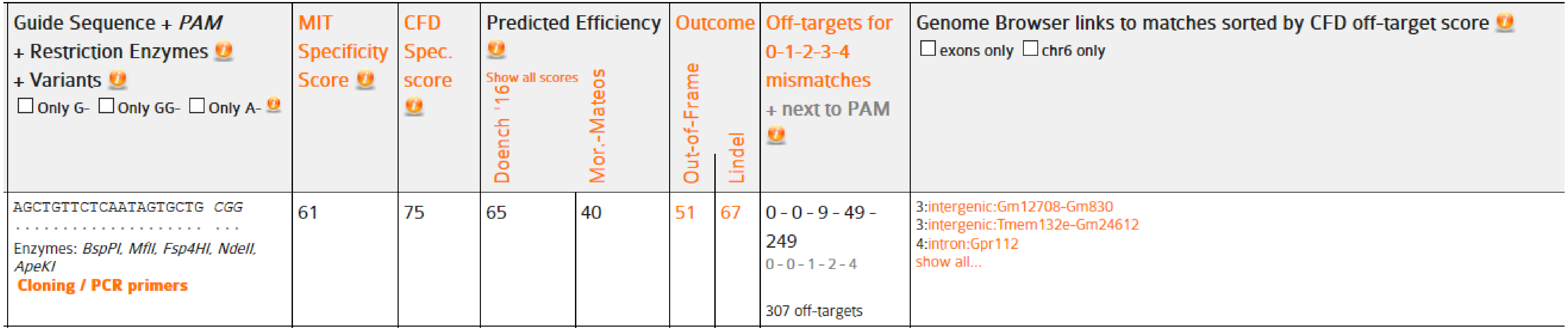
Grin2b-201 ENSMUST00000053880.12

The distance between R187 and C395 is 624 nts in the mRNA

Strategy for the introduction of R187C:

Selection of the best sgRNA

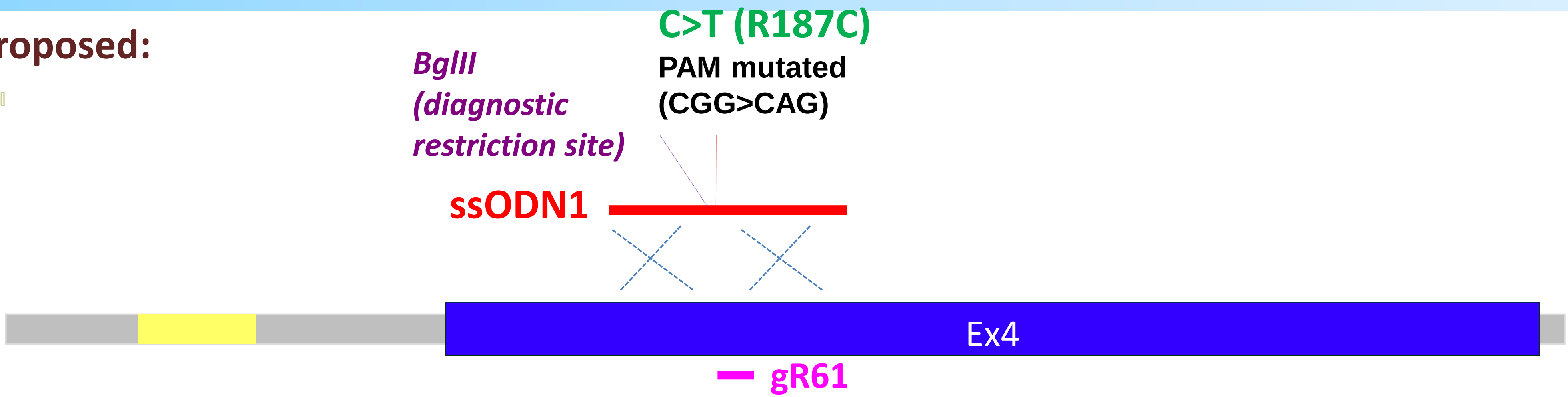
http://crispor.tefor.net/crispor.py?batchId=tFl8Ef7PKveL4oEDtHr8



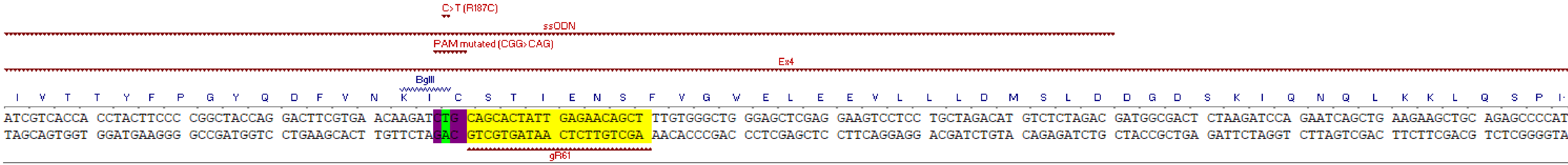
Guide Sequence + *PAM* + Restriction Enzymes + Variants ☐ Only G- ☐ Only GG- ☐ Only A-	MIT Specificity Score	CFD Spec. score	Predicted Efficiency Show all scores		Outcome		Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score ☐ exons only ☐ chr6 only
			Doench '16	Mor.-Mateos	Out-of-Frame	Lindel		
AGCTGTTCTCAATAGTGCTG *CGG* .... Enzymes: *BspPI, MflI, Fsp4HI, NdeII, ApeKI* **Cloning / PCR primers**	61	75	65	40	51	67	0 - 0 - 9 - 49 - 249 0 - 0 - 1 - 2 - 4 307 off-targets	3:intergenic:Gm12708-Gm830 3:intergenic:Tmem132e-Gm24612 4:intron:Gpr112 show all...

Strategy for the introduction of R187C

Approach proposed:



Sequence of the allele after HDR



```
 I   V   T   T   Y  F   P   G   Y   Q   D  F   V   N   K   I   C   S   T   I   E   N   S   F   V   G   W   E   L   E   E   V   L   L   L   D   M   S   L   D   D   G   D   S   K   I   Q   N   Q   L   K   K   L   Q   S   P  I
ATCGTCACCA CCTACTTCCC CGGCTACCAG GACTTCGTGA ACAAGATCTG CAGCACTATT GAGAACAGCT TTGTGGGCTG GGAGCTCGAG GAAGTCCTCC TGCTAGACAT GTCTCTAGAC GATGGCGACT CTAAGATCCA GAATCAGCTG AAGAAGCTGC AGAGCCCCAT
TAGCAGTGGT GGATGAAGGG GCCGATGGTC CTGAAGCACT TGTTCTAGAC GTCGTGATAA CTCTTGTCGA AACACCCGAC CCTCGAGCTC CTTCAGGAGG ACGATCTGTA CAGAGATCTG CTACCGCTGA GATTCTAGGT CTTAGTCGAC TTCTTCGACG TCTCGGGGTA
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R187C mutation as asked

A new diagnostic restriction site is create after HDR (BglII), this site will ease genotyping

PAM sequence (NGG) is mutated into an NAG which could lead to a new double strand break(DSB) (see Zhang et al Sci Rep. 2014 Jun 23;4:5405. doi: 10.1038/srep05405).

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Genotyping protocol

Line Kus7403-11-PM

F1 genotype

Sequence with PCR F1-R1

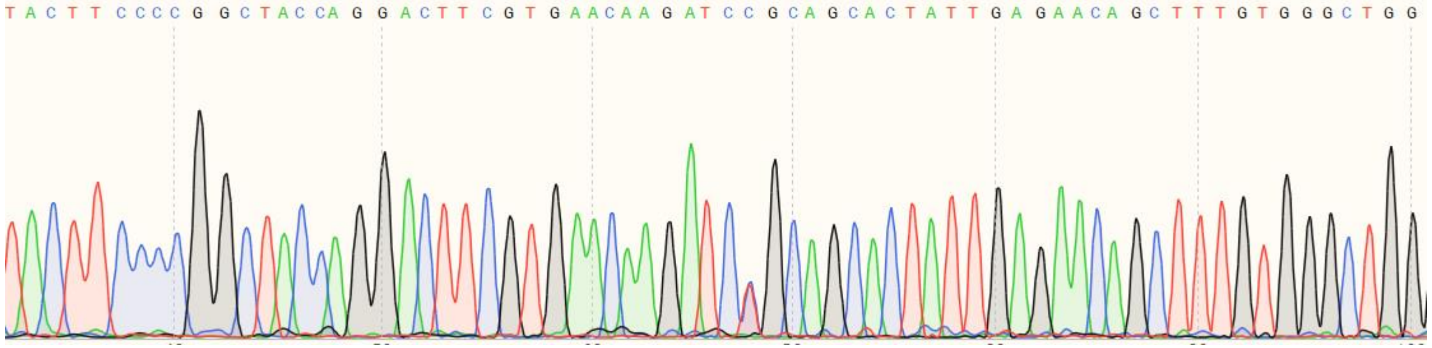
Oligo F1 and R1 in orange

The expected mutation is in green

The BglII restriction site is in italic

TCTGTCATGCTCAACATCATGGAAGAATACGACTGGTACATCTTCTCCATCGTCACCACCTACTTCCCCGGCTACCAGGACTTCGTGAACA*AGATCT*GCAGC
ACTATTGAGAACAGCTTTGTGGGCTGGGAGCTCGAGGAAGTCCTCCTGCTAGACATGTCTCTAGACGATGGCGACTCTAAGATCCAGAATCAGCTGAAGA
AGCTGCAGAGCCCCATCATTCTCCTCTACTGCACAAAGGAAGAAGCCACCTACATCTTCGAAGTAGCTAACTCAGTTGGGCTGACTGGCTACGGCTACACA
TGGATCGTGCCGAGTCTGGTGGCGGGGGATACGGACACGGTGCCTTCAGAGTTCCCCACGGGGCTCATCTCTGTGTCATATGACGAATGGGACTATGGCC
TTCCTGCCAGAGTGAGAGATGGGATTGCCATC

Sanger sequencing



```
TACTTCCCCGGCTACCAGGACTTCGTGAACAAGATCTGCAGCACTATTGAGAACAGCTTTGTGGGCTGG
  Y  F  P  G  Y  Q  D  F  V  N  K  I  C  S  T  I  E  N  S  F  V  G  W
```

The line Kus7403-11-PM is now established.

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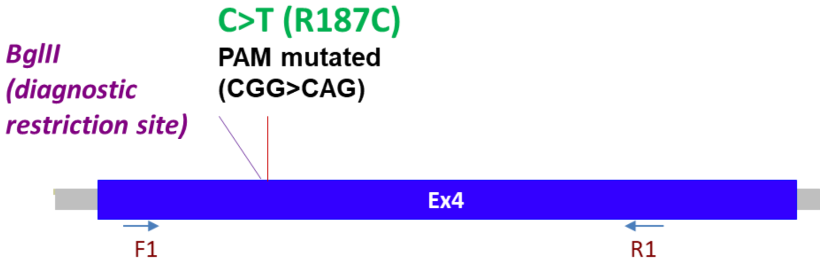
Genotyping protocol

GENOTYPING INSTRUCTIONS



BglII (diagnostic restriction site)

C>T (R187C)

PAM mutated (CGG>CAG)

Ex4

F1

R1

PCR genotyping primers and sequence

Primer ref.	Sequence	Amplification product size WT	Sizes observed if BglII restriction present (associated with asked PM)
F1	TCTGTCATGCTCAACATCATGGAAG	435 bps	94 + 341 bps
R1	GATGGCAATCCCATCTCTCACTCTG		

Sequence with PCR F1-R1

Oligo F1 and R1 in orange

The expected mutation is in green

The BglII restriction site is in italic

TCTGTCATGCTCAACATCATGGAAGAATACGACTGGTACATCTTCTCCATCGTCACCACCTACTTCCCCGGCTACCAGGACTTCGTGAACA*AGATC**T***GCAGC
ACTATTGAGAACAGCTTTGTGGGCTGGGAGCTCGAGGAAGTCCTCCTGCTAGACATGTCTCTAGACGATGGCGACTCTAAGATCCAGAATCAGCTGAAGA
AGCTGCAGAGCCCCATCATTCTCCTCTACTGCACAAAGGAAGAAGCCACCTACATCTTCGAAGTAGCTAACTCAGTTGGGCTGACTGGCTACGGCTACACA
TGGATCGTGCCGAGTCTGGTGGCGGGGGATACGGACACGGTGCCTTCAGAGTTCCCCACGGGGCTCATCTCTGTGTCATATGACGAATGGGACTATGGCC
TTCCTGCCAGAGTGAGAGATGGGATTGCCATC

GENOTYPING INSTRUCTIONS

PCR Protocol

This section describes the composition of the mix and the cycling conditions used for genotyping F0 and F1 genotyping.

Reagents:	Volume (per sample):
Phusion HS (Thermo Scientific) 5X Buffer	4 µl
10mM dNTP	0.4 µl
5’ primer (100 µM)	0.1 µl
3’ primer (100 µM)	0.1 µl
DNA (lysate 1/10)	2 µl
Phusion Hot Start II	0.2 µl
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