phenomin
EXCELLENCE IN MOUSE PHENOGENOMICS

INVESTISSEMENTS D'AVENIR

R105Q KI in Orc1 by CRISPR-Cas9 approach and genotyping protocol

Kus6784 / IM6784

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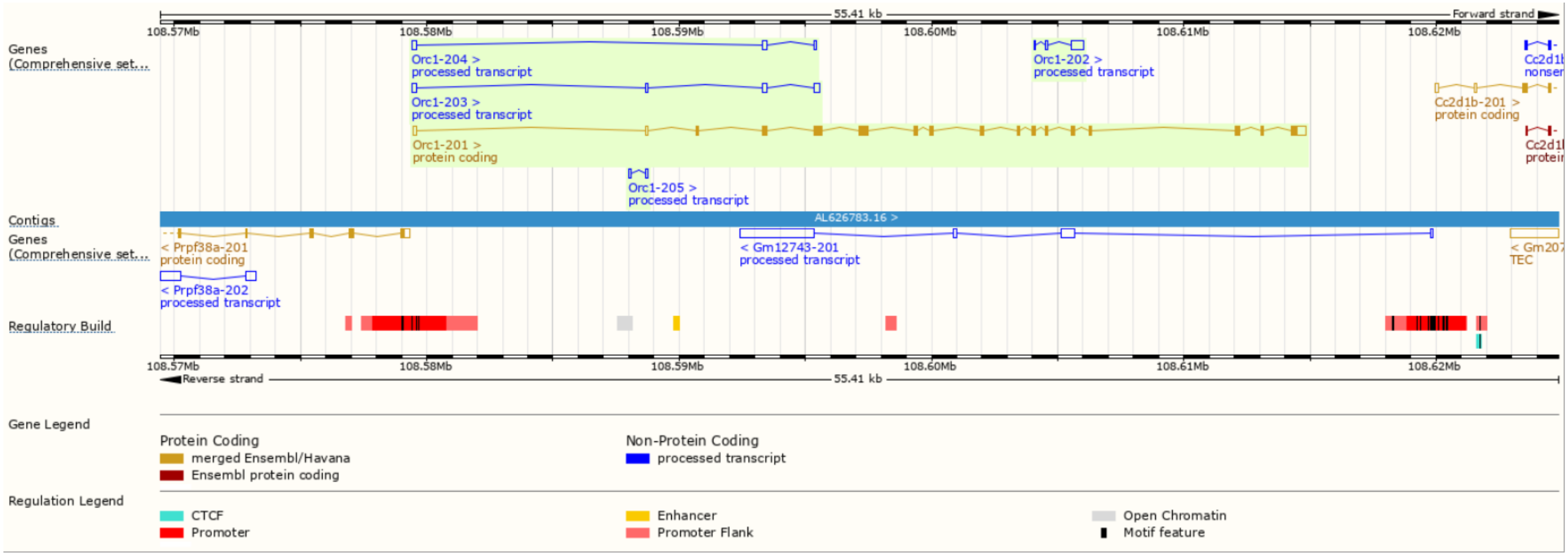
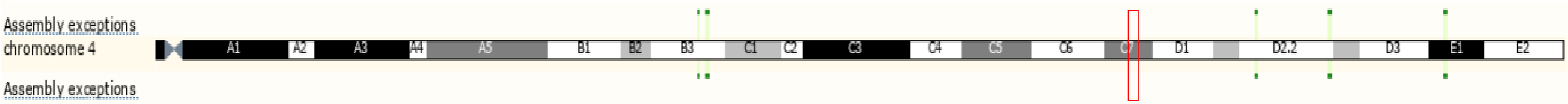
Orc1 Mouse Genomic locus

Gene: Orc1 ENSMUSG00000028587



Chromosome 4: 108,579,423-108,614,833



Orc1 mRNAs and proteins

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	RefSeq
Orc1-201	ENSMUST00000102744.3	3014	840aa	Protein coding	CCDS18453	Q9Z1N2	NM_011015 NP_035145
Orc1-202	ENSMUST00000129931.1	670	No protein	Processed transcript	-	-	-
Orc1-203	ENSMUST00000130162.1	679	No protein	Processed transcript	-	-	-
Orc1-204	ENSMUST00000139772.7	443	No protein	Processed transcript	-	-	-
Orc1-205	ENSMUST00000143497.1	173	No protein	Processed transcript	-	-	-

ORC1 human

```
         Y*     K YR    *         SY B           Y    YSV *Y  R
421 TTGCTTGAGTTGTTCGAAGATGACTCTGATCCTCCTCCTAAGAAACGTGCTCGAGTACAG    480
202 TTGCTTGAGTTGTTCGAAGATGACTCTGATCCTCCTCCTAAGAAACGTGCTCGAGTACAG    261
 68 -L--L--E--L--F--E--D--D--S--D--P--P--P--K--K--R--A--R--V--Q-    87

        Y    YR                K        HRR     RYY  K MR      Y
481 TGGTTTGTCCGATTCTGTGAAGTCCCTGCCTGTAAACGGCATTTGTTGGGCCGGAAGCCT    540
262 TGGTTTGTCCGATTCTGTGAAGTCCCTGCCTGTAAACGGCATTTGTTGGGCCGGAAGCCT    321
 88 -W--F--V--R--F--C--E--V--P--A--C--K--R--H--L--L--G--R--K--P-    107

    *  R        R   Y  SR   KR    SYR          *           YR RK
541 GGTGCACAGGAAATATTCTGGTATGATTACCCGGCCTGTGACAGCAACATTAATGCGGAG    600
322 GGTGCACAGGAAATATTCTGGTATGATTACCCGGCCTGTGACAGCAACATTAATGCGGAG    381
108 -G--A--Q--E--I--F--W--Y--D--Y--P--A--C--D--S--N--I--N--A--E-    127
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Human ORC1 (Query) vs Mouse Orc1 (Subject)

```
Query  1    MAHYPTRLKTRKTYSWVGRPLLDRKLHYQTYREMCVKTEGCSTEIHIQIGQFVLIEGDDD  60
            M  Y TR KTR+T+SWVGRPL +RK   Q YRE+C+K    S EIHI++GQFVLI+G+D+
Sbjct  1    MPSYLTRQKTRQTFSWVGRPLPNRKQFQQMYREICMKINDGS-EIHIKVGQFVLIQGEDN  59

Query  61   ENPYVAKLLELFEDDSDPPPKKRARVQWFVRFCEVPACKRHLLGRKPGAQEIFWYDYPAC  120
            + PYVAKL+ELF++ ++ PPKK ARVQWFVRF E+P  KRHLLGR P AQEIFWYD
Sbjct  60   KKPYVAKLIELFQNGAEVPPKKCARVQWFVRFLEIPVSKRHLLGRSPPAQEIFWYDCSDW  119

Query  121  DSNINAETIIGLVRVIPLAPKDVVPTNLKNEKTLFVKLSWNEKKFRPLSSELFAELNKPQ  180
            D+ IN ETIIG V+V+ LAP++V+P + K+E+TLFVKLSWN+K F PL  E+ A L + Q
Sbjct  120  DNKINVETIIGPVQVVALAPEEVIPVDQKSEETLFVKLSWNKKDFAPLPPEVLAAL-REQ  178

Query  181  ESAAKCQKPVRAKSKSAESPSWTPAEHVAKRIESRHSASKSRQTPTHPLTPRARKRLELG  240
            E + + QKP++AK K+ +SP+    E   K I+S HS SK  QTP + + P A+K LEL
Sbjct  179  EDSPEWQKPLKAKIKNVKSPARNTTEQEVKGIKSNHSTSKFHQTPANIVIPNAKKSLELD  238

Query  241  NLG-----NPQMSQQTSCASLDSPGRIKRKVAFSEITSPSKRSQPDKLQTLSP--ALKAP  293
             LG     N + S+++SC SLD     KR+ AFSE TSP K+  P+K + + P  AL+
Sbjct  239  GLGFTRKPNTRWSKKSSCDSLDYQKTSKRRAAFSETTSPPKK--PNKPREIKPSSALETR  296

Query  294  EKTRETGLSYTEDDKKASPEHRIILRTRIAASKTIDIREERTLTPISGGQRSSVVPSVIL  353
             K  +T        +    +  ++LR R  A  T  +  + TL+PI  G RSSVVPS  L
Sbjct  297  VKNGQT--------QPFCAKSSVVLRARNPAMTTTKLGVDNTLSPIRNGLRSSVVPSGGL  348

Query  354  KPENIKKRDAKEAKAQNEATSTPHRIRRKSSVLTMNRIRQQLRFLGNSKSDQEEKEILPA  413
             P  I+ R AKE +   E   T  R+ RKSS+LT+ RIRQQL  L     DQE
Sbjct  349  TPVYIR-RKAKEQETHKEPIRT-SRVHRKSSLLTLKRIRQQLCLLDGDDRDQE------E  400

Query  414  AEISDSSSDEEEASTPPLPRRAPRTVSRNLRSSLKSSLHTLTKVPKKSLKPRTPRCAAPQ  473
             E  DS S+EE+     LP R     SR        +  T +K P+K+ KPRTP  A PQ
Sbjct  401  EESVDSESEEEDEFISSLPTRNSLGQSR--------TRQTPSKSPQKNPKPRTPHRATPQ  452

Query  474  IRSRSLAAQEPASVLEEARLRLHVSAVPESLPCREQEFQDIYNFVESKLLDHTGGCMYIS  533
            IR R+LA QEPAS LEEARLRLHVSAVP+SLPCREQEFQDIY+FVESKLLD TGGCMYIS
Sbjct  453  IRDRNLAVQEPASALEEARLRLHVSAVPDSLPCREQEFQDIYSFVESKLLDGTGGCMYIS  512

Query  534  GVPGTGKTATVHEVIRCLQQAAQANDVPPFQYIEVNGMKLTEPHQVYVQILQKLTGQKAT  593
            GVPGTGKTATVHEVIRCLQQAA+ +DVPPFQY+EVNGMKLTEPHQVYVQIL+KLTGQKAT
Sbjct  513  GVPGTGKTATVHEVIRCLQQAAETDDVPPFQYVEVNGMKLTEPHQVYVQILKKLTGQKAT  572

Query  594  ANHAAELLAKQFCTRGSPQETTVLLVDELDLLWTHKQDIMYNLFDWPTHKEARLVVLAIA  653
            ANHAAELLAKQFC +GS +ETTVLLVDELDLLWTHKQD+MYNLFDWPTHK A L+VL IA
Sbjct  573  ANHAAELLAKQFCGQGSQKETTVLLVDELDLLWTHKQDVMYNLFDWPTHKGAHLIVLTIA  632

Query  654  NTMDLPERIMMNRVSSRLGLTRMCFQPYTYSQLQQILRSRLKHLKAFEDDAIQLVARKVA  713
            NTMDLPERIMMNRVSSRLGLTRM FQPY++SQL+QIL SRL++L+AFEDDAIQLVARKVA
Sbjct  633  NTMDLPERIMMNRVSSRLGLTRMSFQPYSHSQLKQILVSRLRNLRAFEDDAIQLVARKVA  692

Query  714  ALSGDARRCLDICRRATEICEFSQQKPDSPGLVTIAHSMEAVDEMFSSSYITAIKNSSVL  773
            ALSGDARRCLDICRRATEICE S  + DS  LVT+AH MEA+DEMFSSSYITAIKNSSV+
Sbjct  693  ALSGDARRCLDICRRATEICELSHLRGDSLSLVTVAHLMEAIDEMFSSSYITAIKNSSVV  752

Query  774  EQSFLRAILAEFRRSGLEEATFQQIYSQHVALCRMEGLPYPTMSETMAVCSHLGSCRLLL  833
            EQSFLRAI+AEFRRSGLEEATFQQIYSQHVALCRMEGLPYPTMSETMAVCS LGSCRLLL
Sbjct  753  EQSFLRAIIAEFRRSGLEEATFQQIYSQHVALCRMEGLPYPTMSETMAVCSRLGSCRLLL  812
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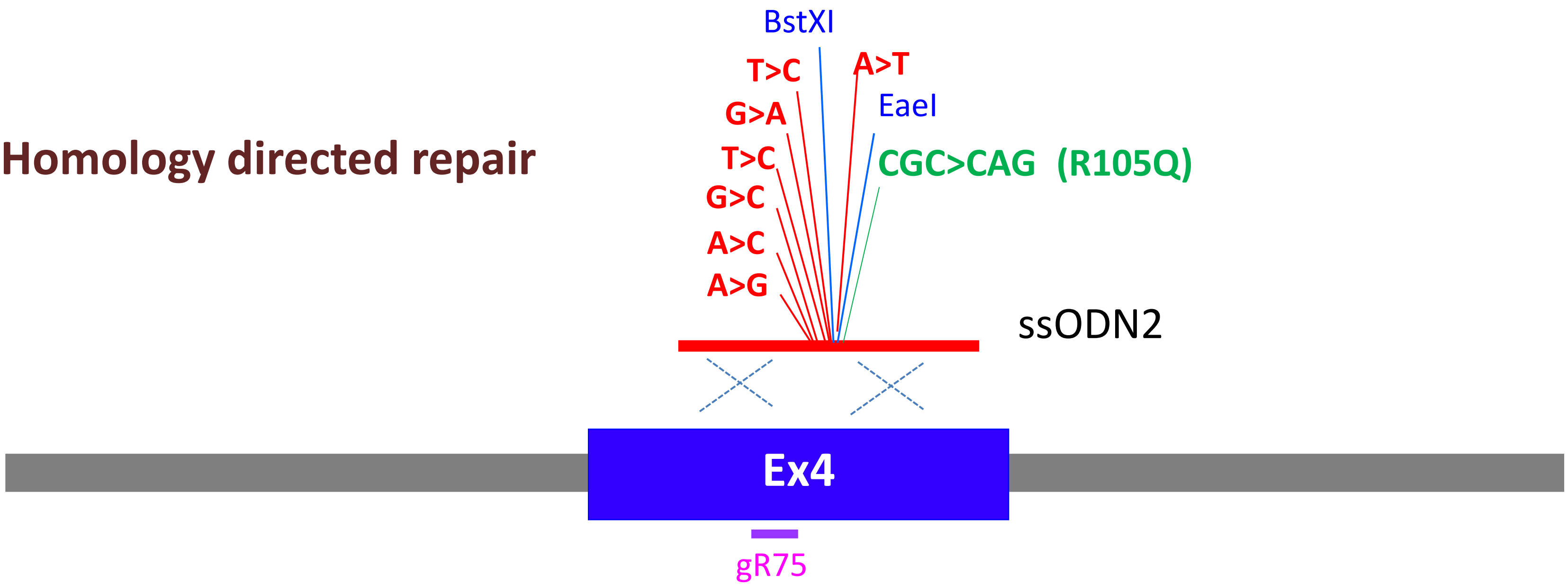
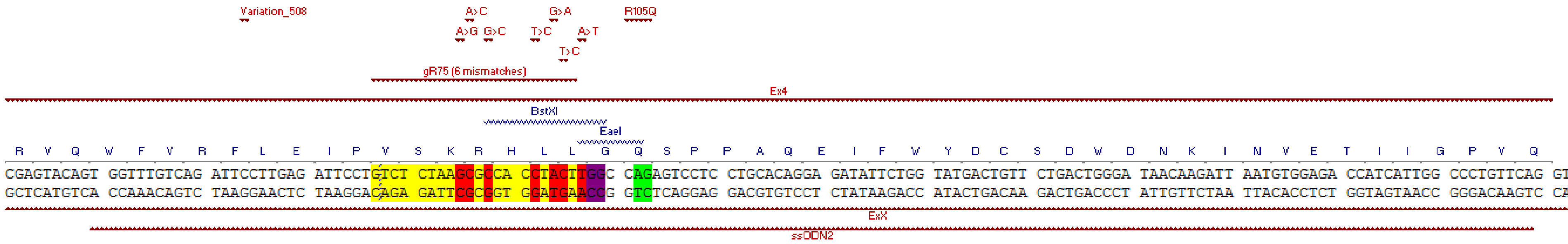
Proposal: introduction of the R105Q PM in Orc1

Homology directed repair



Expected allele



```
 R  V  Q  W  F  V  R  F  L  E  I  P  V  S  K  R  H  L  L  G  Q  S  P  P  A  Q  E  I  F  W  Y  D  C  S  D  W  D  N  K  I  N  V  E  T  I  I  G  P  V  Q
CGAGTACAGT GGTTTGTCAG ATTCCTTGAG ATTCCTGTCT CTAAGCGCCA CCTACTTGGC CAGAGTCCTC CTGCACAGGA GATATTCTGG TATGACTGTT CTGACTGGGA TAACAAGATT AATGTGGAGA CCATCATTGG CCCTGTTCAG GT
GCTCATGTCA CCAAACAGTC TAAGGAACTC TAAGGACAGA GATTCGCGGT GGATGAACCG GTCTCAGGAG GACGTGTCCT CTATAAGACC ATACTGACAA GACTGACCCT ATTGTTCTAA TTACACCTCT GGTAGTAACC GGGACAAGTC CA
```

New diagnostic restriction sites : BstXI, EaeI (best choice)

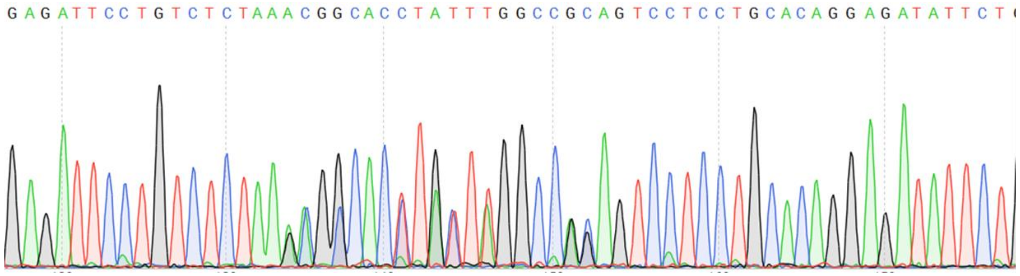
Line Kus6784-38-PM

F1 genotype

Sequence with PCR F1-R1
Oligo F and R in orange
Silent mutations
Asked PMs in green
Diagnostic restriction site in italic

GTTGTGTAGTGTTTGACACGTAGGCAATATTCAGTGCTGTATTGTTTGCCTTACTATTTAATCTTCCCGCCCTCCCCAATCCTCCTAG
GAGCTGAAGTTCCTCCCAAGAAATGTGCACGAGTACAGTGGTTTGTCAGATTCCTTGAGATTCCTGTCTCTAAGCGCCACCTACTT
G*GCCAG*AGTCCTCCTGCACAGGAGATATTCTGGTATGACTGTTCTGACTGGGATAACAAGATTAATGTGGAGACCATCATTGGCCC
TGTTCAGGTTCGTGCCTTAAATCCTGCTTCTATCTTTAGCACCCACACTGCATGGCTTCAGAAATATTACTAGTTCTAATTTGAATTC
TGTTCTAATTATCTGTTTGGCTGCTTGCCCCAGCACACAGATACTGACAGCATAGAACATAATGCTCTGGGCT

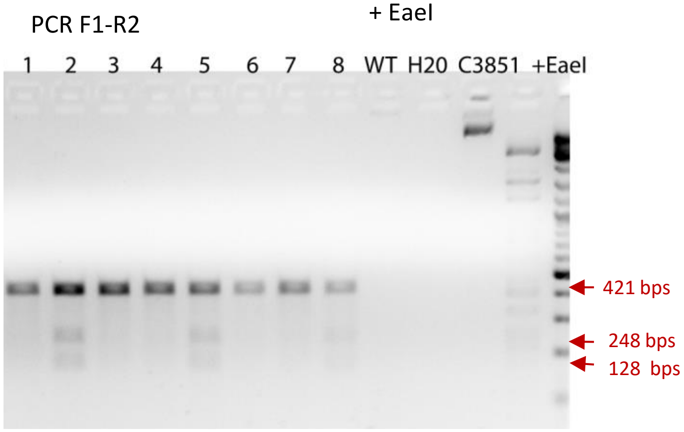
Sanger sequencing



GAGATTCCTGTCTCTAAGCGCCACCTACTTGGCCAGAGTCCTCCTGCACAGGAGATATTC
E I P V S K R H L L G Q S P P A Q E I F

FO	Nb F1 born	F1 heterozygote	
		M	F
Kus7015-25	46	6	6

F1 genotyping



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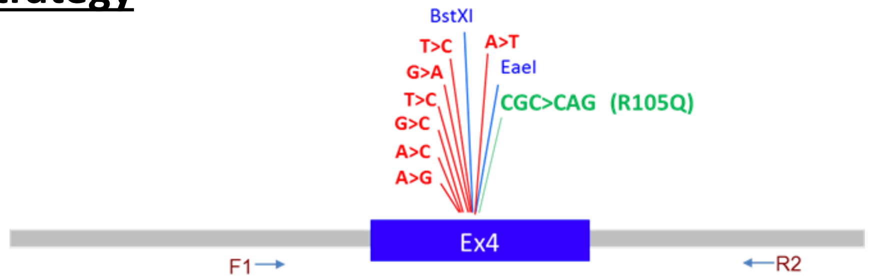
GENOTYPING INSTRUCTIONS

PCR genotyping strategy



Primer ref.	Sequence	Amplification product size WT	Expected size if BstUI diagnostic restriction is present
F1	GTTGTGTAGTGTTTGACACGTAGGC	421 bps	248 bps + 173 bps
R2	AGCCCAGAGCATTATGTTCTATGCT		

Sequence of the PCR product (with mutations introduced)

GTTGTGTAGTGTTTGACACGTAGGCAATATTCAGTGCTGTATTGTTTGCCTTACTATTTAATCTTCCCGCCCTCCCCAATCCTCCT
AGGAGCTGAAGTTCCTCCCAAGAAATGTGCACGAGTACAGTGGTTTGTCAGATTCCTTGAGATTCCTGTCTCTAAGCGCCAC
CTACTTG*GCCAG*AGTCCTCCTGCACAGGAGATATTCTGGTATGACTGTTCTGACTGGGATAACAAGATTAATGTGGAGACCAT
CATTGGCCCTGTTCAGGTTCGTGCCTTAAATCCTGCTTCTATCTTTAGCACCCACACTGCATGGCTTCAGAAATATTACTAGTTC
TAATTTGAATTCTGTTCTAATTATCTGTTTGGCTGCTTGCCCCAGCACACAGATACTGACAGCATAGAACATAATGCTCTGGGCT

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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 EaeI	0,2 µl
H_20	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.