

**Name of Mouse model or mutation:**

PLA2G6-R636Q-EM1-B6N

PLA2G6-R636Q-EM2-B6N

PLA2G6-R636Q-EM3-B6N

**Description:**

Point mutation model made using CRISPR/Cas9.

**Type of mutation:**

SNP: R636Q

**Sequence details****WT**

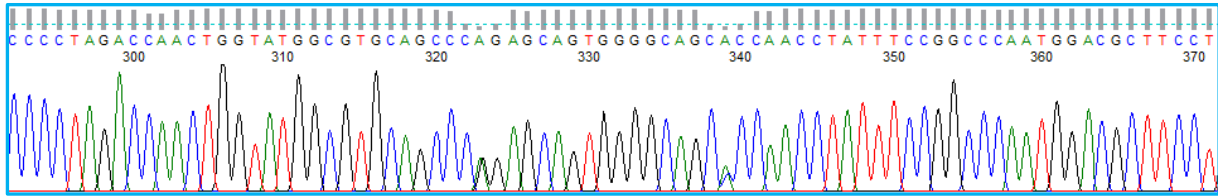
AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTCAGAGCTGAGCGCGTGTGCTGGGGGGGA  
GGGCTGGAGGAAGTTGTGGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCAT  
GTCAGTCCCCTGGCCTTAGGTGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCAC  
CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCCTTCTCCTGTGCTC  
TCCTACCCAGCCTGAGATCTGAAGGGATAGGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCAA  
CTGGTATGGCGTGCAGCCCGAGCAGTGGGGCAGCCCCAACCTATTTCCGGCCCAATGGACGCTTC  
CTGGATGGAGGGCTGCTGGCCAACAACCCACACTGGATGCCATGACTGAAATCCATGAGTACAAT  
CAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGA  
TGAGAACAGTTTTGGTGTGTGCCTTACCTATTCAACACTCCTGGAGGGTCTGATGGGCAGAGTCCG  
TGCTAGGAGAATTTGAGGCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGG  
CTGCTGTTAGTACAGGCTGTGTACCTGATGCTACCCAGCCGGGACACACCCTTGCCTGCAGAGCTT  
ACGTTCTACTTGAAGACTCTATACACAACAGTCTGGGCATGTCAGGTCTGAGAGCGCACCCCTG  
CGGAGGCAGGAGGGGGAGAGTCTGTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTG  
GAACTATCCTGAGGCCTCTGGGTGCAAGACAGTGCAGAGGCAGGGAGTCAAGGACAGGCAGTGGC  
CCTCACCCAAGTCGTCAGAGCAGGGTGACAAGGCCACGTGCCAAGGGAGGTGATCTGACCCTGCC  
TCAGCTTCCAATCTTCAGCTAGTGACAGTGCTTGTCTTGATGCCCTGGGACTCAGGGGGCTGTTG  
CCTGTGCCATACTTGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCCAGTCCAGTCATGGGG  
AACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT

**Mutant**

AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTCAGAGCTGAGCGCGTGTGCTGGGGGGGA  
GGGCTGGAGGAAGTTGTGGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCAT  
GTCAGTCCCCTGGCCTTAGGTGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCAC  
CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCCTTCTCCTGTGCTC  
TCCTACCCAGCCTGAGATCTGAAGGGATAGGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCAA  
CTGGTATGGCGTGCAGCCCAGAGCAGTGGGGCAGCACCAACCTATTTCCGGCCCAATGGACGCTTC

CTGGATGGAGGGCTGCTGGCCAACAACCCACACTGGATGCCATGACTGAAATCCATGAGTACAAT  
CAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGA  
TGAGAACAGTTTTGGTGTGTGCCTTACCTATTCAACACTCCTGGAGGGTCTGATGGGCAGAGTCCG  
TGCTAGGAGAATTTTGAGGCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGG  
CTGCTGTTAGTACAGGCTGTGTACCTGATGCTACCCAGCCGGGACACACCCTTGCCCTGCAGAGCTT  
ACGTTCTACTTGAAGACTCTATACACAACAGTCCTGGGCATGTCAGGTCTGAGAGCGCACCCCTG  
CGGAGGCAGGAGGGGGAGAGTCTGTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTG  
GAACTATCCTGAGGCCTCTGGGTGCAAGACAGTGCAGAGGCAGGGAGTCAAGGACAGGCAGTGGC  
CCTCACCCAAGTCGTCAGAGCAGGGTGACAAGGCCACGTGCCAAGGGAGGTGATCTGACCCTGCC  
TCAGCTTCCAATCTTCAGCTAGTGACAGTGCTTGTCTTGATGCCCTGGGGACTCAGGGGGCTGTTG  
CCTGTGCCATACTTGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCCAGTCCAGTCATGGGG  
AACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT

**PLA2G6-R636Q-EM1-B6N Heterozygous F1 animal sequence trace:**



## Nucleotide Alignment:

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                *           20           *           40           *           60           *           80           *           100          *           120          *           140          *
Pla2g6_WT      : AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTGACAGCTGAGCGCTGTGCTGGGGGGAGGGCTGGAGGAAGTTGTGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCATGTCAGTGCCTGGCCTTAGG
Pla2g6_R636Q  : AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTGACAGCTGAGCGCTGTGCTGGGGGGAGGGCTGGAGGAAGTTGTGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCATGTCAGTGCCTGGCCTTAGG
                AAACATTAACCTGAAGCCACCGACTCAGCCTGCAGGTGACAGCTGAGCGCTGTGCTGGGGGGAGGGCTGGAGGAAGTTGTGGGGATGGAGAGTCACAGACAAGGCACAGGATGCCCTCCCCGCCATGTCAGTGCCTGGCCTTAGG

                160          *           180          *           200          *           220          *           240          *           260          *           280          *           300
Pla2g6_WT      : TGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCACCTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCTGACTCCCTTCTTCTGTGCTCTCCACCCAGCCTGAGATCTGAAGGGATAGGCCTT
Pla2g6_R636Q  : TGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCACCTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCTGACTCCCTTCTTCTGTGCTCTCCACCCAGCCTGAGATCTGAAGGGATAGGCCTT
                TGGCTAAGGACAGGTGTCATTGAAGCCTGTCCACAGTGACACACCACCTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCTGACTCCCTTCTTCTGTGCTCTCCACCCAGCCTGAGATCTGAAGGGATAGGCCTT

                *           320          *           340          *           360          *           380          *           400          *           420          *           440          *
Pla2g6_WT      : ATGTCATTGTCCCCTTCCCCACCCCTAGACCAACTGGTATGGCGTGCAGCCCAGCAGTGGGGCAGCCCAACCTATTTCCGGCCCAATGGACGCTTCCTGGATGGAGGGCTGTGGCCAACAACCCACACTGGATGCCATGACTGAA
Pla2g6_R636Q  : ATGTCATTGTCCCCTTCCCCACCCCTAGACCAACTGGTATGGCGTGCAGCCCAGCAGTGGGGCAGCCCAACCTATTTCCGGCCCAATGGACGCTTCCTGGATGGAGGGCTGTGGCCAACAACCCACACTGGATGCCATGACTGAA
                ATGTCATTGTCCCCTTCCCCACCCCTAGACCAACTGGTATGGCGTGCAGCCCAGCAGTGGGGCAGCCCAACCTATTTCCGGCCCAATGGACGCTTCCTGGATGGAGGGCTGTGGCCAACAACCCACACTGGATGCCATGACTGAA

                460          *           480          *           500          *           520          *           540          *           560          *           580          *           600
Pla2g6_WT      : ATCCATGAGTACAATCAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGATGAGAACAGTTTTGGTGTGTGCCTTACCTATTCAACTCCTGGAGGGTCTGATGGGCAGAGTCCGTG
Pla2g6_R636Q  : ATCCATGAGTACAATCAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGATGAGAACAGTTTTGGTGTGTGCCTTACCTATTCAACTCCTGGAGGGTCTGATGGGCAGAGTCCGTG
                ATCCATGAGTACAATCAGGACATGATCCGCAAGGTGAGAGCCTTCTCAGGTCATGGCCTGGACATGACCACACACTGCAGATGAGAACAGTTTTGGTGTGTGCCTTACCTATTCAACTCCTGGAGGGTCTGATGGGCAGAGTCCGTG

                *           620          *           640          *           660          *           680          *           700          *           720          *           740          *
Pla2g6_WT      : CTAGGAGAATTTTGAAGCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGGCTGCTGTAGTACAGGCTGTGATACCTACCCAGCCGGACACACCCTTGCCCTGCAGAGCTTACGTTCCACTTGAAGACT
Pla2g6_R636Q  : CTAGGAGAATTTTGAAGCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGGCTGCTGTAGTACAGGCTGTGATACCTACCCAGCCGGACACACCCTTGCCCTGCAGAGCTTACGTTCCACTTGAAGACT
                CTAGGAGAATTTTGAAGCCCATGCATGCTGAGTACAGAGAAGTCTGAGATGAGTGGCCAGCGGCTGCTGTAGTACAGGCTGTGATACCTACCCAGCCGGACACACCCTTGCCCTGCAGAGCTTACGTTCCACTTGAAGACT

                760          *           780          *           800          *           820          *           840          *           860          *           880          *           900
Pla2g6_WT      : CTATACACACAACAGTCTGGGCATGTCAGGTCTGAGAGCGCACCCCTGCGGAGGCAGGAGGGGAGAGTCTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTGGAACTATCCTGAGGCCCTCTGGGTGCAAGACAGTGCAGA
Pla2g6_R636Q  : CTATACACACAACAGTCTGGGCATGTCAGGTCTGAGAGCGCACCCCTGCGGAGGCAGGAGGGGAGAGTCTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTGGAACTATCCTGAGGCCCTCTGGGTGCAAGACAGTGCAGA
                CTATACACACAACAGTCTGGGCATGTCAGGTCTGAGAGCGCACCCCTGCGGAGGCAGGAGGGGAGAGTCTGTGAAAGGGTTGAGAACTGGTGAAGGGCTCCTGAGTTTGGAACTATCCTGAGGCCCTCTGGGTGCAAGACAGTGCAGA

                *           920          *           940          *           960          *           980          *           1000          *           1020          *           1040          *
Pla2g6_WT      : GGCAGGGAGTCAAGGACAGGCAGTGGCCCTACCCCAAGTCTGACAGCAGGGTGACAAGGCCACGTCGCAAGGGAGGTGATCTGACCTGCCTCAGCTTTCCAATCTTCAGCTAGTGACAGTGTCTTGATGCCCTGGGGACTCAG
Pla2g6_R636Q  : GGCAGGGAGTCAAGGACAGGCAGTGGCCCTACCCCAAGTCTGACAGCAGGGTGACAAGGCCACGTCGCAAGGGAGGTGATCTGACCTGCCTCAGCTTTCCAATCTTCAGCTAGTGACAGTGTCTTGATGCCCTGGGGACTCAG
                GGCAGGGAGTCAAGGACAGGCAGTGGCCCTACCCCAAGTCTGACAGCAGGGTGACAAGGCCACGTCGCAAGGGAGGTGATCTGACCTGCCTCAGCTTTCCAATCTTCAGCTAGTGACAGTGTCTTGATGCCCTGGGGACTCAG

                1060          *           1080          *           1100          *           1120          *           1140          *           1160          *           1180          *
Pla2g6_WT      : GGGGCTGTGCTTGGCATACTTGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCAGTCCAGTCATGGGGAACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT
Pla2g6_R636Q  : GGGGCTGTGCTTGGCATACTTGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCAGTCCAGTCATGGGGAACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT
                GGGGCTGTGCTTGGCATACTTGAAGGCATCTGTCTAGGTGTGCATTGCTGGCTTCCCAGTCCAGTCATGGGGAACCGAGATGCACCTTTGGTAGGTAGGGTGGGCATCCTGAAGGAGTTCTAGCAGCCTCAGACAT
    
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## Predicted Protein Alignment:

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                *           20           *           40           *
Pla2g6_WT      : QLVWRAARSSGAAPTYFRPNGRFLDGGLLANNPTLDAMTEIHEYNDMIRK
Pla2g6_R636Q  : QLVWRAARSSGAAPTYFRPNGRFLDGGLLANNPTLDAMTEIHEYNDMIRK
                QLVWRAARSSGAAPTYFRPNGRFLDGGLLANNPTLDAMTEIHEYNDMIRK
    
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### QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_Pla2g6_F4 primer (5'-3')	AAACATTAACCTGAAGCCACCG
Geno_Pla2g6_R4 primer (5'-3')	ATGTCTGAGGCTGCTAGAACTC
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1191
Mutant product size (bp)	1191
Notes	Sequence with primers: Geno_Pla2g6_F2 primer (5'-3': GGAAGTTGTGGGGGATGGAG) and Geno_Pla2g6_R1 primer (5'-3': GGAGCCCTTCACCAGTTCTC)

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

### Copy counting by ddPCR

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Pla2g6-R636Q-UNI1
Forward Primer (5'-3')	CACGCCATACCAGTTGGTCTA
Reverse Primer (5'-3')	TCCTACCCAGCCTGAGATC
Probe (5'-3')	TGAAGGGATAGGCCTTATGTCATTGTCC
Label	FAM-BHQ1

This ddPCR assay is universal to both the WT allele and the mutant allele of the gene. WT controls are expected to call at 2 copies and a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	Pla2g6-R636Q-MUT1
Forward Primer (5'-3')	AGCAGCCCTCCATCCAGGAAG
Reverse Primer (5'-3')	TGGCGTGCAGCCCA
Probe (5'-3')	CCAACCTATTTCCGGCCCAATGGA
Label	FAM-BHQ1

This ddPCR assay is unique to the mutant allele of the gene as it sits across the mutated region. WT controls are expected to call at 0 copies and a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCAGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.



## Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; R636Q in exon ENSMUSE00000261149 of *PLA2G6*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

## qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

An Allelic Discrimination assay is used to detect two possible variants of a Single Nucleotide Polymorphism (SNP). It is a multiplexed assay (with two primer/probe pairs) with data being collected at the completion of the PCR process. The relative level of fluorescence from each probe is used to determine the genotype of an animal.

Samples for this line are genotyped using the following primers and probe:

- Forward and reverse primers common to both Wild Type (WT) and mutant alleles
- WT probe binding to the WT base mutated in the mutant allele.
- Mutant probe binding to the SNP.



## PLA2G6-R636Q Allelic Discrimination assay

### PLA2G6-R636Q WT sequence

CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC  
TCCTACC**CAGCCTGAGATCTGAAGGGATA**GGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCA  
ACTGGTATGGCGTGC**AGCCCgGAGCAGTG**GGGCAGCcCCA**ACCTATTTCCGGCCCAATGG**ACGCTT  
CCTGGATGGAGGGCTGCTGGCCAACAACCCACACTGGATGCCATGACTGAAATCCATGAGTACAA

### PLA2G6-R636Q mutant sequence

CTTTCATCAGGATAGCAAGACCCTGCAGCAAGGCCTTGTGGGTCCTGACTCCCCTTCTTCCTGTGCTC  
TCCTACC**CAGCCTGAGATCTGAAGGGATA**GGCCTTATGTCATTGTCCCCTTCCCCACCCCTAGACCA  
ACTGGTATGGCGT**TGCAGCCCaGAGCA**GTGGGGCAGCaCCA**ACCTATTTCCGGCCCAATGG**ACGCTT  
CCTGGATGGAGGGCTGCTGGCCAACAACCCACACTGGATGCCATGACTGAAATCCATGAGTACAA

### SNP details:

WT=G

MUT=A

Lower case letters denote SNP position.

Probe sequence is in bold and shaded grey.

Primer sequences are in bold and underlined.

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
PLA2G6-R636Q_F	n/a	<b><u>CAGCCTGAGATCTGAAGGGATA</u></b>	n/a	Common forward primer
PLA2G6-R636Q_WT_PROBE	FAM	<b><u>CACTGCTCCGGGCT</u></b>	BHQ-plus	Wild type Probe
PLA2G6-R636Q_Mutant_PROBE	TET	<b><u>TGCTCTGGGCTGCA</u></b>	BHQ-plus	Mutant probe
PLA2G6-R636Q_R	n/a	<b><u>CCATTGGGCCGGAATAGGT</u></b>	n/a	WT Reverse



## DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

## qPCR master mix

1X

ABI GTX Taqman master mix	5 $\mu$ l
Assay (Probes 5 $\mu$ M each & Primers 15 $\mu$ M each)	2 $\mu$ l
ddH <sub>2</sub> O	0.5 $\mu$ l
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5 $\mu$ l

## qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500

95°C for 20 sec  
Then 40 cycles of;  
95°C for 3 sec  
60°C for 30 sec

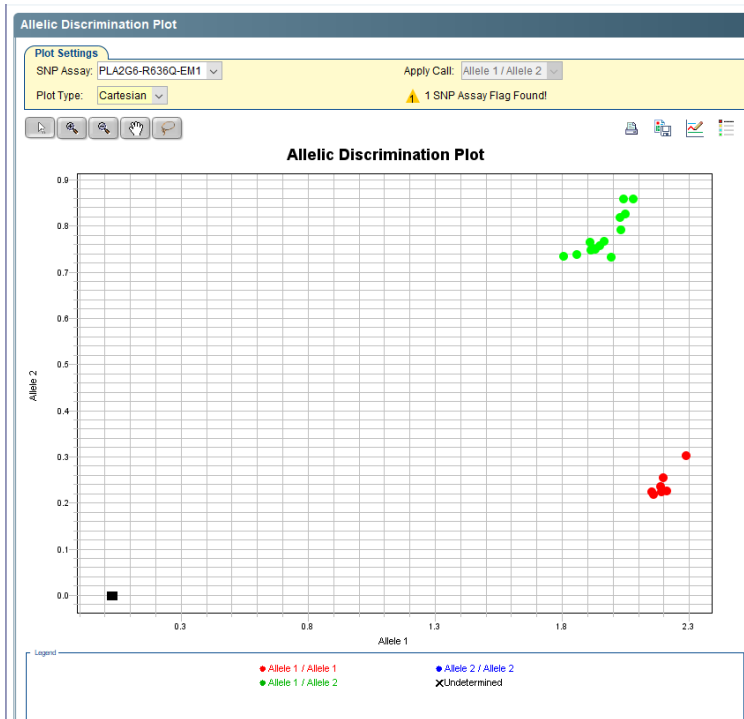




## Analysis

The results are analysed using 7500 software v2.0.6 from Applied Biosystems

### PLA2G6-R636Q Allelic Discrimination assay results (Task 294774 results)



	1	2	3	4	5	6	7	8	9	10	11	12
A	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...
B	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...	PLA2G6-R636Q-EM1-BEN PLA2G6-R636Q-E...							
C	het PLA2G6-R636Q-E...	het PLA2G6-R636Q-E...	wt PLA2G6-R636Q-E...	wt PLA2G6-R636Q-E...	ntc PLA2G6-R636Q-E...	ntc PLA2G6-R636Q-E...						

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