# PLCG2_HEPD0636_2_D04

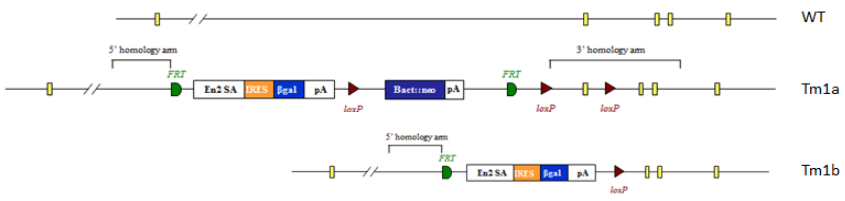
## EUCOMM/KOMP-CSD ‘Knockout-First’assay design

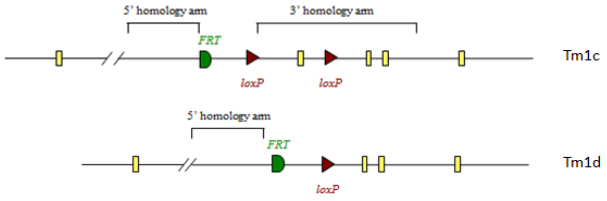
In order to genotype animals generated from the EUCOMM/KOMP-CSD ‘Knockout-First’ ES cell resource, a combination of mutant assays looking for sequences occurring in the targeting cassette, and WT specific assays may need to be performed.

For the majority of lines produced from these large scale gene targeting projects, the Knockout-First-Reporter Tagged Insertion (Promoter Driven Cassette) is used. As well as gene specific elements, the targeting cassette contains many common elements e.g. encoding beta-galactosidase (lacZ gene), neomycin-resistance (neo gene) and loxP sites. During homologous recombination, these sequences are inserted into the genome, resulting in a mutant allele. As a by-product of this process, short sections of genomic DNA are deleted. These deleted sequences can subsequently be used to design a genotyping assay that will detect ONLY the wild-type allele and not the Knockout-First mutant allele (Tm1a). This is known as a break point loss of allele (BP-LOA) assay.

Animals containing the EUCOMM Tm1a mutant allele may be crossed to mice expressing Cre recombinase. The progeny from these matings will carry a deletion of the sequence flanked by the lox P sites, resulting in the removal of the critical region of the gene, creating a Tm1b allele. A different loss of allele assay based around the critical region (CR-LOA) is required to genotype these mice. The CR-LOA assay along with Neo, LacZ and Cre assays may also be used at different points when generating Tm1b containing animals. Tm1c animals are generated by mating Tm1a carrying animals to mice expressing Flp recombinase. The resultant progeny still contain the critical region but no longer express Neo or LacZ as the region between the FRT sites is deleted. To remove the critical region, the progeny now should be mated to mice expressing Cre recombinase which then generates offspring containing the Tm1d allele.

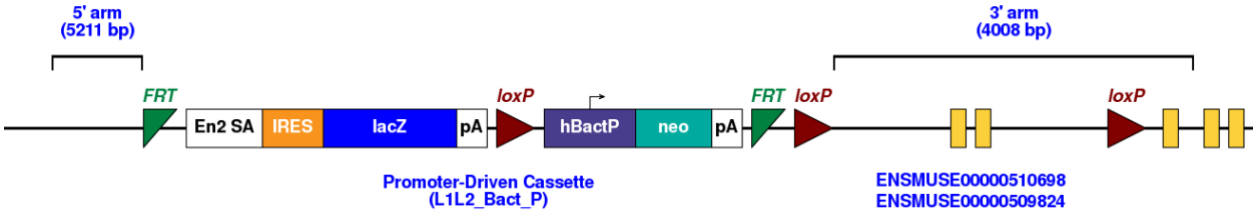
To genotype animals produced from the EUCOMM/KOMP-CSD ES cell resource, we typically use real time qPCR and copy count the various alleles. This assay design sheet will give an outline how to genotype these lines, either by using gel or qPCR based technologies.



# PLCG2-Tm1a

## Samples supplied are carrying the following allele




Details for the tm1a allele can be found below

http://www.knockoutmouse.org/martsearch/project/46041

**QC at Harwell identified that this clone has lost the 3' loxP site. As a result it is a TM1E line and is non-conditional. The genotyping assay below will still confirm the TM1E line**

# Tm1a Genotyping

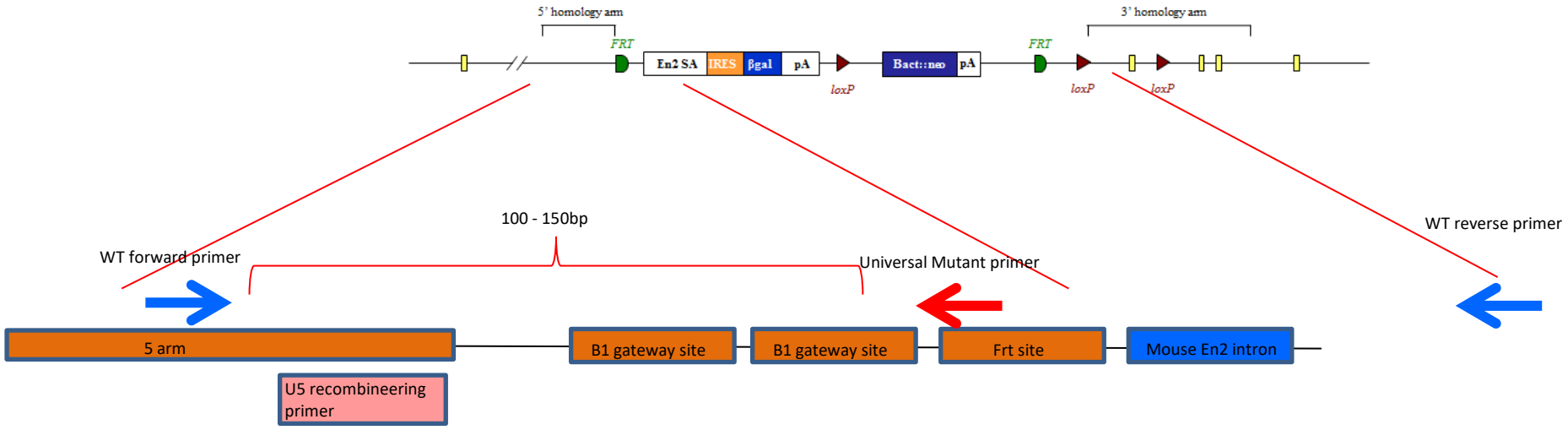
## PLCG2 Gel based Tm1a

Tm1a gel based assays are designed using a universal mutant reverse primer that sits in the sequence just after the 5' homology arm

Tm1a-5mut-R1 GAACTTCGGAATAGGAACTTCG

A forward primer is designed to the 5' homology arm and designed to give a mutant specific band, typically between 100-150bp that will only be present if the cassette is present.

A WT reverse primer is designed to the critical region and should give a product of >200bp. If the mutant cassette is present the product between the two primers is too large to PCR under standard conditions.



# Tm1a Genotyping

PLCG2 'Knockout'-First and WT sequences

5'homology arm (last 300bp)

ATTGGCAAAGCACAAAGCTTTAGTTTTCTGTGTCTGTCTTGGGAAGGGGATGCTGTGAAGTAGGGAATCACCAAAGCCTTTGTGTCAACCAGCTTTTCATTGTGACAACACAGCAG
AGACAACCCACTAAGATATGTTTGGTTTTGATCCTTGGTTTTGAAGGTTTCAGCCCATGGCCACTTGGCTCAGTTGTCCCTGGGCTGTGGTGACACACGGAGGAGGGGAACGTGTG
GTGAAGCACAGGTGCCCATCTCATGGTGGCCAGGAAGGGGAGTGTAGGTGGAACGAGGGAAGGAGGAT

Mutant 5'sequence location of Tm1a-5mut-R1 (mutant reverse primer)

AAGGCGCATAACGATACCACGATATCAACAAGTTTGTACAAAAAAGCAGGCTGGCGCCGGAACCGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAAC
CCTTTCCCACACCACCCTCCACACTTGCCCCAAACACTGCCAAC

3'homology arm (1st 300bp)

TATCTGCAAGCATATAAATATTTGTTCATATACATTCTTCTATATGCCCTTCACGTGGATTGTTTTCAGTGTGATATGTGTGCATAGGACCCAGGGCTGGGCCACTACCCCTCCCG
CAATGAACCAACACCTTCCCACATATACCACTCTTTCTTCTCAGCTGGTTGGGAATTAGTGATGGGGAGTTGTCCTTTATGGAGGCCTCACTTGATCTCATGGATGCTCCCTCGGG
GCTCAGACAGGGGTCTTCAGCCACAGGTGGAGTCTTGTTACCAAGCAGCCTTGTGAGGATGTTGGGGA

# Tm1a Genotyping

Critical region

TCTTGTCTAGCACTCCTAATGACCCATCAGGTTCTAAACCTGTCACTTGACCAGTTCATCGACAAGGCCTGGACTCTGATCCAGTCACCTCCTCATGGCCCCACTTCTGATGGCTG
TTTTCAGTGTCGGGTTCAAGCTTCTGACAGACACCTGAGTGTTGGGGCACATTTAACGATCACACCATAGCACCCTTCCTCCTGGGCCGCCTGGGAGGAGGCAGATGGCCTGGTGG
CTTTTGGTTTCCTGTCCACACTCAGGAGAATTGGACCCTGAGCTCAGATGGGACTCTGGTCAGAGATGGACTCACAGCTGTCTTCTAGGACCGAGGTGCTGGGGTCCCAGGGCTGC
AGGGCGGGGCTCTATCCTCTCCTGCAGGGAACTGGGGAATCTGACAAGCTTTATTTGTTTTCTCTCAGTGGACATCATGGAGATAAAGGAAATCCGTCCGGGGAAGAACTCCAAGG
ACTTTGAGCGAGCCAAGGCTGTCCGCCACAAGGCAGAATGTTGCTTCACCATCCTCTACGGCACCCAGTTTGTCCTCAGCACGCTCAGTTTGGCAAGTGGGTGCACATTTGTCTGG
ACATCCCTCCGCCCACCCCCCTGCCACCTCCTCCTCCTTCTGAGAGAGATCATACTTCATGTCTGCTAATGCATGCTCTGGGAACCCTGGTCTTGGGTCCCCATCCTACTGCTTTG
TAGCTTGTGTCTTTGGGCATGGGTATTTAACCTTTCCAAGTTGCAGTTTGCTTGGCTGTGACCTGGGGACAGCGATGTGGCTTTCTCCTTGGGACGTTGTGAGAAGTATGTTACCT
GTCTGCCTGGCTCAAGTTGAGAGTTAAATGAAGGTAGTTAACATGGTCACTGAGAGATAGGCTCTGCTGGACAAACATGTCACTTGTTTCCTACAGTGGTTGCTGCACAATAAACA
CTCATCTGAATGACCTTGGGTACATATGCAGGCACAGAGCACACACATATATGCATGGGCATACATGAACATAGCACATATGGATGCATGCACACACATGCATGTATATAGTATGC
AAGCATGCATGCGTGCACACAGCATACACATACACACAGCAATACATATATGCATGTGCACACACAGCATACATTCATATGTATCCATACAGCACAAACATGTATGCCCACAGTAT
ATACATGCATGGCTGCATACAGCAATACATGTGTGCACATATGGGCATAACACATGCATGCATGAACATGACACACACGCAACACACATTCTTGAACACAGTAAACATGTAACATA
TACGCATAGCACATACAATGCACACACAGCACAAAATGCATGCACATACCACACATGCAAAAGCACACAGATGAATCCACATATCAATAAACACATGTACATGCATATAACTATCA
CATATGCCTCTGCACAGCACACATATGGATGCATGCACATAGCACACATGGATGTACATATAAAACACTTATGCAGAACTCACATATGCACACAGCACATACATGTACACACTACA
CACATGCCTATACACATGACACAGCATACACATCTCACATATGTACATGTATAAATACACATGTGAGAACGTATAAACTTACATATCCGGAGATGGGATTTCTGGCCATCTCAATG
GTATATCCATCTAGAGCTCAGAGGTAGAGCTACCATTCACAAGGTCCCAGATTCCAGCCCCCAGCAGAAACAACAAGAGTCACCCAGAGTTCACTGCCCCCTCTCCAATTCTGTCT
ACAGCGGACTCAAAGGAGGATGCGGTGAAGTGGCTCTCTGGTTTGAAGATCCTACACCAGGAAGCGATGAGTGCATCCACCCCCACAATGATTGAGAGGTACACAACCCACTGGCA
TCATCCTCCTTTGTTCTAGGGTCTTCGGGAATAGACCAAAGGGAGAAAATGGGACATCGAGTCCCCAAGTTCATCAGTGCCCTGTGTGCAGCAGCCCCTGATAGTGGCTAAGAACT
CTCCAGTGCTCCACATAAGATCCTTTCTCCCTAGTGTTGAGCCTTGACCTTTGGCGTGAAGA

Tm1a 5’ mutant sequence

ATTGGCAAAGCACAAAGCTTTAGTTTTCTGTGTCTGTCTTGGGAAGGGGATGCTGTGAAGTAGGGAATCACCAAAGCCTTTGTGTCAACCAGCTTTTCATTGTGACAACACAGCAG
AGACAACCCACTAAGATATGTTTGGTTTTGATCCTTGGTTTTGAAGGTTTCAGCCCATGGCCACTTGGCTCAGTTGTCCCTGGGCTGTGGTGACACACGGAGGAGGGGAACGTGTG
GTGAAGCACAGGTGCCCATCTCATGGTGGCCAGGAAGGGGAGTGTAGGTGGAACGAGGGAAGGAGGATAAGGCGCATAACGATACCACGATATCAACAAGTTTGTACAAAAAAGCA
GGCTGGCGCCGGAACCGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAACCCTTTCCCACACCACCCTCCACACTTGCCCCAAACACTGCCAAC

# Tm1a Genotyping

## PLCG2 'Knockout'-First and WT sequences

Genomic sequence

TTAGTCACTGGCATGGGTGGTACTCTTAGCAACACCCAGAGAGGTCTCCAGTGAGCATCAGTCCCTCTCCTGCTATGATAGAGTAATCTGGGTCTTATCCCTAAAGACCAGTTCCA
TTGGCAAAGCACAAAGCTTTAGTTTTCTGTGTCTGTCTTGGGAAGGGGATGCTGTGAAGTAGGGAATCACCAAAGCCTTTGTGTCAACCAGCTTTTCATTGTGACAACACAGCAGA
GACAACCCACTAAGATATGTTTGGTTTTGATCCTTGGTTTTGAAGGTTTCAGCCCATGGCCACTTGGCTCAGTTGTCCCTGGGCTGTGGTGACACACGGAGGAGGGGAACGTGTGG
TGAAGCACAGGTGCCCATCTCATGGTGGCCAGGAAGGGGAGTGTAGGTGGAACGAGGGAAGGAGGATGAAGGAATGATTTCCTTCCGGCCAGACCCTACCTCTTGTCTAGCACTCC
TAATGACCCATCAGGTTCTAAACCTGTCACTTGACCAGTTCATCGACAAGGCCTGGACTCTGATCCAGTCACCTCCTCATGGCCCCACTTCTGATGGCTGTTTTCAGTGTCGGGTT
CAAGCTTCTGACAGACACCTGAGTGTTGGGGCACATTTAACGATCACACCATAGCACCCTTCCTCCTGGGCCGCCTGGGAGGAGGCAGATGGCCTGGTGGCTTTTGGTTTCCTGTC
CACACTCAGGAGAATTGGACCCTGAGCTCAGATGGGACTCTGGTCAGAGATGGACTCACAGCTGTCTTCTAGGACCGAGGTGCTGGGGTCCCAGGGCTGCAGGGCGGGGCTCTATC
CTCTCCTGCAGGGAACTGGGGAATCTGACAAGCTTTATTTGTTTTCTCTCAG**TGGACATCATGGAGATAAAGGAAATCCGTCCGGGGAAGAACTCCAAGGACTTTGAGCGAGCCAA**
**GGCTGTCCGCCACAAGGCAGAATGTTGCTTCACCATCCTCTACGGCACCCAGTTTGTCCTCAGCACGCTCAGTTTGGCAA**GTGGGTGCACATTTGTCTGGACATCCCTCCGCCCAC
CCCCCTGCCACCTCCTCCTCCTTCTGAGAGAGATCATACTTCATGTCTGCTAATGCATGCTCTGGGAACCCTGGTCTTGGGTCCCCATCCTACTGCTTTGTAGCTTGTGTCTTTGG
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ATACAGCAATACATGTGTGCACATATGGGCATAACACATGCATGCATGAACATGACACACACGCAACACACATTCTTGAACACAGTAAACATGTAACATATACGCATAGCACATAC
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GCACACATATGGATGCATGCACATAGCACACATGGATGTACATATAAAACACTTATGCAGAACTCACATATGCACACAGCACATACATGTACACACTACACACATGCCTATACACA
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CTCAGAGGTAGAGCTACCATTCACAAGGTCCCAGATTCCAGCCCCCAGCAGAAACAACAAGAGTCACCCAGAGTTCACTGCCCCCTCTCCAATTCTGTCTACAG**CGGACTCAAAGG**
**AGGATGCGGTGAAGTGGCTCTCTGGTTTGAAGATCCTACACCAGGAAGCGATGAGTGCATCCACCCCCACAATGATTGAGAG**GTACACAACCCACTGGCATCATCCTCCTTTGTTC
TAGGGTCTTCGGGAATAGACCAAAGGGAGAAAATGGGACATCGAGTCCCCAAGTTCATCAGTGCCCTGTGTGCAGCAGCCCCTGATAGTGGCTAAGAACTCTCCAGTGCTCCACAT
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TCCCGCAATGAACCAACACCTTCCCACATATACCACTCTTTCTTCTCAGCTGGTTGGGAATTAGTGATGGGGAGTTGTCCTTTATGGAGGCCTCACTTGATCTCATGGATGCTCCC
TCGGGGCTCAGACAGGGGTCTTCAGCCACAGGTGGAGTCTTGTTACCAAGCAGCCTTGTGAGGATGTTGGGGACCCCTTCTCCTTTTGGGGACTTTGTCCTCATTCCCTAGA

# Tm1a Genotyping

Tm1a gel based assays are designed using a universal mutant reverse primer that sits in the sequence just after the 5' homology arm

## PLCG2 tm1a gel based primers

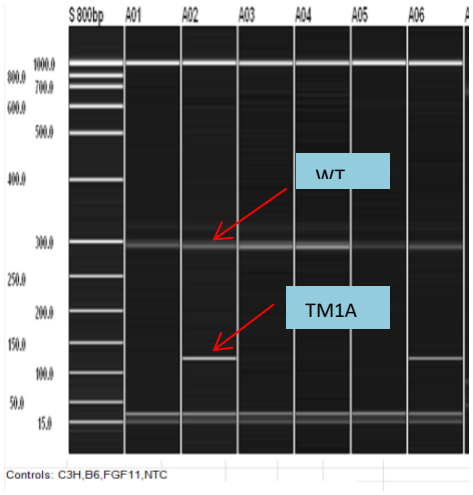
| | |
|---|---|
| PLCG2-5arm-WTF | TGTAGGTGGAACGAGGGAAG |
| PLCG2-Crit-WTR | AGGAAGGGTGCTATGGTGTG |
| Tm1a-5mut-R1 | GAACTTCGGAATAGGAACTTCG |

| | |
|---|---|
| WT band | 255 |
| Mutant band | 111 |

## PCR mix

| | |
|---|---|
| KAPA Taq PCR master mix | 5µl |
| 5arm-WTF | 0.5µl |
| Crit-WTR | 0.5µl |
| Tm1a-5mut-R1 | 0.5µl |
| $H_2O$ | 2.5µl |
| DNA | 1µl |

## Example of a generic TM1A assay




## Cycling conditions

**60TM30FA**

1. 95ºC 1min.
2. 95ºC 10sec.
3. **60**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC for ever
8. end