

## Tm1a – Knockout-First Allele

The tm1a allele was originally designed to be a knockout by splicing the cDNA to a LacZ cassette. The cassette was then inserted upstream of a critical exon to create a null allele of the gene. The diagram below is a generic example of a promoter-driven cassette;



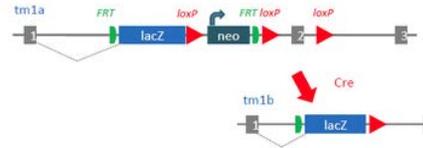
However for many genes skipping over the LacZ cassette restored gene expression to some extent. There is no way of knowing whether the tm1a for your particular gene will be a knockout, a wild-type (phenotypically) or perhaps a hypomorph. This will have to be checked by the end user.

As a result this allele should not be used for conditional experiments as mice carrying the tm1a allele can have their own phenotype.

Nevertheless, the tm1a allele is very versatile as it can be converted into tm1b or tm1c alleles.

## Tm1b – LacZ Tagged Null Allele

The tm1b allele is produced by deletion of the critical exon and the neomycin cassette using a Cre recombinase that recognises loxP sites.



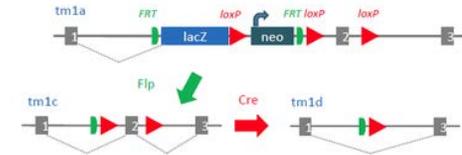
This allele is considered a true knockout as skipping over of the LacZ cassette will no longer restore gene expression.

The cassette expresses LacZ in tissues where the gene of interest is knocked out. Beta-galactosidase staining can be used to follow the tissue expression of your gene of interest.

This allele can be difficult to use if your gene has an essential housekeeping function and the homozygote null is lethal.

## Tm1c – Conditional Ready Allele

The tm1c allele is produced by deletion of the LacZ reporter and Neomycin selection cassette using a site-specific Flp recombinase that recognises FRT sites.



The tm1c mouse is returned to a phenotypically wild-type state, and the exons are spliced together as normal. However the critical exon(s) is still flanked by loxP sites.

A tissue specific cre-deleter of your choice can now be used to create a tissue specific knockout of the gene (**tm1d allele**, tissue specific null allele).

## Summary

	Allele	Pros	Cons
Tm1a	Knockout-first allele	Very versatile can be converted into tm1b and tm1c.	May not be a true knockout.
Tm1b	Null allele	Gene knockout. Expresses LacZ in tissue where the gene is expressed.	Homozygotes can be lethal for genes with essential housekeeping functions.
Tm1c	Conditional allele	Ready for use in tissue specific KO experiments.	No LacZ expression.