Transgenes (TG)

Basic Vector TG	Can target anywhere
TG with some homology	Target to least homologous region
Promoter--Vector TG	Target at promoter-vector junction
Mouse cDNA	Target at exon-exon junction (Be aware of intron size)
Human TG	Target to least homologous region

SNPs (MUT)

Basic SNP	Center SNPs in reporter oligos.
SNP based off AA change given	Locate AA change. Center SNPs in reporter oligos.
rs#	Locate rs#. Ensure reporter 1 is BL6 sequence. Center SNPs in reporter oligos.

Both reporters MUST be on the same strand or the assay will not work.

Humanized Models (KO)

Standard humanized KO model	Identify endogenous human junction and include 2-3 mismatches per oligo (WT and Mutant) if possible.
Humanized model with FRT or LoxP	Target flanking the FRT/LoxP which allows you to avoid homology

Knockouts (KO)

Standard KO Model	Target KO assay at 5' or 3' junction
Small CRISPR deletion	Balance KO probe across new junction
Large CRISPR deletion	Flank new junction

Designs for corresponding WT will be noted in the wildtype section.

KO landmarks

Start/Stop	Design WT at start or stop (depending on model). KO probe will be a generic.
Coding Region	Design anywhere in coding region. KO probe will be a generic
Restri-ction Site	Design according to restriction sites. KO probe will be a generic.
Coordi-nates	Design according to coordi-nates. KO probe will be a generic.

When in doubt - SEQUENCE.

Wildtypes (WT)

WT for small insertion (<400bp)	Balance probe (40/30) or do allele specific primer.
WT for large insertion (>400bp)	Can flank insertion site or have a probe across insertion site but do NOT need to balance.
WT where some bases were deleted (either via CRISPR or upon insertion of vector)	Target probe within deleted bases. Primers can be on either side.

Floxed (FL or MD)

Floxed allele with vector	Flank LoxP, use tearpin, or break the hairpin.
Floxed allele with minimal vector	Use tearpin or break the hairpin
Floxed allele when unsure which LoxP	Flank LoxP or do tearpin
Marker deleted floxed allele	Flank FRT/LoxP

Identify which LoxP you have. If you are breaking the hairpin you MUST know which LoxP to ensure you break in the correct direction.

Excised (EX)

EX with vector	Flank remaining LoxP or tearpin.
EX with minimal vector	Tearpin or can flank LoxP if large enough deletion created by Cre recombination.

CRISPR

NHEJ	Flank new junction created as long as it is >400bp
HDR	Must target 1 oligo outside of ssODN to be site specific.

Remember that CRISPR rules can apply to a variety of models including SNPs, Conditional lines, and even traditional KOs. Please be aware if you are designing for CRISPR and utilize our best practices.

By **scarlettnicole87**

cheatography.com/scarlettnicole87/

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