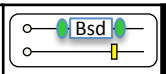
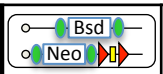
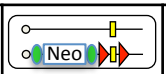
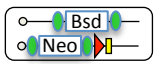
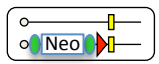
Homozygous Standard Workflow – Non-Essential genes [**NE1**]

Bsd first wt/wt -> tm1 -> tm1a -> tm1/tm1a

FEP: *SEP/PIQ:*

Diagram:							
Genotype:		**wt/wt**	**tm1/wt**	**tm1/tm1a**	**wt/tm1a**	**tm1/tm1e**	**wt/tm1e**
LOA CRIT	- OHT	2	1	1	2	1	1
LOA TAM	+ OHT	2	1	0	1	1	1
LOA DEL	- OHT	2	1	0	1	0	1
BSD		0	1	1	0	1	0

FEP:

tm1/wt is what you want

wt/wt is total failure of electroporation

* No final loxp site with Bsd vector so no benefit in doing + OHT assay?

SEP:

tm1/tm1a is what you want

wt/tm1a is best possible control but rare

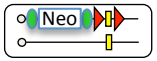
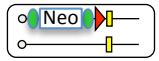
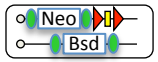
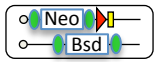
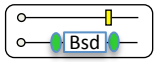
tm1/wt is second best control and very likely

wt/wt is total failure of both electroporations but still a useful control

Homozygous Standard Workflow – Non-Essential genes [**NE1a**]

Neo first wt/wt -> tm1a -> tm1 -> tm1a/tm1

FEP: *SEP/PIQ:*

Diagram:									
Genotype:		**wt/wt**	**tm1a/wt**	**tm1e/wt**	**tm1a/tm1**	**tm1e/tm1**	**wt/tm1**	**tm1a/off target Bsd**	**off target Neo/tm1**
LOA CRIT	- OHT	2	2	2	1	1	1	2	1
LOA TAM	+ OHT	2	1	2	0	1	1	1	1
LOA DEL *	- OHT	2	1	1	0	0	1	1	1
NEO		0	1	1	1	1	0	1	1
BSD **		0	0	0	1	1	1	1	1

FEP:

tm1a/wt is what you want

tm1e/wt is where most of the cassette goes in but the final loxp does not

wt/wt is total failure of electroporation

* LOA DEL assay optional at FEP stage

** No Bsd resistance in cassette so no benefit to performing the Bsd assay at FEP stage

SEP:

tm1a/tm1 is what you want

wt/tm1 is best possible control but rare

tm1a/wt is second best control and very likely

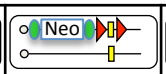
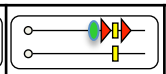
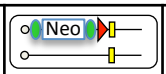
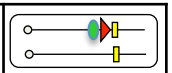
wt/wt is total failure of both electroporations but still a useful control

off target affects suggested by data, may not be real

Homozygous Standard Workflow – Essential genes [**E**]

Neo first wt/wt -> tm1a -> (Dox) -> tm1 -> tm1c/tm1

FEP:

Diagram:						
Genotype:		**wt/wt**	**tm1a/wt**	**tm1c/wt**	**tm1e/wt**	**tm1f/wt**
LOA CRIT	- OHT	2	2	2	2	2
LOA TAM	+ OHT	2	1	1	2	2
LOA DEL	- OHT	2	1	1	1	1
NEO		0	1	0	1	0

FEP:

tm1c/wt is what you want.

tm1a/wt is where the Dox does *not* excise the Neo resistance.

tm1f/wt is where the Dox does excise the Neo resistance but the final loxp site was *not* inserted.

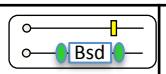
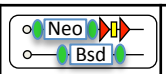
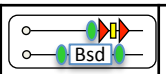
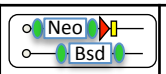
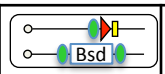
tm1e/wt is where the Dox does *not* excise the Neo resistance *and* the final loxp was not inserted.

wt/wt is total failure of electroporation.

Homozygous Standard Workflow – Essential genes [**E**]

Neo first wt/wt -> tm1a -> (Dox) -> tm1 -> tm1c/tm1

SEP/PIQ:

Diagram:								
Genotype:		**wt/tm1**	**tm1a/tm1**	**tm1c/tm1**	**tm1e/tm1**	**tm1f/tm1**	**tm1a/off target Bsd**	**off target Neo/tm1**
LOA CRIT	- OHT	1	1	1	1	1	2	1
LOA TAM	+ OHT	1	0	0	1	1	1	1
LOA DEL	- OHT	1	0	0	0	0	1	1
NEO		0	1	0	1	0	1	1
BSD		1	1	1	1	1	1	1

SEP:

tm1c/tm1 is what you want

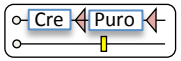
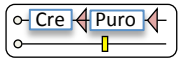
tm1c/wt shows the SEP failed and is a good control

wt/tm1 is also a good control

Heterozygous Cre Knockin Standard Workflow – No Dre [**CreKi**]

wt/wt -> tm1 -> tm1/wt

EP/PIQ:

Diagram:				
Genotype:	**wt/wt**	**tm1/wt**	**tm1 lrpcr/wt**	**wt/crepuro off target wt**
CRE	0	1	1	1
LOA DEL	2	1	X	2
PURO	0	1	1	1
LOA CRIT	2	1	1	2
LRPCR	X	X	Pass	X

EP:

tm1/wt is what you want

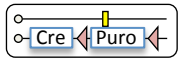
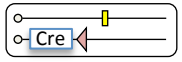
wt/wt is total failure of electroporation

In addition to these tests they must all pass chromosome tests Chry and Chr8, and if the LOA DEL test fails then LRPCR primer bands can be checked

Heterozygous Cre Knockin Standard Workflow – Dre [**CreKiDre**]

wt/wt -> tm1 -> (Dre) -> tm1.1/wt

EP/PIQ:

Diagram:							
Genotype:	**wt/wt**	**tm1/wt**	**tm1.1/wt**	**tm1 lrpcr/wt**	**tm1.1 lrpcr/ wt**	**wt crepuro off target/ wt/**	**wt cre off target/wt**
CRE	0	1	1	1	1	1	1
LOA DEL	2	1	1	X	X	2	2
PURO	0	1	0	1	0	1	0
LOA CRIT	2	1	1	X	X	2	2
LR PCR	X	X	X	Pass	Pass	X	X

EP:

tm1.1/wt is what you want

tm1/wt is a failure of the *Dre* recombinase step

wt/wt is total failure of electroporation

lrpcr check is done if LOA DEL is absent or fails

In addition to these tests they must all pass
chromosome tests Chry and Chr8a (pass or passb).