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

Bsd first tm1 -> tm1a -> tm1/tm1a

First Electroporation:

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

tm1/wt is what you want

wt/wt is total failure of electroporation

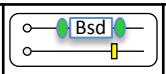
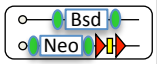
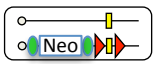
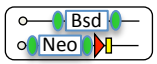
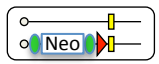
* No final loxp site with Bsd vector so no benefit in doing + OHT assay

** No Neo resistance in cassette so no benefit in doing Neo assay

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

Bsd first tm1 -> tm1a -> tm1/tm1a

Second Electroporation:

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
NEO		0	0	1	1	1	1
BSD		0	1	1	0	1	0

tm1/tm1a is what you want

wt/tm1a is best possible control but rare

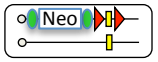
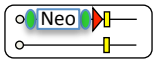
tm1/wt is second best control and very likely

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

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

Neo first tm1a -> tm1 -> tm1a/tm1

First Electroporation:

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

tm1a/wt is what you want

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

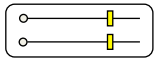
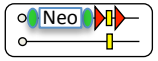
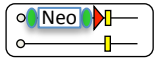
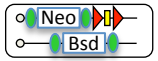
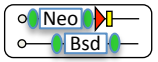
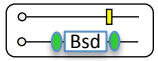
wt/wt is total failure of electroporation

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

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

Neo first tm1a -> tm1 -> tm1a/tm1

Second Electroporation:

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

tm1a/tm1 is what you want

wt/tm1 is best possible control but rare

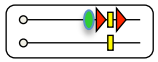
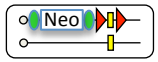
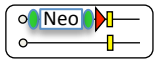
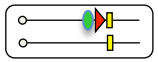
tm1a/wt is second best control and very likely

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

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

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

First Electroporation:

Diagram:						
Genotype:		**wt/wt**	**tm1c/wt**	**tm1a/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	0	1	1	0
BSD **		0	0	0	0	0

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

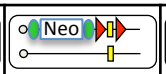
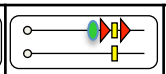
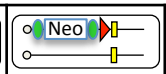
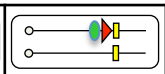
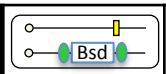
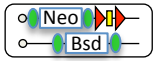
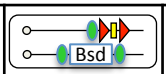
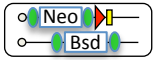
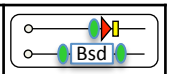
wt/wt is total failure of electroporation

** No Bsd resistance in cassette so no benefit to doing Bsd assay

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

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

Second Electroporation:

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

tm1c/tm1 is what you want

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

wt/tm1 is also a good control