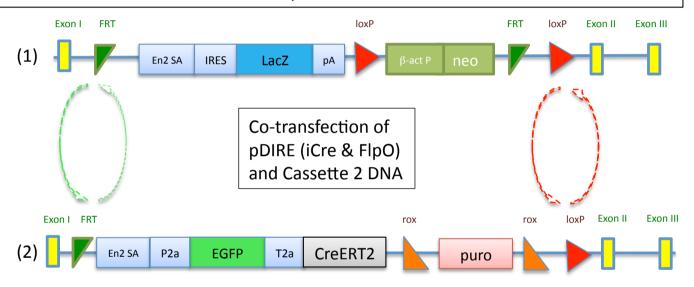
## **GUDMAP2 Strategy for Production of Reporter Strains**

Strategy: The GUDMAP2 reporter strain production is based on the use of pre-existing targeted gene loci from the KOMP-CSD/EUCOMM ES cell clone library. ES cell clones will be modified by swapping the targeted allele with a multifunctional cassette comprising the fluorescent reporter, EGFP, and the tamoxifen-inducible recombinase, CreERT2, using a process known as dual-recombinase-mediated cassette exchange (dRMCE). Novel multifunctional reporter cassettes used with dRMCE are being developed at the Sanger Institute and mouse strains produced in GUDMAP2 will complement efforts by EUCOMMTOOLS. Following modification and verification of the modified allele, ES cell clones will be used for construction of chimeras and the derivation of novel reporter strains.

## Steps:

- Procuring ES Cell clones comprising KOMP-CSD/ EUCOMM targeted loci as shown in (1).
- 2. Modify ES cell clones with the multifunctional cassette (2) by dRMCE.
- 3. Verification of modified loci by DNA sequence analysis.
- 4. Generation of chimeras, germ line testing and breeding F1 generation.
- Distribution of F1 animals to nominating investigators, GUDMAP2 consortium, and MMRRC/Jax for colony expansion and archiving.



## **Further Considerations:**

- 1. The objective of GUDMAP2 is to produce 5 reporter strains in year 1. Current plans call for production of 20 more strains between 2012-2016.
- 2. Nomination process for the first 5 genes expected to be completed by early 2012. To nominate please visit the <u>GUDMAP Reporter Strain Nominations</u> page.
- 3. GUDMAP2 reporter cassette is currently fine-tuned and validated. Depending on functionality and efficacy, final cassette may slightly differ from cassette shown in (2).