Gene targeting and exchange vector guidelines

These guidelines describe quality control assurances for designing gene targeting vectors.

Keywords: exchange cassette | gene target

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Quality control assurances for gene targeting vectors

1. At a minimum, all cloning junctions for each new targeting vector need to be sequenced across to verify correct ligation.
2. All loxP and FRT sites need to be sequence confirmed, even if they have been derived from a plasmid that is known to contain sequences that have previously been shown to recombine correctly.
3. The orientation of all selection cassettes, usually neomycin and thymidine kinase, needs to be determined, preferably by DNA sequencing as primers are often placed in these sequences for analysis of the targeted clones.
4. The DNA sequence files must be reviewed with another laboratory member, preferably Kathy, then saved for future review if necessary.
5. A detailed map that is of publication quality of the plasmid needs to be prepared, reviewed with Mark, and then both the map and plasmid DNA (uncut) needs to be provided to Kathy to be stocked.
6. A probe for southern blot hybridization needs to be isolated and tested with the enzyme(s) that will be used when screening electroporated cells. It is best to do the southern blots using 129SvEvTac DNA as this is the strain used to generate the TL-1 ES cells which we prefer to use. Any primers that are necessary for confirming the presence of vital loxP sites need to also be synthesized and tested.

Quality control assurances for exchange vectors

1. At a minimum, all cloning junctions for each new exchange vector need to be sequenced across to verify correct ligation.
2. The orientation of all selection cassettes, typically hygromycin, need to be known as primers are often placed in these sequences.
3. The DNA sequence files must be reviewed with another laboratory member, preferably Kathy, then saved for future analysis if necessary.
4. A detailed map that is of publication quality needs to be prepared, reviewed with Mark, and then both the map and plasmid DNA (uncut) needs to be provided to Kathy to be stocked.
5. Screening strategies and the necessary primers for confirming correct exchange need to be determined, synthesized and tested prior to electroporation. If necessary, PCR amplification conditions for certain primer pairs may also need to be optimized.

Written by Kathy Shelton, 8/4/2003