

GENE TRAP FLIPPING - INFECTION OF MURINE EMBRYONIC STEM CELLS

Gene trap cassettes of ES cell clones are integrated either in sense or in antisense, leading to disruptive or non-disruptive integrations, respectively. As loxP and frt sites flank the gene trap cassette, it is possible to reverse the orientation, e.g. by transiently infecting cells with a Cre/Flp-expressing plasmid. The generated isogenic sister clone can be used as perfect internal control.

For the production of retrovirus containing the Cre/Flp expressing plasmid, please see *Retrovirus Production Protocol* at https://www.haplobank.at/e-commerce/control/haplobank_resource. The Cre/Flp-expressing plasmid should be linked w/ a FACS-able color and/or a selection marker (e.g. MLP-mCherry-Cre-puro), to evaluate infection efficiency and/or select for infected cells.

INFECTION OF MURINE EMBRYONIC STEM CELLS

- ES cells are propagated by our standard procedures and should be passaged several times prior to infection
- 3-4h prior to infection seed about 250'000 cells per 6-well or 50'000 cells per 24-well for infection in triplicates
- Carefully take off the viral supernatant from the packaging cells and filter through 0.45µm filter, optional: to increase infection efficiency add to viral supernatant 3mM JQ1 1: 60'000 and 8mg/mL Polybrene 1:4'000
- Alternatively: if you use a frozen virus stock (see *Retrovirus Production Protocol*), thaw aliquots (e.g in a 37°C water bath) and add media according to previously determined dilution factor. Add JQ1 and Polybrene if required.
- Distribute viral supernatant on ESCs
- 8-12h post infection exchange media. Check infection efficiency by FACS 48h post infection or change to selection medium and select until selection control wells are empty (with puro it takes 3-4 days)
- Confirm the successful inversion of the gene trap cassette by inversion PCR (please see *Inversion PCR Protocol* at https://www.haplobank.at/e-commerce/control/haplobank_resource)