Haplobank

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Gene trap flipping: Infection of murine embryonic stem cells with retrovirus

Gene trap cassettes of ES cell clones are integrated either in sense or antisense integration, 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 w/ a Cre/Flp-expressing plasmid. The thereby generated isogenic sister clone can be used as perfect internal control.

For the production of retrovirus containing the Cre/Flp-expressing plasmid, please see the protocol 'Production of Retrovirus for gene trap flipping'. 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 suck off viral supernatant from 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 protocol 'Production of Retrovirus'), 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 PCR (please see protocol 'Inversion PCR')