Ex vivo transduction of murine murine whole bone marrow for gene transfer to hematopoietic stem cells

- Harvest bone marrow from mouse femurs and tibias using 1cc 27G 5/8" syringe needles and flush with Iscove's Modified Dulbecco's Media (IMDM) w/out any supplements into tubes.
- Spin down cell pellet at ~1200rpm, aspirate supernatent and hemolyze with hemolytic buffer for ~2min, spin down and aspirate super. Can repeat hemolysis step if cell pellet isn't clear of red blood cells.
    - **Hemolytic buffer (1000ml)**:
        - 8.3g Ammonium chloride (FW = 53.49)
        - 1.0g Sodium bicarbonate (FW = 84.01)
        - 200μl EDTA (0.5M stock)
        - ddw (or DEPC treated water) up to 1000ml
        - Filter sterilize
- After hemolyzing cells, resuspend in HSC media (IMDM, 10%FBS, 1%pen-strep, 50ng/ml mSCF) ***Can culture HSCs with additional factors: IL-11, Flt-3L, IL-3, TPO, IL-6 pending on needs of prolonged *ex vivo* culture.
- Filter cell suspension through 35μm mesh cell strainer tubes (5ml polystyrene), count and aliquot to CH296 treated non-TC treated plates for transduction or T25 flask for O/N pre-stimulation.
- For pre-stimulation, add total cell fraction after straining and culture O/N in T25 flask in HSC media. Following day can then aliquot to CH296 treated non-TC plates for transduction.
    - **CH296 (Retronectin coating):**
        - Reconstitute CH296 (Takara Bio) with 1ml DI or DEPC treated water and filter sterilize through 0.22μm syringe filter.
        - Add 19ml sterile PBS to 1ml of reconstituted CH296 to yield 25μg/ml stock.
        - Coat **non-TC treated** plates for >2hrs at RT with 400ul of stock solution
        - Aspirate off CH296 and replace with 2%BSA in Hank's Balanced Salt Solution (HBSS) and incubate for 30min at RT
        - Aspirate off BSA/HBSS and add HBSS+2.5% Hepes pH7.0 (7.05) and leave at 4^{o}C until use.
        - Just prior to use, rinse 2x with PBS and plate cells in treated wells
- Add protamine sulfate (final [c] = 4ug/ml) to HSC media and transduce with lentivector containing transgene of interest at varying multiplicities of infection (MOI) and/or time points at 37C.
- Following transduction (1 – 24h), remove cells in supernatent fraction and trypsinze remaining adherent cells. Combine and add 2ml PBS (or 2%FBS-PBS) for wash step. Spin for 5-10min at 1200rpm
- Repeat wash step with PBS or 2%FBS-PBS)
- Aspirate supernatent and resuspend for downstream analysis (FACS) or further experiments.
    - **Injection:**
        - Resuspend cells in 150ul HBSS and keep on ice until injection.
    - **Fluorescence Activated Cell Sorting (FACS):**
        - Resuspend cells in >300ul 2%FBS-PBS + propidium iodide (PI) solution (1:1000 of 1mg/ml stock) ***If using PE-conjugated antibody, DO NOT add PI solution, will interfere with fluorescent read out.