- Read the following article: Lutolf et al. Perturbation of single hematopoietic stem cell fates in artificial niches, *Integrative Biology* (2008), and answer the following questions. For some questions, you may need to look into referenced papers or outside material for additional information. [http://pubs.rsc.org/en/Content/ArticleLanding/2009/IB/b815718a#!divAbstract](http://pubs.rsc.org/en/Content/ArticleLanding/2009/IB/b815718a#!divAbstract)

a) (2 points) For the isolation of hematopoietic stem cells (HSCs) in this work, a panel of protein markers is labeled with antibodies and used for FACS-based enrichment. What does it mean that the HSCs are Lineage (Lin) negative?

(2 points) Lineage-negative means that the HSCs do not express a panel of protein surface markers present on mature blood lineage cells. (did not need to include specific markers, but they include: CD3 (t lymphocyte), B220 (b lymphocyte), CD11b (monocyte), Ly-6G (granulocyte), Ter-119 (erythroid))

b) (2 points) To confirm the function of sorted HSCs, the investigators test the reconstitution efficiency in mice. What 2 (redundant) labels do they use to evaluate this?

(1 point) GFP
(1 point) Ly5.1

c) (4 points) The reconstitution efficiencies of 2 different sorted populations (LKS and LKS-CD150) are directly compared in Figure 2B. What does the data in Figure 2B suggest is the benefit of performing the additional CD150 step? What is a potential reason underlying this benefit?

(2 points) The data in Figure 2B suggests that LKS-CD150 cells are more efficient at reconstituting mice than LKS cells since fewer LKS-CD150 cells are required for 100% reconstitution, and generally LKS-CD150 cells give higher reconstitution.

(2 points) The addition of the CD150 to the cocktail increases the concentration of the reconstitution mediating cell type within the purified population, compared to the LKS strategy alone.
d) (2 points) What key property of polyethylene glycol (PEG) made it useful for cell seeding into the microwells and use with migratory cells?

(2 points) PEG hydrogels are non-adhesive for cells, which would prevent cell adhesion on the outside of the microwells, and would also prevent migration out of the microwells.

e) (2 points) What was the process for investigating single cell function within the microwells (i.e. how did they load single cells into the wells, and analyze single cells)?

(1 point) Cells were randomly sedimented into microwells. (cell density was selected to get a large number of wells with single cells although many wells with 0 cells or >1 cell were also present).

(1 point) In the analysis, the authors only analyzed wells that were originally seeded with a single cells. Other wells were simply ignored.

f) (3 points) On average, the time to 1st division was longer for LKS-CD150 cells compared to LKS cells (Figure 3). How does this finding correlate with what is believed to be a difference between stem cells and progenitor cells?

(1 point) A longer time to 1st division for LKS-CD150 cells suggests that these cells are less proliferative (or more quiescent) than LKS cells.

(2 points) This difference suggests that LKS-CD150 cells are most likely stem cells and the LKS cells are most likely progenitor cells (or transit amplifying progenitor cells).

g) (3 points) In the initial experiments utilizing unmodified PEG microwells (no conjugated proteins), what experiment (performed by the authors) suggested that LKS-CD150 cells were not receiving adequate signals within the microwell for self-renewal?

(3 points) The authors transplanted LKS-CD150 cells cultured in unmodified microwells into irradiated recipients and these cells DID NOT reconstitute recipient mice.
h) (2 points) What is an FC-chimeric protein?

(2 points) An FC-chimeric protein is a fusion protein, specifically a fusion of the FC region of an antibody (immunoglobulin) and another protein of interest.

i) (2 points) What modification to the PEG microwells makes it possible to present FC-chimeric proteins within the microwells?

(2 points) The PEG microwells were modified to present ProteinA, which has 4 binding sites for the FC region of immunoglobulins, and thus will present the FC-chimeric proteins to cells within the microwells.

j) (4 points) Compared to basal conditions, what is the effect of (I) Wnt3a, (II) IGF-2, (III) thrombopoietin (TPO), and (IV) N-Cadherin (N-Cad) on the in vitro proliferation of HSCs?

(1 point) (I) Wnt3a: Decreased

(1 point) (II) IGF-2: No effect

(1 point) (III) TPO: Increased

(1 point) (IV) N-cad: Decreased (+ more asynchronous, also acceptable)

k) (3 points) In Figure 6, the investigators perform secondary transplants to follow-up analysis of the reconstitution in primary transplant recipients. In these experiments, bone marrow from primary recipients is transferred to secondary lethally irradiated recipients. What does this secondary transplant experiment demonstrate that is not demonstrated by the primary transplant data?

(3 points) Secondary transplantation experiments are a more rigorous demonstration of reconstitution, by demonstrating that the cells transplanted into the primary recipient can contribute to **long-term self-renewal**.
i) (3 points) In Figure 7, the investigators select cells from individual wells based on the number of divisions observed in the wells, and then perform reconstitution experiments with these defined sets of cells. What information does this data provide that was not provided by the experiment in Figure 6?

(3 points) By selecting individual wells they could directly examine the differences in reconstitution efficiencies for cells that underwent 0, 1, or more cell divisions within the various modified microwell environments.

m) (2 points) Overall, what is the primary benefit of performing time-lapse assessment of individual HSC compared to bulk analysis of multicellular HSC populations?

(1 point) This approach allows you to examine the number PLUS TIMING of cell divisions for individual HSCs.

(1 point) You can additionally correlate the number and timing of cell divisions with functional measures (e.g. reconstitution).