Sysbio tissue culture meeting notes, Oct 2016

Core facilities for Sysbio: Flow cytometry and sorting, Microfluidics, Microscopy

Opportunities for Collaboration:

- Adam Palmer in the Sorger lab has a lentivirus library of barcodes; this can be used to track sub-clonal evolution

- Alex Ng in the Church lab has a lentivirus library of transcription factors as well as a library of differentiated cells (namely neurons, muscle-like, and blood vessel cell lines)

Discussion points:

1. **What are the most difficult questions and methods that we need help with?**

- Culturing zebrafish cells; problems achieving adherence despite adding serum to media

- Automated experiments at the Sorger lab;
    - The D300 from HP automates drug printing (nL - uL drops);
    - A new machine automates cell inoculation;
    - The Operetta automates imaging and quantification of fluorescent protein localization;
    - **Contact Clarence Yapp for training: LHRRB room 302; 617 432 1893**

- Cell genetic engineering:
    - Transposon/lentiviral/transfections are commonly performed followed by clonal selection by FACS (single cell sorting) and growing sorted cells in conditioned media or on a bed of irradiated feeder cells and a suite of inhibitors;
    - Genetic engineering is performed to mark proteins for localization experiments;
    - One idea to overcome the problem of certain cell lines not able to grow from single cells is to engineer kill switches into feeder cells so that they can be selected away once the desired cells have divided enough; the limitation is that the desired cells have to possess a permanently expressed selection marker

2. **What kind of experiments would we do if we could do anything? What cell line models would we use?**

- Biannual tissue culture departmental meetings to connect tissue culture scientists and refresh the departmental database (see next)

- Departmental tissue culture database and mailing list, including a listserv for experimental troubleshooting aka the Experimental Spam Listserv with options for daily or weekly digests
    - Physical resources such as plasmids and cell lines with basic annotations about experimental usage
    - Protocols
    - Stephanie Terrizzi will provide the template used at Brown
    - Include the departmental shared strains, as well as strains from each lab; this requires contribution from each lab's point person for tissue culture; contribution will earn access to the database

- Automated media changing and passaging as compared to asking colleagues for favors

- Large-scale experiments; overcoming having to handle hundreds of plates by using multiplex screens
    - The Broad Institute Genetic Perturbation Platform has genome-wide CRISPR libraries
    - Using suspension cells instead of adherent; not using trypsin in passaging will eventually select for non-adherent cells (cells are dislodged by flask banging)

- Barcoded lineage tracking e.g. phylogenetic mapping via CRISPR mutations (Schier, Science, 2016)

For reference:

1. Techniques used in the department:
    a. CRISPR KO and editing
    b. Transfections (lipofectamine and lentiviral)
    c. Flow cytometry
    d. Fluorescence microscopy, multiplex immunofluorescence
    e. Lineage tracing by DNA barcodes
    f. Single and combination drug response profiling, using live cell imaging
    g. RNA sequencing
    h. Proteomics
    i. hiPSC transgenesis, differentiation
    j. Organoid culture
    k. Long-term timelapse confocal imaging
    l. Harvesting primary tissue from mice
2. Cell lines in use in the department:
    a. HEK293 (T)
    b. U2OS
    c. U87 brain
    d. B-cell lymphomas
    e. Melanoma cell lines
    f. Human iPSC
    g. Primary murine intestine
3. Research questions in the department
    a. How to make cells easier to engineer
    b. How combination chemotherapy suppresses drug resistance evolution; understanding mechanisms of adaptive resistance to cancer drugs; understanding oncogene addiction
    c. Rapid and efficient methods to convert human stem cells into primary-like cells and organoids
    d. How signaling and mechanics regulate the stability of the intestinal stem cell niche

Attendees from the Flow Core, Lab Ops, Church, Klein, Lahav, Megason, Silver, and Sorger labs:

Alina Chan yachan@hms.harvard.edu; Stephanie Terrizzi stephanie_terrizzi@hms.harvard.edu; Dan Lu dan.lu.camb@gmail.com; Peng Fei Xu pengfei_xu@hms.harvard.edu; Luca Gerosa luca.gerosa@gmail.com; Samantha Keough samantha_keough@hms.harvard.edu; Janelle Vultaggio janelle_vultaggio@hms.harvard.edu; Neeti Mittal neeti_mittal@hms.harvard.edu; Kyogo Kawaguchi kyogo_kawaguchi@hms.harvard.edu; Alex Ng alexhmng@gmail.com; Adam Palmer acpalmer@gmail.com; Sherry Gao sherrygao@college.harvard.edu