Project title:
Design of flexible reporters for transcription factor expression and activity in developing lymphocytes

A full-time undergraduate co-op student/research assistant position (4-12 months) is available in the Zandstra Stem Cell Bioengineering laboratory, which is in the School of Biomedical Engineering located within the Biomedical Research Centre, and is affiliated with the Michael Smith Laboratories, at the University of British Columbia's Vancouver campus. The successful candidates will join our world-class research team **to develop a flexible reporter system to track the expression of key genes as stem cells are differentiated into T cells *in vitro***. Our highly successful multidisciplinary program integrates researchers in stem cell biology, biological computation, synthetic biology, developmental biology, and regenerative medicine, with the goal of developing a platform for scalable *in vitro* production of T cells from stem cells for eventual use as off-the-shelf cell therapies. Our research program is based on understanding how individual cells make developmental decisions by studying multiscale interactions between cells, their internal regulatory networks, and the external microenvironment, and then mobilizing our findings to generate therapeutically relevant T cells from stem cells.

Details on the project and position:

Successful outcomes from this project will facilitate expansion of our existing *in vitro* T cell development program and eventual clinical translation. A critical aspect of engineering T cell development is understanding the stage-specific expression of key transcription factors that drive cell fate decisions. Typical reporter systems for such genes are inflexible and can preclude simultaneous measurements of the expression of other important genes or fractionation of live cell sub-populations. We therefore need a flexible reporter system that accurately reports the expression of cytosolic and nuclear proteins while minimally perturbing existing measurement channels.

We anticipate that a flexible reporter system will enable us to better measure and ultimately guide the development of T cells from stem cells. A promising approach to achieve this involves expressing a truncated, membrane-bound receptor fused to an epitope tag. The tag can then be stained with an antibody conjugated to any fluorophore of interest at any time point desired during the differentiation of stem cells to T cells. This reporter can then be integrated into stem cells such that it is co-expressed with genes of interest, or have their expression activated by the proteins encoded in such genes. We will investigate the quantitative properties of the reporters, including the precision at which they can report on the expression or activity of target genes. We will also investigate novel gene editing strategies to efficiently knock-in the reporters to specific genomic loci.

The candidate will have the opportunity to assist in developing the new reporter system using protein engineering and synthetic biology approaches. They will also run experiments, measure reporter activities in stem cells and developing T cells, and analyze the resulting data. Specifically, the student will be involved in aspects of the project that may include:

- Protein engineering/genetic design/molecular cloning of flexible reporter constructs
- Culture and CRISPR-based engineering of stem cells, including validation of reporter knock-ins
- Quantitative flow cytometry measurement and analysis of reporter activities in relation to their targeted genes
- Measurement of reporter activities for key target genes during *in vitro* T cell differentiation

Relevant literature:

Tewary, M., Shakiba, N., and Zandstra, P. W. (2018) Stem cell bioengineering: building from stem cell biology. Nat. Rev. Genet. 19, 595–614.

Shukla, S., Langley, M. A., Singh, J., Edgar, J. M., Mohtashami, M., Zúñiga-Pflücker, J. C., and Zandstra, P. W. (2017) Progenitor T-cell differentiation from hematopoietic stem cells using Delta-like-4 and VCAM-1. Nat. Methods 14, 531–538.

Iriguchi, S., Yasui, Y., Kawai, Y., Arima, S., Kunitomo, M., Sato, T., Ueda, T., Minagawa, A., Mishima, Y., Yanagawa, N., Baba, Y., Miyake, Y., Nakayama, K., Takiguchi, M., Shinohara, T., Nakatsura, T., Yasukawa, M., Kassai, Y., Hayashi, A., and Kaneko, S. (2021) A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. Nat. Commun. 1–15.

Wroblewska, A., Dhainaut, M., Ben-Zvi, B., Rose, S. A., Park, E. S., Amir, E. A. D., Bektesevic, A., Baccarini, A., Merad, M., Rahman, A. H., and Brown, B. D. (2018) Protein Barcodes Enable High-Dimensional Single-Cell CRISPR Screens. *Cell 175*, 1141-1155.

Duportet, X., Wroblewska, L., Guye, P., Li, Y., Eyquem, J., Rieders, J., Rimchala, T., Batt, G., and Weiss, R. (2014) A platform for rapid prototyping of synthetic gene networks in mammalian cells. Nucleic Acids Res. 42, 13440–13451.

Steyer, B., Bu, Q., Cory, E., Jiang, K., Duong, S., Sinha, D., Steltzer, S., Gamm, D., Chang, Q., and Saha, K. (2018) Scarless Genome Editing of Human Pluripotent Stem Cells via Transient Puromycin Selection. Stem Cell Reports 10, 642–654.

Haupt, A., Grancharova, T., Arakaki, J., Fuqua, M. A., Roberts, B., and Gunawardane, R. N. (2018) Endogenous protein tagging in human induced pluripotent stem cells using CRISPR/Cas9. J. Vis. Exp. 2018, 1–9.

Roberts, B., Hendershott, M. C., Arakaki, J., Gerbin, K. A., Malik, H., Nelson, A., Gehring, J., Hookway, C., Ludmann, S. A., Yang, R., Haupt, A., Grancharova, T., Valencia, V., Fuqua, M. A., Tucker, A., Rafelski, S. M., and Gunawardane, R. N. (2019) Fluorescent Gene Tagging of Transcriptionally Silent Genes in hiPSCs. Stem Cell Reports 12, 1145–1158.

Anzalone, A. V., Koblan, L. W., and Liu, D. R. (2020) Genome editing with CRISPR–Cas nucleases, base editors, transposases and prime editors. Nat. Biotechnol. 38, 824–844.

Notes:

1) This position is suitable for an independent, resourceful, highly self-motivated candidate with relevant experience.
2) Position will be between 4 and 12 months duration, to start in May 2021; duration to be decided at the outset however extensions may be possible
3) No vacation time is provided as vacation pay is provided in lieu; however if the candidate has any scheduling constraints please discuss with us
4) We encourage successful applicants to also apply for external award funding as appropriate and eligible e.g. an NSERC undergraduate summer research award (USRA)

(https://students.ubc.ca/career/campus-experiences/nserc-undergraduate-student-research-awards) and/or a Centre for Blood Research-School of Biomedical Engineering USRA (https://www.bme.ubc.ca/research/funding-opportunities/); either one of these awards can be held at one time concurrent with a co-op position. Note that the NSERC USRA program requires a minimum period of continuous duration of work.

5) The salary will be $2500/month full-time (based on 35 hours work/week). Salary will be pro-rated for any partial months worked, and is inclusive of any award funding received.

Ideal candidates would have experience in some or all of the below:

- Cell biology or biochemistry (stem cell biology lab experience and/or experience working in the field of hematopoiesis research is an asset)
- Mammalian cell culture and aseptic technique
- Molecular cloning
- Genetic engineering
- Computer programming skills (working knowledge of MATLAB or Python is an asset)
- Data analysis (statistical methods are an asset)
- Flow cytometry

Individuals must also:

- Work well in a goal-oriented team environment;
- Be highly self-motivated and engaged in research
- Possess excellent communication skills – both verbal and written;
- Be open to instruction and constructive criticism on the project and their capabilities
- Have the ability to work semi-independently and organize own workload under supervision
- Keep meticulous records of experiments and data, report on research progress and outcomes openly within the team, and maintain research confidentiality
- Demonstrate an ability to design and analyze experiments, review experimental methodologies in response to feedback
- Have the ability to acquire and update knowledge in their specialized area and implement relevant technologies to advance the project

For further information about these projects and to apply, please also send us your application package ***as one PDF file*** via email at **zandstra.lab@ubc.ca** to include

- Email subject line: “Engineering flexible receptors for T cells 2021 co-op/RA student application”
- Cover letter
- Dates and duration of your availability (preferred start date, for how many months)
- CV
- Copy of all university transcripts (require English translations where applicable; originals must sent prior to acceptance of offer)
- Contact information for 3 references

For further information on our research and team, please visit our website and Twitter account:

https://www.stemcellbioengineering.ca/

https://twitter.com/StemCellBioEng

We will consider applications until the position is filled, at which time we will remove the job posting on our lab website - https://www.stemcellbioengineering.ca/careers/

We regret that we can only contact those applicants who are selected for further consideration.
