Project title: Engineering genetic sensors to track T cell differentiation in vitro

An undergraduate summer co-op student/research assistant position 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 genetic sensor and 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 gene regulatory networks, and the external microenvironment, and then utilize our findings to generate therapeutically relevant T cells from stem cells.

Details on the project and position:

Successful outcomes from this project will support and expand our existing clinically-relevant *in vitro* T cell development program. A critical aspect of engineering T cell development is to understand the dynamic expression profiles of key stage-specific transcription factors (TFs) that direct cell fate decisions. Typical reporter systems for such genes are inflexible and can preclude simultaneous measurements of other important genes or fractionation of live cell sub-populations. We therefore need a more flexible reporter system that accurately senses and reports the expression of TFs, is compatible with live cell sorting, and minimally interferes with existing measurement channels.

We anticipate that a flexible sensor/reporter system will enable us to better measure and ultimately guide *in vitro* differentiation of T cells from stem cells. A promising strategy involves expressing a truncated, membrane-bound receptor fused to an epitope tag. The tag can be stained with an antibody conjugated to any fluorophore at any time point during the differentiation of stem cells to T cells. This reporter can be integrated into stem cells and induced by either sensing the mRNA expression or functional activity of target genes. We will investigate quantitative properties of the sensors, including their accuracy and precision. Finally, if successful, the sensors will be used to drive the expression of transgenes to enhance T cell differentiation.

The candidate will have the opportunity to assist in developing the new sensor/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 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
- Writing methods and results for publication in a scientific journal

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.
- Michaels, Y. S., Edgar, J. M., Major, M. C., Castle, E. L., Zimmerman, C., Yin, T., Hagner, A., Lau, C., Ibañez-rios, M. I., Knapp, D. J. H. F., and Zandstra, P. W. (2021) DLL4 and VCAM1 enhance the emergence of T cell-competent hematopoietic progenitors from human pluripotent stem cells. *bioRxiv*.
- 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.
- 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.
- 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.
- Jiang, K., Koob, J., Chen, X. D., Krajeski, R. N., Zhang, Y., Villiger, L., Zhou, W., Abudayyeh, O. O., Chen, F., and Gootenberg, J. S. (2022) Programmable eukaryotic protein expression with RNA sensors. bioRxiv.
- Kaseniit, K. E., Katz, N., Kolber, N. S., Call, C. C., Wengier, D. L., Will, B., Sattely, E. S., and Gao, X. J. (2022) Modular and programmable RNA sensing using ADAR editing in living cells.

Notes:

1) This position is suitable for an independent, resourceful, highly self-motivated candidate with relevant experience
2) You should be currently enrolled in an undergraduate engineering or science degree program at the time of application
3) Position will be 4 months duration, to start in early May 2022. There is a possibility for extension (NB for co-op program applicants)
4) No vacation time is provided as vacation pay is provided in lieu; however if the candidate wishes to minimally alter the start or end dates, please discuss with us
5) The salary will be $2600/month full-time (based on 35 hours work/week and includes 4% vacation pay). Salary will be pro-rated for any partial months worked, and is inclusive of any award funding received.
6) We encourage students to apply for external awards e.g. SBME Synergy summer studentship (note application deadline is Mar 18th) https://www.bme.ubc.ca/undergraduate/sbme-synergy-summer-studentship-program/

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

To apply, please choose the most appropriate method(s) for you, and note any deadlines:

1) **If you are applying through the UBC co-op program**, then please search their website for our Job Posting and follow the instructions there
    a. This opportunity will be posted through both the UBC Engineering and Science co-op programs
2) **If you are applying through the UBC Work Learn International Undergraduate Research Awards program** (WLIURA), then apply via UBC Careers Online, search for Job ID 898858, here:
    a. https://ubc-csm.symplicity.com/index.php/pid926632?
    b. This opportunity will be posted from March 7 to March 20, 2022 only
    c. More information is here: https://students.ubc.ca/career/campus-experiences/undergraduate-research/work-learn-international-undergraduate-research-awards
3) please send us your complete application package ***as one PDF file*** via email at **zandstra.lab@ubc.ca** to include:

- Email subject line: "Engineering genetic sensors to track T cell differentiation in vitro 2022 co-op/RA student application"
- Cover letter
- Dates of your availability (start and end dates, prefer 4 month position, or longer)
- CV
- Copy of all university transcripts (require English translations where applicable; originals must sent prior to acceptance of offer)
- Contact information for 3 references

- Let us know if you are looking for a co-op position and/or are eligible for the WLIURA.

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 on a rolling basis until the position is filled, at which time we will note this on the job posting on our lab website here - https://www.stemcellbioengineering.ca/careers/

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