CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**In-situ lineage tracking with 1 million inducible DNA barcodes**
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Programme	Early Detection
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Laboratory Location	Hutchison MRC Research Centre

Project Outline	**Aims and objectives** The stem cells maintaining blood, skin, intestine and other tissues, are in a state of constant turnover and thus accumulate genetic alterations, some of which lead to clonal expansions and cancer [1]. Understanding this requires the ability to measure the population dynamics that occur during tissue maintenance. Here we propose to build an in-situ lineage tracking tool that can inducibly generate millions of DNA barcode combinations, allowing one to track millions of cell lineages in parallel with exquisite precision using next-generation sequencing. Unlike previous semi-quantitative approaches [2], this technology will have the ability to quantitatively track the clonal dynamics associated with tissue maintenance in-vivo, and yield insights into how homeostasis is achieved and how it breaks down in the earliest stages of cancer. Our previous work in S. Cerevisiae has demonstrated that site-specific DNA barcoding based on the cre-lox system and quantitative tracking of lineage dynamics can be used to gain insight into how mutations arise, expand and compete in large cellular populations [3]. Further development of this technology in partnership long-term collaborator Sasha Levy now makes it possible to generate barcode diversity in-situ, without the need for transformation of a plasmid library. This modified technology will make use of 3 tandem loxP "landing pads" each of which (upon induction of Cre) can irreversibly incorporate one of ~100 unique barcode sequences stored in three independent tandem arrays elsewhere in the genome. For this MRes rotation project that we plan to scale-up this technology to robustly generate 1 million unique barcode combinations in yeast. This will demonstrate that this technology has the ability to trace cell lineages in vivo with single-cell precision, opening up major unanswered questions in stem cell biology and cancer onset to interrogation.
Experimental plan	The student will first build long tandem array constructs composed of ~100 barcodes separated by loxP sites and integrate this construct into the genome of a yeast strain that already contains a cre-lox landing pad using standard homologous recombination. The student will then investigate how the diversity of barcodes that can be induced with this construct depends on the induction conditions and genomic location of the tandem array. Once this has been optimized, the student will integrate a further two tandem arrays and attempt to achieve a diversity in excess of 1 million unique barcodes, which will be carefully quantified using a custom designed 2-step PCR protocol that uses unique molecular identifiers (UMIs) to tag individual DNA molecules.

Main Techniques	• cloning • transformations • cre-lox recombination • ultra deep DNA sequencing • sequencing pipelines and error correction • statistical analyses of time series data
Key References	**Young, A. L., Challen, G. A., Birmann, B. M. & Druley,** *T. E. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. Nature Communications 7, 12484 (2016).* **Sun, J.** *et al. Clonal dynamics of native haematopoiesis. Nature 514, 322–327 (2014).* **Levy, S. F**. *et al. Quantitative evolutionary dynamics using high-resolution lineage tracking. Nature 519, 181–186 (2015).*