Advancing homology-directed repair driven CRISPR technologies in zebrafish

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Project description:
The rise of CRISPR technology for targeted genome editing has led to huge advancements in the ways we can study gene function and create animal models, particularly in highly genetically tractable model organisms such as the zebrafish. CRISPR genome editing technology works by targeting a nuclease (typically Cas9) to a specific genomic region where double-strand breaks (DSBs) are induced. These DNA breaks are typically repaired by competing pathways, most commonly by error prone non-homologous end-joining (NHEJ) (often introducing insertions and deletions (indels) that disrupt gene function) or, more rarely, by homology-directed repair (HDR), which uses a homologous region of DNA as a repair template. HDR can be used to target single nucleotide changes or to insert tags into the genome at precise locations, however, this system remains relatively inefficient and prone to confounding errors when compared to NHEJ.

This project aims to implement new advancements to CRISPR HDR technology to improve the efficiency rates for targeting precise genetic locations using the highly amenable and advantageous zebrafish model. The student will design guide RNAs (gRNAs) and single-stranded oligodeoxynucleotide donor templates (ssODN) for several different targets in the zebrafish genome and implement current published advances in the technology [1-6] to maximise efficiency of targeting. Targeting several different genetic regions of interest will maximise the success of the project, demonstrate the wide applicability of these advancements for researchers using CRISPR technology and provide the student with highly transferable and desirable genome editing skills. Genetic regions to be targeted include engineering single base substitutions in potassium ion channels of the heart which cause cardiac arrythmias, introducing point mutations in transcription factors associated with neurocristopathies and inserting inducible tissue specific Cre expression from loci expressed at different stages in stem/progenitor cells in the embryo. Once zebrafish lines carrying the correct targeted genetic change are generated these can be phenotyped via methods such as light sheet live imaging of the heart of larval fish to assess parameters including heart rate, contraction and stroke volume [7].

References: [1] Zhang Y et al. J Biol Chem. 2018. 293: 6611. [2] Richardson C et al. Nat Biotechnol. 2016. 34: 339. [3] Savic N et al. Elife. 2018. 7: e33761. [4] Nambiar TS et al. Nat Commun. 2019. 10(1): 3395. [5] Jayavaradhan R et al. Nat Commun. 2019. 10(1): 2866. [6] Canny MD et al. Nat Biotechnol. 2018. 36: 95. [7] Lin KY et al. Anal Chem. 2014, 86(4): 2213.