# Donnelly Centre TechTalks

Informal trainee-organized seminars and discussions to foster collaboration within the Donnelly Centre via the exchange of expertise and equipment for experimental techniques

## Analzying the binding specificities of RNA-binding proteins in vitro using RHT-SELEX and RNAcompete

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Current estimates suggest that over 1,500 human RNA-binding proteins (RBPs) are involved in post-transcriptional gene regulation. Although most RBPs recognize RNA elements through canonical RNA-binding domains (RBDs), many do not. Interestingly, the latter include a variety of transcription factors and metabolic enzymes that are typically not associated with RNA-binding function. A major obstacle in post-transcriptional gene reguation is that the RNA-binding specificities for most RBPs are not well defined. In the Hughes' Lab, we address this issue using several integrated biochemical and computational systems including — RNA high throughput systematic evolution of ligands by exponential enrichment (RHT-SELEX) and RNAcompete. RNAcompete has been used to determine the RNA-binding preferences of hundreds of RBPs to short linear RNA sequences whereas RHT-SELEX is a newer system which is capable of modeling the binding of RBPs to both linear and structured RNA target sites. RNAcompete incorporates two highly optimized systems — protein purification and custom DNA library generation — that may be of interest to the research community at large: we routinely purify GST-tagged proteins at a ~90% success rate and have collaborated with other labs to generate diverse short DNA libraries that can be used for a variety of downstream applications. RHT-SELEX can also be modified to use DNA and methylated DNA targets for researchers that are interested in analyzing the binding specificity of DNA-binding proteins of interest.

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