IMPLEMENTING A HIGH-THROUGHPUT PARALLEL CRISPRI SCREENING PLATFORM TO IDENTIFY FUNCTIONAL LNCRNAS

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Technological advances in RNA-seq have led to the view that the human genome is pervasively transcribed, resulting in the production of thousands of long non-coding RNAs (lncRNAs). A few lncRNAs are now recognized as key components of diverse physiological processes. However, molecular genetics lacks a more comprehensive view of lncRNome functionality and the mechanisms through which lncRNAs operate. Current high- throughput approaches to study lncRNA function (i.e. pooled CRISPR library screens) are typically limited to a single phenotypic readout. We created a platform enabling serial cellular and molecular observations to screen for functional lncRNAs in a high-throughput and parallel manner using CRISPR interference (CRISPRi). We applied PCR, in vitro transcription and bead-based purification for high-throughput production of single gRNAs in 96-well plate format. These gRNAs were transfected in cells growing in 96-well culture plates and expressing dCas9. Cells were monitored in real-time using the Incucyte to quantify growth and proliferation. Subsequently, cells were lysed and lysates were used to construct RNA-seq libraries in order to generate a molecular profile for each condition. A proof-of-concept screen including 20 lncRNA targets and 10 single gRNAs per target demonstrated the feasibility of our screen and revealed genes and pathways differentially expressed upon lncRNA knockdown. We are currently expanding the number of lncRNA targets with the aim to silence every lncRNA gene expressed in our model cell line, thus revealing the cellular and molecular phenotypes associated to several hundreds of lncRNAs. Moreover, additional dCas9 models are being generated to probe lncRNA functions in various disease-relevant model systems including asthma, COPD and cancer.