Implementing a high-throughput parallel CRISPRi screening platform to identify functional lncRNAs

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Aims

- lncRNAs represent the majority of human transcriptome diversity but the fraction of lncRNAs that are functional remains elusive
- Pooled CRISPR-screens have revealed collections of functional lncRNAs but are focused on a single cellular phenotype typically cell proliferation)
- We developed an arrayed CRISPRi screening platform for high-throughput and unbiased characterization of lncRNA function
- Catalogs of functional lncRNAs will be used to study associations with various lncRNA features

lncRNA selection

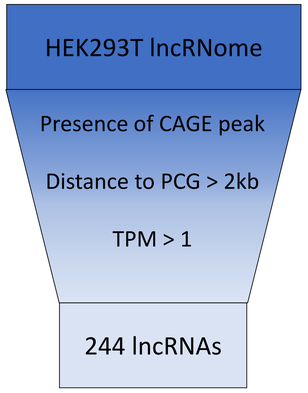
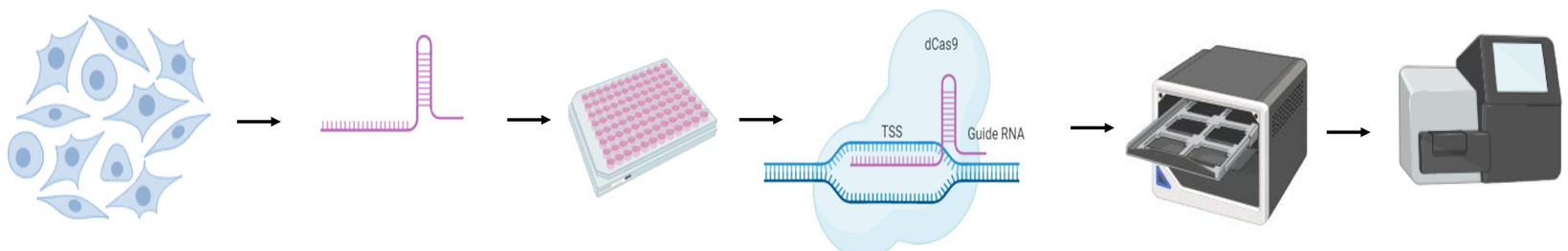


Figure 1. Selection of 244 target lncRNAs in HEK293T cells. 72 were analyzed in the first batch.

Workflow



1. dCas9 HEK293T cells

2. gRNA production PCR & IVT

3. gRNA transfection in 96 well format

4. Knock down of lncRNAs of interest

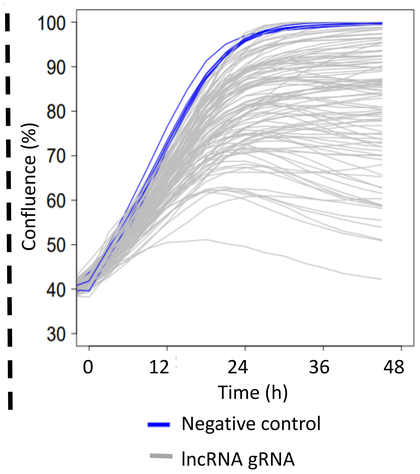
5. Cellular read-out: real-time monitoring

6. Molecular read-out: RNA sequencing

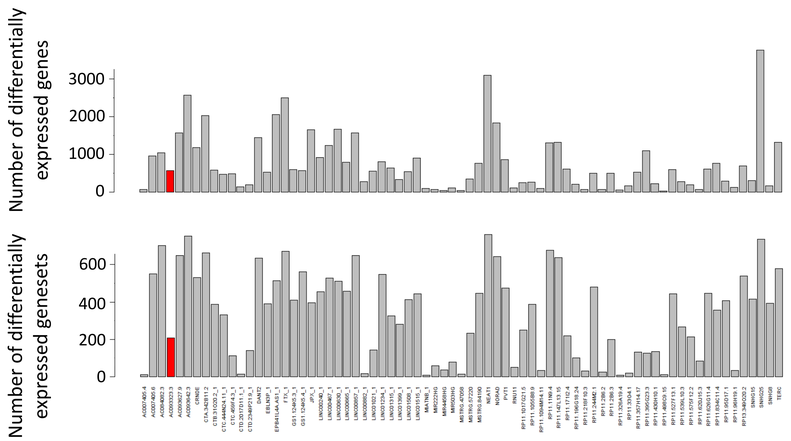
Results

Intermediate results for the first batch of 72 lncRNAs

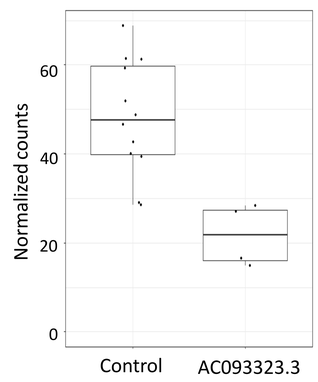
Cell proliferation



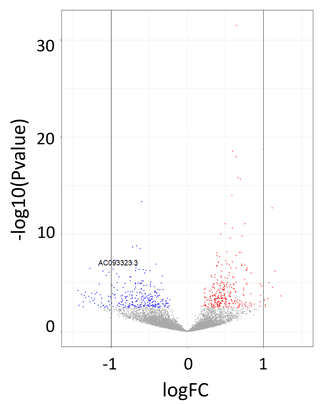
Differentially expressed genes and gene sets upon lncRNA knock down



Knock down of lncRNA (AC093323.3)



Genes differentially expressed upon AC093323.3 knock down



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Conclusions

We established an arrayed CRISPR interference screening platform based on high-throughput gRNA production, live cell imaging and shallow RNA-sequencing of cell lysates. This platform enables a systematic evaluation of lncRNAs at the cellular and molecular level, allowing us to prioritize functional lncRNAs in the model system under investigation. Results presented here demonstrate the feasibility of our approach and reveal dozens of lncRNAs that affect the HEK293T transcriptome upon knockdown. Differentially expressed genes are currently evaluated in more detail in order to remove potential gRNA off-targets. Once functional lncRNAs are selected, we will investigate associations with various features, including conservation, splicing (efficiency), enhancer-association, expression abundance, expression specificity etc. Similar analysis will be performed in other model cell lines.