

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

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# Important to investigate the function of lncRNAs

- 15 000-100 000 lncRNAs vs 21 000 protein coding genes
- Minor part studied on a functional level
- Are they all functional?

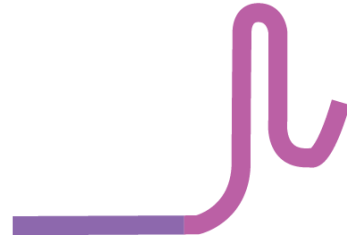
-> Need for a high-throughput platform to probe functionality

# Workflow of a high-throughput parallel CRISPRi screen

1. dCas9-KRAB-MeCP2 expressing cells



2. Production of hundreds of gRNA by PCR & IVT



3. gRNA transfection



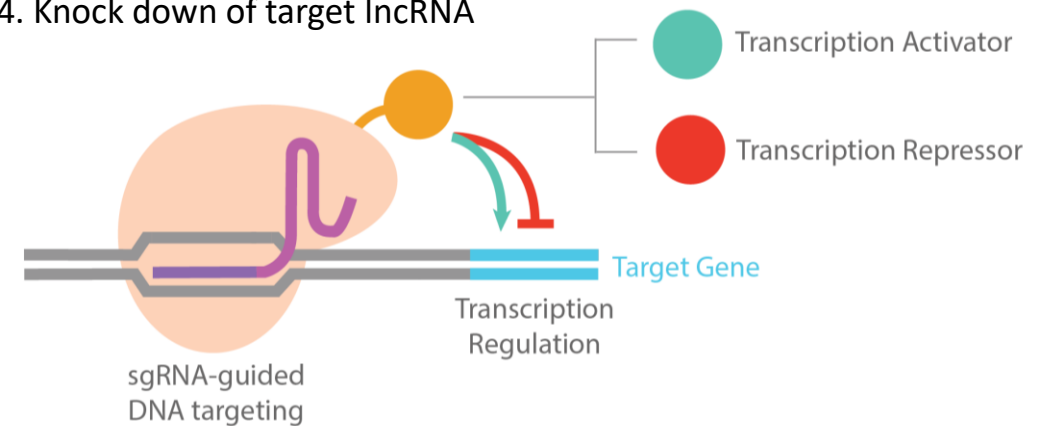
6. Molecular read-out



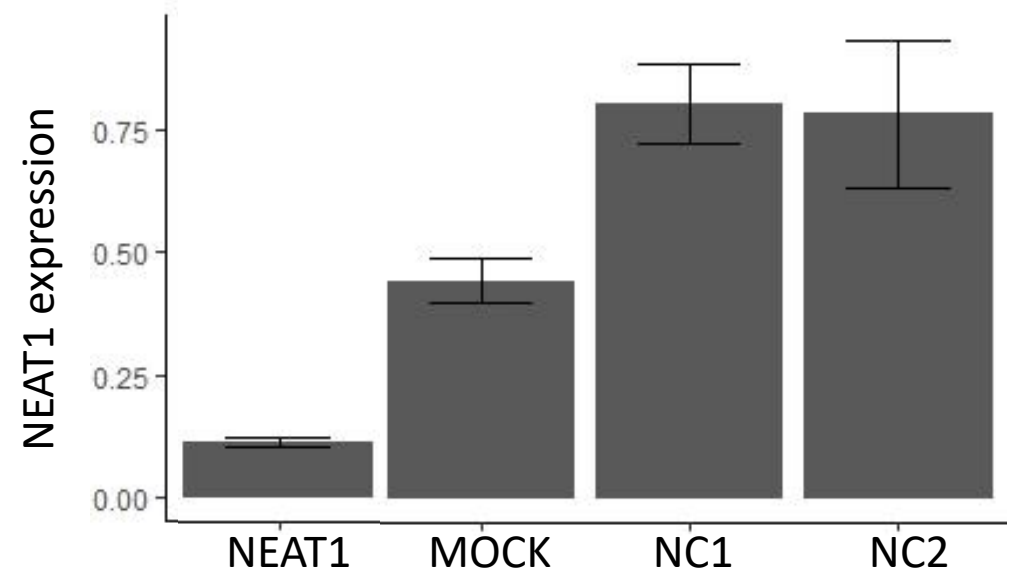
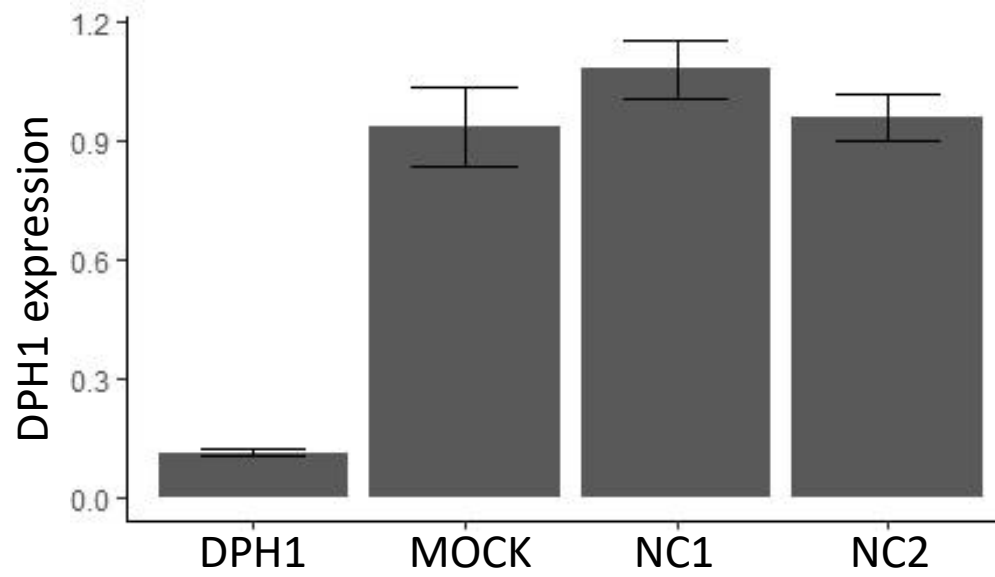
5. Cellular read-out



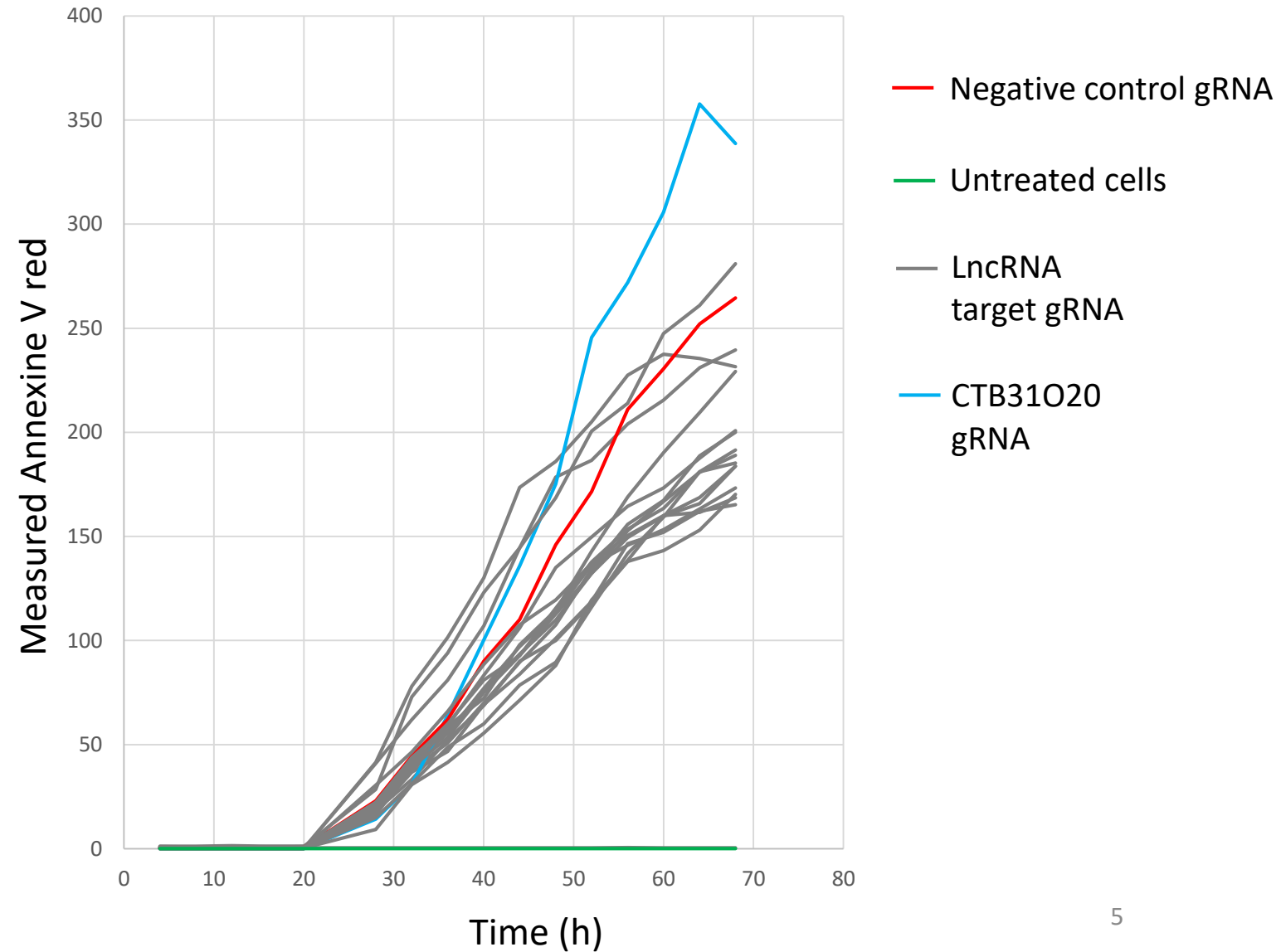
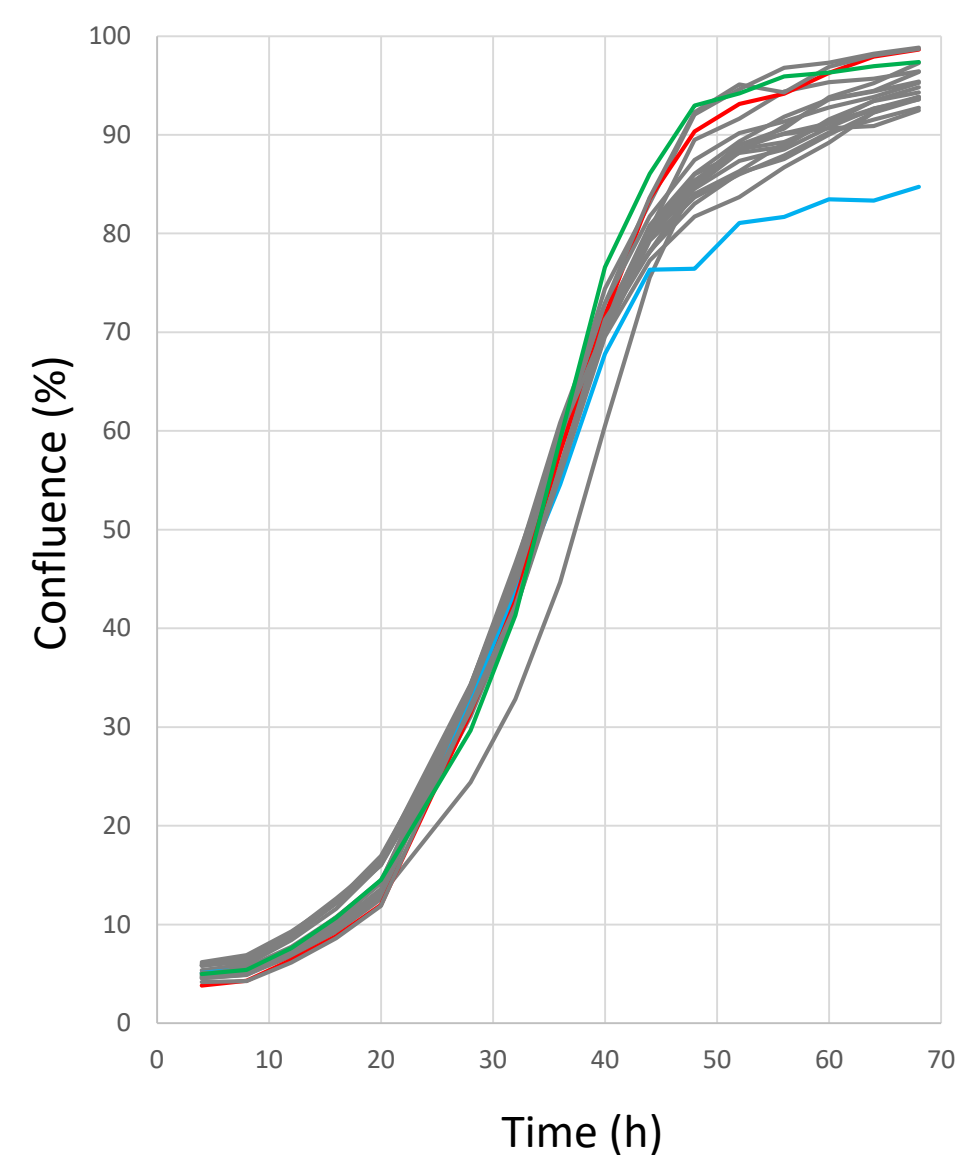
4. Knock down of target lncRNA



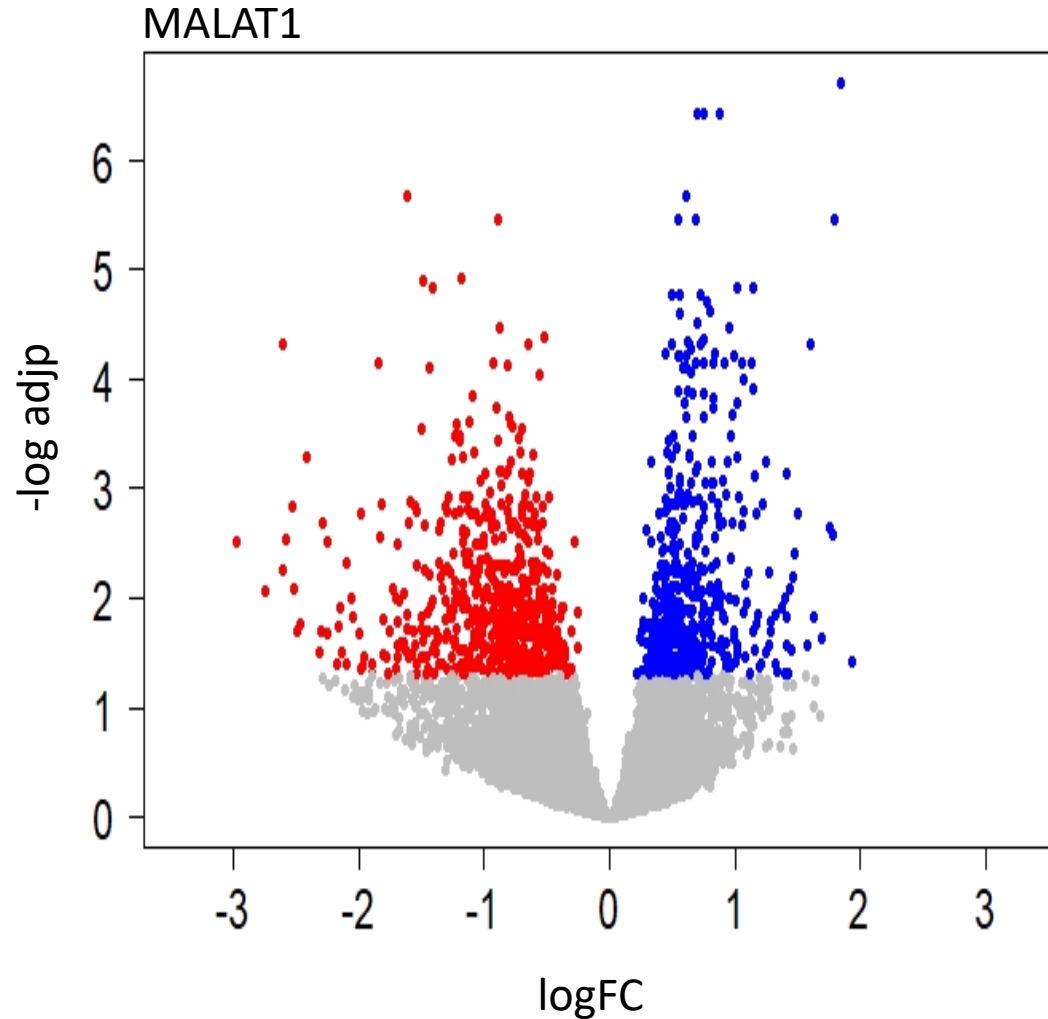
# Functional CRISPRi machinery



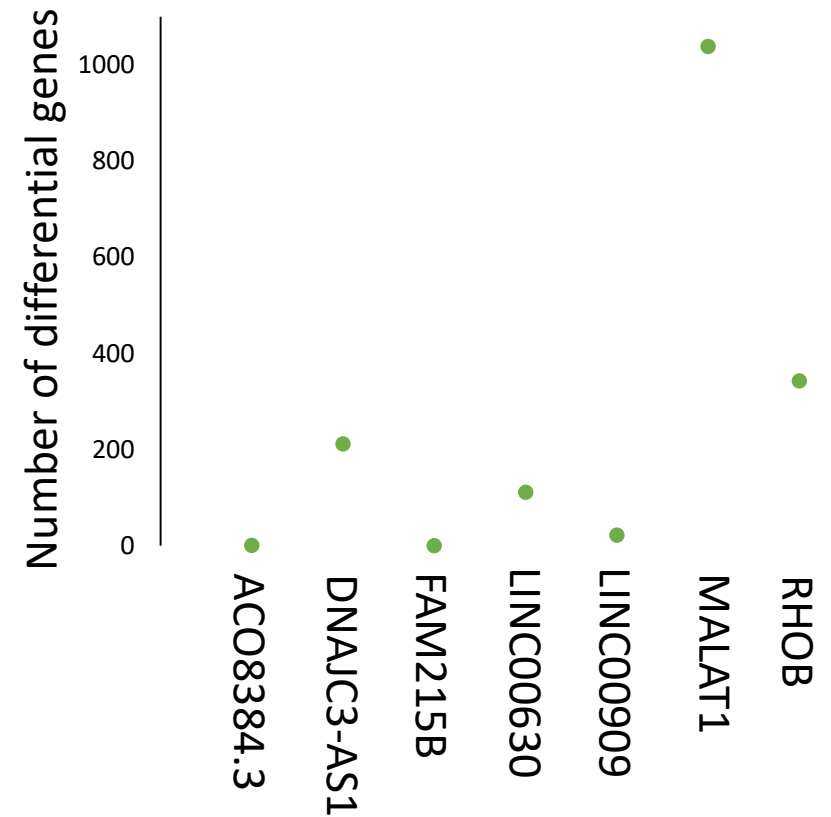
# Cellular read-out: Real-time monitoring reveals that lncRNAs affect proliferation and/or apoptosis



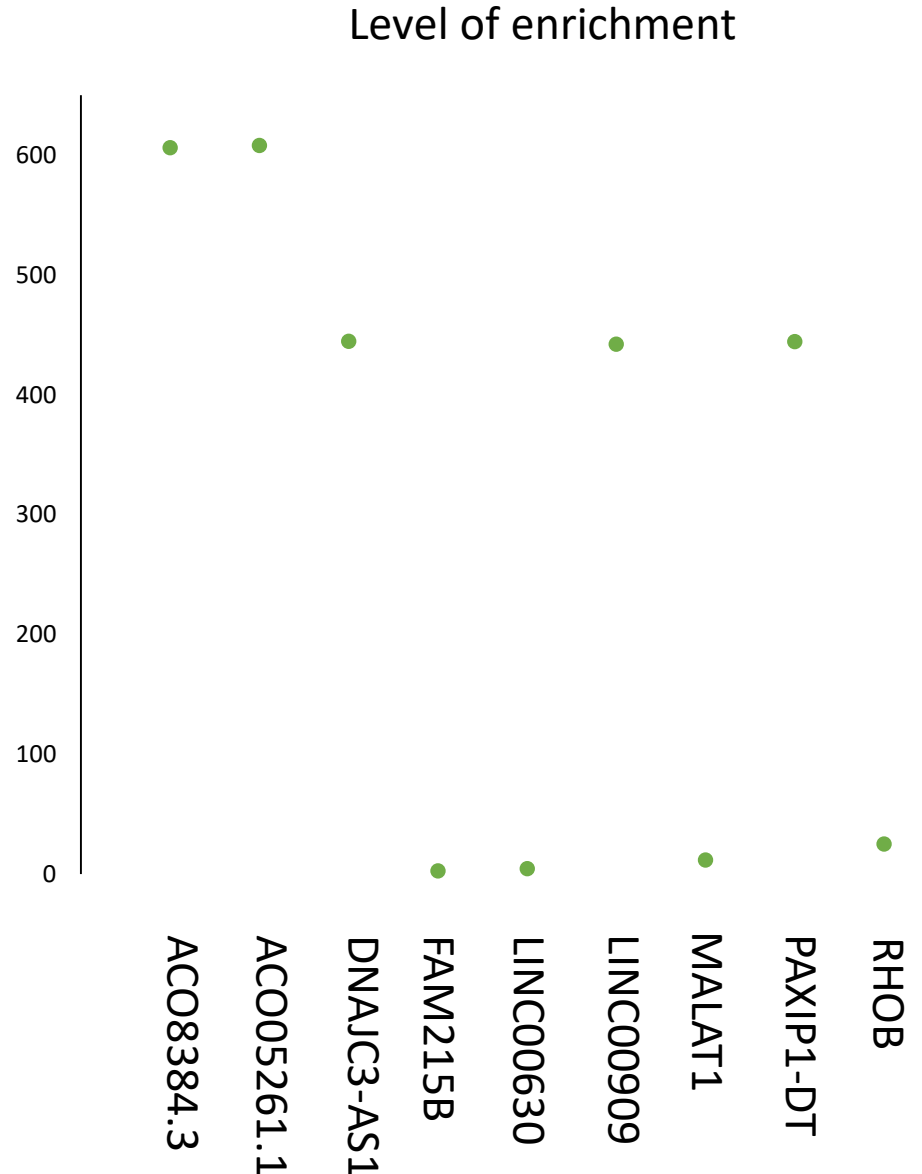
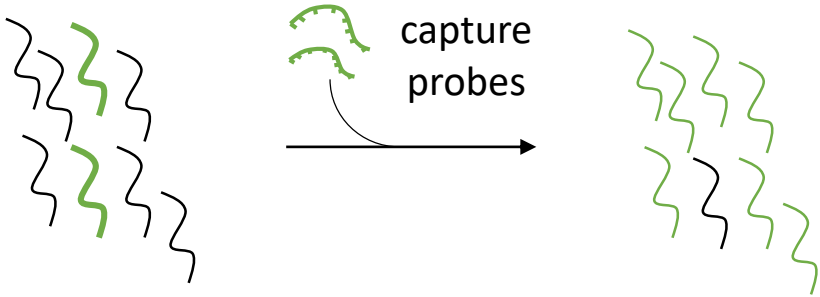
# Molecular read-out: Differentially expressed genes were detected after KD



- 1038 differentially expressed genes with MALAT1 KD
- 12 significantly affected gene sets with MALAT1 KD (GSEA analysis)



# Using capture sequencing to detect KD of lncRNAs



# What is next?

- Plan to screen for an extra 144 targets in HEK293T
- What with difficult to transfect cell lines
  - High-throughput lentiviral gRNA production
- Not limited to study lncRNAs
- Set-up an arrayed screen in different cell lines
  - Respiratory diseases
  - Immuno-ocology
  - ....



# Acknowledgement

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