A HIGH-THROUGHPUT CRISPRi SCREENING PLATFORM TO UNRAVEL FUNCTIONAL LONG NON-CODING RNAS

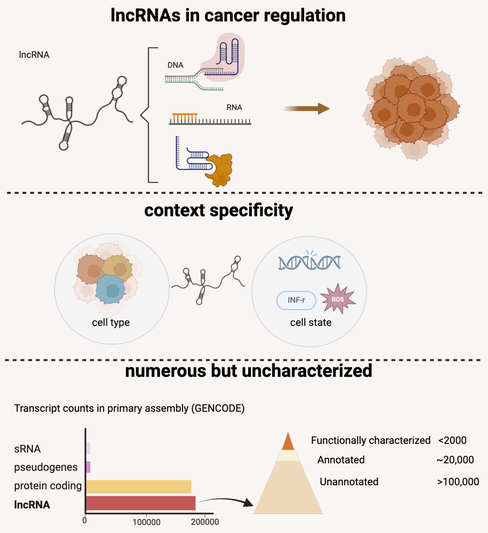
Xin Wang[1,2], Eric J. de Bony[1,2], Wim Trypsteen [1,2], Ramiro Martinez[1,2], Tim Van De Looverbosch[3], Winnok De Vos[3], Pieter Mestdagh[1,2]

[1] OncoRNALab, Center for Medical Genetics (CMGG), Ghent University, Ghent, Belgium,
[2] Cancer Research Institute Ghent (CRIG), Ghent University, Ghent, Belgium,
[3] Laboratory of Cell Biology and Histology, Department of Veterinary Sciences and Health Sciences, University of Antwerp, Antwerp, Belgium

Connect with me:
LinkedIn (Scan QR Code)
xinxwang.wang@ugent.be

GHENT UNIVERSITY

INTRODUCTION

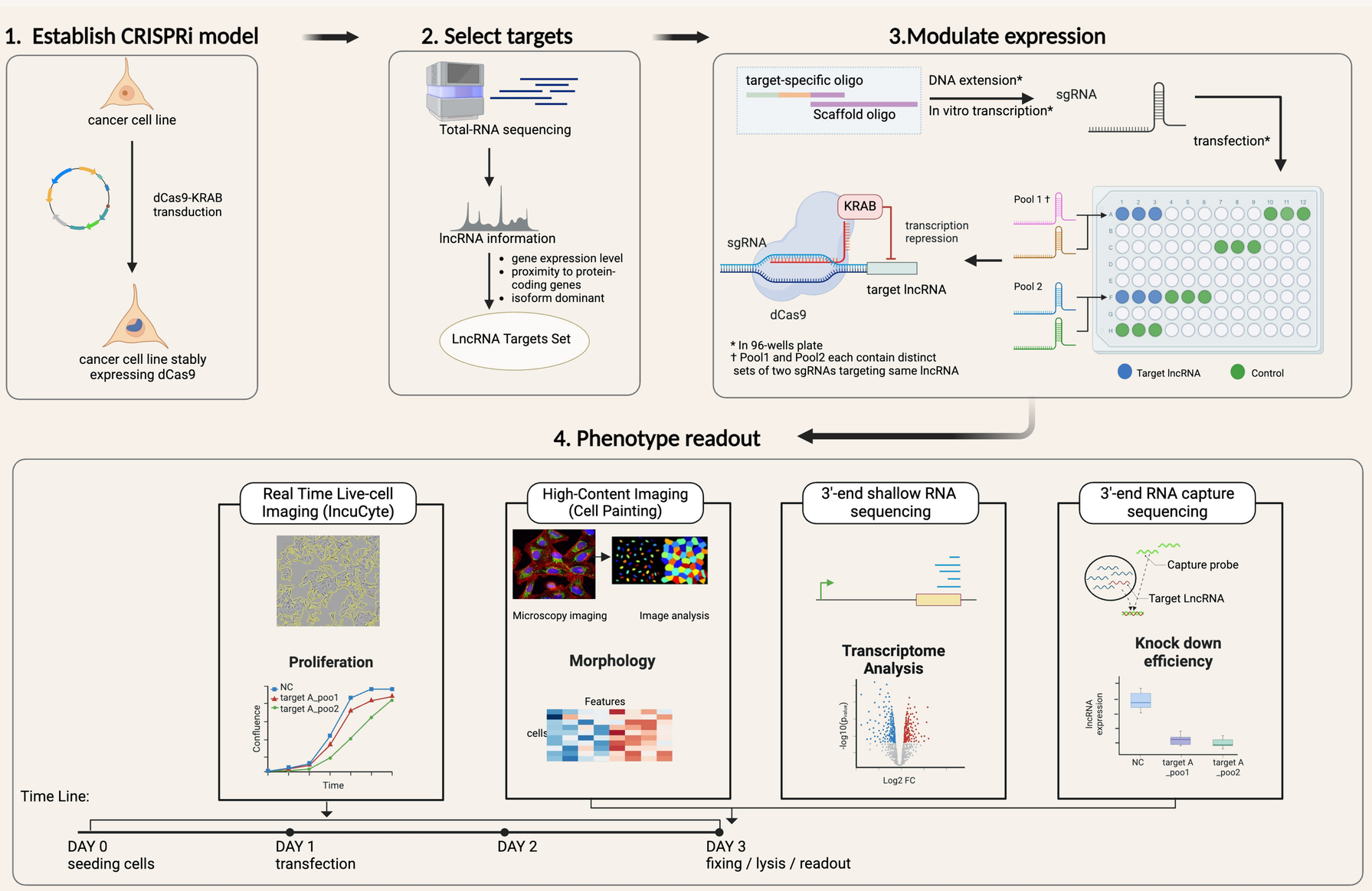


- Despite the critical roles of long noncoding RNAs (lncRNAs) in gene regulation and cancer, only a small fraction have been functionally annotated.
- The lack of efficient high-throughput screening further limits our understanding of their biological significance in cancer.
- Our work primarily focuses on developing a high-throughput CRISPRi screening platform to systematically identify functional lncRNAs in tumor biology.

OBJECTIVE

Develop a high-throughput CRISPRi screening platform that integrates CRISPRi-mediated silencing with multiple phenotypic readouts, enabling systematic identification of functional lncRNAs across diverse cell types and states in tumor biology.

METHODS

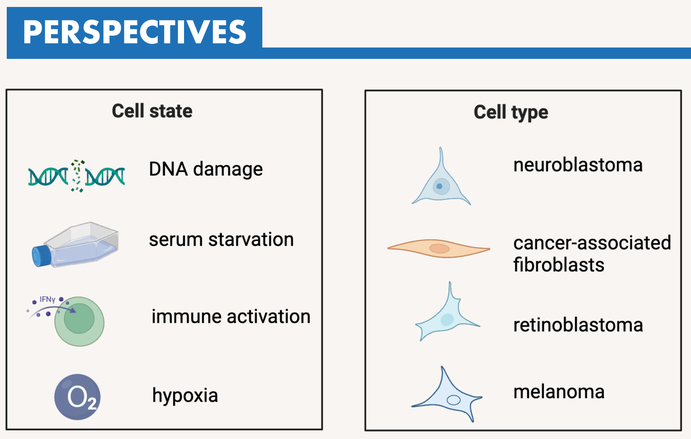


RESLUTS

Figure 1. **Differentially abundant genes upon lncRNA knockdown in the neuroblastoma cell line Kelly.**
Differential gene analysis from 3′ end shallow sequencing identified several potential target lncRNAs, with RP11-14O19.2 emerging as a particularly strong candidate in the neuroblastoma cell line Kelly.

Figure 2. **Molecular phenotype of lncRNA RP11-14O19.2 knockdown in Kelly cells.**
(A) Volcano plot displaying the differentially expressed genes following RP11-14O19.2 knockdown.
(B) Bubble plot depicting Gene Set Enrichment Analysis (GSEA) results, showing that the significantly enriched gene sets are predominantly associated with translation-related processes.

PERSPECTIVES



- We aim to expand this high-throughput CRISPRi screening platform by integrating diverse perturbations to systematically explore functional lncRNAs across various cellular contexts.
- In addition, we plan to apply the platform to a broader range of cancer cell models to uncover novel lncRNAs.

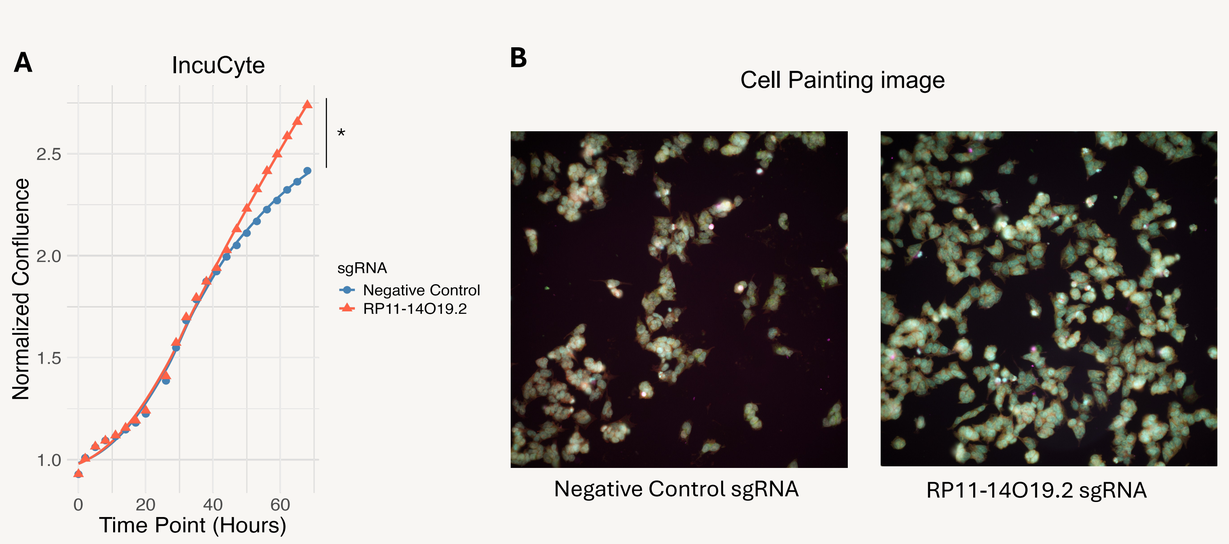


Figure 3. **Cellular phenotype o of lncRNA RP11-14O19.2 knockdown in Kelly cells.**
(A) Cell confluence measured by IncuCyte live-cell imaging over time, showing enhanced proliferation following RP11-14O19.2 knockdown ($p < 0.05$).
(B) Representative Cell Painting images from control and RP11-14O19.2 knockdown conditions, showing visual differences in cell morphology and density.

CONCLUSION

- We developed a high-throughput CRISPRi platform that enables efficient knockdown of lncRNAs and integrates diverse phenotypic readouts—including cell proliferation, transcriptomics, and morphology—to systematically identify functional lncRNAs.
- The platform demonstrates compatibility with various cancer cell lines and cellular states, enabling the exploration of context-specific lncRNA functions.
- This integrative approach lays a foundation for constructing functional models of lncRNA regulation, advancing our understanding of their roles in tumor biology.

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