Direct Fibroblast Reprograming with CRISPRa

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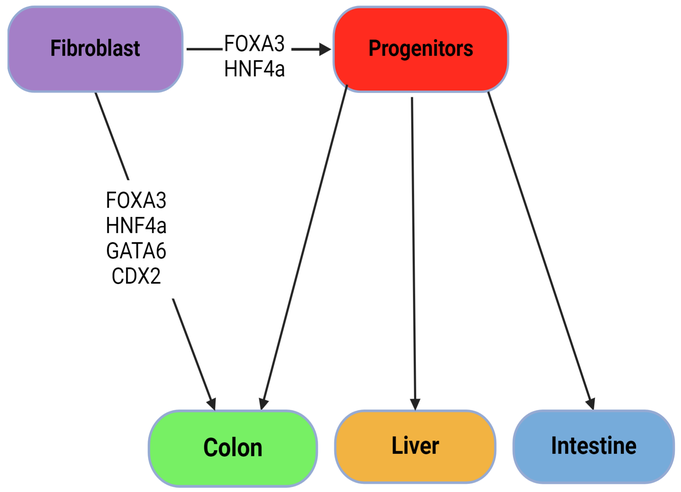
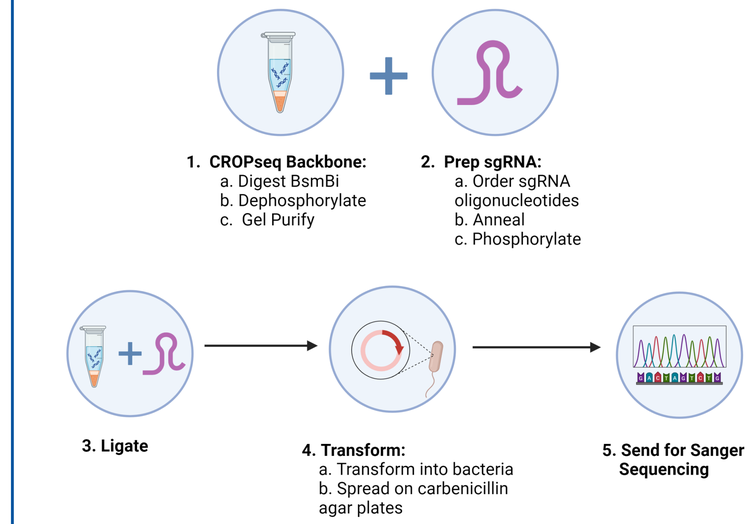
Introduction

- **Fibroblast** (connective tissue cells) **reprogramming** has previously been done by inserting the whole gene with lentivirus, which is time-consuming and inefficient due to the complex imperfect process of introducing entirely new genetic material [1] [2].
- **CRISPR activation** (CRISPRa) will be able to reprogram fibroblast faster and cheaper by simply overexpressing existing genes [3].
- **Single guide RNAs** (sgRNAs) direct the CRISPRa system to genomic locations which then uses a **dead Cas9 protein** to activate (but not cut) **transcription factors** such as FOXA3 and HNF4a, driving the conversion of fibroblasts cells into progenitor cells [4].
- With the addition of transcription factors GATA6 and CDX2, **the progenitor cells develop into colon organoids**, which can then be studied and safely tested on [5].
- The primary objective of this research is to create sgRNA plasmids that target the transcription factors to facilitate the conversion process [6].



Methods: Cloning sgRNA Plasmids

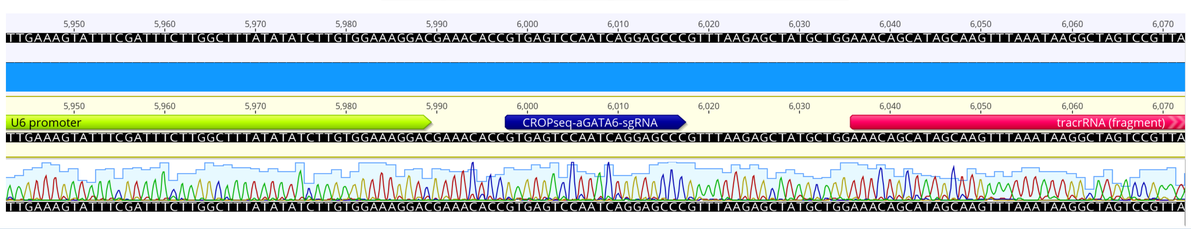


Results

While the Sanger sequences for GATA6 and CDX2 were successful, the Sanger sequence for HNF4a and FOXA3 were not. This is probably due to a contaminated insert.

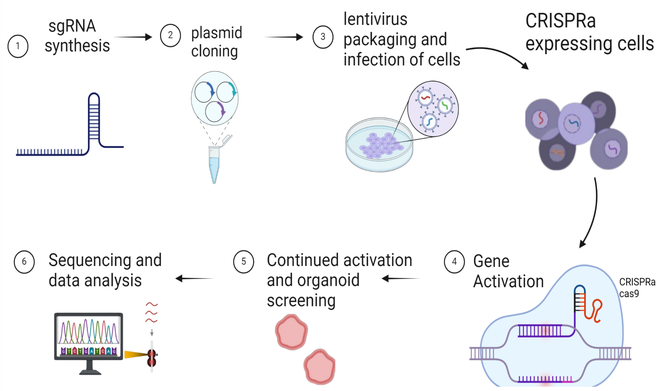
	Expected DNA Sequence	Actual DNA Sequence *N=Unidentified Base
GATA6	GTGAGTCCAATCAGGAGCCC	GTGAGTCCAATCAGGAGCCC
CDX2	AATGCAAATTATGTTTCGAG	AATGCAAATTATGTTTCGAG
FOXA3	GTCTCCTGGCGATCCCGCAG	CACTCGGCCCTCCNGTGTTG
HNF4a	GCCCAGCCTATCCACCGGCG	CC--CCNGCCCTNCNCCGG

Example of Sanger Sequence Results (GATA6)



Future Work

Next steps are to repeat the FOXA3 and HNF4a sgRNA plasmid cloning and continue with future plans.



Further work will harness CRISPRa libraries to determine which transcription factors can develop into other organoids for disease research and therapeutic applications.

References

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