Investigating the contribution of DNA looping in proto-oncogene expression in blood malignancies

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1 Background and Aims

Background

• Haematological cancers begin in blood forming tissues such as the bone marrow or immune cells e.g. white blood cells.

• In some blood cancers a genomic rearrangement occurs and a super-enhancer from one gene moves location and switches on a different gene that promotes cancer progression(1).

• This process has recently been identified as a possible drug target to treat cancers containing this rearrangement.

Aims

• Use a technique called CRISPR-Cas9, which allows genome editing, to disrupt an interaction between the gene CCND1 and the super-enhancer, then observe the effect on cancer cells.

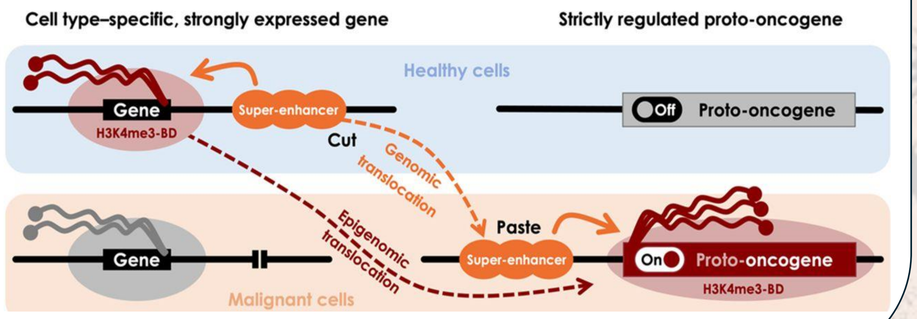
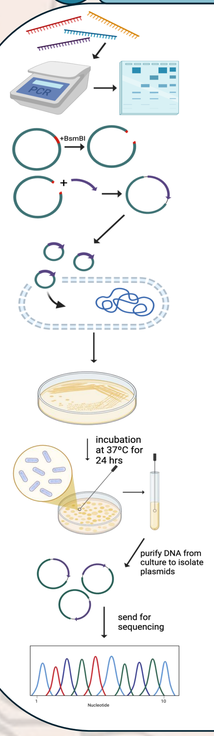


Figure 1: Image shows how the translocation leads to cancer(1)

2 Methods



1. Designed a set of guide RNAs that bind to CCND1. Optimised and validated the primers and guides using polymerase chain reaction (PCR) and gel electrophoresis.

2. Once a set of guides validated, cloned the guides into a LentiV2 plasmid cut open using the BsmBI enzyme.

3. Transformed bacteria to contain the plasmid, spread on an agar plate and incubated overnight.

4. Transferred colonies into liquid LB and then isolated the plasmid DNA to be sent for sequencing.

CRISPR-Cas9

Clustered
Regulatory
Interspaced
Short
Palindromic
Repeats

• CRISPR-Cas9 is a modern gene-editing tool adapted from a bacterial immune system.

• In gene editing, we use guide RNAs to target specific DNA sequences, and the enzyme Cas9 to make precise cuts, like genetic scissors.

• The cell's DNA repair mechanisms then allow for genome modification at the cut site.

3 Results

Guide Validation

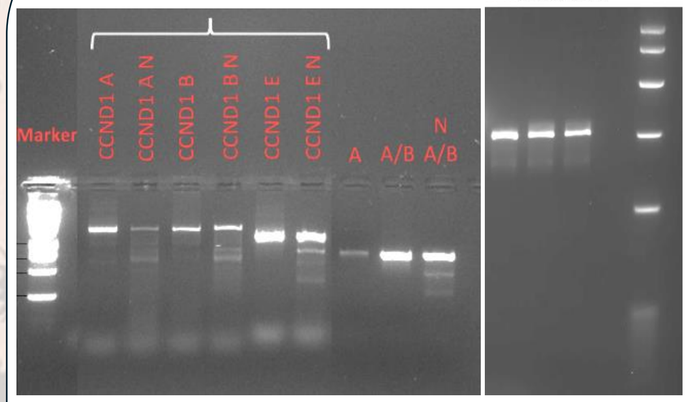


Figure 2: Guide validation was done to confirm the guides were binding a single target. In the left image, the CCND1E guide shows successful validation with 3 bands when nucleated (N) and 1 when not. The right image shows that guides CCND1A and CCND1B also validated using a different method, indicated by 2 bands when nucleated. A/B and HPRT act as controls.

Plasmid Sequencing

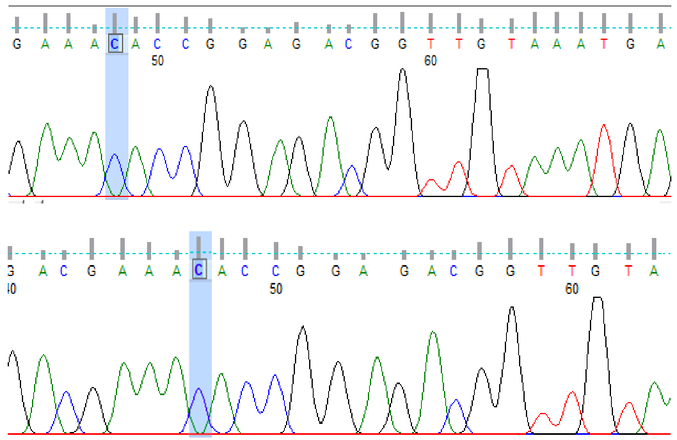


Figure 3: After cloning a guide into the plasmid, sequencing revealed the original plasmid sequence at the highlighted position C, indicating the guide sequence was not successfully inserted.

4 Conclusions

• The first PCR guide validation confirmed CCND1E as a suitable CRISPR-Cas9 guide.

• A second validation using an alternate method confirmed CCND1A and CCND1B as additional viable guides for CRISPR.

• Sequencing post-cloning revealed that the guide sequence did not replace the original plasmid's sequence as expected.

Next Steps

• Next steps involve repeating cloning and bacterial transformation, possibly with protocol adjustments.

• One recurring issue encountered in the project was a low yield of plasmid DNA. However, the team has since implemented a new kit that achieves better results for future work.

• Once cloning succeeds, the plasmid yield will be increased by growing the transformed bacteria.

• The lentivirus will then be modified to carry the plasmid with the guide and Cas9, enabling myeloma cell transduction. The cells can then be used to assess any changes in CCND1 expression.

5 Acknowledgements

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6 References

(1) Mikulasova et al, Genome Research, 2022.

(2) Images in methods section produced using Biorender.