Investigating the role of *RIPPLY3* in childhood B-cell acute lymphoblastic leukaemia (B-ALL)

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Introduction

- B-ALL is a type of blood cancer that starts from the white blood cells in the bone marrow and causes the accumulation of B lymphoblasts which disarms our immune system.
- There is a genetic subtype of B-ALL characterized by aneuploidy which refers to the gain or loss of whole chromosomes and can lead to relapse.
- Chromosome 21 is the most common gained chromosome in B-ALL.
- *RIPPLY3* is a gene in chromosome 21 that is suspected to be involved in promoting leukemia.

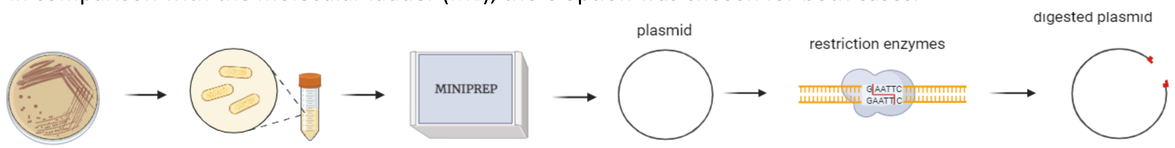
Aims

- Design and construct reagents for cloning the *RIPPLY3* genomic region using the natural Transformation Associated Recombination (TAR) machinery of yeast.

Results and Methods

- Different hooks were amplified from genomic DNA by Polymerase Chain Reaction (PCR) and analysed by gel electrophoresis which uses an agarose matrix and an electrical field to separate DNA fragments by size.

Figure 1 and 2. Gel electrophoresis images for the 3' and 5' hook. The expected size for 3' C was 158 base pairs (bp) and for 5' C 98 bp. In comparison with the molecular ladder (ML), the C option was chosen for both cases.



- A167 plasmid DNA was purified from *E.coli* with a Qiagen miniprep kit after picking individual colonies from the agar plates and growing them in liquid cultures under ampicillin selection.
- The plasmid and 3' hook were digested using restriction enzymes.

- The linearised plasmid was isolated by gel electrophoresis and was then extracted from the gel.
- The 3' hook was then cloned onto the linearised plasmid in T7 DNA ligation reactions, performed with different ratios of hook:plasmid.
- The cloned plasmids were transformed into competent *E.coli* DH10B cells which were then grown on ampicillin agar plates and screened using PCR amplification and gel electrophoresis.

References

1. Lee NCO, Petrov NS, Larionov V, Kouprina N. Assembly of Multiple Full-Size Genes or Genomic DNA Fragments on Human Artificial Chromosomes Using the Iterative Integration System. Curr Protoc. 2021; 1(12):e316, 2. Lee NCO, Petrov NS, Larionov V, Kouprina N. Assembly of Multiple Full-Size Genes or Genomic DNA Fragments on Human Artificial Chromosomes Using the Iterative Integration System. Curr Protoc. 2021;1(12):e316, 3. Haas OA, Borkhardt A, Hyperdiploidy: the longest known, most prevalent, and most enigmatic form of acute lymphoblastic leukemia in children. Leukemia. 2022;**36** 2769–2783

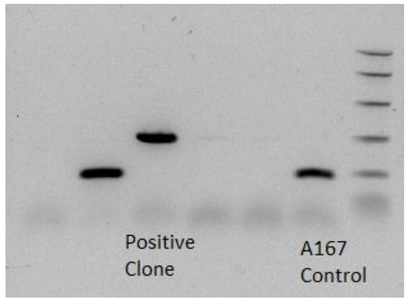


Figure 3; Gel image of PCR products amplified from A167 after cloning, using primers that flank the 3' hook insertion site. By comparison with the un-cloned control plasmid, one clone has a larger product indicating that the 3' hook has been successfully cloned into the linearised plasmid.

sgRNA targets and High Molecular Weight genomic DNA preparation

- High Molecular Weight (HMW) genomic DNA was purified from 2 million B lymphoblastoids.
- sgRNA templates and targets were designed using UCSC, PRIMER3 and the snapgene bioinformatics web tools
- The sgRNAs were synthesised using in-vitro transcription and tested for cleavage efficiency in CRISPR-Cas9 reactions using target sequences amplified from genomic DNA by PCR.
- By adding the sgRNAs to the prepared HMW genomic DNA, cuts are introduced around the genomic region that *RIPPLY3* is located in.
- This region would later be recombined with the linearised plasmid, that has both hooks attached to it, in yeast spheroblasts using their natural TAR system.

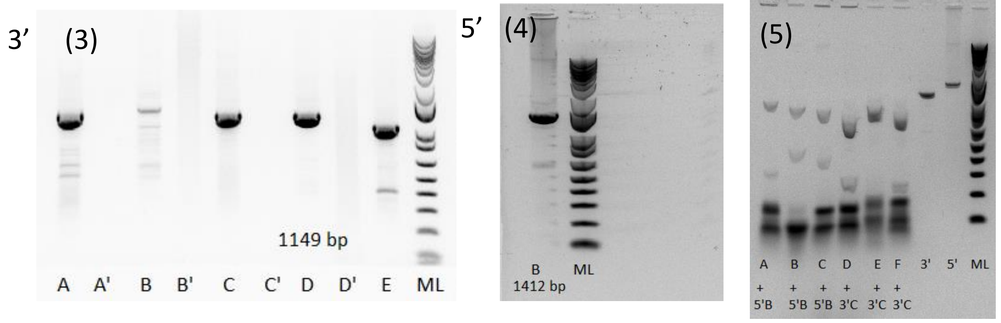


Figure 3,4 and 5: Gel electrophoresis images. The expected size for target 3'D was 1149 bp (Figure 3) and for 5'B 1412 bp (Figure 4). The sgRNAs are tested in CRISPR-Cas9 reactions with the chosen targets. There are two products in each case indicating the sgRNAs have successfully cut the targets and are ready to be used on genomic DNA (Figure 5).

Conclusion and Future steps

- In summary, the 3' hook for *RIPPLY3* was successfully cloned.
- In the future, the 5' hook will also be cloned and the next phase of the experiment which is the cloning of the *RIPPLY3* genomic region in yeast spheroblasts will be carried out.
- Once cloned, *RIPPLY3* will be transferred to the Human Artificial Chromosome (HAC) and ultimately expressed in blood cells that can be later on used for the generation of novel animal models that carry an array of identified oncogenes and can be used into research for new treatment for B-ALL

Scan to follow a step-by-step plasmid linearisation in the lab!

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