

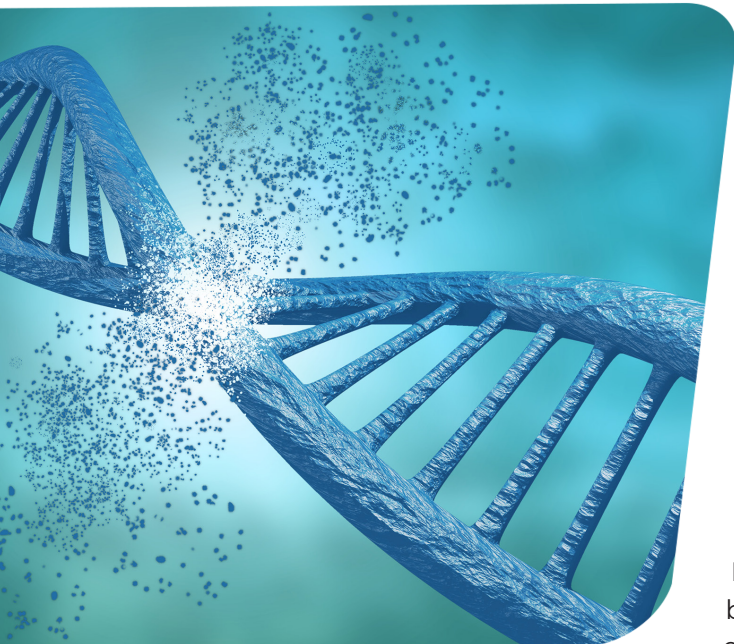
# Single-cell DNA Analysis of a Novel Acute Erythroid Leukemia (AEL) CRISPR Induced Mouse Model

## Abstract

Acute erythroid leukemia (AEL) is a rare, high-risk form of acute myeloid leukemia (AML) that has a distinct morphology and mutational spectrum. To better understand the biology of the mutations in the progression of AEL, mouse models of AEL were generated using a multiplexed genome editing approach of hematopoietic stem and progenitor cells (HSPCs). Single-cell DNA sequencing using the Tapestri Platform was used to analyze serially transplanted tumors for the precise genome edits induced by CRISPR/Cas9 and for the co-occurrence of secondary mutations. By combining this elegant CRISPR strategy with single-cell DNA sequencing, the investigators revealed the clonal architecture that led to the propagation and evolution of subsequent tumors. The Tapestri Platform is the only high-throughput system that can profile mouse DNA for SNVs and indels at the single-cell level. And now, with Tapestri Designer 2.0, a custom single-cell DNA mouse panel is easily designed to sequence any genomic DNA target across the entire mouse (mm10) or human (hg9) genome, a new feature that will aid researchers in identifying both on and off-target genome editing events.

## Takeaways

- A novel mouse model of AEL was generated using a multiplex CRISPR gene editing strategy
- Tapestri Designer 2.0 enables the design of a large panel of mouse genes to determine CRISPR induced and spontaneous secondary mutations in single cells
- Single-cell data revealed the clonal architecture of the fittest clones that led to propagation in subsequent tumors



## Materials & Methods

Six lentiviral pools of RFP-gRNAs targeting combinations of genes found to be mutated in AEL (*Trp53*, *Tet2*, *Dnmt3a*, *Asx1l*, *Rb1*, *Stag2*, *Ezh2*, *Nfix*, *Bcor*, *Ppm1d*) were used to infect HSPCs from Cas9-GFP mice, and then transplanted into recipient mice. Analyzing one of the resultant mouse models of AEL, primary tumors with edits in *Trp53* and *Dnmt3a* were serially transplanted into secondary, tertiary, and quaternary mice. Leukemia cells from each transplanted mouse were analyzed with the Tapestri Platform using a custom DNA panel of 30 mouse genes with 75 amplicons directed at CRISPR targeted DNA edit sites and secondary AEL genes of interest. The CRISPR/mouse single-cell DNA custom panel was designed using Mission Bio's White Glove Service, which is now replaced by the newly released [Tapestri Designer 2.0 software](#) that enables whole genome coverage for both mouse and human.

## Results

### Generation of leukemia mouse models using multiplex CRISPR/Cas9

Based on previously published genomic data in AEL<sup>1</sup> (Figure 1a) and to examine the role and cooperativity of the different alterations in leukemogenesis, Dr. Ilaria Iacobucci from Dr. Mullighan’s lab at St. Jude Children’s Research Hospital used multiplex CRISPR/Cas9-based genome editing to generate tumors in mouse models (Figure 1b). Six different lentiviral pools of guide RNAs (gRNAs) targeting key mutated genes in human AEL were generated and used to transduce Cas9-eGFP-mouse HSPCs. Guide RNAs were designed to generate loss-of-function mutations and edited cells were transplanted into mice and analyzed for tumor development.

Looking more closely at one of the six pools of gRNAs that targeted the epigenetic and tumor suppression genes, *Trp53*, *Bcor* and *Dnmt3a*, two models of AEL emerged. These results illustrate that mouse models of leukemias were reliably generated using an informed strategy of CRISPR-Cas9 induced co-occurring mutations in HSPCs. Subsequent single-cell DNA sequencing experiments in one

of the AEL mouse models (Figure 2 shown in the red box) with mutations in *Trp53* and *Dnmt3a* in the primary tumor, enabled the dissection of clonal heterogeneity and tumor evolution in serial passages in mice.

### Single-cell DNA sequencing of AEL mouse model reveals genetic basis for tumor evolution

The evolution of tumors often involves the accumulation of mutations that lead to better clonal survival. To investigate whether this evolution is driven by specific genetic alterations or combinations thereof, the researchers turned to single-cell DNA sequencing of serially transplanted tumors. To determine which mutations were present in the primary tumor, and tumors that evolved through serial transplantation, a single-cell sequencing amplicon panel of 30 mouse genes was designed. Genes were selected based on the genomic locations targeted by CRISPR/Cas9 and recurrent sites of acquired somatic mutations (Figure 3a). Single-cell DNA sequencing not only revealed spontaneously occurring mutations at the clonal level, but also validated edits generated through CRISPR-Cas9 directly from the DNA.

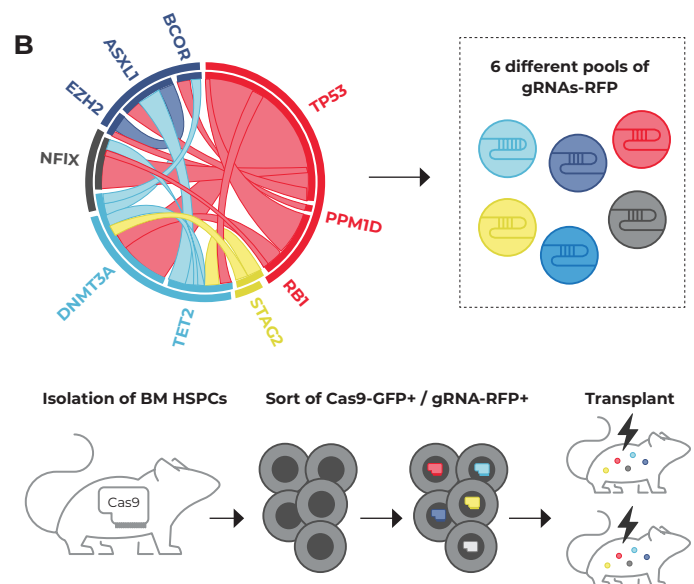
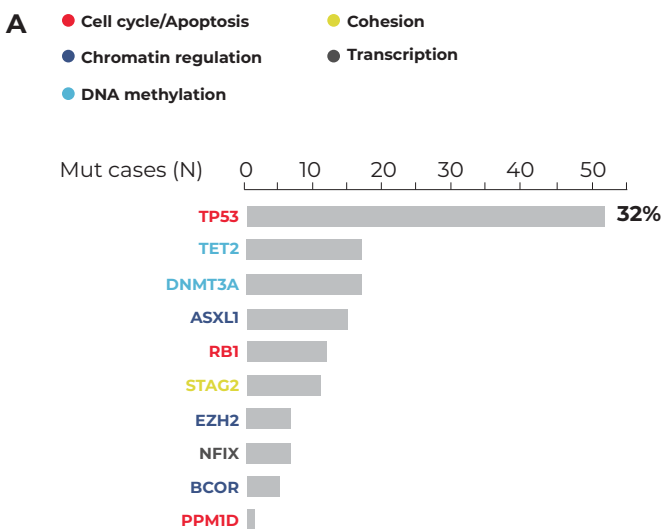


Figure 1 - Frequency of commonly mutated genes in human AEL (A) and pattern of co-occurrence that was used to inform various combinations of RFP-gRNAs for multiplex gene editing of Cas9-GFP mouse HSPCs using CRISPR-Cas-9 (B-upper). Edited HSPCs were sorted for RFP and GFP and then transplanted into mice for subsequent analysis of tumors (B-lower).

Typically, cells containing reporters attached to gRNA and Cas9, in this case RFP and GFP, are assumed to contain both the gRNA and Cas9. However, this alone does not verify that the cells are edited for the desired mutation. In addition, there were many different mutations that were induced through genome editing with the gRNA targeting *Trp53* (Figure 3b). These results highlight the power of using targeted single-cell DNA sequencing to directly confirm the edits that are made to the DNA using genome editing strategies for thousands of single cells.

The primary tumor from the AEL model was serially transplanted into secondary, tertiary, and quaternary mice. Tumor samples were collected at each timepoint and analyzed by single-cell DNA sequencing on the TapeStri Platform (Figure 4a). The primary tumor was found to have two clonal populations, one with mutations in *Trp53*, *Dnmt3a*, and *Kif1a* (Figure 4b, lime clone) and the other that acquired an additional mutation in a fourth gene, *Ptpn11* (Figure 4b, blue clone). In the secondary tumor, the later clone gained mutations in *Kit*, *Fancd2*, and *Nudt18*, which persisted as the dominant clone in the tertiary and quaternary tumors. Single-cell DNA-Seq revealed the precise genetic basis for the phenotypic evolution of this mouse model of AEL, confirm the genome edits that were made directly from the DNA, and show that primary and secondary mutations in multiple genes co-occurred within the same single cells.

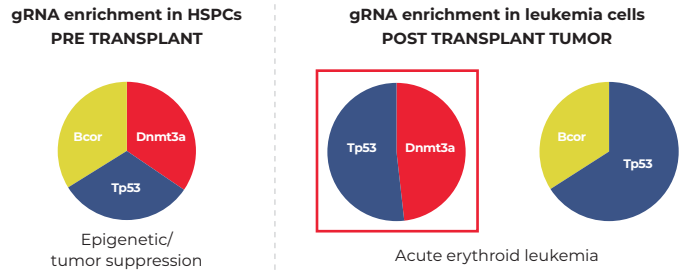


Figure 2 - One of the six pools of gRNAs targeting *Trp53*, *Bcor* and *Dnmt3a* was used to edit HSPCs that were subsequently transplanted into mice (left). Different AEL tumors were induced with different combinations of initiating mutations. The red box indicates the tumor used for serial transplantation expanded upon in Figure 4.

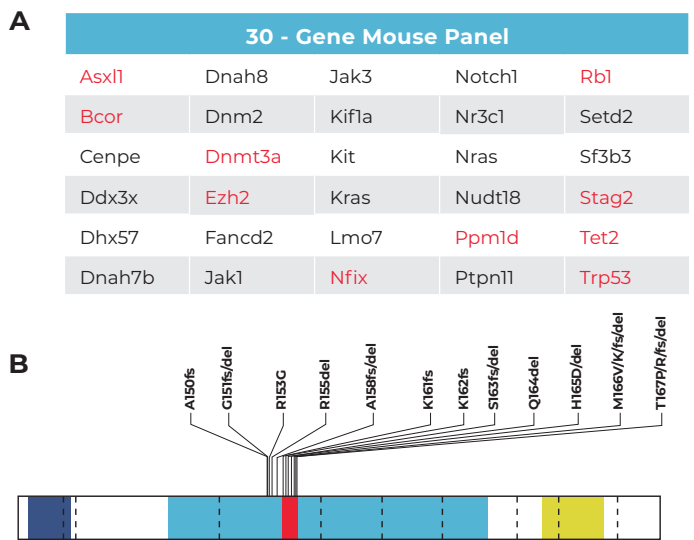


Figure 3 - Panel of amplicons designed to sequence 30 genes related to AEL. Red genes were mutated through CRISPR gene editing in the original mouse models and black genes are of interest for secondary mutations (A). *Trp53* mutations induced by CRISPR-Cas9 illustrates abundance of different mutations generated from the gRNA design shown by the red box (B).

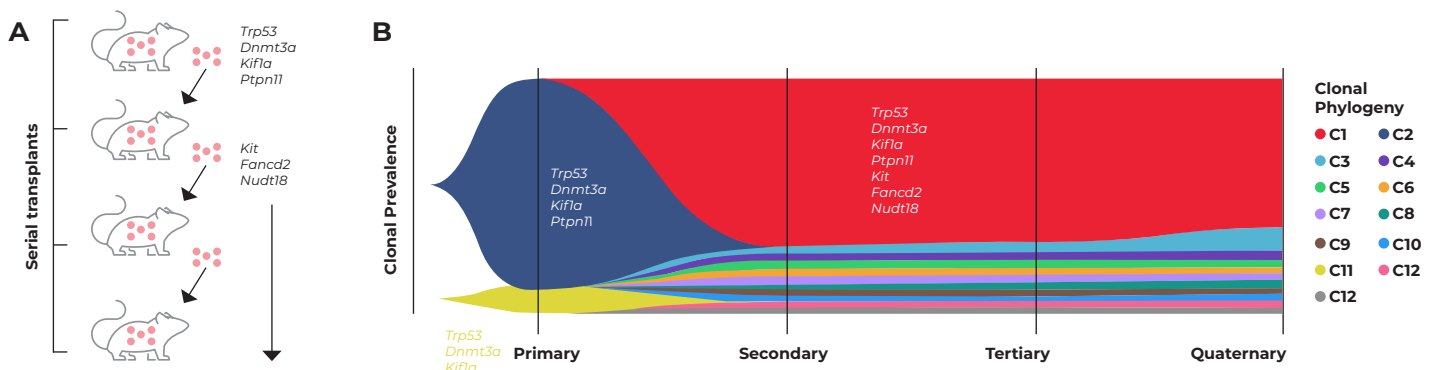


Figure 4 - The primary tumor induced through gene editing of *TP53* and *DNMT3A* was serially transplanted into secondary, tertiary, and quaternary mice (A). Single-cell DNA sequencing revealed that the primary tumor also had mutations in *KIF1A* and *PTPN11* giving rise to two clonal populations (B). Evolution within the secondary tumor gave rise to a dominant population with the addition of mutations in *FANCD2* and *NUDT18*, which persisted throughout the tertiary and quaternary tumors.

## Conclusion

Mouse models of AEL were successfully generated through multiplex CRISPR/Cas9 editing of genes found to be mutated in human AEL patients. Tumor evolution was precisely visualized through single-cell DNA sequencing, which enabled detection and quantification of many loss-of-function mutations in a large panel of genes, and also determined which mutations co-occur in the same cells to unravel the clonal architecture within each tumor. In addition, edits induced by multiplex CRISPR in the primary tumor were fully resolved at the DNA level and with single cell-

resolution and not inferred based on reporters or bulk sequencing. The Tapestri Platform is available for high-throughput single-cell DNA sequencing experiments across the entire mouse and human genomes, and targeted panels are designed in minutes using Tapestri Designer 2.0 software. These fast custom panel designs enable researchers to interrogate gene edits directly from the DNA for any genome editing system, and enable generation of animal models of disease as shown here and in a recent publication<sup>2</sup>, as well as support for the development and production of cell and gene therapies.

## References

1. Iacobucci, et. al., Modeling and targeting of erythroleukemia by hematopoietic genome editing, *Blood*, 2021.
2. Ten Hacken, et. al., High throughput single-cell detection of multiplex CRISPR-edited gene modifications, *Genome Biology*, 2020.