Rare CRISPR BCL-2 KO Cells Discovered Using Levitation Technology

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Abstract

The use of CRISPR/Cas9 is a key research tool for the study of gene function and role in cancer. In combination with magnetic levitation technology, the ability to enrich for rare genetically modified clones becomes achievable where otherwise impossible with standard techniques such as cell sorting. Previously shown, hypodiploid B cell acute lymphoblastic leukemic (B-ALL) cell lines, including the NALM-16 cell line, were highly sensitive to inhibition of BCL-2 by Venetoclax *in vitro*. However, inhibition of BCL-2 is not complete and residual activity remains present in some cells, leading to therapeutic resistance over time.

In this study using the LeviCell system, we enriched for surviving NALM-16 cells that had BCL-2 knocked out via CRISPR-mediated gene editing. We show here that dead or dying cells post CRISPR modification can mask the ability to effectively identify and isolate surviving clones. Without the use of the LeviCell to quickly and gently enrich these rare surviving cells, these clones would be unavailable for further study.

Levitation Technology Background

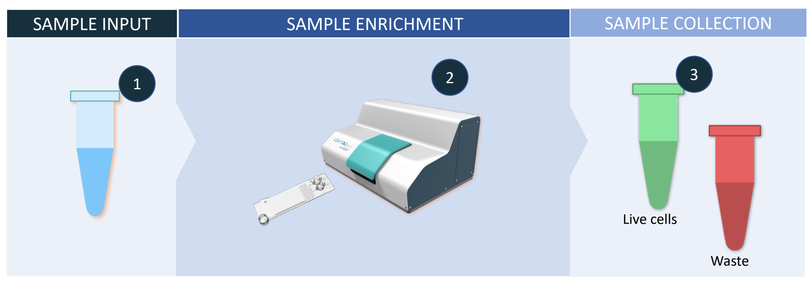


Figure 1:

Step 1: A sample consisting of a mixture of cells is introduced to the system through an inlet well.

Step 2: LeviCell technology separates live from dead cells and debris according to intrinsic cellular properties resulting in different levitation heights within the magnetic field (shown in more detail in Figure 2).

Cells of different viability equilibrate at different levitation heights. As the sample flows through the system, different cell types can be imaged, counted, and collected.

Step 3: Following final equilibration of the sample (5-20 minutes), cells transition to a bifurcated channel that collects viable cells in the top chamber for harvest and further study.

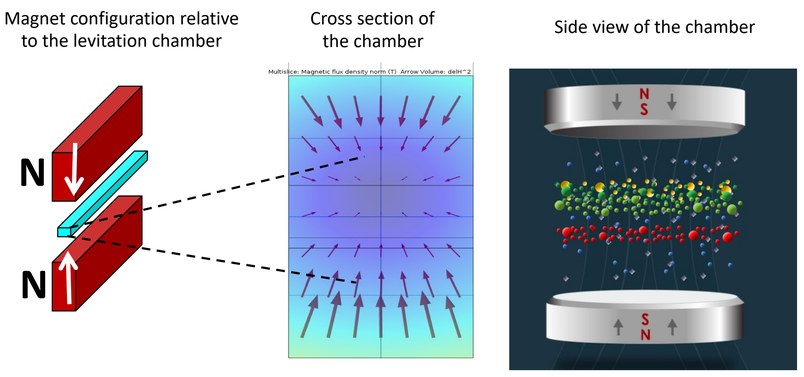


Figure 2: Within the cross-section of the levitation chamber, cells reach their final levitation height determined by a combination of cell buoyancy, magnetic forces created within a paramagnetic fluid, and gravity. The side view shows different cell populations at different levitation heights.

Benefits of the LeviCell System

Simple & Efficient

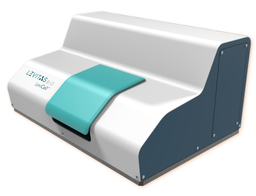
With Levitation technology, there are no complicated fluidics or preparation steps. Simply load the sample and LeviCell does the rest.

Undamaged Cells

Unlike all other methods, levitation is gentle on the cells and does not damage or affect them further.

Unlabeled

Remove bias from cell analysis by eliminating labels and dyes. Catch cells based on what they are, not what marker they express.



Live Cell Enrichment of NALM-16 Cell Line

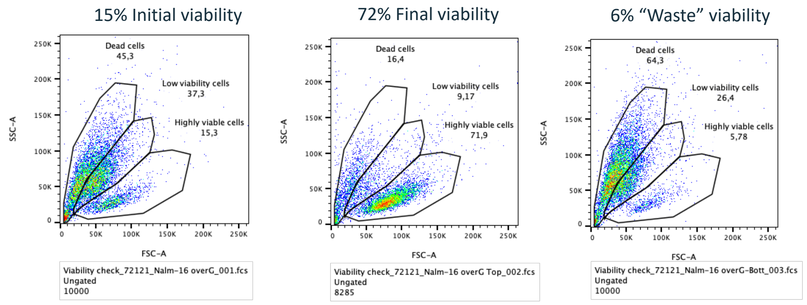
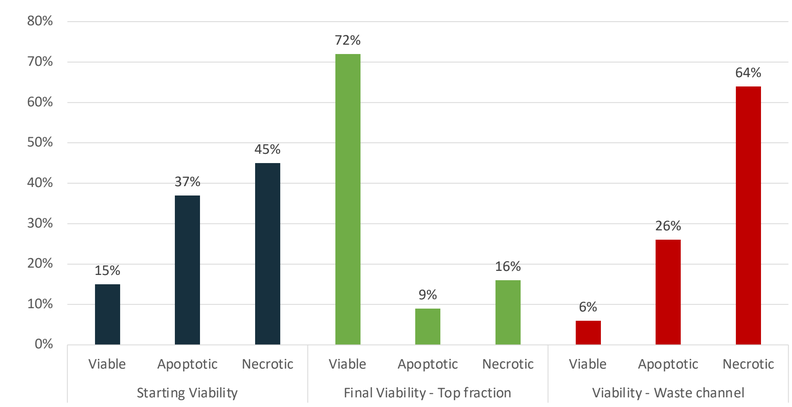


Figure 3: NALM-16 cell line had an initial viability of 15%. After running on the LeviCell, viability increased to 72% based on FSC x SSC stringent gating.



CRISPR Workflow & Analysis

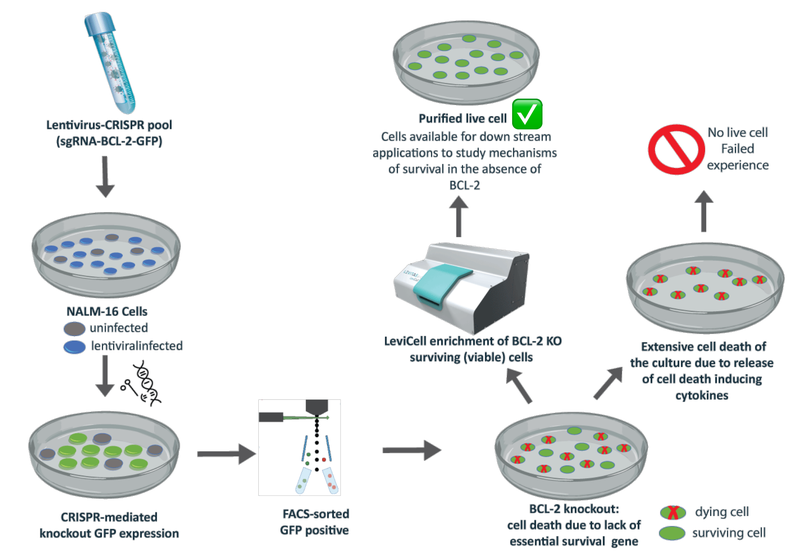


Figure 4. CRISPR Lentiviral library was used to transduce NALM-16 cells. Upon successful transduction, cells express a GFP reporter gene. BCL-2 gene knock-out may or may not result in cell death. Cells were then analyzed via flow cytometry for GFP to confirm transduction and viability.

CRISPR Transduced Marker Selection

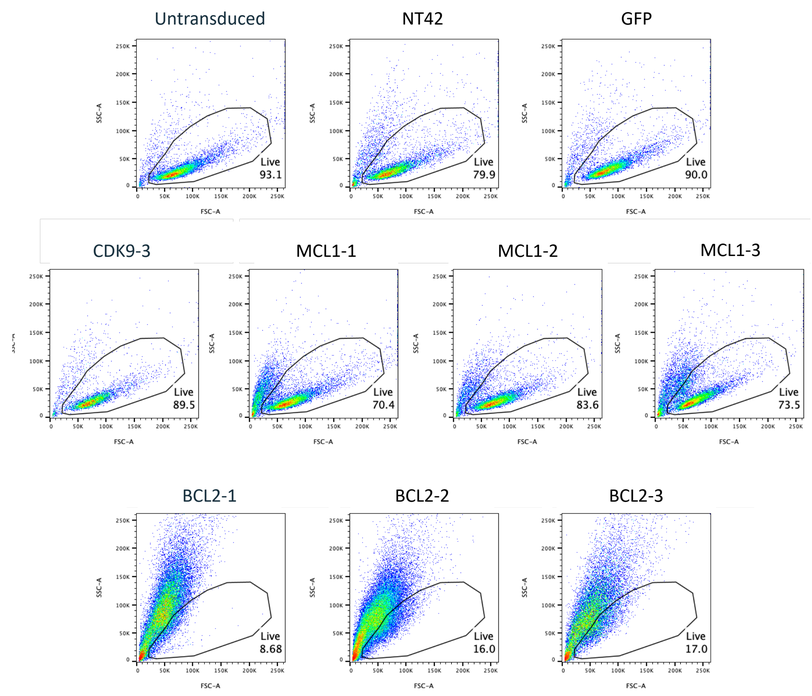
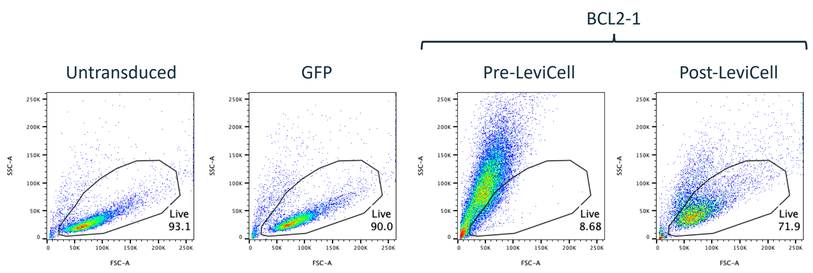
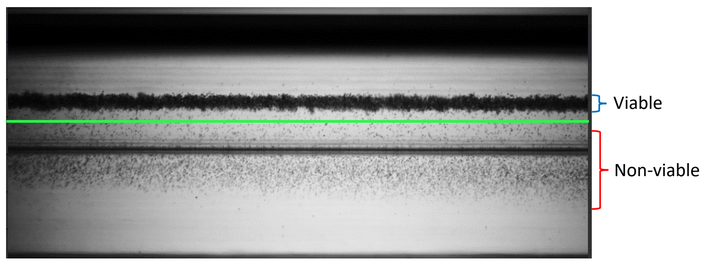


Figure 5: NALM-16 cells were CRISPR transduced knocking out several candidate genes and using a GFP reporter as confirmation of successful transduction. After Day 2, cells were analyzed via flow cytometry to assess cell survival. Untransduced NALM-16 cells were run as a gating control. Knock-out of CDK9 or MCL1 had minor or moderate effects, respectively, on cell survival whereas BCL-2 knock-out resulted in high levels of cell death.

CRISPR-transduced BCL-2 resistant clones recovered using LeviCell



48.7% Initial Viability. Input 750,000 total cells.



ViaCell Counts

LeviCell	Sample ID	Viability (%)
Input	BCL-2 Input	48.7
Top Fraction	BCL2-Viable	71
Waste	BCL2-Non-Viable	35

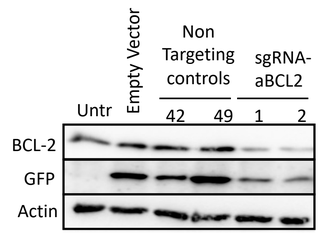


Figure 6: After viability check using flow cytometry, 2 of 3 CRISPR transduced BCL-2 samples were subsequently run on the LeviCell system to enrich for live cells (BCL2-1 shown). Analysis by flow cytometry indicated that the percentage of live cells was extremely low and obscured by the high prevalence of dead cells. Enrichment of live cells on the LeviCell allows further analysis of the rare BCL-2 KO cells. Western blot analysis was performed to analyze protein expression of the 2 CRISPR BCL-2 transduced samples and GFP plus Actin expression

Populations exhibiting differential levitation heights can be resolved

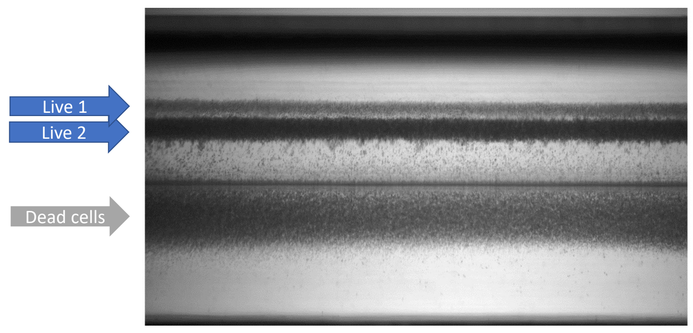


Figure 7: CRISPR transduced cells were run directly on LeviCell where two distinct live bands were identified after final equilibration. These two populations are distinct enough from each other that they can be serially retrieved independently and potentially reveal differences that may occur during the CRISPR transduction process or cell response post transduction. Further investigation of these cells are ongoing.

Conclusions

- Levitation technology provides a gentle and quick (5-20 min) method to isolate live cells post CRISPR transduction.
- Levitation technology can enrich low percentage populations of live cells otherwise unseen or difficult to isolate through other methods.
- Levitation technology quickly and gently removes the dead cell milieu at the earliest possible moment to prevent unnecessary cell death of rare, surviving cells.
- Levitation technology is a novel method that can reveal biological phenotypic differences not seen in standard methods.
- Levitation technology offers:
  - (A) Single instrument for a variety of applications
  - (B) Simple 3-step workflow with minimal hands-on time
  - (C) Enrichment with no expensive reagents or consumables
  - (D) Compatibility with small volumes and rare samples

References

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