RESEARCH CASE STUDY

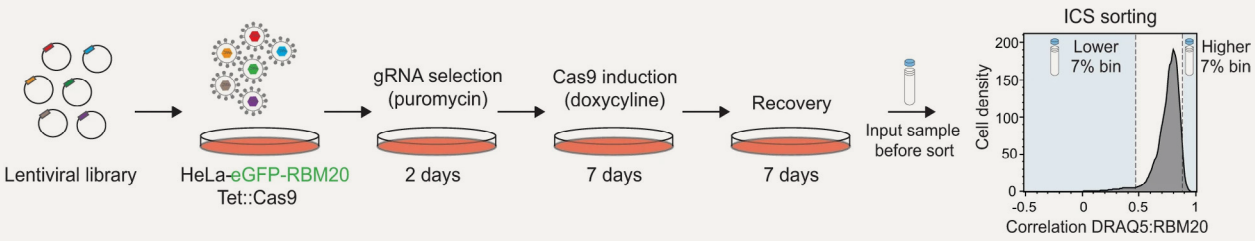
From CRISPR screens to drug discovery targets:

See how BD CellView™ Image Technology in the BD FACSDiscover™ Platform facilitates the discovery of targets in cardiomyopathy

SYNOPSIS: Dilated cardiomyopathy (DCM) is a heart muscle disease where the heart becomes enlarged and has difficulty pumping blood. Scientists have found that nearly half of DCM cases are genetic, with several gene variants linked to the disease. One such gene encodes the RNA-binding motif protein 20 (RBM20), which is involved in regulating alternative splicing. Normally, RBM20 localizes to the nucleus, but mutations in this gene can cause the protein to stay in the cytoplasm where it can form granules and worsen disease.

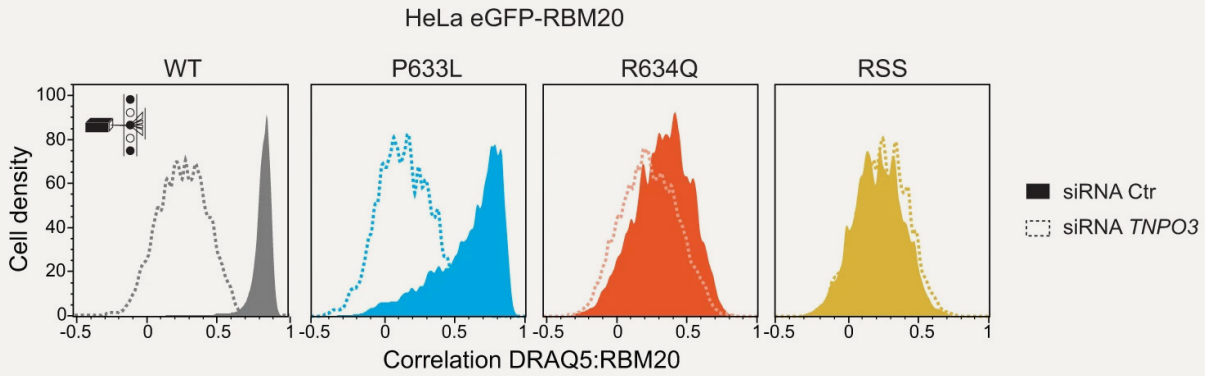
> Achieving genome-wide scale without the image-enabled cell sorting technology would have been very challenging, if at all possible.

IN A RECENT STUDY, researchers at the European Molecular Biology Laboratory (EMBL) used BD CellView™ Image Technology for image-enabled cell sorting (ICS) to combine genome-wide CRISPR screening, fluorescence imaging, and fluorescence activated cell sorting to capture patterns in protein mislocalization. The team screened 18,408 genes using a pooled CRISPR knockout library in HeLa reporter cells expressing eGFP-RBM20. Using BD CellView™ Image Technology, they could quantify nuclear RMB20 localization and sort cells based on localization to the nucleus vs. cytoplasm. By analyzing guide RNAs (gRNAs) after sorting, the team identified Transportin-3 (TNPO3) as a positive regulator for nuclear import. They found that normally TNPO3 interacts with RBM20, but mutations in RBM20 can prevent this from happening, resulting in cytoplasmic localization of RBM20. Lastly, the team validated this finding in cell and animal models by overexpressing TNPO3 to enhance the interaction between TNPO3 and RBM20. They showed that this overexpression can at least partially restore RMB20 nuclear localization. These results identified TNPO3 as a novel target for developing therapeutics for RMB20-based DCM.



The ICS screen workflow begins by introducing the fluorescently labeled RBM20 into HeLa cells followed by the CRISPR knockout screen. Cells with cytoplasmic RBM20 (lower and higher bin) and cells with nuclear localization (main peak in histogram) were recovered using image-enabled cell sorting to allow isolation of gRNAs and identification of potential therapeutic targets.

Image credit: Figure 3A. Kornienko et al. 2023. CC BY 4.0.



Different RBM20 variants have different nuclear localizations as demonstrated by the correlation in the fluorescence signal of DRAQ5 (nuclear stain) and GFP (tag expressed on RBM20). Wild-type RBM20 localized to the nucleus while inhibition with siRNA TNPO3 decreased its nuclear localization. Two mutants, P633L and R634Q, had reduced nuclear localization, which was further decreased with inhibition from siRNA TNPO3. The RSS mutant was fully cytoplasmic and its localization did not change with siRNA TNPO3 inhibition.

Image credit: Figure 4D. Kornienko et al. 2023. CC BY 4.0.

CONCLUSION: Image-based sorting enriches rare phenotypes that would have otherwise been missed using bulk methods or fluorescence intensity-only cytometry. In this study, BD CellView™ Image Technology allowed researchers to combine genome-wide CRISPR screening with image-based cell sorting to identify the mechanism behind protein mislocalization. Studies such as these enable researchers to discover new targets for drug discovery.

Publications: Kornienko, J., Rodríguez-Martínez, M., Fenzl, K. et al. Mislocalization of pathogenic RBM20 variants in dilated cardiomyopathy is caused by loss-of-interaction with Transportin-3. *Nat Commun* 14, 4312 (2023). https://doi.org/10.1038/s41467-023-39965-6