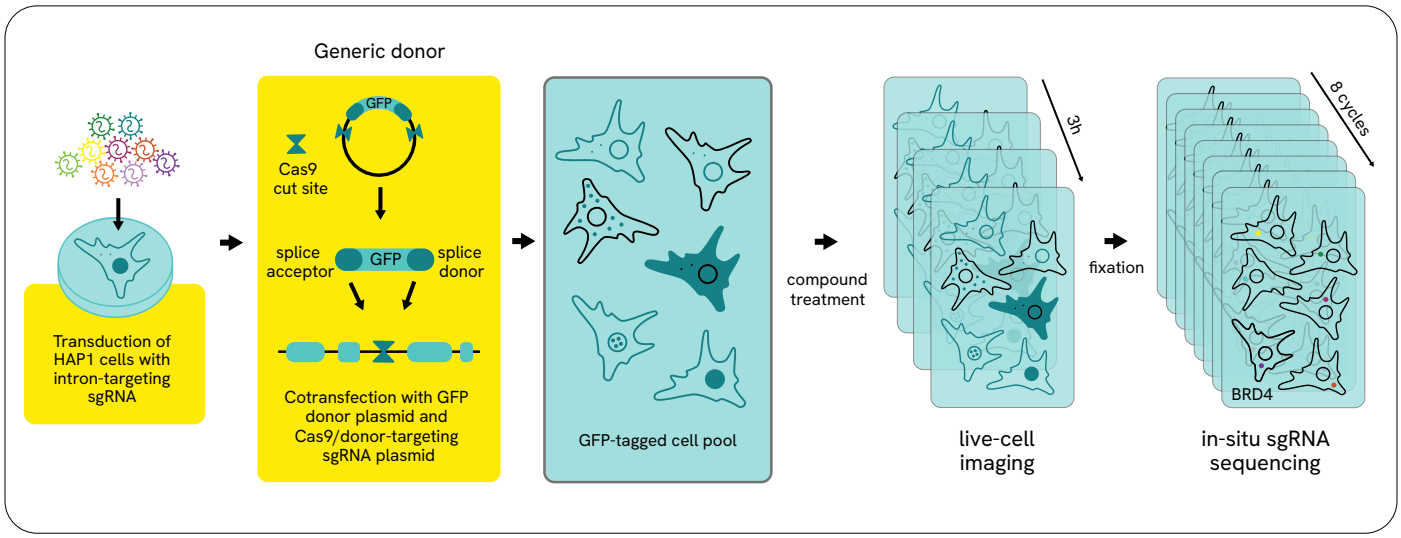
CASE STUDY

A scalable method to monitor protein levels and localizations in cells.

Pooled protein tagging, cellular imaging, and in-situ sequencing to identify cellular response to drug treatment

The levels and subcellular locations of proteins within a cell regulate important aspects of many cellular processes and can become targets of therapeutic intervention. Endogenous labeling of a protein with a fluorescent tag is a powerful technique for understanding protein function in a living cell through visualization by live-cell imaging. It can also help to characterize the effects in response to cellular perturbation. However, current techniques are limited by the number of proteins that can be studied and are not amenable to unbiased discovery approaches.

Researchers at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences have developed a scalable approach to study the level and localization of hundreds of proteins in parallel in response to different perturbations. Their method uses CRISPR-Cas9-based intron tagging to generate cell pools expressing hundreds of GFP-fusion proteins from their endogenous promoters. Protein localization changes can then be monitored by time-lapse microscopy followed by clone identification using in situ sequencing. With this approach, the team identified several proteins impacted by treatment that had previously not been recognized by other high-throughput methods.



Methods and Results

Pooled GFP Pooled GFP intron tagging of metabolic enzymes

To generate a pool of GFP-tagged cells, the researchers transduced HAP1 cells with an intron-targeting sgRNA library comprising over 14,000 sgRNAs targeting over 11,614 introns of 2,387 metabolic enzyme genes. Cells were cotransfected with a GFP donor plasmid and a plasmid expressing Cas9 and the donor-targeting sgRNA. The donor plasmid contained the fluorescent tag flanked by generic sgRNA targeting sites, splice acceptor and donor sites, and 20 amino-acid linkers. Transfected cells were selected using blasticidin for 24 hours and GFP-positive cells were sorted six days after transfection.

To obtain clonal cell lines, they used single-cell dilution where cells were seeded in 96-well cell culture plates, expanded, and suspensions transferred to 96-well PhenoPlate imaging plates and corresponding 96-well culture plates. Cells on the imaging plates were imaged using the Opera Phenix™ high-content imaging system. The team then performed multiplexed amplicon sequencing of the sgRNA regions in the corresponding clones on the culture plates to identify the intron-targeting sgRNAs expressed in imaged cells. Overall, they isolated 335 clonal cell lines for which they could identify integrated sgRNAs, indicating a single-tagged protein. Of these, 36 had no previous localization data (as annotated on The Human Protein Atlas) available for the tagged protein, demonstrating how pooled protein tagging can be used to characterize the localization of previously unannotated proteins.

Compound screening on cell pools followed by in-situ sequencing

In the next stage of their investigation, the researchers treated the diverse pool of cells expressing GFP-tagged proteins with the BRD4-targeting PROTAC dBET6 to explore whether the compound changed the levels of localization of the tagged proteins. BRD4 is a transcriptional regulator that plays a key role during embryogenesis and cancer development. Using the Opera Phenix high-content imaging system, the team was able to track the dynamics of GFP-tagged proteins over 3 hours following treatment in approximately 7,000 cells in a single well on a 384-well plate. To identify the sgRNA sequence of proteins affected by the treatment, cells were fixed and a two-color in situ sequencing protocol was developed using the Opera Phenix. Eight cycles of nucleotide incorporation and imaging were sufficient to assign sgRNA sequences.

The researchers not only identified known targets of BRD4 using this method, but they also discovered that the compound had an effect on the localization of several proteins that had previously not been reported. When the team treated the cell pool with methotrexate (MTX), a metabolite used to treat cancer, autoimmune diseases, and ectopic pregnancies, they also observed known and novel changes to the localization of several proteins in the cell pool.

Conclusion

The present study showed that the generation of targeted GFP-tagged cell pools using CRISPR-Cas9-based intron tagging enables the identification of known and novel cellular responses to perturbations by time-lapse microscopy. Notably, by combining intron tagging with in situ sequencing, the drug treatment can be performed in a pooled format as opposed to arrayed screening, which first requires the isolation and characterization of individually tagged clones. The authors conclude that this approach can easily be applied to other sets of genes beyond metabolic enzymes and potentially in a genome-wide manner to study protein dynamics at scale.

Reference

Reicher A, Koren A, Kubicek S. Pooled protein tagging, cellular imaging, and in situ sequencing for Monitoring Drug Action in real time. Genome Research. 2020;30(12):1846–55.

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