Leveraging a CRISPR-based LAPSE workflow to guide drug targeting strategies

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ABSTRACT

Large scale genetic screening and profiling has enabled nomination of new oncology targets, but developing potent and selective small molecule inhibitors against these targets remains a resource intensive pursuit. In addition, enzymatic inhibition might not necessarily generate the same phenotype achieved through targeted genetic knockout. We have developed the Longitudinal Assay Profiling Specific Edits (LAPSE), which leverages CRISPR technology to make precise edits and track mutant allele frequencies to reveal cell fate. This can be used for confirmation that cancer cell growth specifically requires enzyme activity, or a particular protein-protein interaction can be determined by tracking precision amino acid substitutions or in-frame deletions. In the case of the former, observing enzyme-dead mutations dropout from the allele pool in surviving cells suggests that the pharmacological inhibition of the target would be sufficient for eliciting growth inhibition. In contrast, a catalytically dead mutation that rescues and sustains cancer cell viability observed by allele amplification in the surviving cells suggests that targeted protein degradation would be required for therapeutic effect. We have successfully applied the LAPSE assay to guide the drug discovery efforts of a diverse class of targets, including kinases, methyltransferases and acetyltransferases. Through NGS and single cell analysis, we have increased the resolution of allele profiling, and recently have also expanded the LAPSE technique for *in vivo* target evaluation.

Expanding the spectrum of competitive growth assays

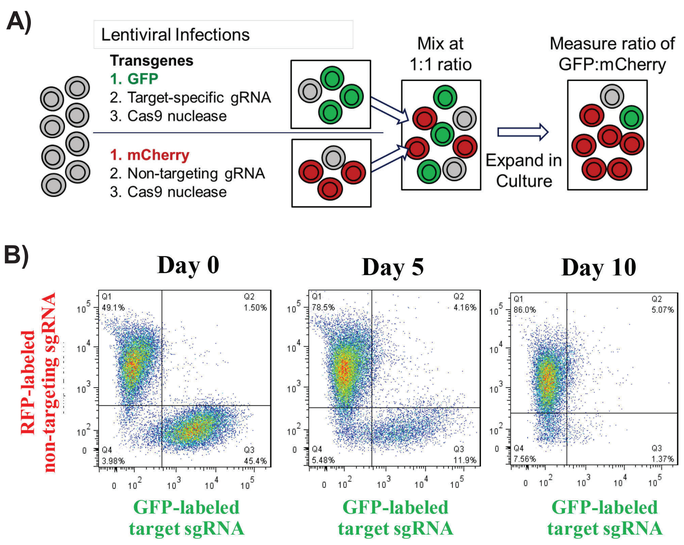
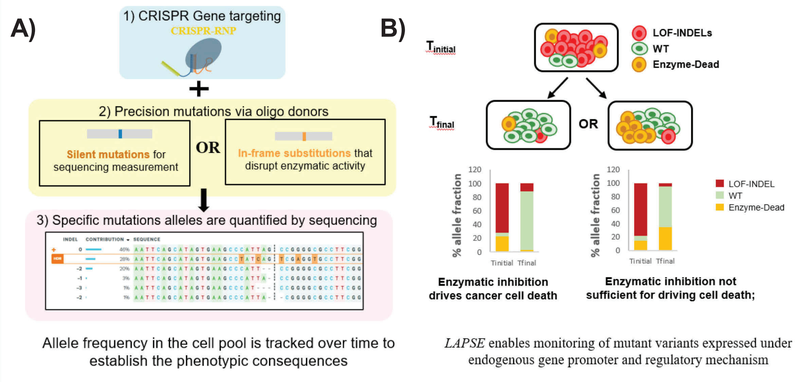


Figure 1: **FACS-based competitive growth assay previously used for target evaluation. A)** Fluorophore-based competitive assay to track growth of cells transduced with target-specific gRNA with cells transduced with non-targeting control gRNA. **B)** Depletion versus spike-in control establishes gene dependency.

LAPSE (Longitudinal Assay Profiling Specific Edits)



Allele frequency in the cell pool is tracked over time to establish the phenotypic consequences

LAPSE enables monitoring of mutant variants expressed under endogenous gene promoter and regulatory mechanism

Figure 2: **The novel LAPSE workflow enables tracking of endogenously engineered mutations for label-free competitive growth assay**.
A) Schematics of the LAPSE technique. CRISPR gene tagging systems is used to generate either silent mutations or in-frame substitutions that disrupt enzymatic activity of a gene. Tracking the specific mutations over time establishes the phenotypic consequences of the engineered mutations. **B)** The LAPSE method can be applied for early target evolution to guide drug targeting strategy.

Applying LAPSE to establish essentiality of MASTL kinase domain activity

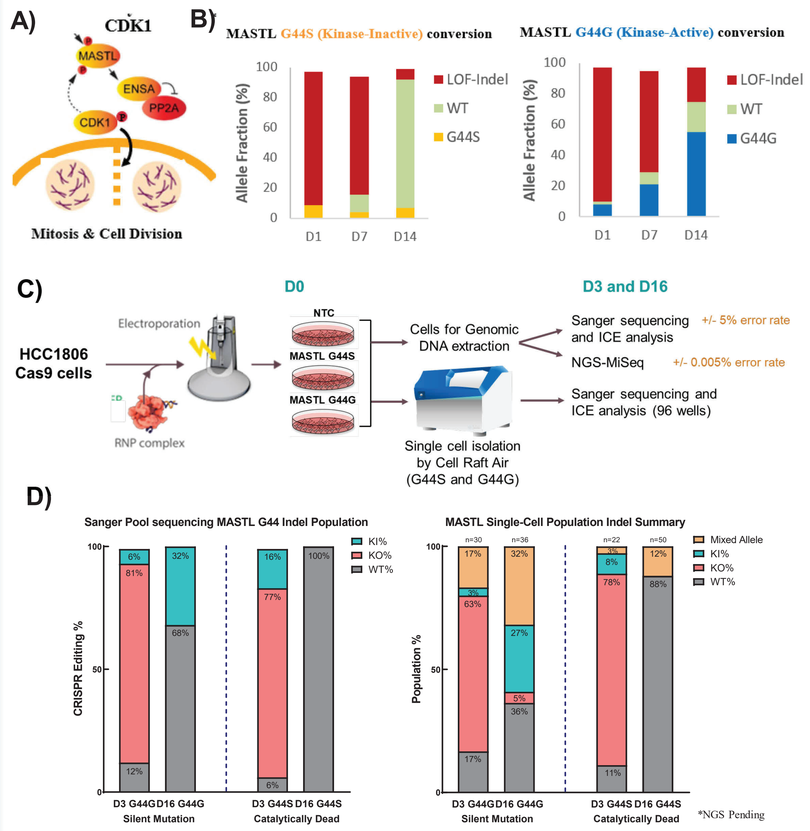


Figure 3: **MASTL kinase activity is required for cancer cell survival. A)** MASTL kinase modulates mitotic progression of cells by blocking the activity of tumor suppressor phosphatase PP2A. **B)** MASTL kinase-inactivating mutation G44S, and not the kinase-active G44G silent mutation, is depleted from the pool, suggesting catalytic inhibition of MASTL is sufficient for achieving anti-cancer benefits. **C)** Workflow for LAPSE experiment with addition of single-cell and NGS analysis. **D)** Single-cell analysis provides allele mutation distribution and shows comparable conclusions on impact of catalytic activity of MASTL on cell fate as the pooled sequencing results.

Compound anchored LAPSE to survey CDK4 mutations promoting resistance to Palbociclib

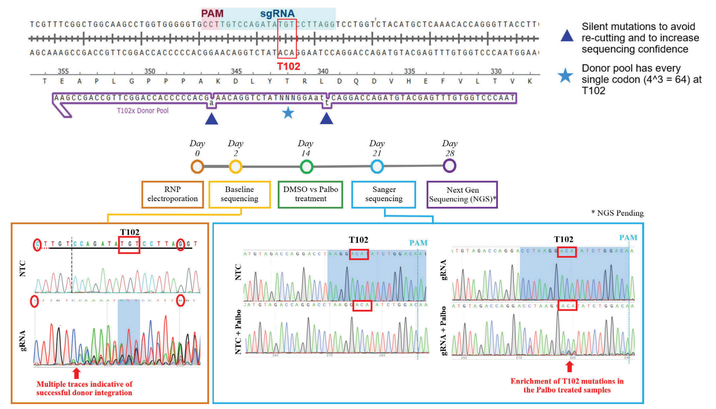


Figure 4: Saturation mutagenesis using LAPSE for discovery of resistance mutations. A) LAPSE reagent design for saturation mutagenesis of CDK4 T102 residue. **B)** Deconvolution of CDK4 T102 mutations enriched post Palbociclib treatment can identify potential mechanism of resistance.

Determining JAK catalytic dependence following αPD1 treatment using *in vivo* LAPSE

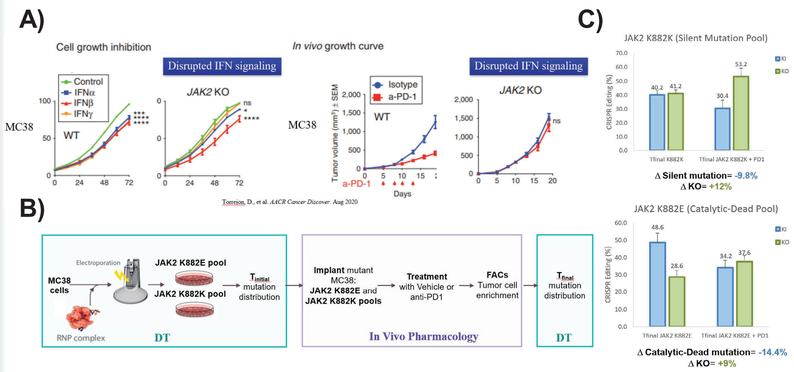


Figure 5: **Applying LAPSE technique for *in vivo* target evaluation.**
A) Loss of JAK2 drives PDx-resistance **B)** *In vivo* LAPSE study design. CRISPR engineered MC38 JAK2 K882E kinase-dead and K882K silent mutation pools were implanted in C57BL/6 mice and treated with isotype control or anti-PD1 inhibitor. Tumors were harvested 12 days post treatment and FACs sorted for tumor cell enrichment. RT-PCR was performed using primers flanking the sgRNA target side and amplicons generated were sequenced to measure final mutation distribution. **C)** JAK2 catalytic activity is dispensable in driving PDx-resistance. JAK2 KO mutation is enriched with α-PD1 treatment. Both JAK2 silent-K882K and catalytic-dead-K882E mutations are depleted after α-PD1 treatment.

Conclusion

- The LAPSE technique can provide a faster timeline than traditional transgene rescue experiments.
- Endogenous gene modification by LAPSE avoids over-expression artifacts.
- Application of the LAPSE method to genes of any size is possible.
- Successful application of the LAPSE workflow has been applied for evaluating diverse oncology targets, including kinases, methyltransferases, GEFs, ATPases etc.
- NGS is currently being performed to increase sequencing depth of the LAPSE technique .