Visualization of in vivo gene editing heterogeneity at the transcript level with the BaseScope™ Duplex RNA *in situ* hybridization assay

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Highlights

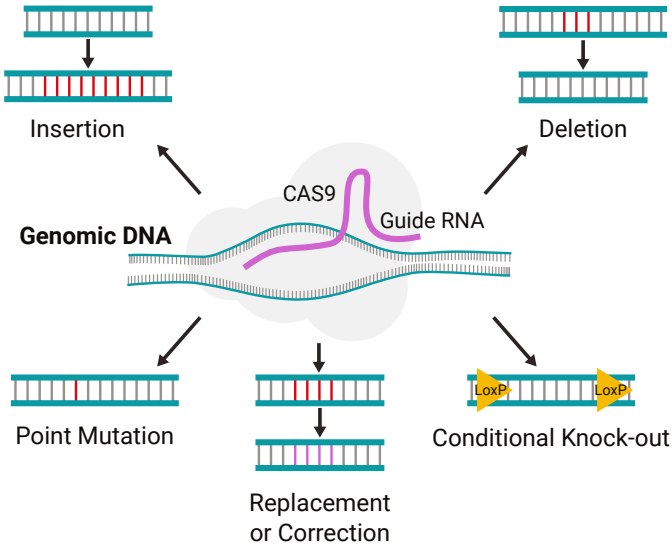
Genome editing, revolutionized by the CRISPR/Cas9 system, is a powerful research tool and promising therapeutic strategy for human genetic diseases. The BaseScope™ RNA *in situ* hybridization (ISH) Assay is the only method available to quantify cell-specific CRISPR-edited transcripts, including single nucleotide changes, in the context of intact fixed tissue. In this report we illustrate one use of the BaseScope™ Duplex Assay to assess the heterogeneity of *in vivo* CRISPR/Cas9 gene editing in the mouse liver. The BaseScope™ assay can be utilized in gene editing applications to:

- Visualize tissue and cellular biodistribution of the gene edited transcripts
- Measure cell-specific Cas9 mRNA expression and guide-RNA in target tissues
- Identify gene edits in specific cell populations by duplexing RNA ISH cell marker probes and edit-specific probes
- Discern if the CRISPR/Cas9-mediated mutation is mono- or bi-allelic with duplex of WT and edited probes

In vivo gene editing in animal models is achieved by plasmid or viral vector-based delivery of the CRISPR/Cas9 molecular machinery. The BaseScope™ RNA ISH Assay is a simple and powerful tool that complements NGS and qPCR in characterization of CRISPR-mediated gene editing. Whereas NGS/qPCR provide quantitative average values of wildtype and edited sequences, they provide no information on heterogeneity or percentage of edited cells within a tissue. The BaseScope™ ISH Assay provides a landscape view of an entire tissue and quantification of wildtype versus edited transcripts with single-cell resolution, where the actual number of cells within the target tissue containing the edited mRNA transcript can be quantified with the BaseScope™ Assay.

The BaseScope™ Assay is similar to the RNAscope® ISH technology[1]. Both achieve single-molecule RNA detection using paired oligo ("ZZ") probes to amplify signal without non-specific background. However, the BaseScope™ probe design and signal amplification system enables single-molecule RNA detection with a 1 ZZ probe and it can differentially detect single nucleotide edits and mutations in intact fixed tissue[2].

Several studies have applied the RNAscope® and BaseScope™ ISH Assays to confirm deletion or knockdown of a target gene with CRISPR/Cas9[4–6]. Han et al.[4] utilized the RNAscope® Assay to determine the effectiveness of multiple guide RNAs in inactivating *Frrs1l* in neurons, while Zallar et al.[5] used the assay to characterize the normal pattern of *Ghsr* expression in the rat brain and



Visualization of an *in vivo* edited transcript in the mouse liver

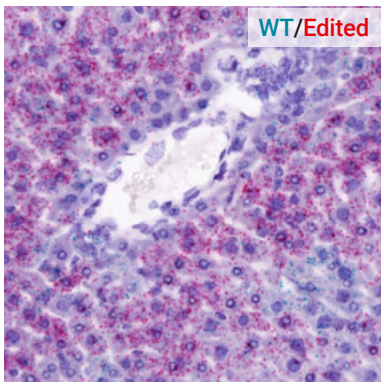


FIGURE 1. Applications of CRISPR-mediated gene editing and visualization by the BaseScope™ Assay. CRISPR-mediated gene editing can be applied for gene deletion, gene insertion, gene replacement or correction, point mutation, or conditional knockout. In vivo gene editing is heterogeneous within the target tissue. The BaseScope™ Assay can be used for cell-specific, simultaneous visualization of wildtype and CRISPR-edited mRNAs as well as Cas9 mRNA and guide RNA in the tissue context.

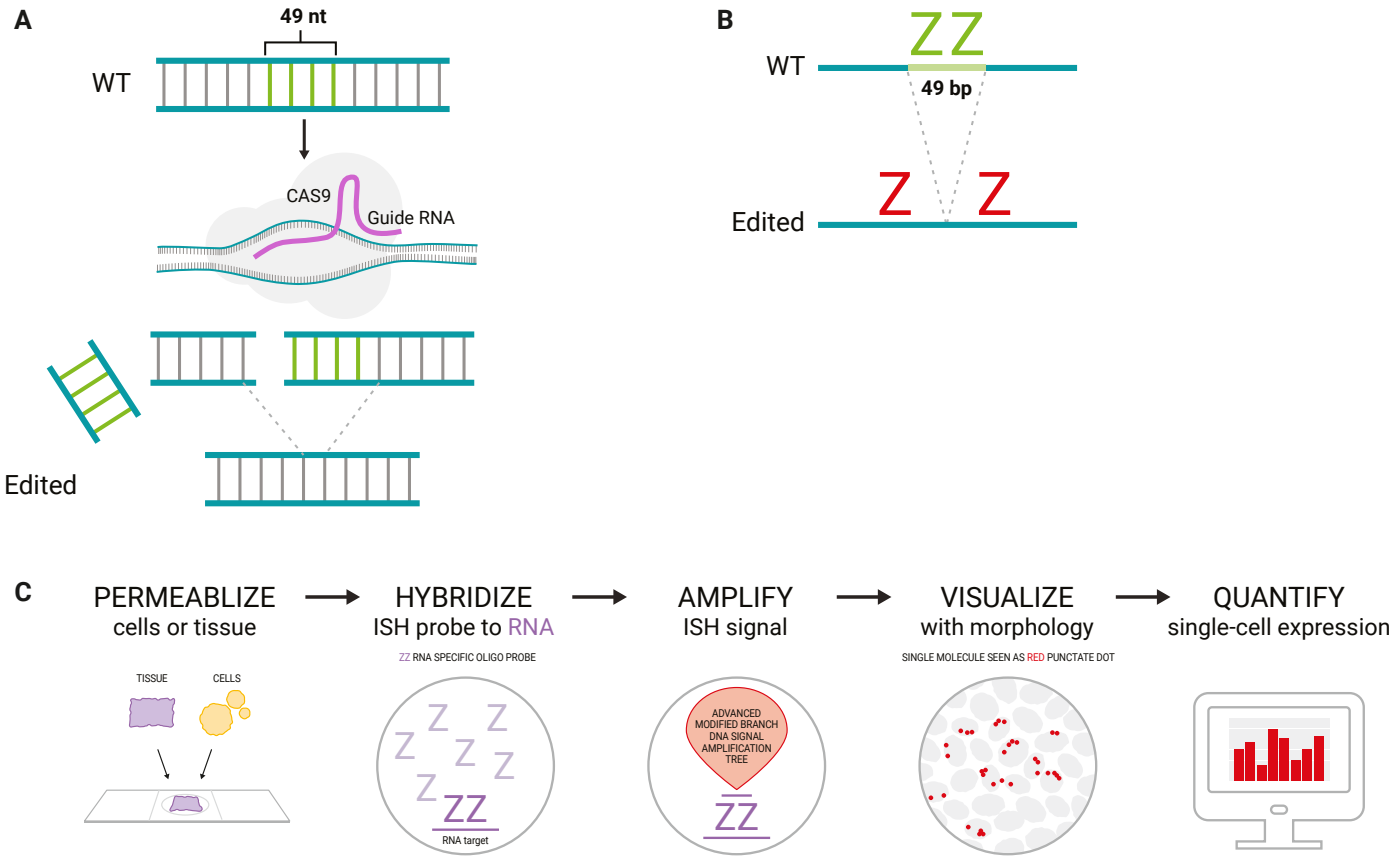


FIGURE 2. CRISPR-mediated nucleotide deletion and BaseScope™ probe design. (A) CRISPR-Cas9 was used to delete 49 nts from "Gene X", resulting in a novel junction sequence labeled "Edited". (B) BaseScope™ paired oligo (1 ZZ) probes were designed to target either the 49 nts in the WT sequence or the novel Edited junction sequence. (C) The BaseScope™ Assay workflow.

then confirm deletion of *Ghsr* in knockout rat brains generated by CRISPR/Cas9 editing. Piwecka et al.[6] deleted the circular RNA Cdr1as locus from the mouse genome using CRISPR/Cas9. In order to show the normal expression pattern in the mouse brain, as well as confirm the absence of expression in the deleted brain, the BaseScope™ Assay was used to specifically target the unique exon junction present in the *Cdr1as* circRNA.

The BaseScope™ Assay has the specificity required to confirm target gene editing in intact fixed tissue. Here we illustrate the application of the BaseScope™ Duplex Assay to confirm cell-type specific gene editing by CRISPR/Cas9 in mouse liver tissue.

Results

In this report we utilized the BaseScope™ Duplex Assay to detect gene edited transcripts in the mouse liver. CRISPR/Cas9 was delivered to the liver in lipid nanoparticles (LNP) and was designed to target and delete 49 nucleotides (nts) from a wild-type (WT) gene sequence, generating a novel sequence junction (Edited) (Figure 2A). The BaseScope™ probes (1 ZZ) were designed to target either the WT sequence or the sequences brought together as a result of the 49 nts deletion (Figure 2B). Either CRISPR/Cas9 or vehicle was delivered to mice *in vivo*, targeting the liver. The BaseScope™ Duplex Assay was performed on FFPE sections of unedited liver and Edited liver (CRISPR/Cas9) to simultaneously detect the WT and Edited sequences. In the unedited liver only the WT signal was detected, whereas in the CRISPR/Cas9-treated liver both WT and Edited signals were detected (Figure 3). WT, Edited, or both signals were detected overwhelmingly in hepatocytes, and no signal was detected for either probe in Kupffer cells (Figure 3). Examination of the portal and central veins showed no detectable signal for either probe in endothelial cells, nor was signal for either probe detected in the cells lining the bile duct (Figure 4). Taken together, these results reveal that the CRISPR/Cas9-mediated deletion was restricted to hepatocytes.

Three single-cell expression profiles were observed in hepatocytes from the CRISPR/Cas9-treated liver: WT only, Edited only, or co-expression of WT and Edited sequences in the same cell (Figure 3). These findings indicate that hepatocytes in CRISPR/Cas9-treated animals are a heterogeneous population of homozygous WT, heterozygous WT/Edited and homozygous Edited.

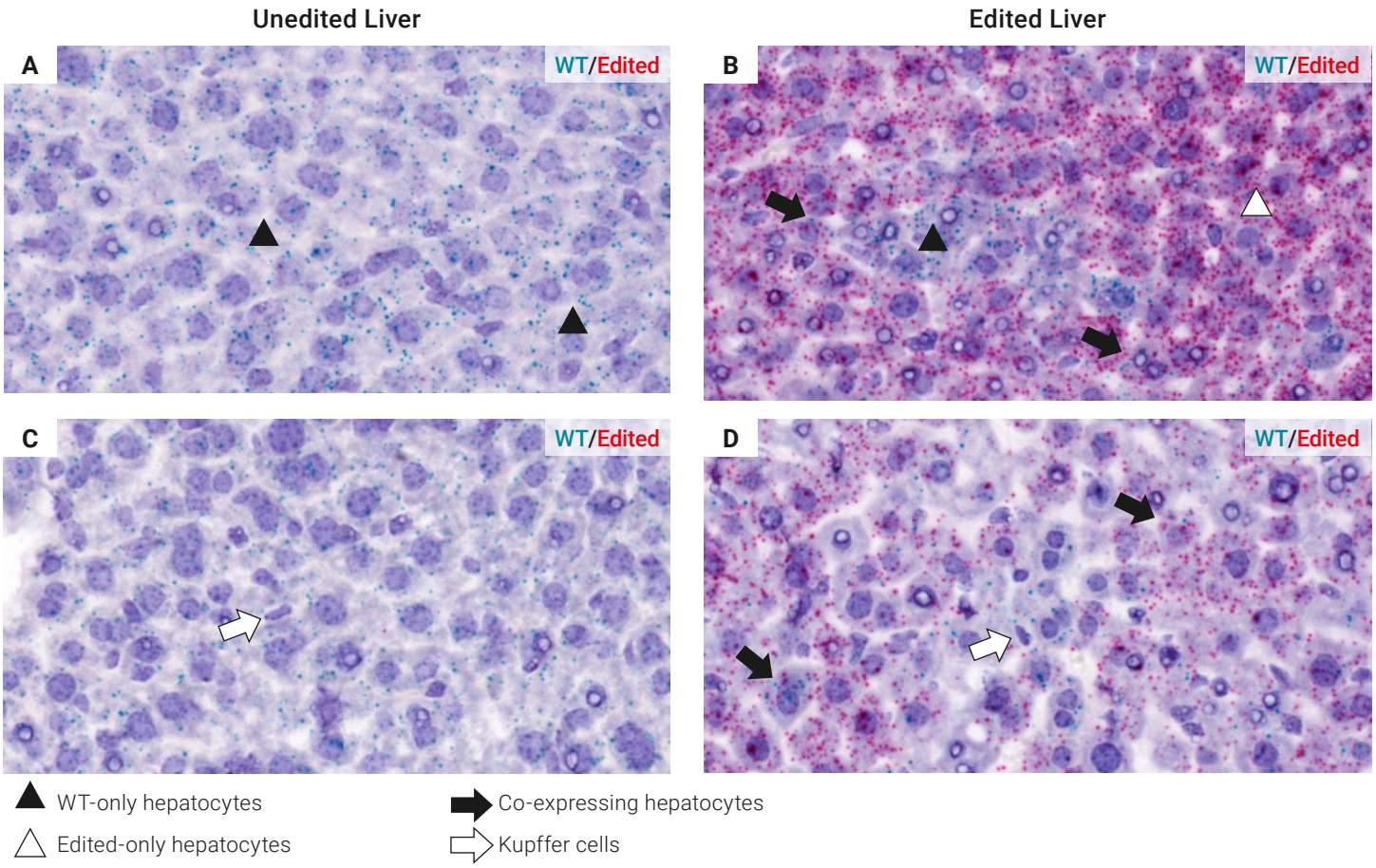


FIGURE 3. Visualization of CRISPR-mediated nucleotide deletion in hepatocytes. The BaseScope™ Duplex Assay was used to visualize the CRISPR-edited Gene X mRNA deletion of 49 nts in mouse liver. The WT sequence (green) was detected in both the unedited liver (A, C) and the Edited liver (B, D), whereas the Edited sequence (red) was detected only in the Edited liver (B, D). Both the WT and Edited sequences were detectable in hepatocytes (A, B; arrowheads). Most hepatocytes expressed either WT only (A, B; black arrowheads) or Edited only (A, B; white arrowheads), however a few cells co-expressed both the WT and Edited sequences (B, D; black arrows). Neither sequence was detected in Kupffer cells (C, D; white arrows).

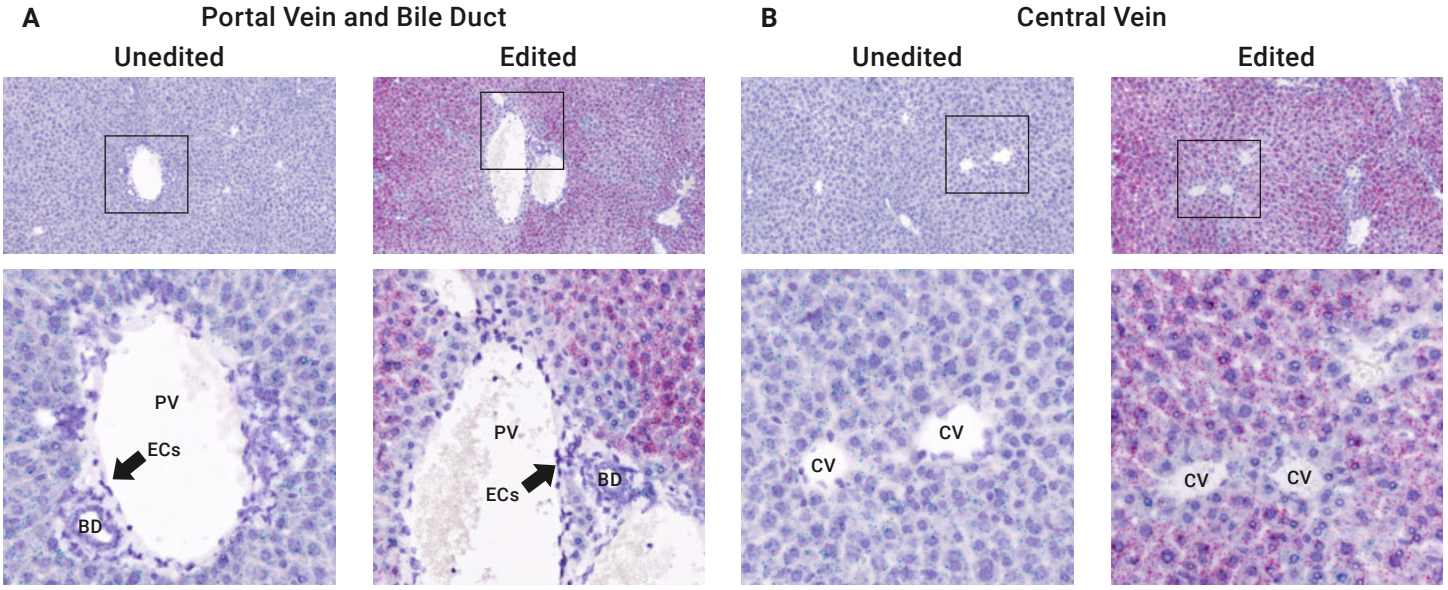


FIGURE 4. CRISPR-mediated deletion was not detected in portal vein, bile ducts, or central vein. The BaseScope™ Duplex Assay revealed that both the WT and Edited sequences were undetectable in the (A) endothelial cells (ECs) of the portal vein (PV) and the bile duct (BD), as well as (B) the central vein (CV).

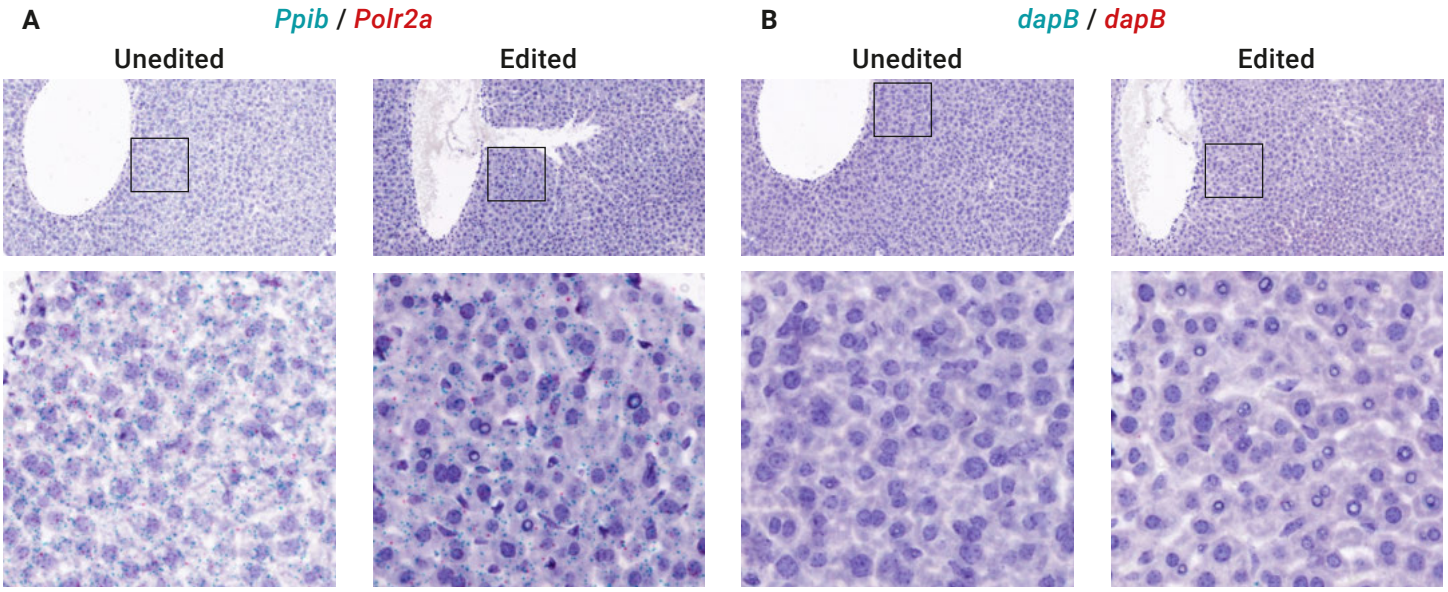


FIGURE 5. Sample quality and assay technical controls. The BaseScope™ Duplex Assay detected the positive controls *Ppib* (green) and *Polr2a* (red) in both the unedited and edited livers (A). Staining with the negative control probe *dapB* did not detect any signal in either sample (B).

Conclusions

Genome editing has been revolutionized by the CRISPR/Cas9 system and is proving to be a powerful research tool in the study of genetic diseases. In this study we illustrate the ability of the BaseScope™ Assay to identify the specific cells targeted by the CRISP/Cas9 system in the mouse liver. WT and Edited transcripts were detected primarily in hepatocytes, with no detection in Kupffer cells or cells of the portal triad. In addition, we could discern the monoallelic or biallelic gene-editing status of cells in CRISPR/Cas9-treated liver. The BaseScope™ Duplex Assay illustrated in this report can be applied easily to any animal model and any target gene. As shown in this report specific probes can detect and quantify the prevalence and heterogeneity of in vivo gene edited transcripts in any intact fixed tissue.

References

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