# Methods for genome-wide detection of single strand breaks induced by gene editors reveals the specificity of SpCas9 nuclease domains and provides comprehensive lists of potential off-targets for Prime Editors

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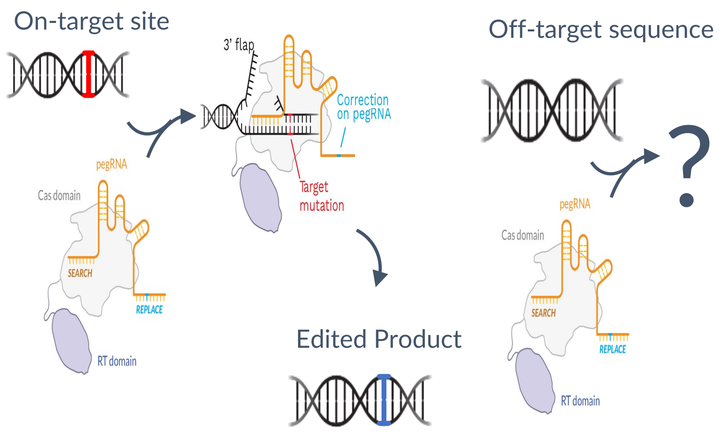
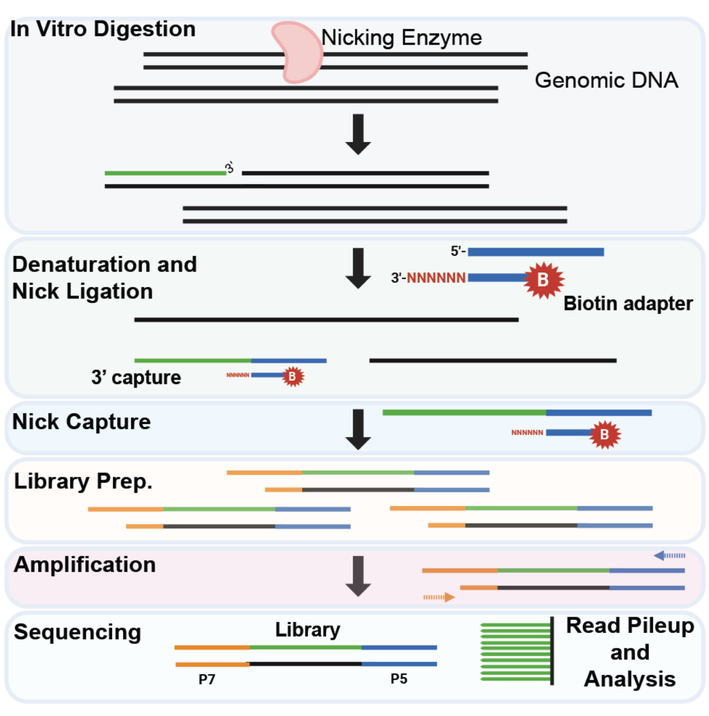
## Background

Gene editing can restore normal function to damaged genes; however, the application of gene editing to the clinic is limited by the potential genotoxicity of off-target events. Prime Editing may have a fundamental safety advantage compared to other gene editing approaches because it does not generate double stranded DNA breaks and any edits it makes requires additional sequence homology and are subject to specificity checks by the cell's DNA repair machinery. While methods to detect off-targets for SpCas9 and nuclease-based editors are more mature, detection and quantification of off-target risk of second generation nickase editors, including Prime Editors, are works in progress.




## Strategy and Methods for detecting potential off-targets

To nominate off-target sites in the genome we use multiple biochemical approaches that detect potential off-target sites recognized by Prime Editor. We have developed the genome-wide single strand break detection method 3-Prime End liGation sequencing (PEG-seq) that can detect the single strand breakome of a gene editor.

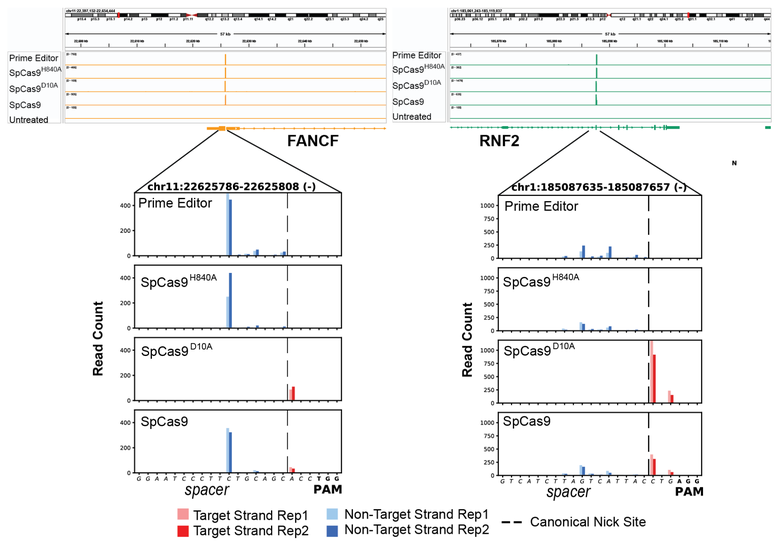



PEG-seq is a proprietary technique that identifies sites in the genome that have been nicked by a Prime Editor RNP. PEG-seq is strand specific and identifies with base-pair resolution the location of nicking events as detailed above.

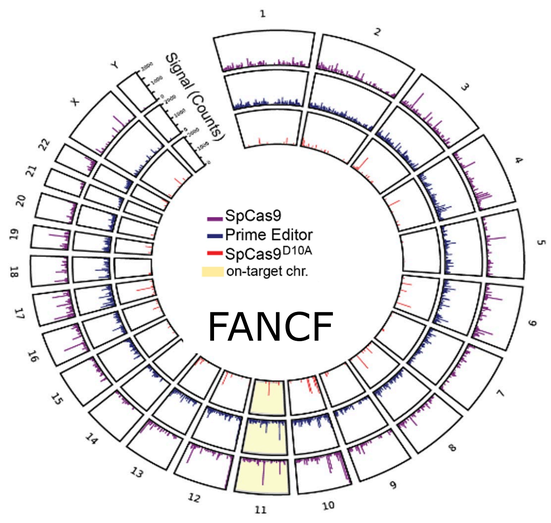
## Results

### PEG-seq identifies sites of DNA nicking genome wide generated by Prime Editor to detect potential off-targets

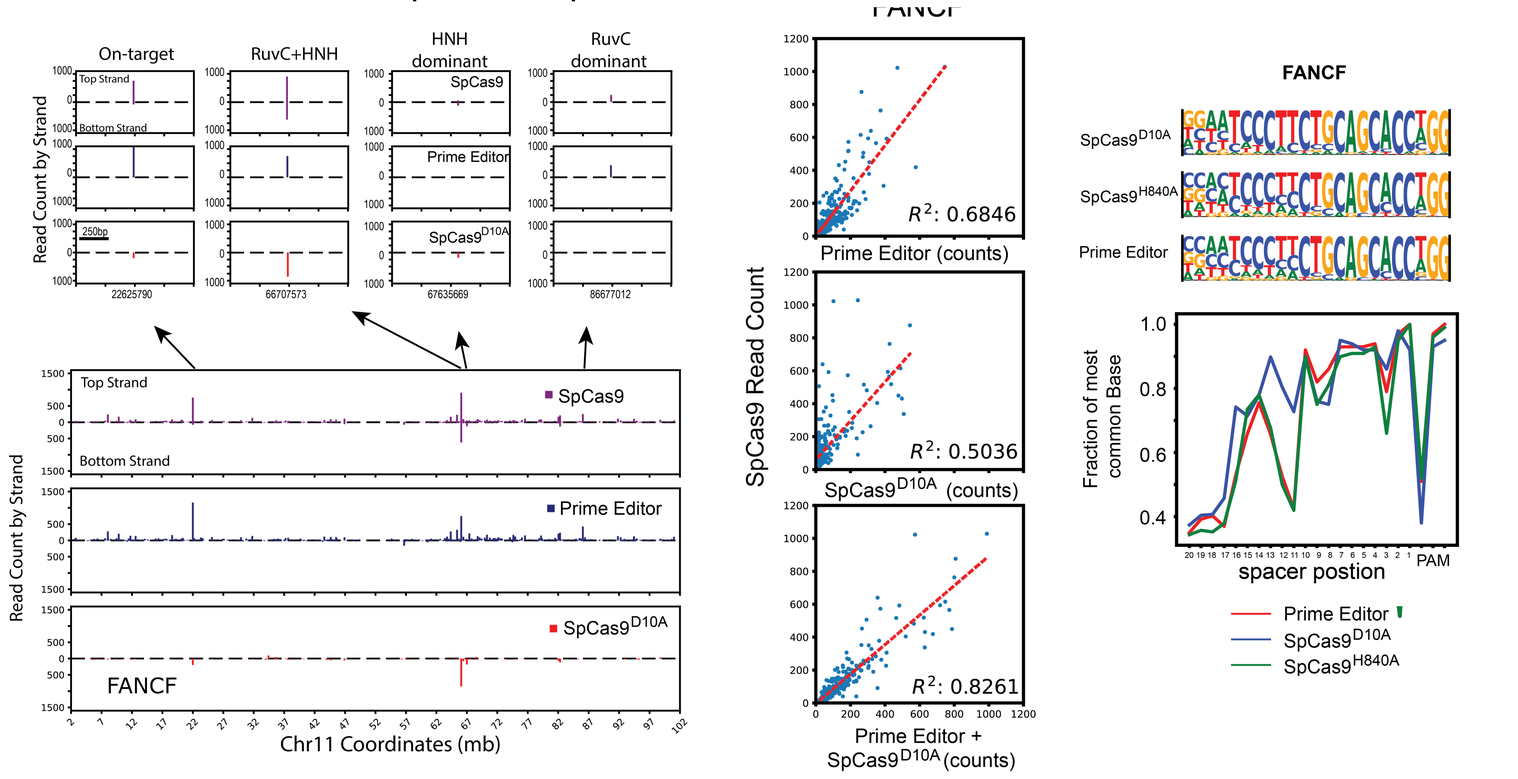
PEG-seq detects nicks with strand and base specificity



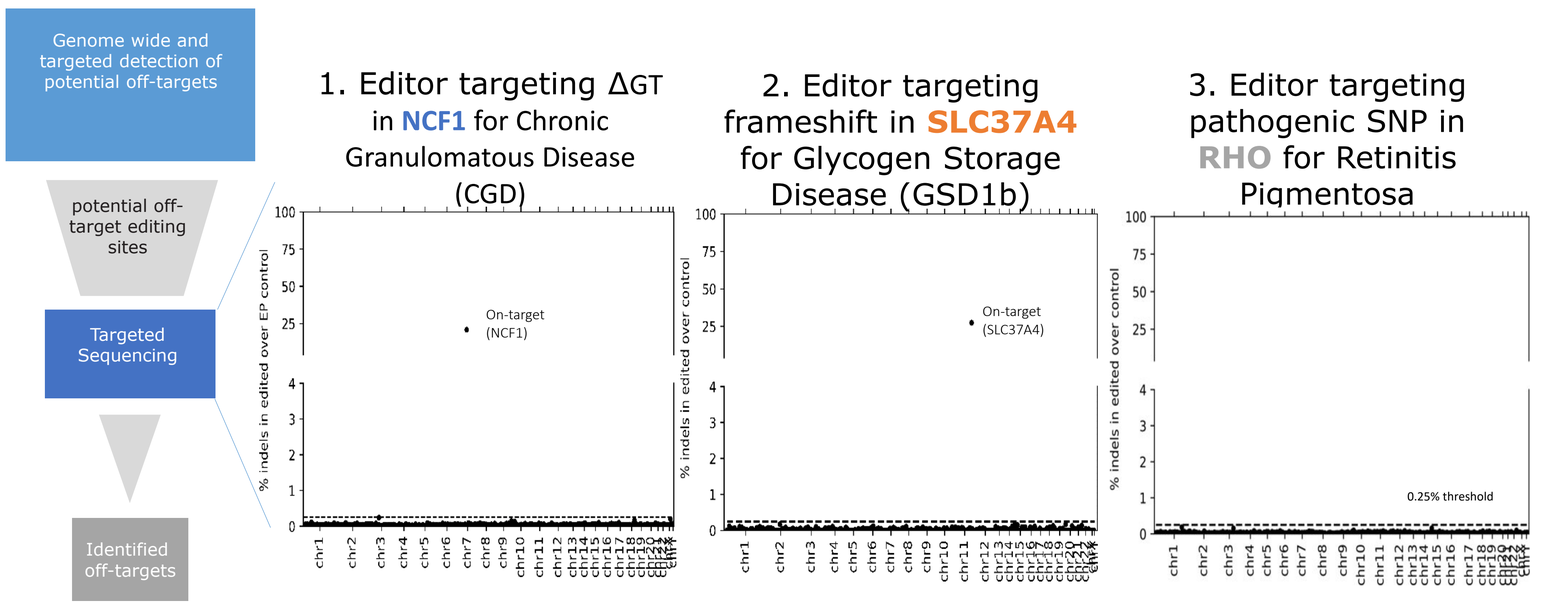

Genome wide detection of potential off-target sites identifies distinct activities of HNH and RuvC




DNA nicking by SpCas9 is explained by the combined activities of SpCas9$^{D10A}$ and Prime Editor (SpCas9$^{H840A}$). RuvC nicking is more frequent has a distinct dependence mismatches in the the spacer sequence.




### Analysis of Prime Edited cells does not identify off-targets in cellulo




Sites identified from biochemical and in silico methods were analyzed with targeted sequencing of cells edited with lead reagents correcting the indicated genes. No off-target events were detected in these three programs.

## Conclusions

A robust pipeline of off-target assays has been developed to evaluate Prime Editors. We show that PEG-seq is a novel strand and base specific method to detect nicking events genome wide and can be used to identify potential sites of off-target activity of Prime Editors.

Deep sequencing of potential off-target sites in edited cells identifies on- but not off-target editing in Prime Edited cells for three lead editors.