Performance and modularity of Horizon's Pin-point™ base editing system characterized by arrayed and pooled screening platforms

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Base editing is a CRISPR-Cas-based technology that allows the introduction of point mutations in the DNA without relying on double strand break formation. Horizon's Pin-point™ base editing technology utilizes an RNA aptamer embedded within the sgRNA to enable the recruitment of a deaminase for highly efficient and precise nucleotide conversion. The resultant modularity of the platform enables a high degree of flexibility that allows fine tuning of critical aspects of editing behaviour.

In this study we report the development and utilization of arrayed and pooled screening platforms to provide comprehensive characterization of editing performance, to benchmark different iterations of the Pin-point system and to assess guide RNA functionality in a high-throughput manner. Firstly, we describe an arrayed screening platform using five different cytidine deaminases and three structurally distinct tracrRNAs, and assess functionality of the Pin-point system architecture at 70 guide-specific genomic sites. Secondly, we present a flexible and adaptable pooled screening reporter platform for high-throughput parallel assessment of >65,000 gRNAs, including 7009 guides targeting know pathogenic SNVs.

Pin-Point Base Editing System

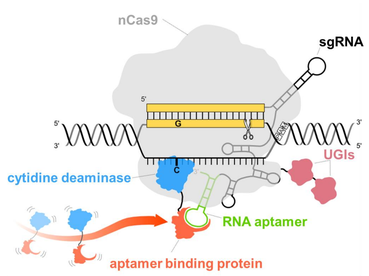
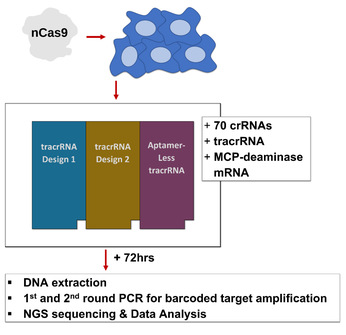


Fig 1. A Schematic of one configuration of the Modular Pin-point Base Editing system: A Cas9 nickase (nCas9) is guided to DNA target site via a guide RNA with an aptameric region engineered into the scaffold. The aptamer recruits a deaminase to the DNA target site via fusion with an aptamer binding protein. The combination of nCas9, an aptamer binding protein fused to a deaminase, and an aptameric guide RNA can be delivered to cells either as lentiviral particles or as mRNA and synthetic gRNA to efficiently base edit a DNA target of interest.

Arrayed Base Editor Screen Design

Fig 2. Arrayed Screen Outline: HEK293 and U2OS cells are transduced with nCas9 fused with polypeptide uracil glycosylase inhibitor (UGI) domains. Characterized clones are reverse transfected in 384-well format with 25 nM crRNA, 25 nM tracrRNA and 50 ng MCP-deaminase mRNA per well. Transfections are performed under conditions to achieve >95% transfection efficiency with minimal impact on cell viability. The gRNA library includes a set of well characterised gRNAs and gRNAs designed to tile the protein coding region of the STAG2 gene (with at least one C to be present within positions 1 to 18 of the 20 bp protospacer). Sequence motif and C positioning are well distributed across all the different gRNAs. Cells are incubated for 72 hrs prior to DNA extraction. Pooled amplicon libraries are sequenced on a MiSeq NGS platform (2x300 PE), and amplicon NGS data are analysed following Horizon's in-house data analysis pipeline.



Arrayed Base Editor Screen Results

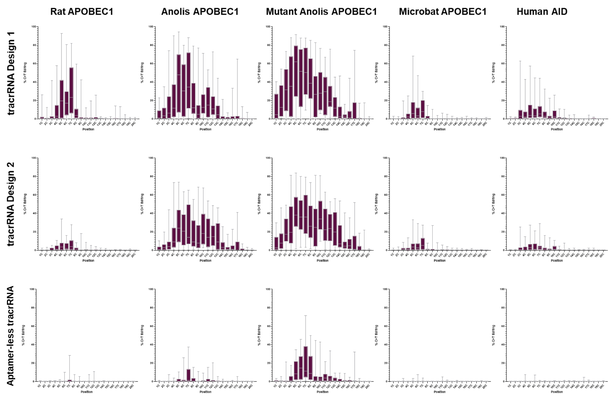


Fig 3. Protospacer Editing: Percentage C>T transitions at each position across the protospacer is plotted as a box plot with the min/max of the distribution. The data show that a wide range of editing can be achieved at each position, and is guide dependent. The screen reveals distinct differences in the editing efficiency and editing window of each of the base editing architectures that are assessed. For example, Anolis APOBEC1 has a greater editing efficiency than the rat protein, while the mutant Anolis deaminase has a greater efficiency and a reduced specificity when compared to the wild-type protein. The tracrRNA design 1 has improved efficiency with all the analysed deaminases as compared to design 2. Aptamer-less editing is minimal for all deaminases with the exception of the mutant Anolis protein.

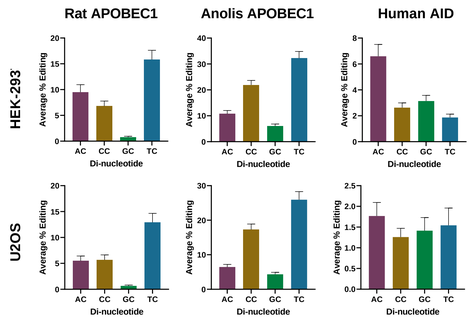


Fig 4. Impact of the Preceding Base on Editing Efficiency: The screening platform allows for an assessment of the impact of sequence context on the editing efficiency with each deaminase. As previously reported for rat APOBEC1, GC context is inhibitory for both rat and anolis variants, whereas TC is the most favorable context. This holds true for all the deaminases tested except AID where AC is the preferred context. Interestingly, Anolis APOBEC1 data shows a stronger inhibitory impact for the AC context than the rat protein.

Pooled Base Editor Screen

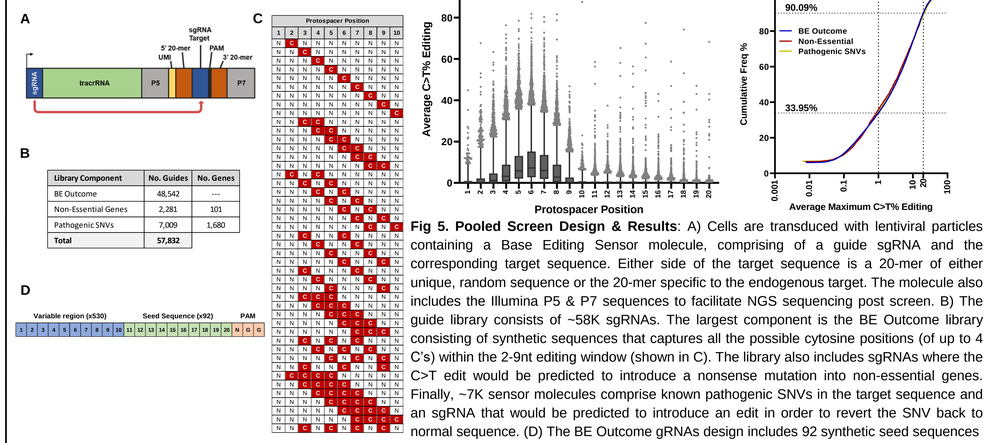


Fig 5. Pooled Screen Design & Results: A) Cells are transduced with lentiviral particles containing a Base Editing Sensor molecule, comprising of a guide sgRNA and the corresponding target sequence. Either side of the target sequence is a 20-mer of either unique, random sequence or the 20-mer specific to the endogenous target. The molecule also includes the Illumina P5 & P7 sequences to facilitate NGS sequencing post screen. B) The guide library consists of ~58K sgRNAs. The largest component is the BE Outcome library consisting of synthetic sequences that captures all the possible cytosine positions (of up to 4 C's) within the 2-9nt editing window (shown in C). The library also includes sgRNAs where the C>T edit would be predicted to introduce a nonsense mutation into non-essential genes. Finally, ~7K sensor molecules comprise known pathogenic SNVs in the target sequence and an sgRNA that would be predicted to introduce an edit in order to revert the SNV back to normal sequence. (D) The BE Outcome gRNAs design includes 92 synthetic seed sequences with 530 variations of position 1-10 with each combination of cytosines represented >10 times with different nucleotides at various positions. (E) The screen was performed in HEK293 cells with rat APOBEC1 and anolis APOBEC1. Outcome was analysed at day 4 after transfection of the Pin-point system components. Preliminary data with rat APOBEC1 of editing across the target molecule reveals that high editing levels can be achieved and that the distribution of the editing is as expected. (F) Analysis of the Maximum C>T editing achieved per guide shows that all three components of the library perform equally.

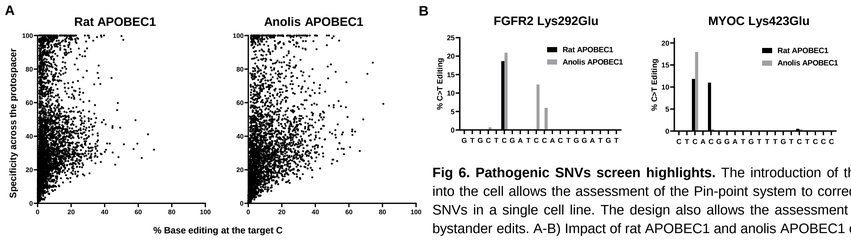


Fig 6. Pathogenic SNVs screen highlights. The introduction of the sensor molecule into the cell allows the assessment of the Pin-point system to correct many pathogenic SNVs in a single cell line. The design also allows the assessment of any unfavorable bystander edits. A-B) Impact of rat APOBEC1 and anolis APOBEC1 on target C editing efficiency and specificity across the protospacer over the pathogenic SNVs library. The rat APOBEC1 data identified 356 guides with over 90% of the editing specifically of the target base while 211 guides reach the same threshold when Pin-point base editor is configured with the anolis APOBEC1. C-D) Two examples of pathogenic SNVs that are specifically edited by one or the other deaminase.

Conclusions

We present the Pin-point base editing platform as a modular system for addressing a diverse range of editing applications. Utilizing an arrayed screening platform and a high-throughput pooled screening platform we are able to:

- Profile the functionality and specificity of different deaminases for editing a DNA target of interest, including known pathogenic SNVs
- Characterize editing behaviour of multiple configurations of the Pin-point platform to identify specific configurations for specific needs
- Characterize features that determine gRNA performance for optimal targeting with the Pin-point system.

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