P0666 Controlling C-to-T editing with TALE base editors

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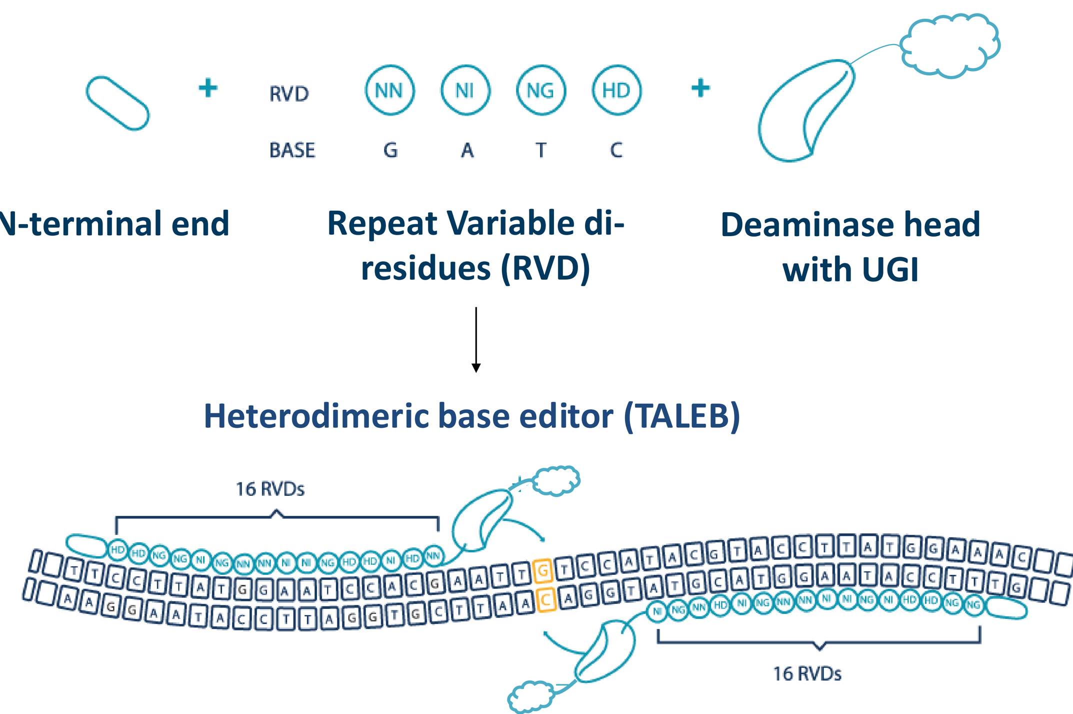
Abstract

TALE base editors (TALEB) are fusions of a transcription activator-like effector domain (TALE), split-DddA deaminase halves, and an uracil glycosylase inhibitor (UGI). The C-to-T class of TALEB edits double strand DNA by converting a cytosine (C) to a thymine (T) via the formation of an uracil intermediate.
We recently developed and applied a strategy that allowed the comprehensive characterization of C-to-T conversion efficiencies within the editing window. This method also takes advantage of a highly precise and efficient TALEN®-mediated ssODN knock-in in primary T cells to assess how target composition and spacer variations affect TALEB activity/efficiency.
Specifically, we highlighted that the composition of bases surrounding the target TC may strongly influence the editing efficiencies. We also demonstrated that different TALEB scaffolds could be used to relax target sequence limitations (increasing the targetable sequence) or be used to decrease, or eliminate, bystander editing within the editing window (increasing specificity), overall allowing TALEB to be fine-tuned for a desired gene editing outcome.
We then applied a range of different technics to assess characteristics of nuclear genome editing. We first focused on on-target editing but also explored the possibility and risk associated with genome wide TALE dependent/independent binding and editing. By using an experimental model relevant for therapeutic application consisting in primary cells (PBMC and HSC) and TALEB mRNA vectorization, we demonstrated that single TALEB arm does not lead to detectable editing (detection limit: 0.1-0.2%).
We further applied hybrid capture assays to probe for off-target editing, in particular region of the genome that were previously highlighted in cells lines, and demonstrated, in our same relevant experimental setup, the absence of detectable editing (detection limit: 0.2%).
Altogether, the datasets obtained in this study enhanced our understanding of TALEB and permits the design of extremely efficient (high editing frequencies and edit purity) and specific (absence of TALE independent off site and very limited, if not absence of, DSB generation) TALEB, compatible with the development of future therapeutic applications.

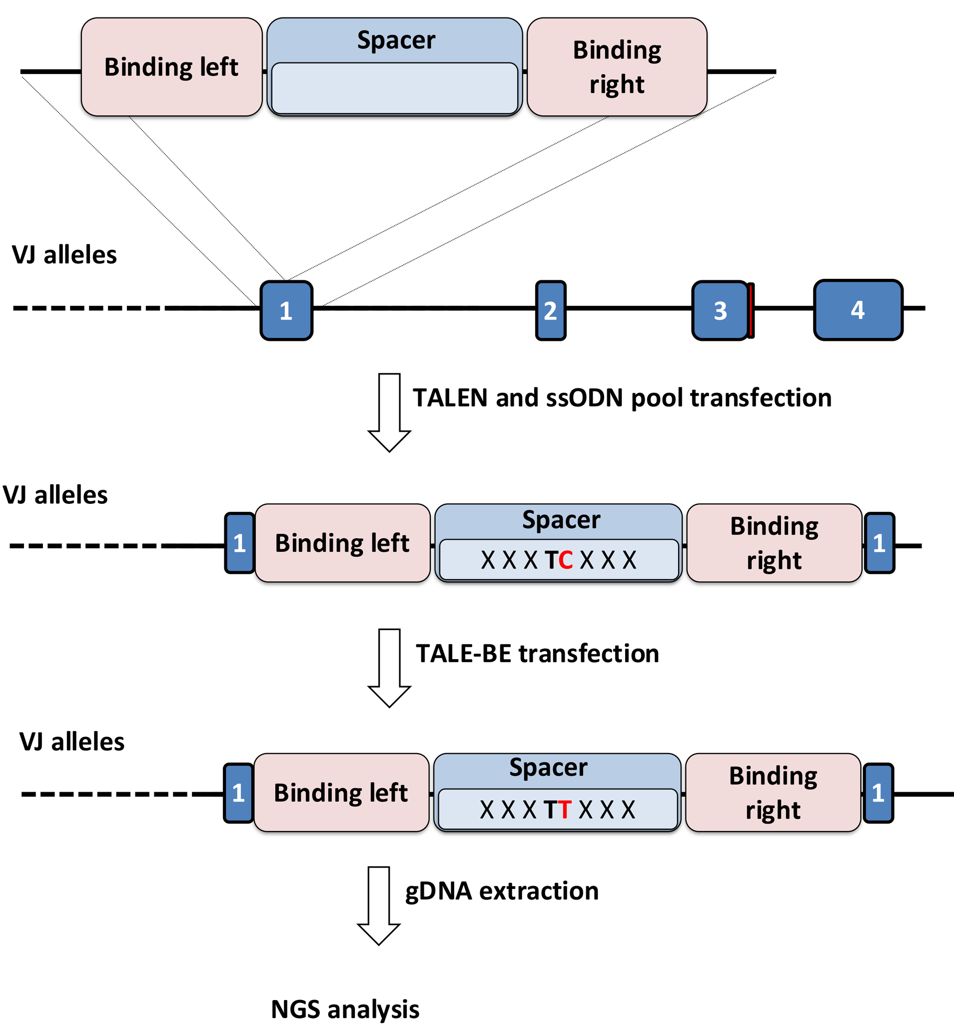
#1 Background

Base editing is a technology that leads to the introduction of point mutations in defined loci of a targeted DNA sequence. Base editors create mutations by deamination of the targeted bases (C), which are then converted into T, during the DNA repair process.



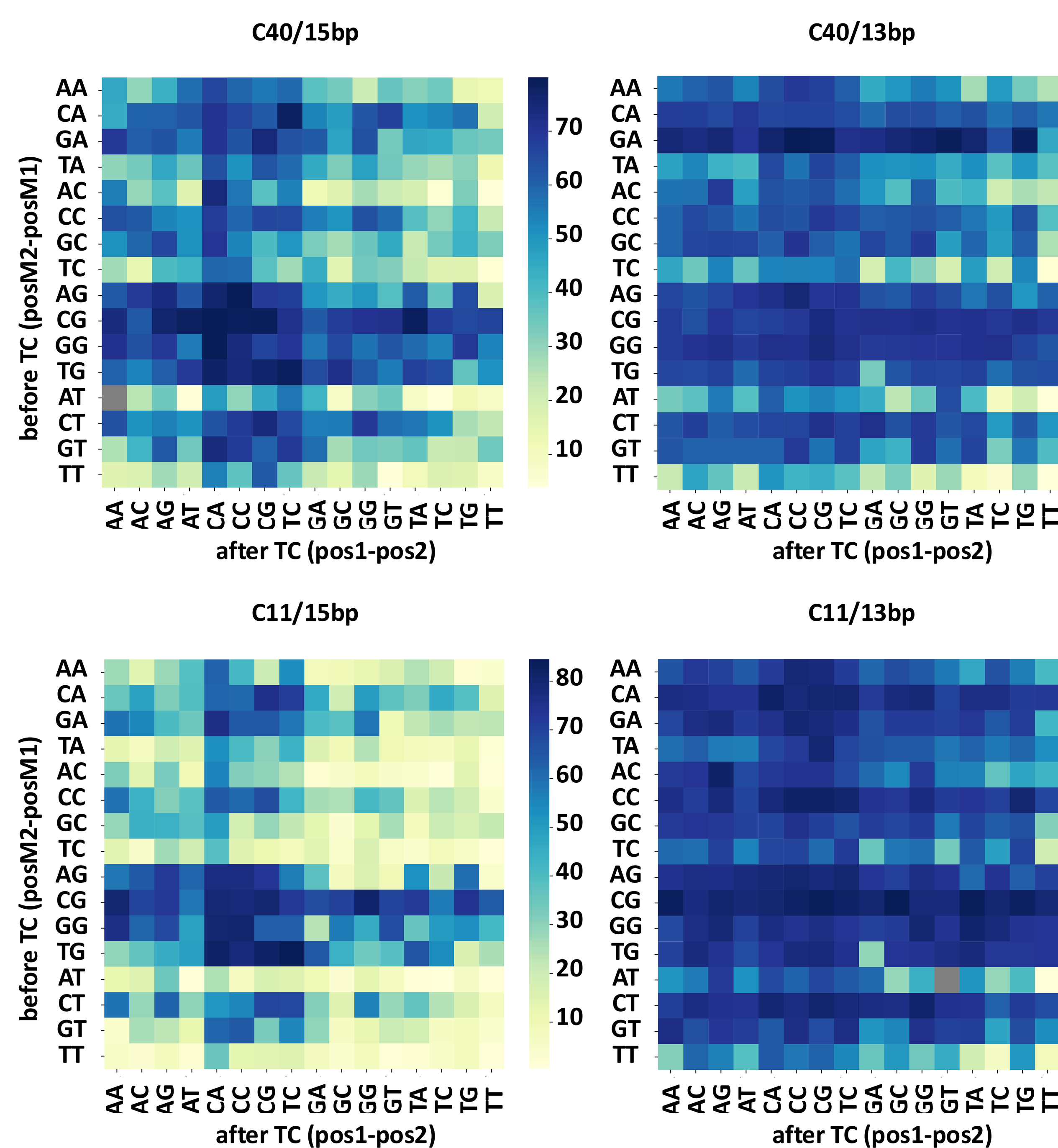
Schematic representation of a TALEB.

#2 Development of a TALEB high throughput screening platform



We developed a system allowing medium to high throughput screening of TALEB *in cellulo*, in a defined genetic environment. Such a cell-based assay enables the exploration of the impact, on editing efficiency, of multiple parameters such as (i) the architecture, (ii) the spacer length (sequence separating the two TALE binding site) and (iii) the sequence composition surrounding the targeted TC.

#3 Defining how TALEB architecture and TC context impact editing efficiencies



In the C11 architecture, editing is more stringent against a suboptimal context, compared to C40. The 15 bp spacer collection in the C40 and C11 architectures show the following nucleotide preferences:

posM2: A = T << G < C
posM1: T = C < A << G

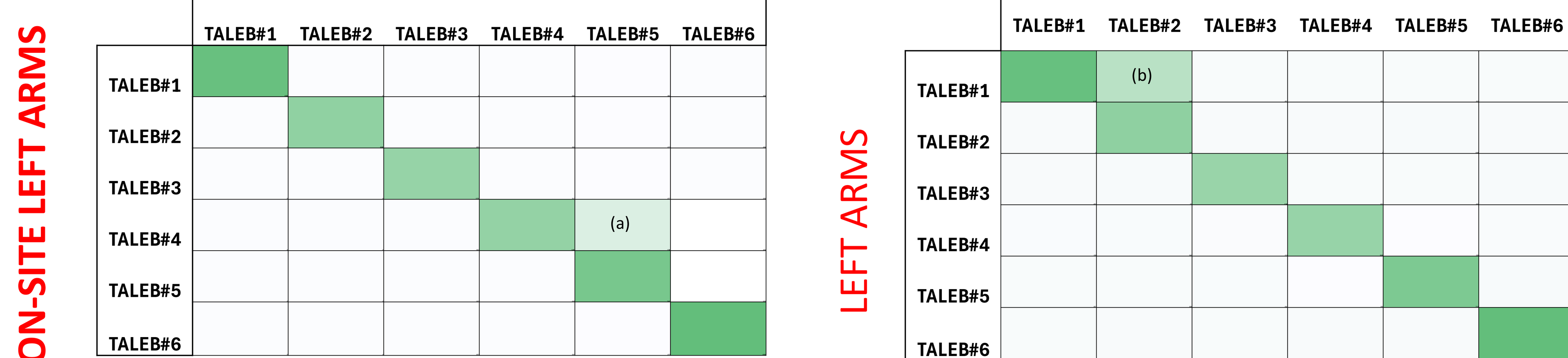
pos1: T < G < A << C
pos2: T < C < G < A

The 13 bp context in the C40 and C11 architectures seem to have less importance for editing efficiency, which shows higher overall editing. However, the 13 bp spacer collection show a similar context preference to the above:

posM2: T < A < G < C
posM1: T < C < A < G

pos1: T < G < A < C
pos2: T < A = C < G

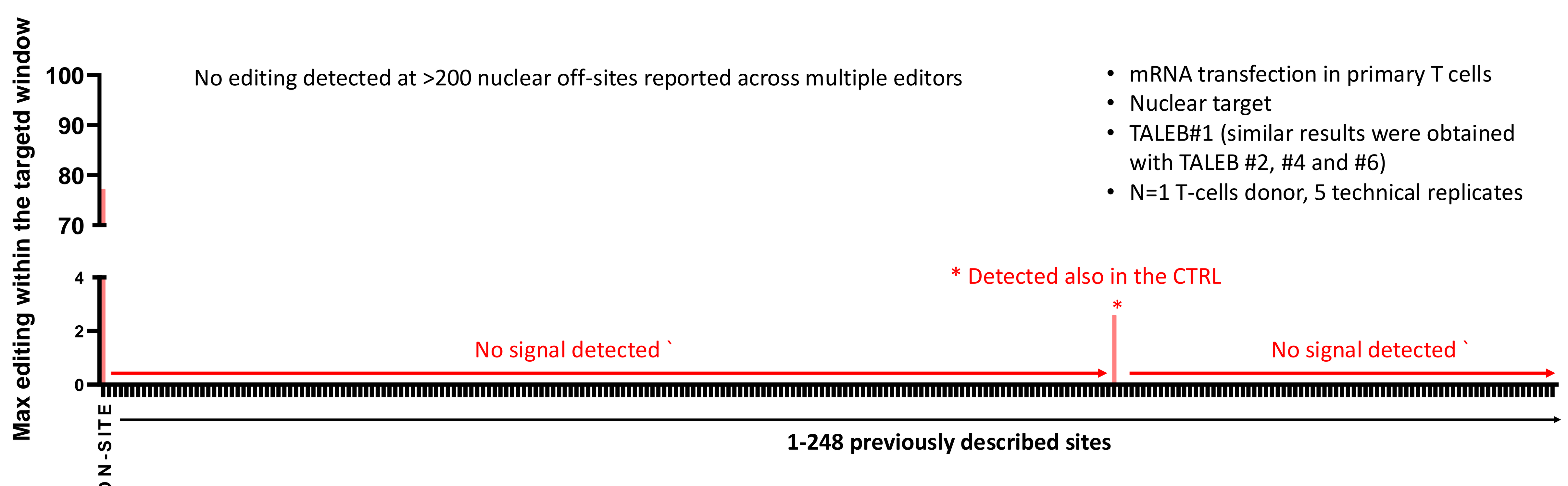
#4 TALEB do not edit site in the nucleus with only one arm binding



- mRNA transfection in primary T cells
- TALEB#1-5= nuclear targets
- TALEB#6= mitochondrial target
- N=1 T-cell donor

No editing detected at multiple sites with one miss-paired TALEB arm (only one arm binding). (a) Expected editing due to high sequence homology between the right arm of TALEB#4 and #5. (b) Expected editing due to the close proximity of the two arms tested.

#5 TALEB do not edit previously described nuclear off-sites



- mRNA transfection in primary T cells
- Nuclear target
- TALEB#1 (similar results were obtained with TALEB #2, #4 and #6)
- N=1 T-cells donor, 5 technical replicates

#6 Conclusions

This data demonstrates that:

- High throughput screening of TALEB allowed identification of optimal combination of TALEB architecture and positioning on the target to maximize the desired editing outcome
- In primary T-cells, when vectorized as mRNA, TALEB require both arms binding to edit the desired nuclear target
- In primary T-cells, when vectorized as mRNA, TALEB do not show editing at off sites previously reported

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