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Supplemental Information

A One-Step PCR-Based Assay to Evaluate the Efficiency and Precision of Genomic DNA-Editing Tools

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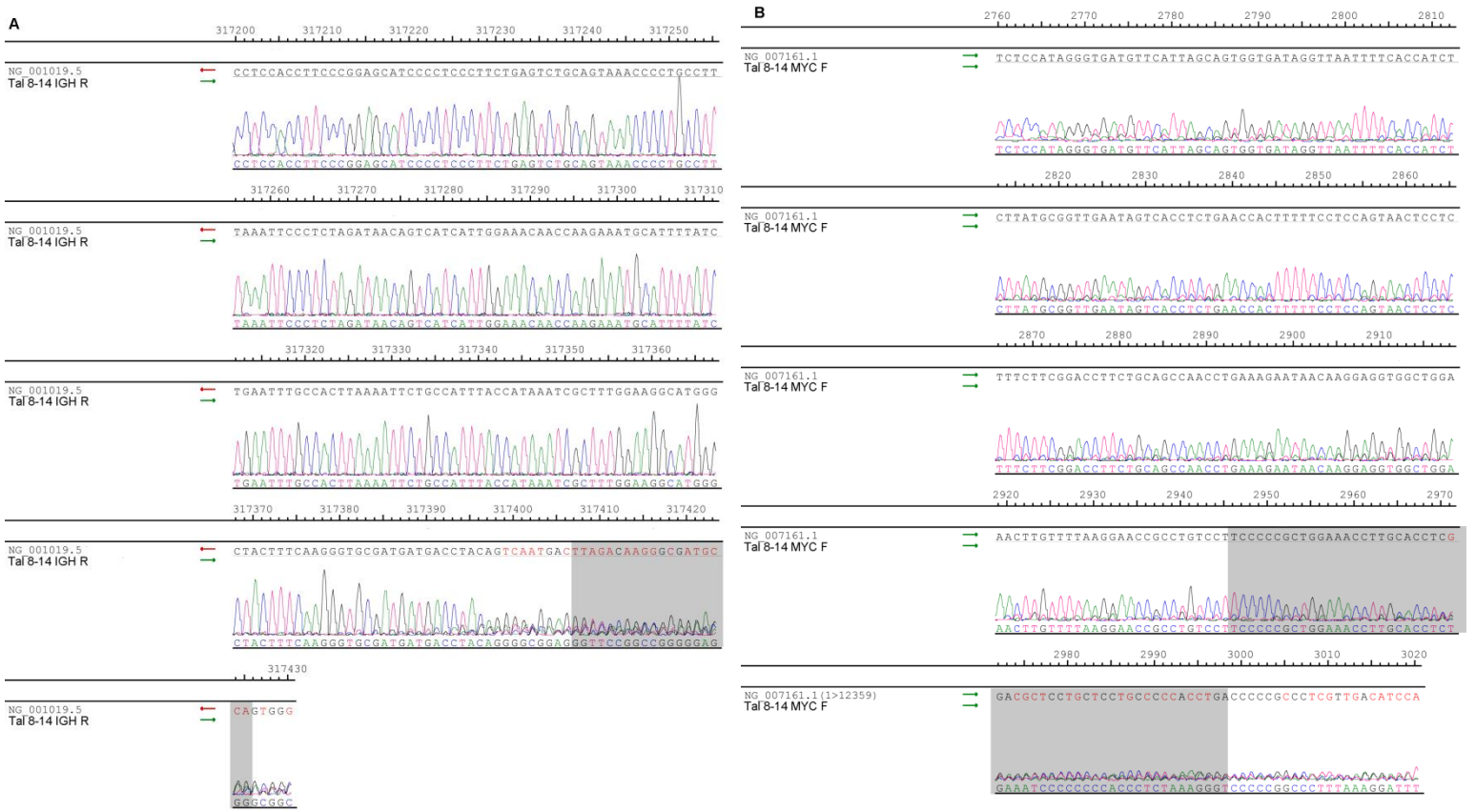
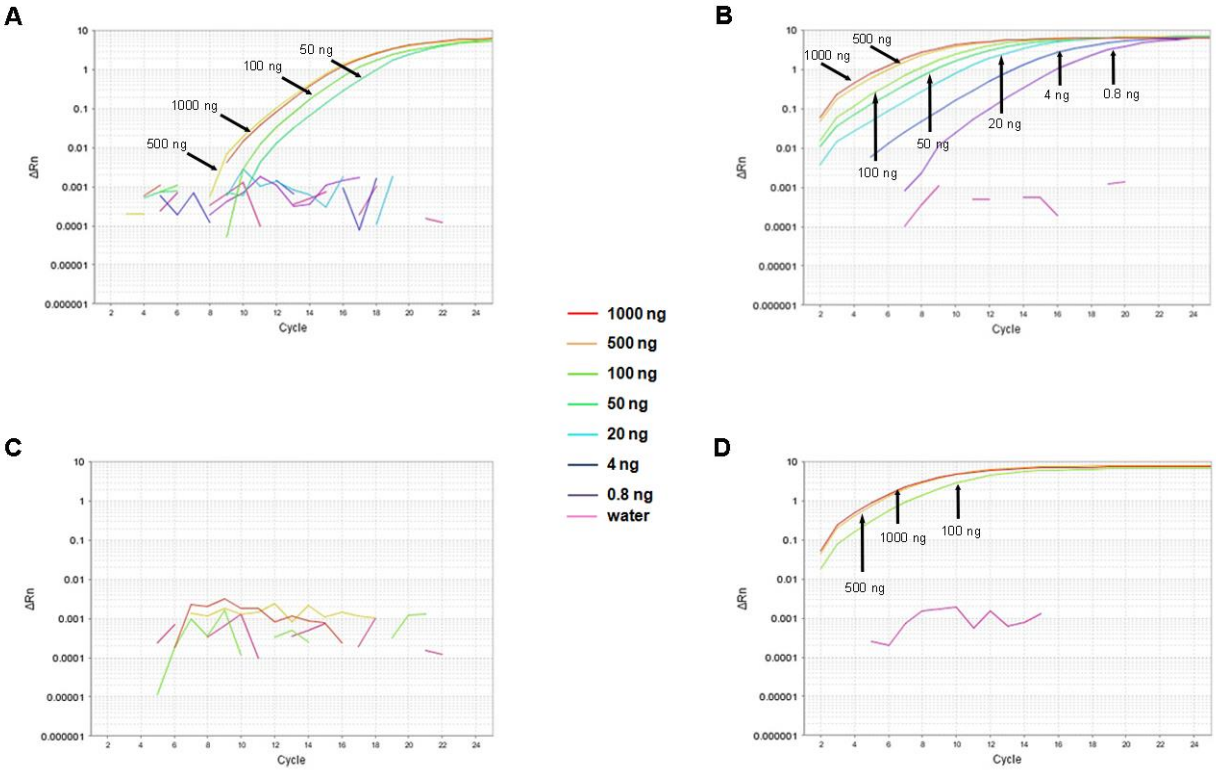
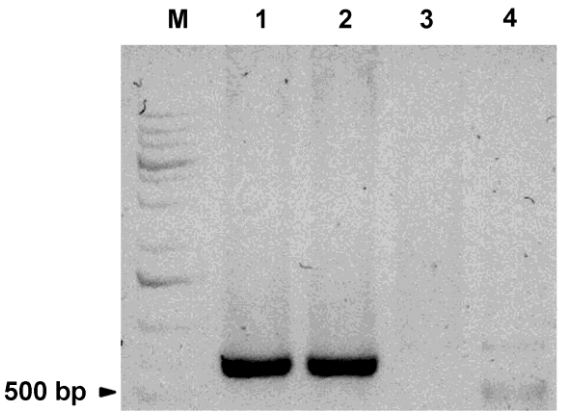


Supplementary Figure 1. PCR detection of rearranged DNA sequence resulting from t(8;14) chromosomal translocation.

Alignment of *IGH* **(A)** or *MYC* **(B)** wild types sequences (NG_001019.5 and NG_007161.1, respectively) with the sequence of the amplicon obtained from DNA extracted from transfected HeLa cells and amplified with MYC F + IGH R primers. In red are highlighted the mismatches between the two sequences and in grey is highlighted the sequence recognized by the TALENs.



Supplementary Figure 2. Nested qPCR analysis of ENIT sensitivity.
Amplification plots of the second step of a nested PCR of serially diluted DNA extracted from TAL8/14-transfected HeLa (10% transfection efficiency) or non-transfected HeLa and amplified in PCR1 with MYC F + IGH R primers or MYC F + MYC R primers, and with the corresponding internal primers in PCR2 (MYC_in F + IGH_in R primers or MYC_in F + MYC_in R primers, respectively). Water was used for negative control of amplification. X-axis: number of PCR cycles; Y axis: the magnitude of the fluorescence signal generated during the PCR over time (ΔRn).
a. PCR2 amplification curves: DNA from TAL8/14-transfected HeLa cells, amplified with mixed MYC F + IGH R primers in PCR1 and MYC_in F + IGH_in R primers in PCR2; **b.** PCR2 amplification curves: DNA from TAL8/14-transfected HeLa cells, amplified with control MYC F + MYC R primers in PCR1 and MYC_in F + MYC_in R primers in PCR2; **c.** PCR2 amplification curves: DNA from untransfected HeLa cells, amplified with mixed MYC F + IGH R primers in PCR1 and MYC_in F + IGH_in R primers in PCR2; **d.** PCR2 amplification curves: DNA from untransfected HeLa cells, amplified with control MYC F + MYC R primers in PCR1 and MYC_in F + MYC_in R primers in PCR2.
The experiment was performed at least three times, and every sample was run in triplicate for both PCR2 and PCR1.



Supplementary Figure 3. Nested ENIT can be used to detect the efficiency of various gene editing tools in different cell types

PCR on DNA extracted from MRC 5 cells transformed with SV40 and transfected or not with the four TALENS recognizing the MYC and IGH regions. The represented amplicons were obtained using the following primer pairs and samples: *Lane 1* - transfected cells MYC F+R, *Lane 2* - transfected cells IGH F+R, *Lane 3* untransfected cells MYC F+ IGH R, *Lane 4* transfected cells MYC F + IGH R. M is the molecular weight marker.

Supplementary table 1. Sensitivity of the existing methods for testing engineered nucleases efficiency

Method	Minimal amount of DNA required for detection, ng	Minimal number of cells required for detection	Reference
T7	100-800 ng	15,000-120,000	[1-4]
Surveyor	50 -200ng	7,500-30,000	[5-6]
RE-PCR	500 ng	75000	[7]

Supplementary references:

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3. Fu, Y, Sander, JD, Reyon, D, Cascio, VM and Joung, JK (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol *32*: 279–284.
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5. **http://www.idtdna.com/pages/docs/default-source/user-guides-and-protocols/userguide-surveyor-standard.pdf**
6. Qiu, P, Shandilya, H, D'Alessio, JM, O'Connor, K, Durocher, J and Gerard, GF (2004). Mutation detection using Surveyor™ nuclease. Biotechniques *36*: 702–707.
7. Xie, K and Yang, Y (2013). RNA-Guided genome editing in plants using a CRISPR-Cas system. Mol. Plant *6*: 1975–1983.