



SELECTED OPPORTUNITIES IN GENOME EDITING

CtIP fusion to Cas9 enhances transgene integration by
homology-dependent repair

(BIO 16366)

CTIP FUSION TO CAS9 ENHANCES TRANSGENE INTEGRATION BY HOMOLOGY-DEPENDENT REPAIR (BIO 16366)

Product factsheet

POC *in vitro*

▶ Product:

- ◆ Nuclease Cas9/CtIP fusion protein

▶ Application:

- ◆ Efficient transgene integration by homology-dependent repair (HDR)

▶ Rational:

- ◆ HDR using an exogenous DNA repair template supports precise genome editing
- ◆ CtIP, a key protein in early steps of homologous recombination that acts as a cofactor for MRE11 endonuclease in triggering DNA end resection

▶ POC:

- ◆ The Cas9-CtIP fusion is simple to use and allows obtaining **2-fold or more efficient transgene integration** than with Cas9 in several experimental systems, including human cell lines, iPS cells and rat zygotes
- ◆ A **minimal N-terminal fragment of CtIP**, designated HDR Enhancer, is sufficient to stimulate HDR and this depends on CDK phosphorylation sites and multimerization domain essential for CtIP activity in homologous recombination.

▶ Patent and publication:

- ◆ Patent - EP 17 305 260.6
- ◆ Charpentier M. *et al.*, Nat Commun. 2018 Mar 19;9(1):1133. doi: 10.1038/s41467-018-03475-7.

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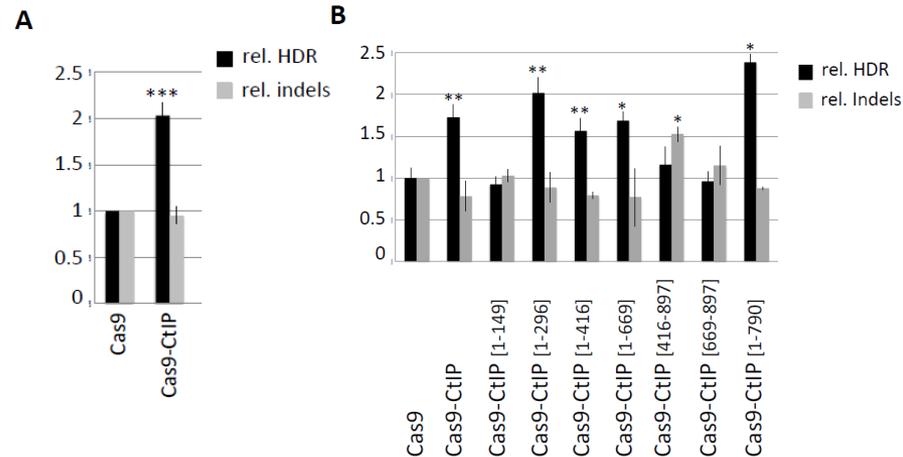
Proof of concept

◆ Forcing CtIP to the DNA break site stimulates transgene integration (A)

- The Cas9-CtIP fusion allowed to stimulate GFP cDNA integration by 2 fold compared to Cas9

◆ Identification of minimal HDR enhancer fragment of CtIP (B)

- Truncated CtIP proteins were fused to Cas9 nuclease and tested in RG37DR cells for GFP transgene integration at the AAVS1 locus using T2 guide RNA.
- N-terminal fragment of CtIP from aa 1 to 296 is sufficient for HDR stimulation. The N-terminal fragment from aa 1 to 296 was coined HE for HDR-Enhancer domain and the corresponding Cas9 fusion designated Cas9-HE.



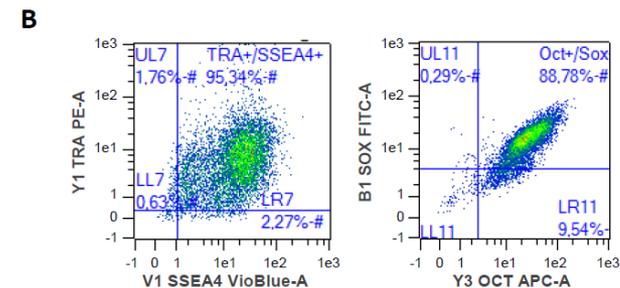
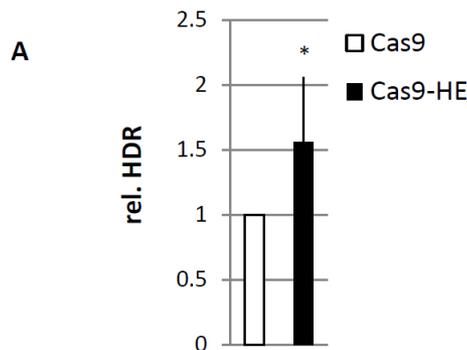
Human RG37DR fibroblasts were transfected with the indicated plasmids and GFP transgene donor with homology arms to the targeted AAVS1 locus. HDR-mediated transgene integration was measured by FACS analysis of GFP-positive cells resulting from targeted GFP transgene integration. Indels at the cleavage site were measured by the T7E1 assay.

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Proof of concept

▶ HDR stimulation in human iPS cells and rat oocytes

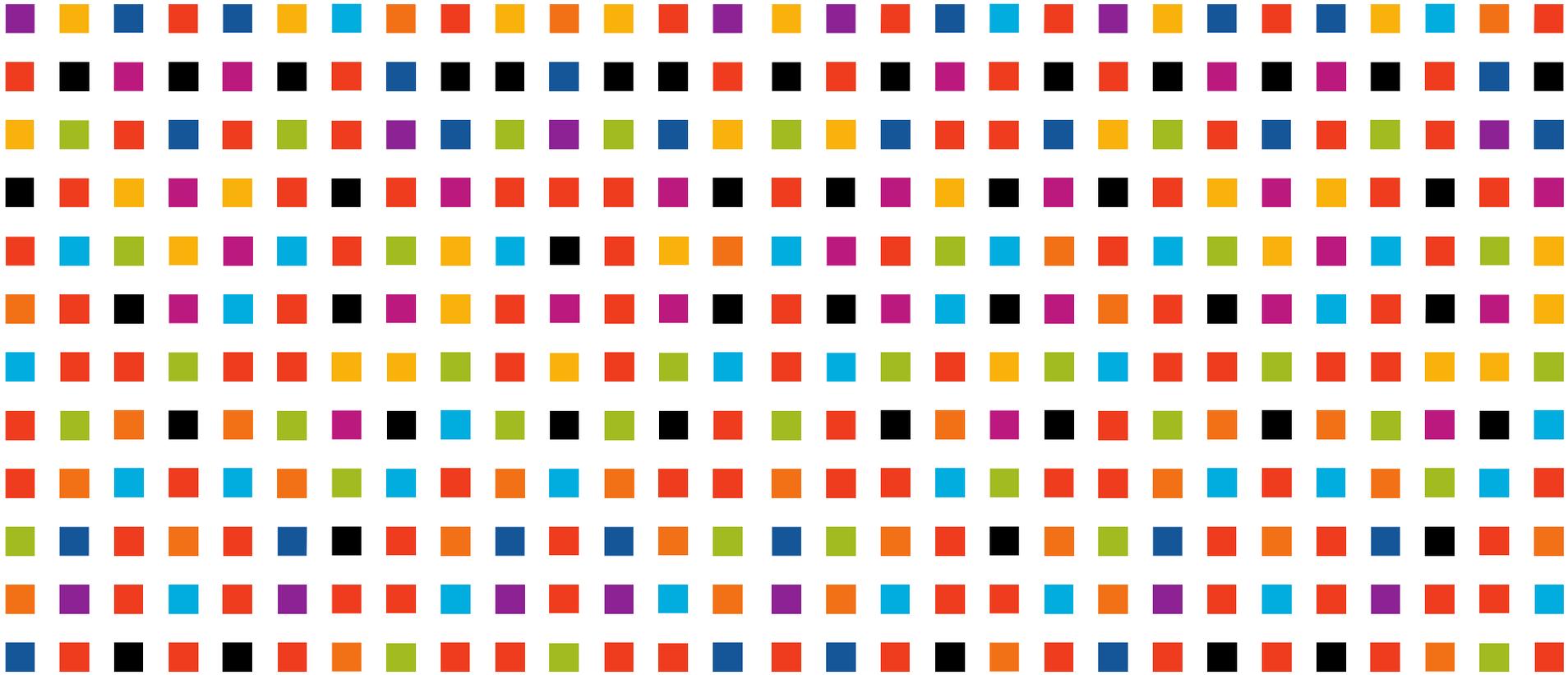
- ◆ Cas9-HE resulted in more efficient HDR than Cas9 in **human iPS cells (A)**
- ◆ Expression of stemness markers was maintained and cardiac differentiation could be efficiently induced (B)



- ◆ Integration by HDR was strongly increased at the Rosa26 locus in **rat zygotes microinjected with Cas9-HE** compared to Cas9 (representing 8 % and 1 % of harvested embryos for Cas9-HE and Cas9, respectively)

Cas9 form	Eggs injected (survival rate %)	Eggs transferred	E14 embryos (% of transferred)	indels + (% of E14)	donor integration+ (% of E14)	HDR+ (% of E14)
Cas9-HE	216 (75%)	154	37 (24%)	38 (78%)	5 (13%)	3 (8 %)
Cas9	284 (77%)	211	84 (39%)	62 (73%)	2 (2%)	1 (1%)

co-microinjection into rat zygotes of a long donor DNA (containing a 4.7 kbp flanked by homology arms of approximately 1 kbp), sgRNAs targeting the Rosa26 locus and Cas9-HE or Cas9 mRNA



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