SELECTED OPPORTUNITIES IN GENOME EDITING

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

(BIO16366)

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. Com., in press

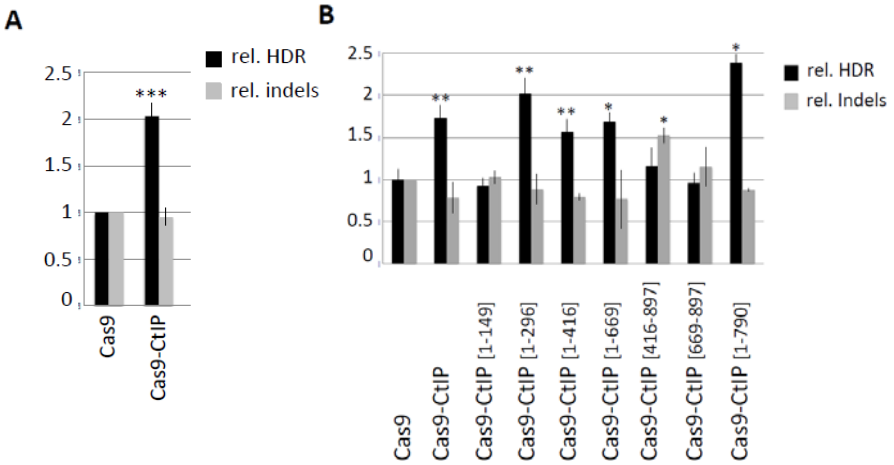
stephanie.olas@inserm-transfert.fr
alexandre.florimond@inserm-transfert.fr

CtIP fusion to Cas9 enhances transgene integration by homology-dependent repair (BIO 16366)

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.



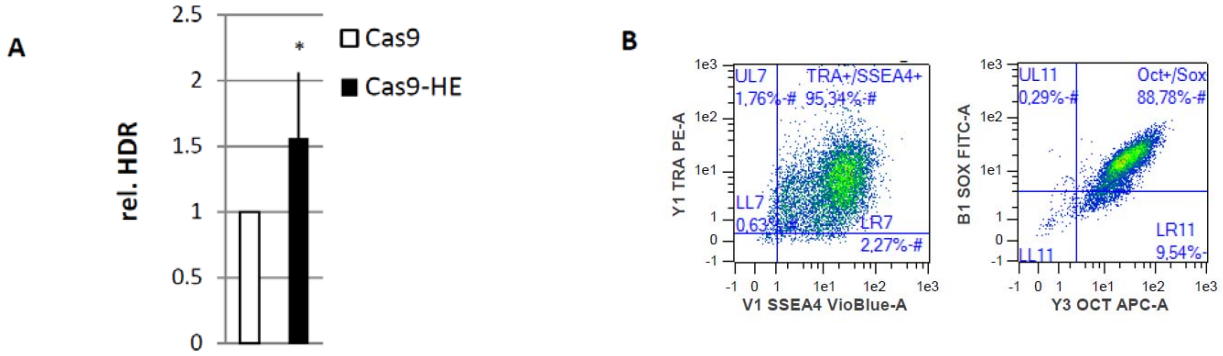
Human RG37DR fibroblasts were transfected with the indicated plasmids and GFP transgene donor with homology arms to the targeted AAVS1 locus. HDR-mediated transgene integration was measured by FACS analysis of GFP-positive cells resulting from targeted GFP transgene integration. Indels at the cleavage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

stephanie.olas@inserm-transfert.fr
alexandre.florimond@inserm-transfert.fr

CtIP fusion to Cas9 enhances transgene integration by homology-dependent repair (BIO 16366)

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)

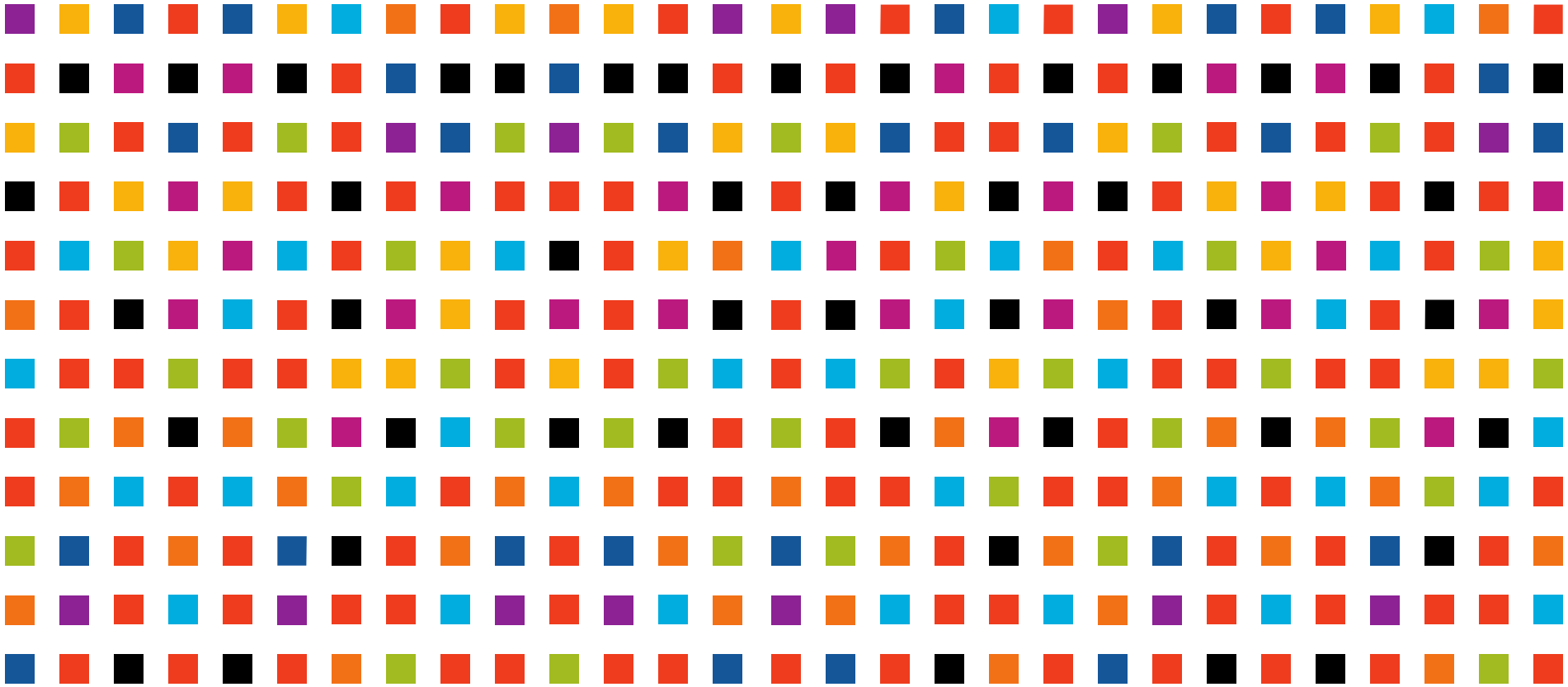


  - Integration by HDR was strongly increased at the Rosa26 locus in **rat zygotes microinjected with Cas9-HE** compared to Cas9 (representing 8 % and 1 % of harvested embryos for Cas9-HE and Cas9, respectively)

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

stephanie.olas@inserm-transfert.fr
alexandre.florimond@inserm-transfert.fr



CONTACTS:

STEPHANIE.OLAS@INSERM-TRANSFERT.FR

ALEXANDRE.FLORIMOND@INSERM-TRANSFERT.FR