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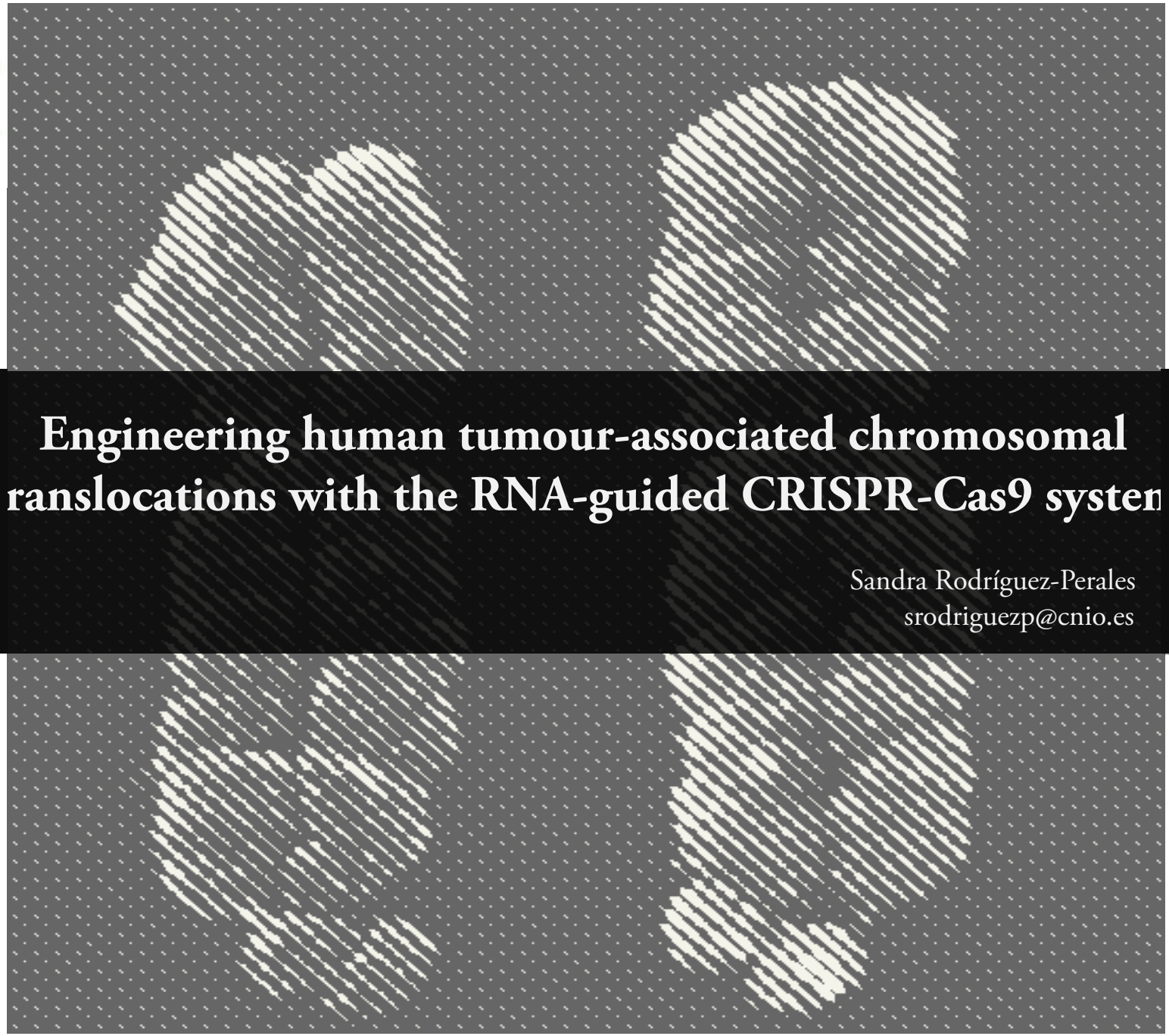


Engineering human tumour-associated chromosomal ranslocations with the RNA-guided CRISPR-Cas9 systen

Sandra Rodríguez-Perales
srodriguezp@cnio.es

4-7 July 2015

10th European Cytogenetics Conference

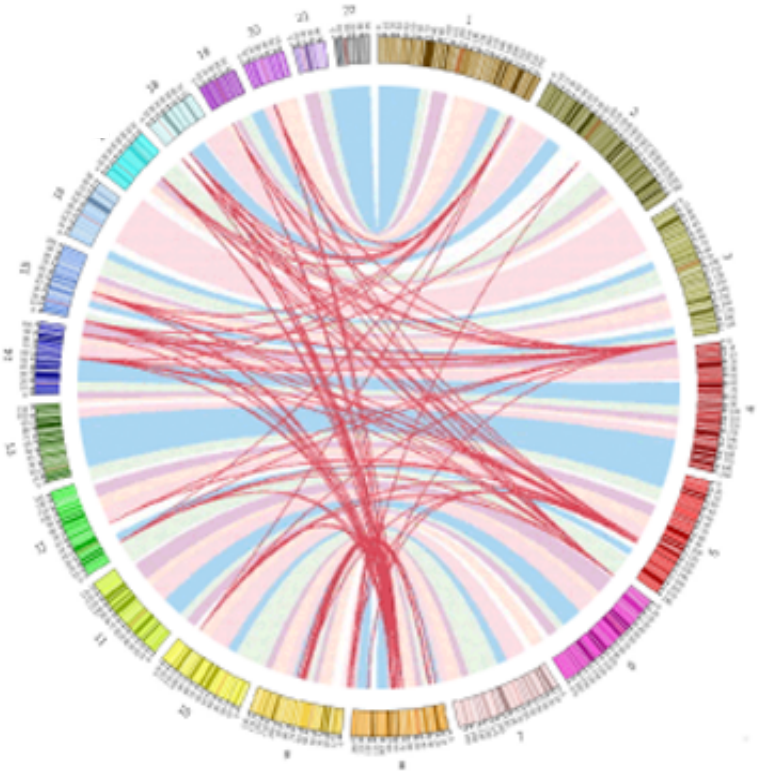
Chromosome Translocation

& CANCER

Chromosome translocations:

primary cause of many cancers

involved in the progression of tumors

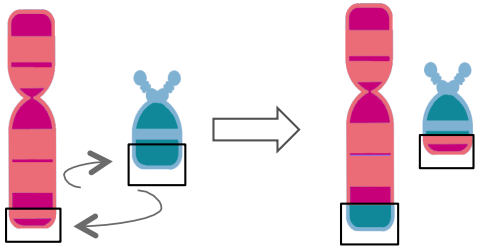


Chromosome Translocation

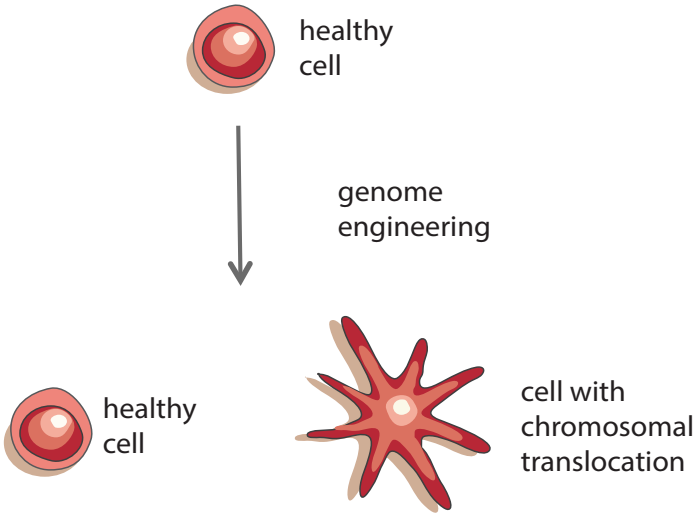
MODELS

The most widely used models:
- Patient-derived cell line
  (accumulate secondary mutations)
- Overexpression model

de novo generation of the translocation



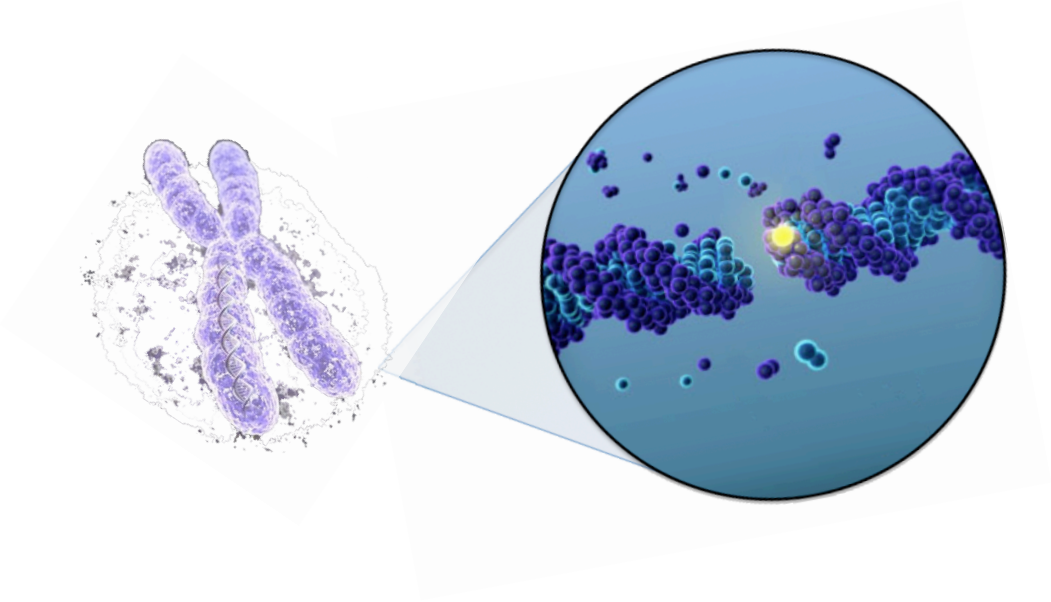
GE MODELS



Chromosome Translocation

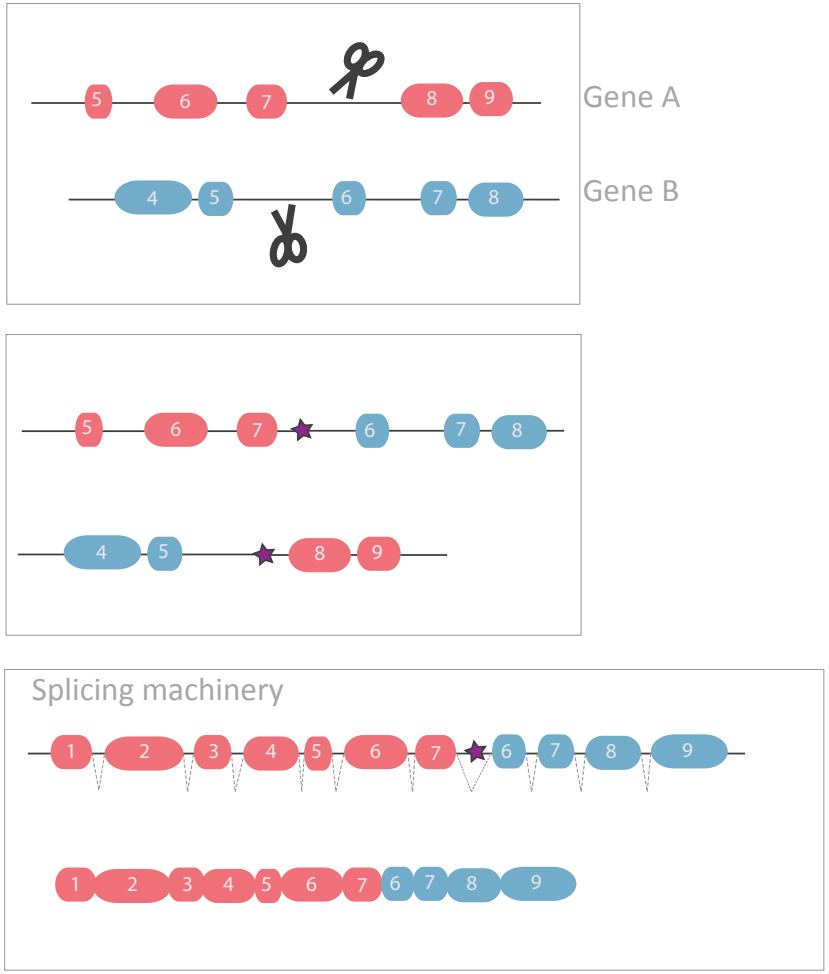
CAUSES

Two double strand breaks (DSBs)

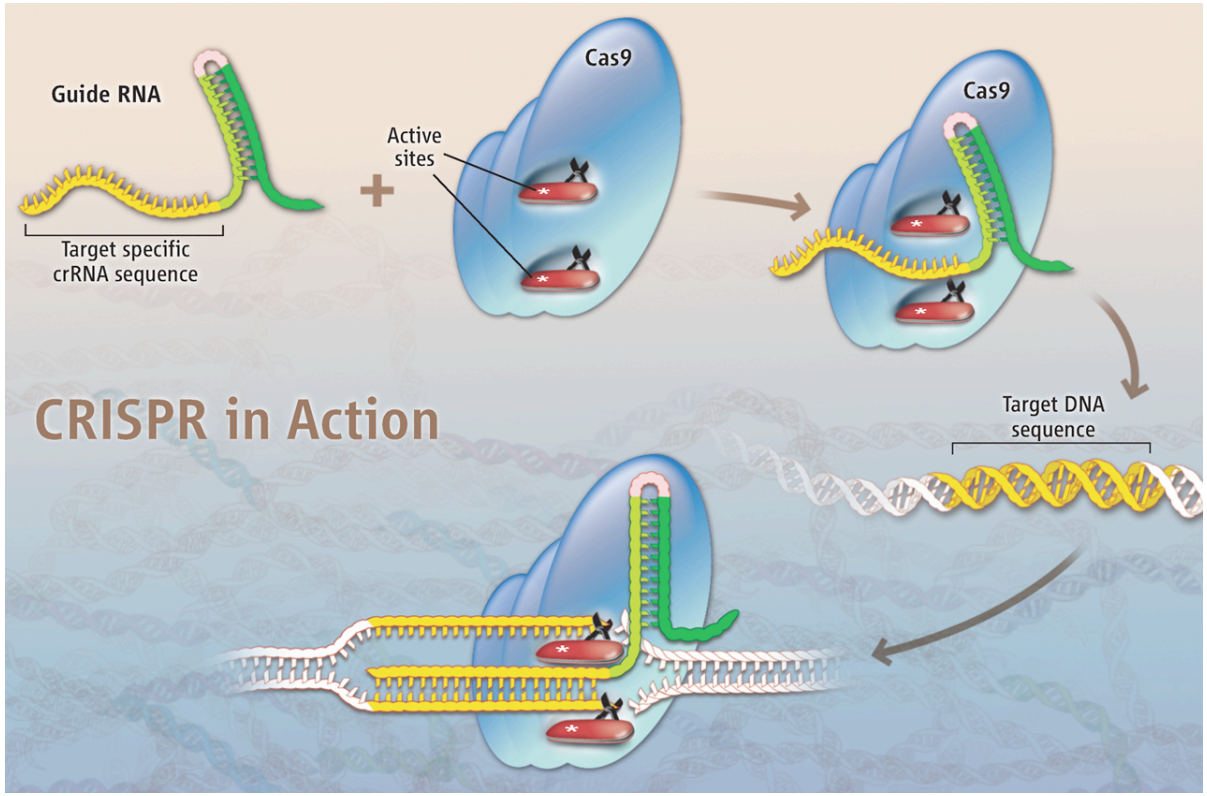


DSBs

DSB introduction within the targeted regions in the genome



CRISPR System

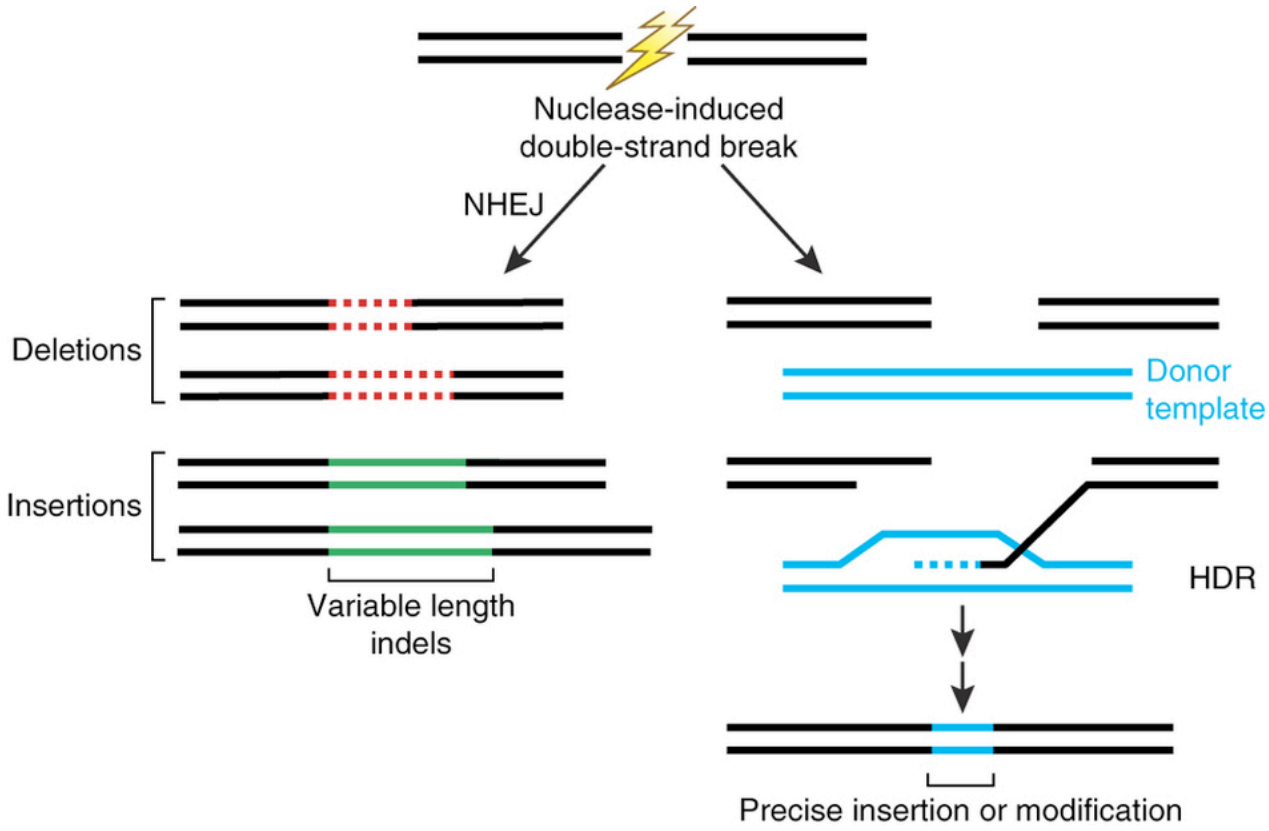


Small guide RNAs (sgRNAs) that lead Cas nucleases to target DNA specific sequences and induce nucleic acid cleavage

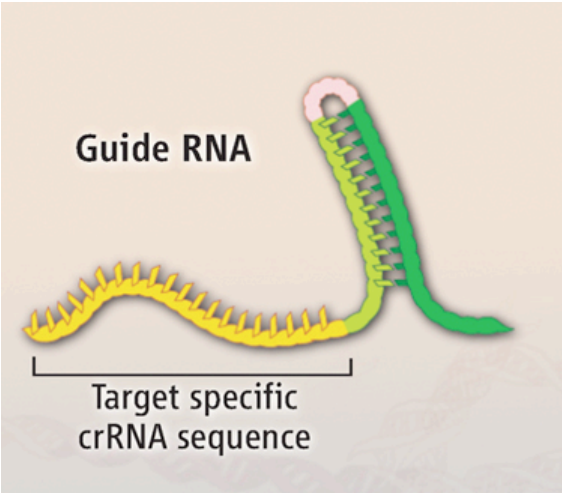
SANDRA RODRIGUEZ-PERALES

Science 20 December 2013:
Vol. 342 no. 6165 pp. 1434-1435

CRISPR System



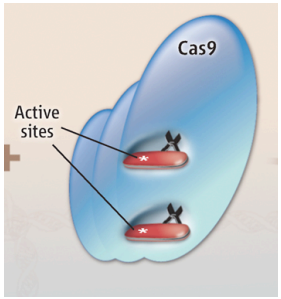
The specificity of DNA recognition is achieved through a short RNA sequence and so by simply altering the composition of this RNA code we can very easily change the specific location to the target genome



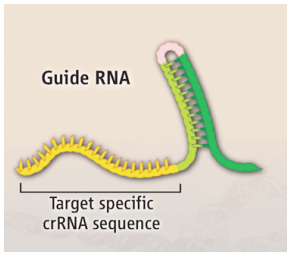
CRISPR System

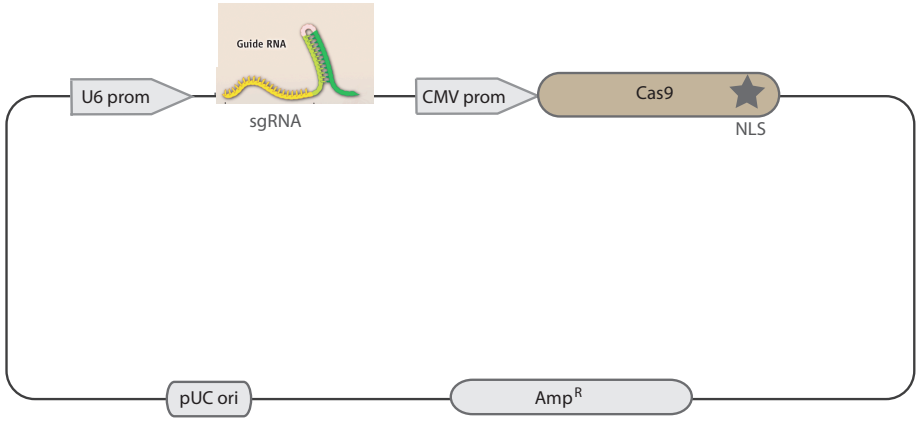
Two components must be introduced into the cell to perform genome editing

The Cas9 nuclease

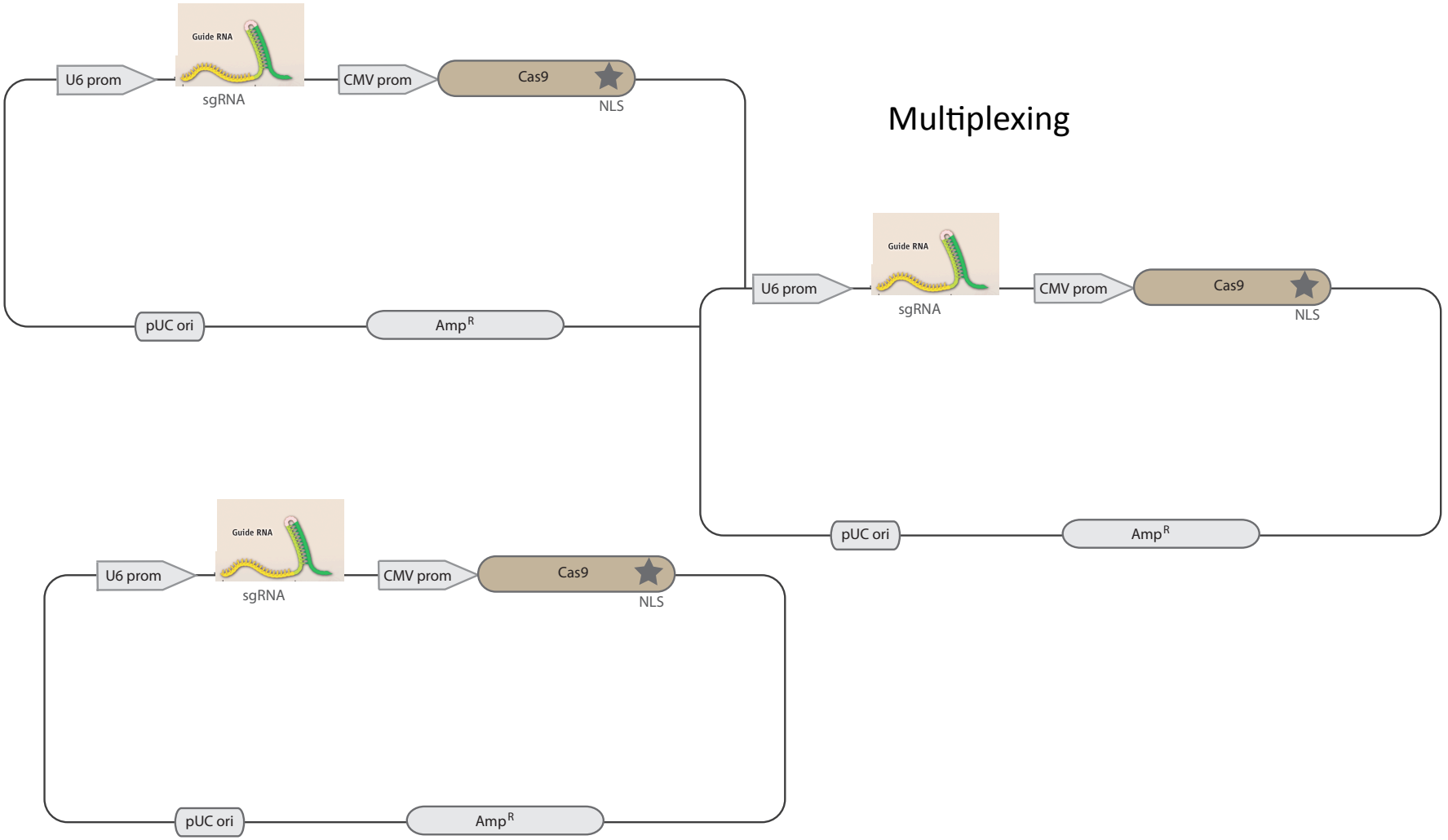


The programmable guide RNA that target at a specific 20nt region



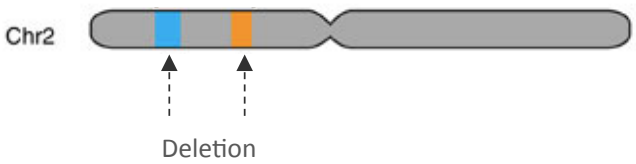


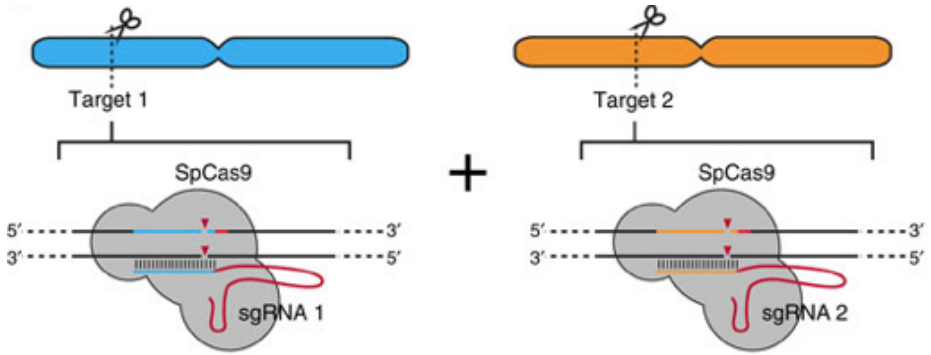
Modified from Church Lab's plasmid (obtained from ADDGENE)

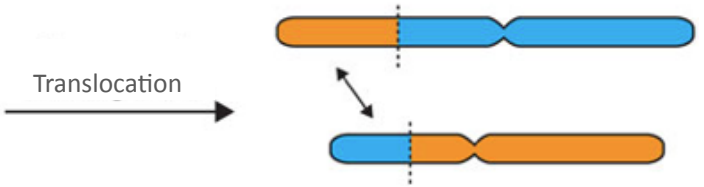


Modified from Church Lab's plasmid (obtained from ADDGENE)

Engineering a Translocation



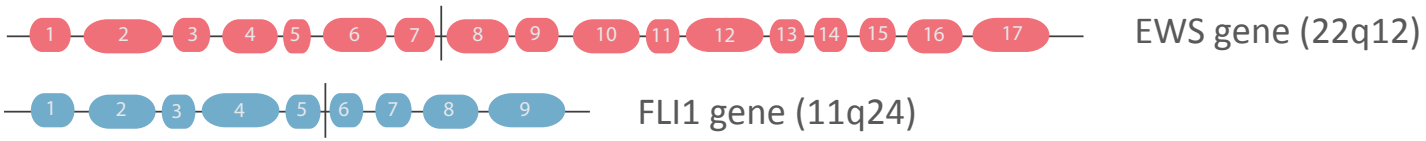




Choi et al; Nat Commun 5 (3728)

Ewing Sarcoma is a prototype of a solid tumor
Occurs in children and adolescents
Arise in tissues of mesenchymal origin

t(11;22)(q24;q12)

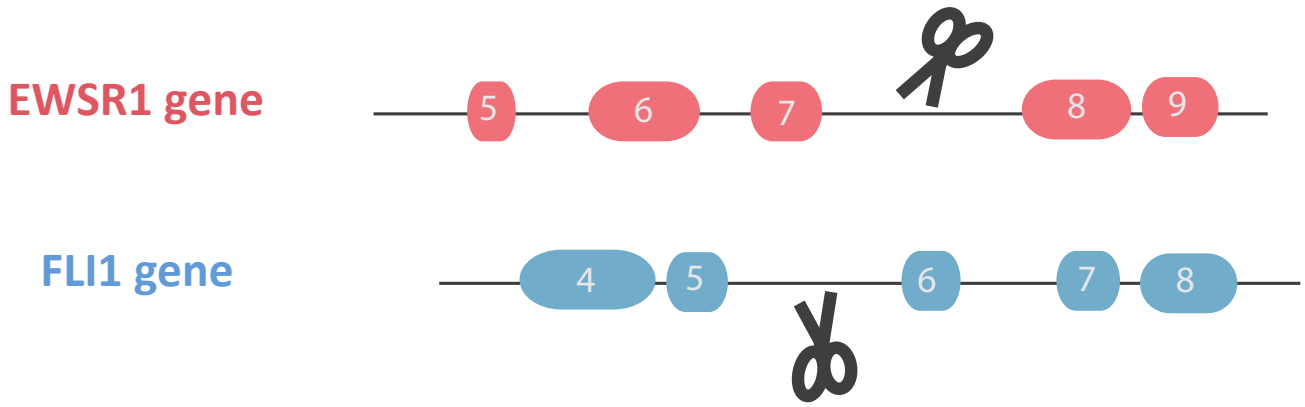


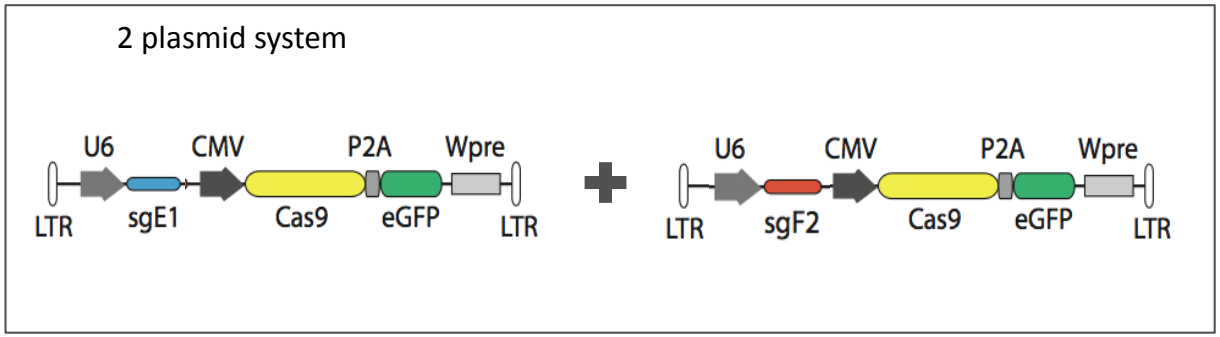
EWS/FLI1 fusion gene

EWSR1-FLI1 chimeric protein (transcription factor)

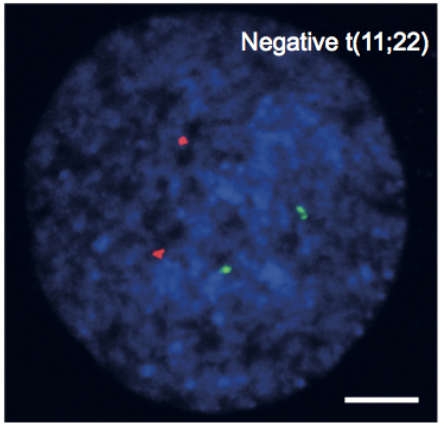
Torres et al, Nature Communications 5; 3964 (2014)

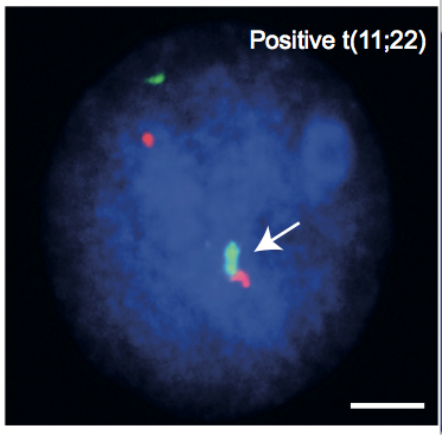
To induce t(11;22)(q24;q12), 2 sgRNAs were designed





To quantify translocations
FISH





FISH probe:
EWS(green)/*FLI1*(red)
Single fusion probe

Total transfected cells, no selection

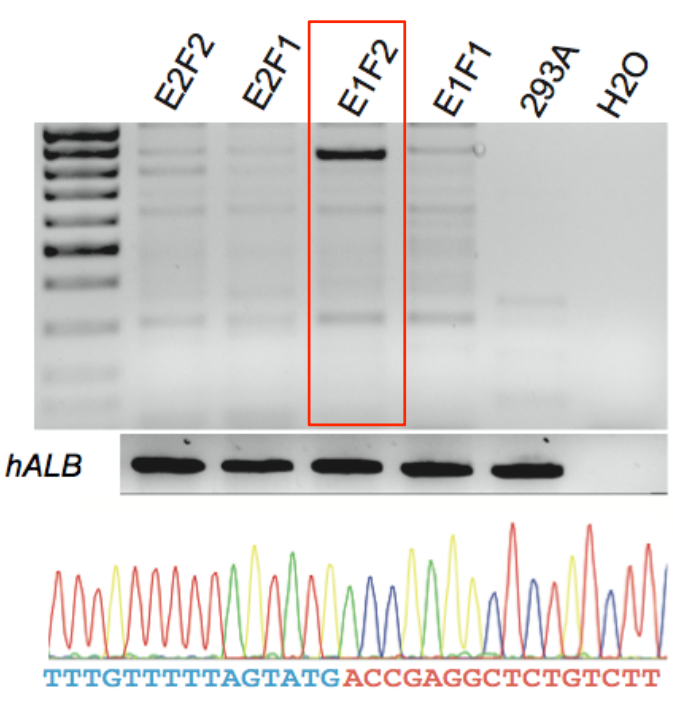
Cell Type	Total cell nuclei	t(11;22)	Frequency (%)
HEK293	285	5	1,75
hMSCs	300	1	0,3

CRISPR efficiency can vary depending on the cell type

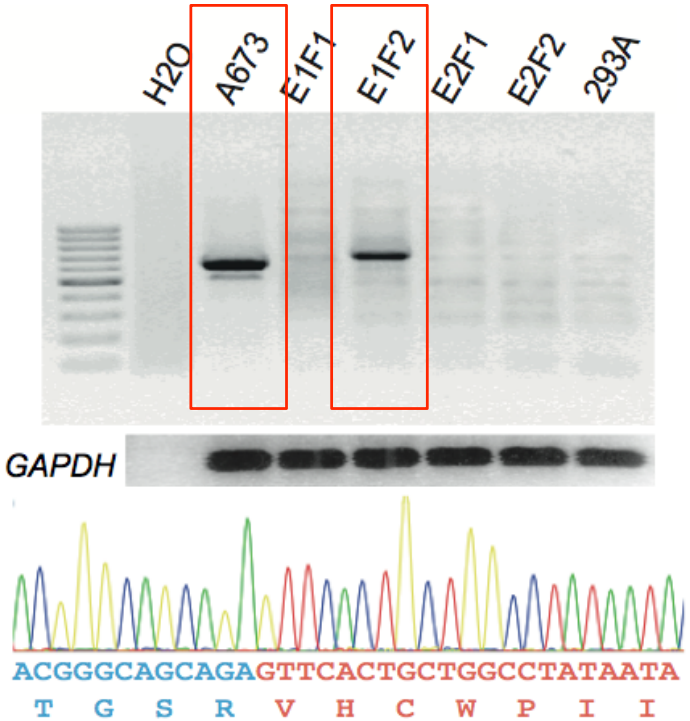
Torres et al, Nature Communications 5; 3964 (2014)

MOLECULAR ANALYSIS

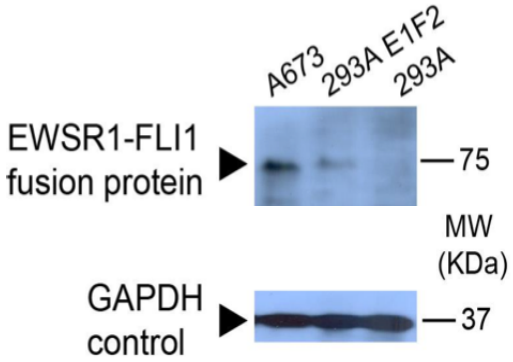
DNA sequencing

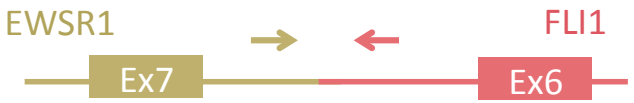


cDNA sequencing (RT-PCR of RNA)



Western blot



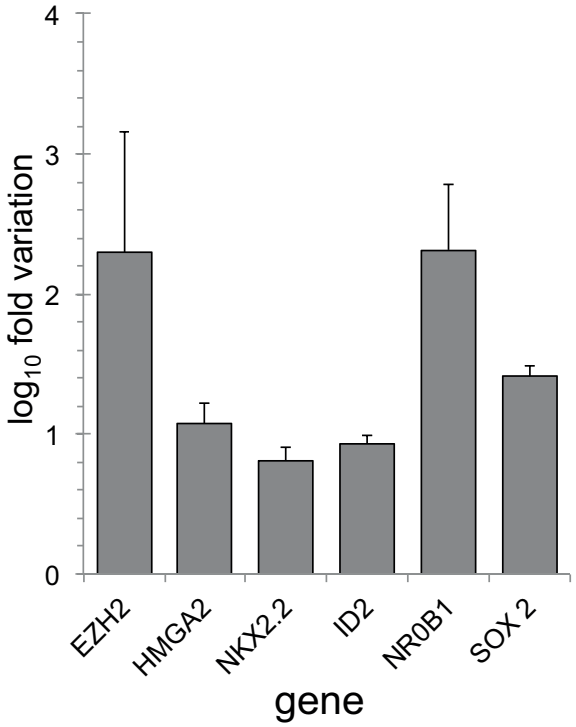


A673: ewing sarcoma cell line

Torres et al, Nature Communications 5; 3964 (2014)

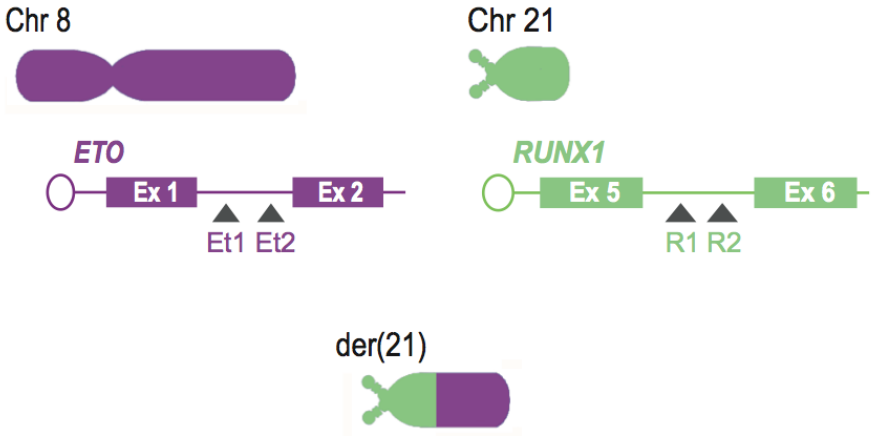
FUNCTIONALITY OF THE FUSION PROTEIN

The levels of expression EWSR1-FLI1 target known genes were quantified by real-time reverse transcription-PCR (quantitative PCR)

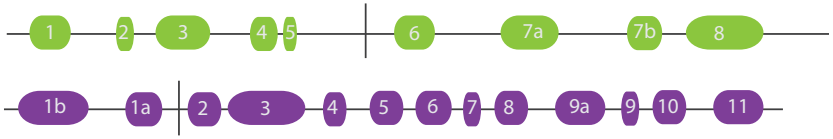


Increased over the normal expression of HEK293 non-t(11;22) cells

Torres et al, Nature Communications 5; 3964 (2014)



The translocation fuses:



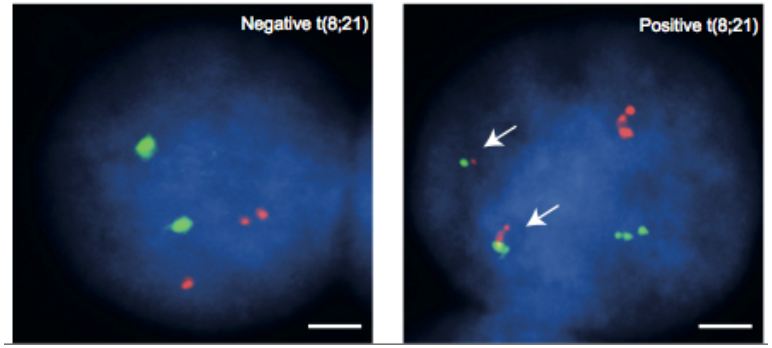
RUNX1 gene on chromosome 21

ETO gene on chromosome 8

RUNX1/ETO fusion gene

Torres et al, Nature Communications 5; 3964 (2014)

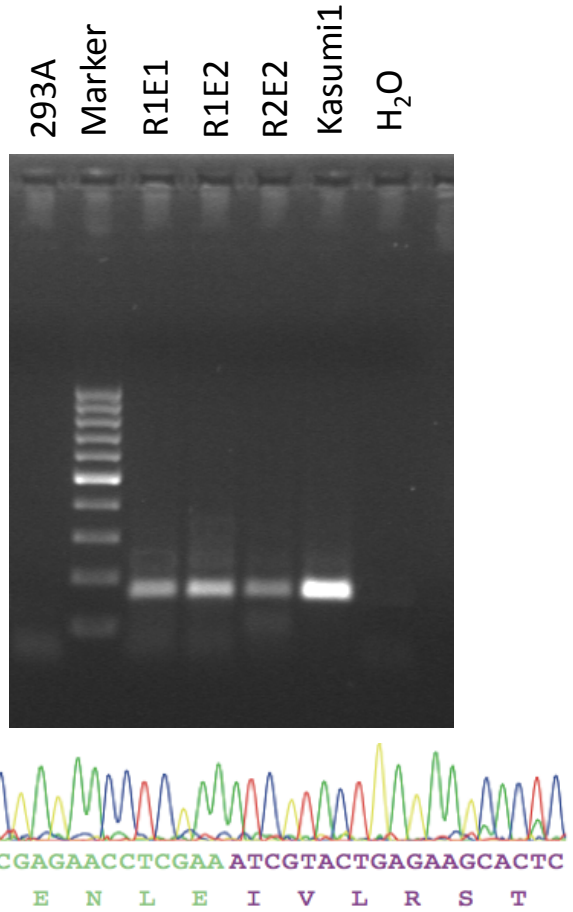
FISH ANALYSIS



Dual fusion probe

Cell type	Total cell nuclei	t(11;22)	Frequency (%)
HEK293	310	12	3,8
CD34+	1200	2	0,16

cDNA sequencing (RT-PCR of RNA)



Torres et al, Nature Communications 5; 3964 (2014)

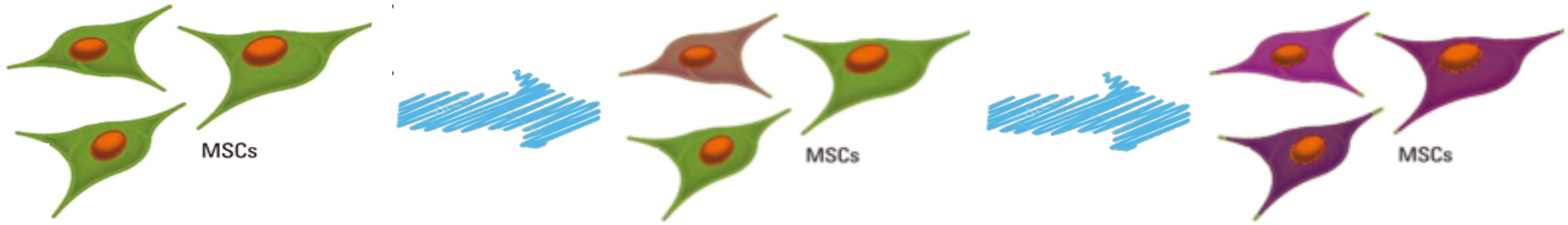
Cell Type	Cell nuclei	t(11;22)	Freq (%)
HEK293	285	5	1,75
hMSCs	300	1	0,3

	Cell nuclei	t(8;21)	Freq (%)
HEK293	310	12	3,8
CD34+	1200	2	0,16

Generation of an Ewing sarcoma human cellular model using the CRISPR system

Hallmark: t(11;22)

Cell type: hMSC



0,3% t(11;22)+ cells

100% t(11;22)+ cells

Torres et al, In press

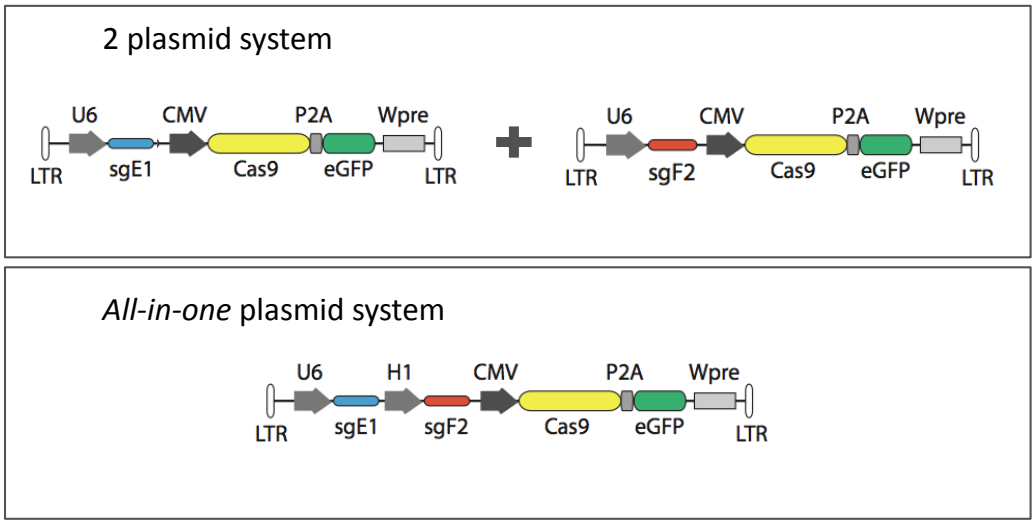
An optimized strategy is needed, especially when mimicking translocations in HUMAN PRIMARY CELLS

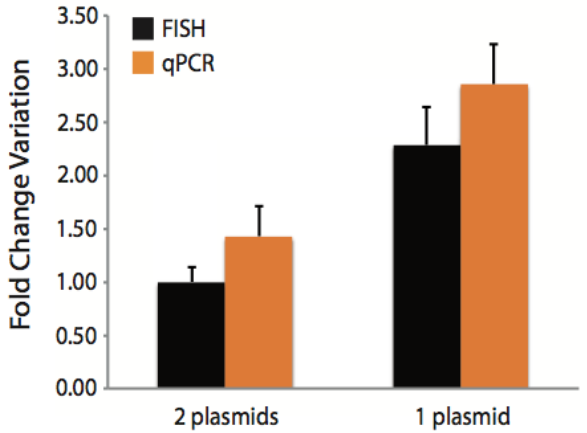
Conditions optimized:

1. CRISPR expression plasmid
2. Amount of transfected plasmid
3. CRISPR delivery system
4. Selection of transfected cells

Torres et al, In press

1.- CRISPR expression plasmid

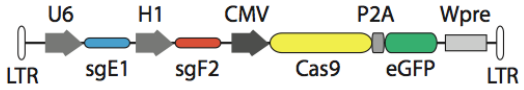


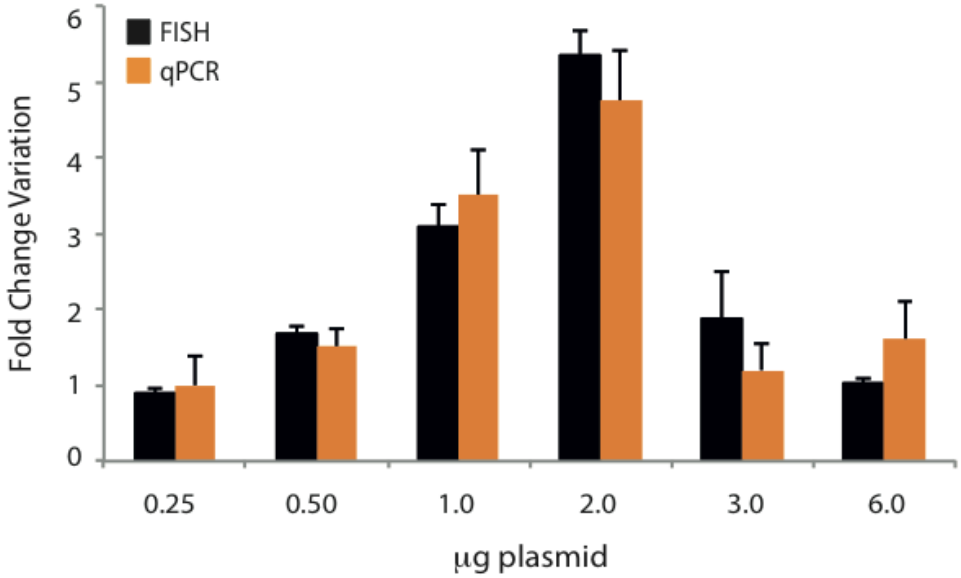


Torres et al, In press

2.- Amount of transfected plasmid

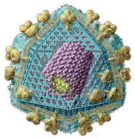
All-in-one plasmid system

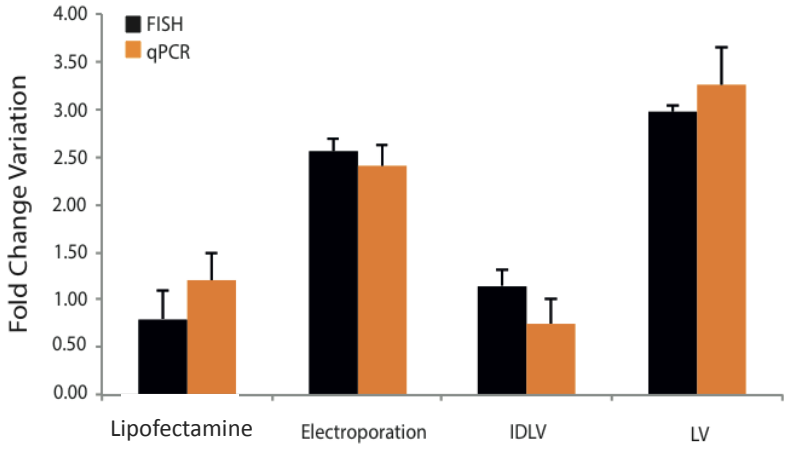




Torres et al, In press

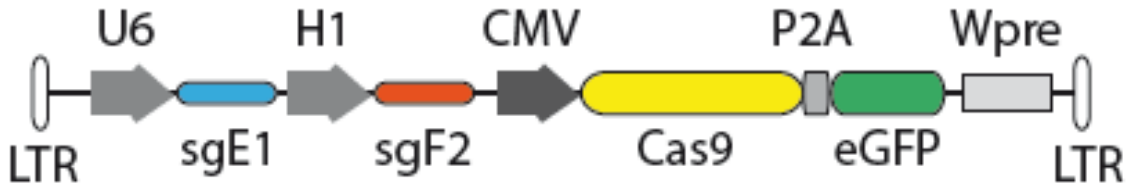
3.- CRISPR delivery system

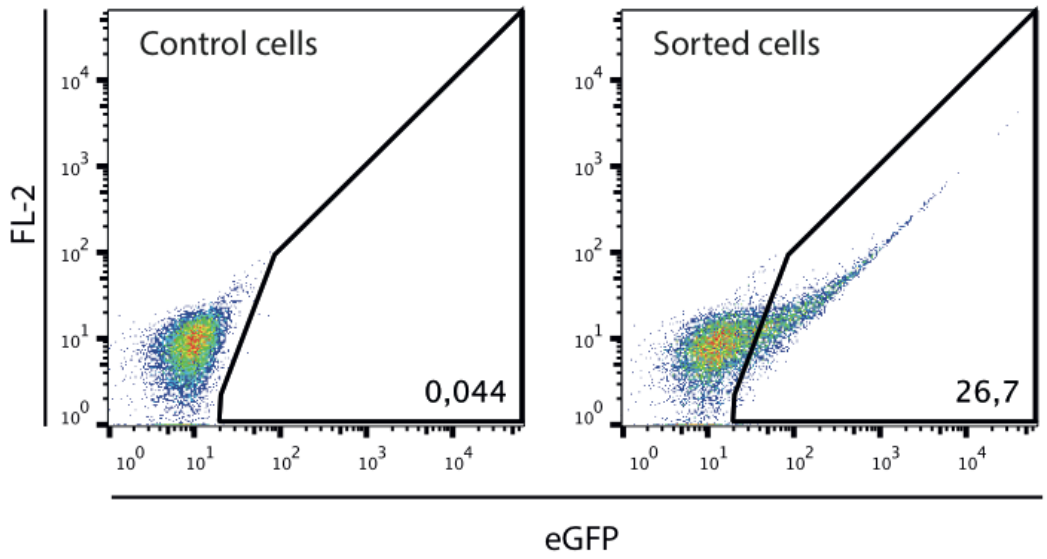
	Neon	Lipofectamine 3000
Electroporation		
Viral	LV	IDLV
		

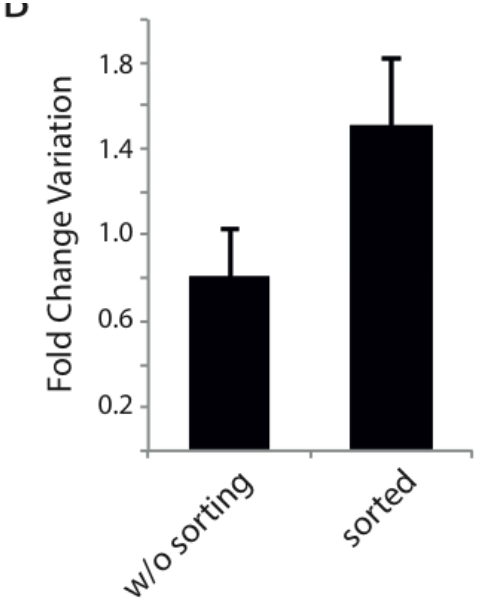


Torres et al, In press

4.- Selection of transfected cells







Torres et al, In press

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