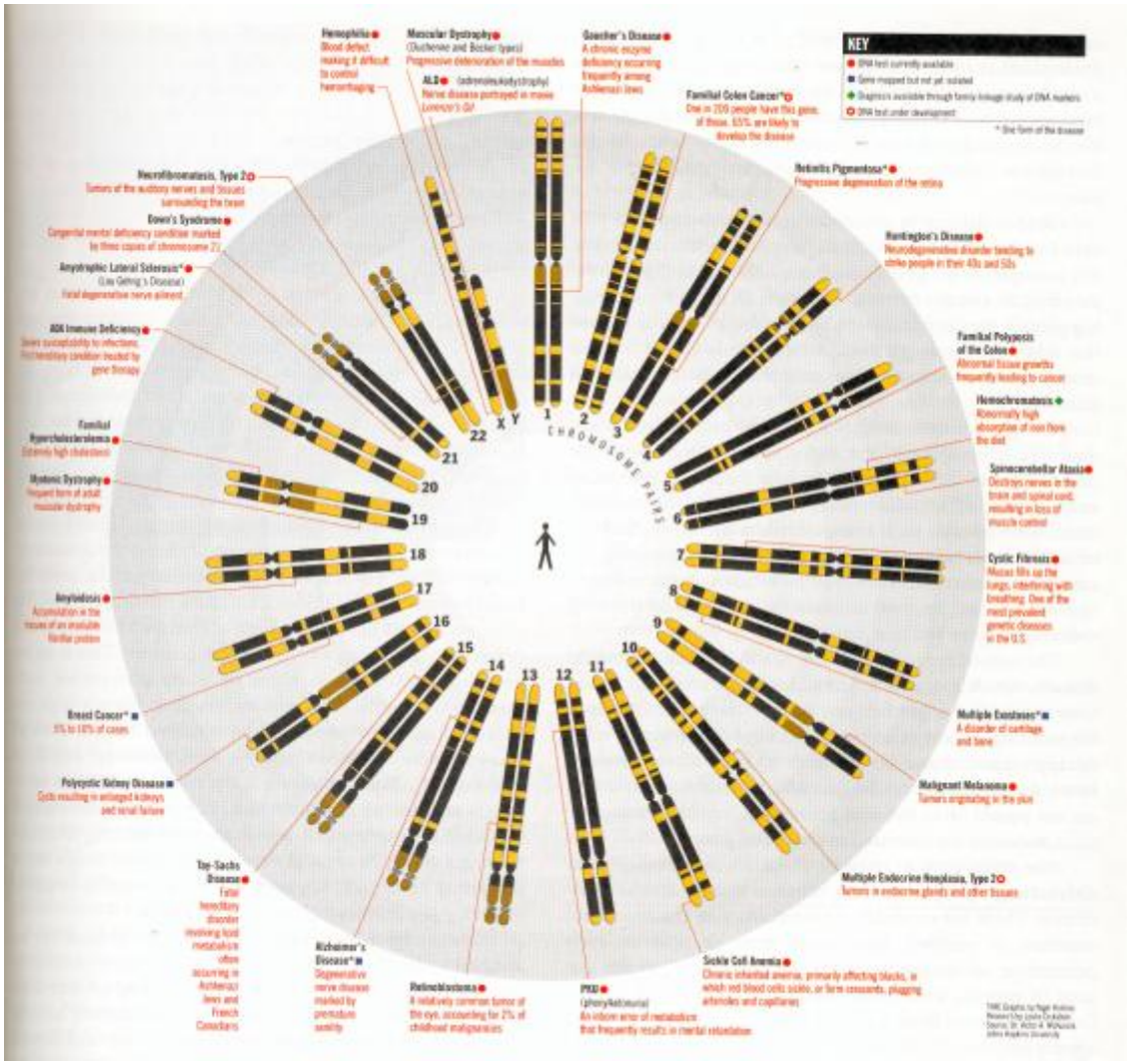
New Tool for Genome Surgery

Presented by Duo Li

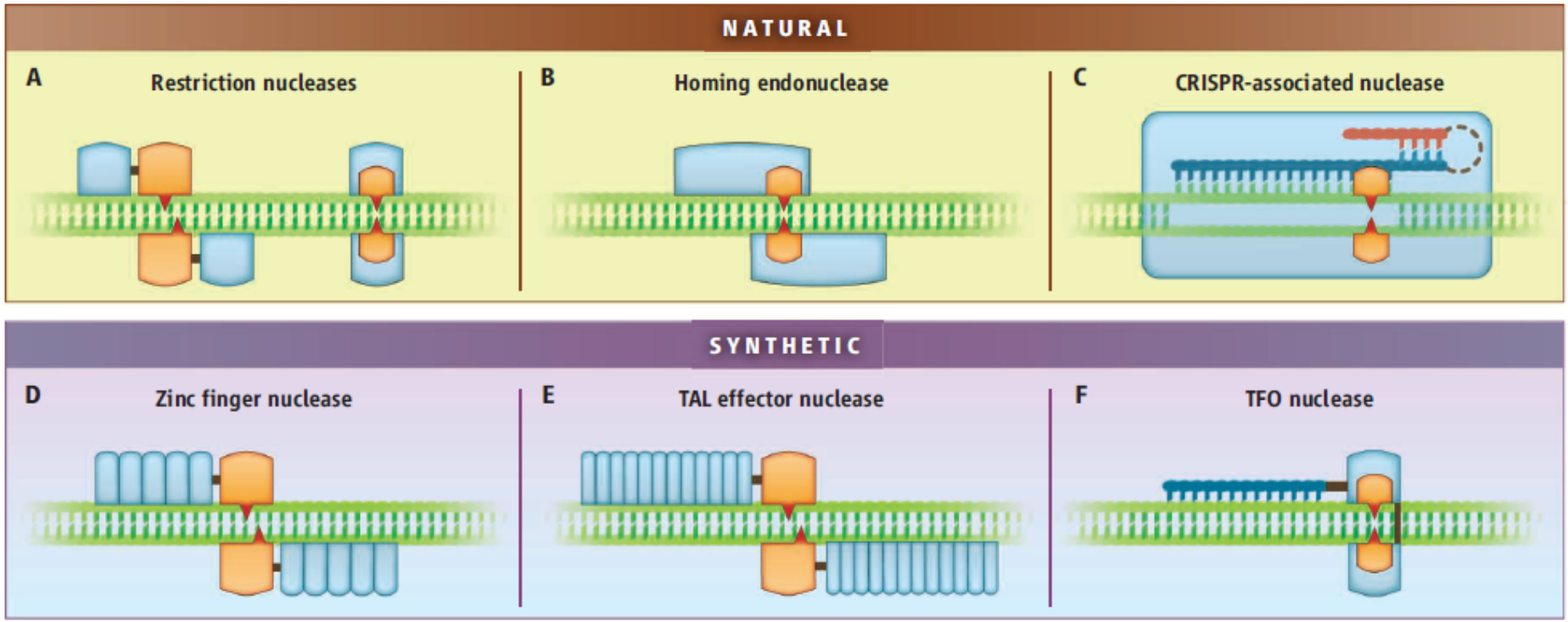
12.03.2013

Location of Various Genetic Disesases



Griffiths AJF, *et al.*

Potential Genome Editing



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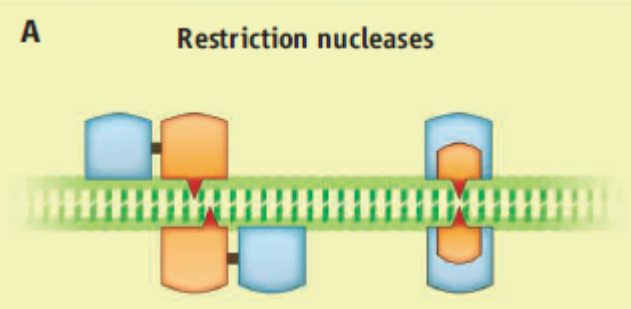
Restriction Nuclease



- Recognize sites very shot, 4-8 bp.
- Occur too frequently in the genome.

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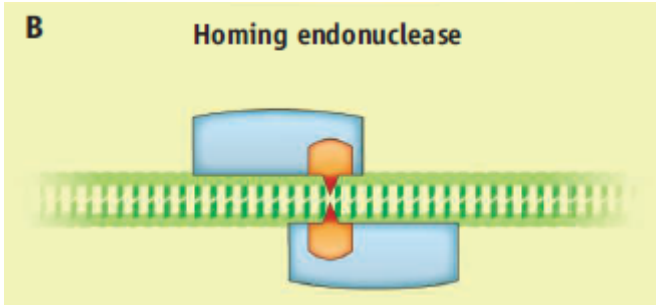
Homing Endonuclease



- Microbial DNA-cleaving enzymes mobilize their own reading frames by generating double strand breaks at specific genomic invasion sites.
- Recognize longer targets, 20-30bp.
- Wide range of fidelity influenced by host constraints on the coding sequence of the target gene.
- Results in the insertion, deletion, mutation or correction of DNA sequences.
- Specificity require laborious protein design.

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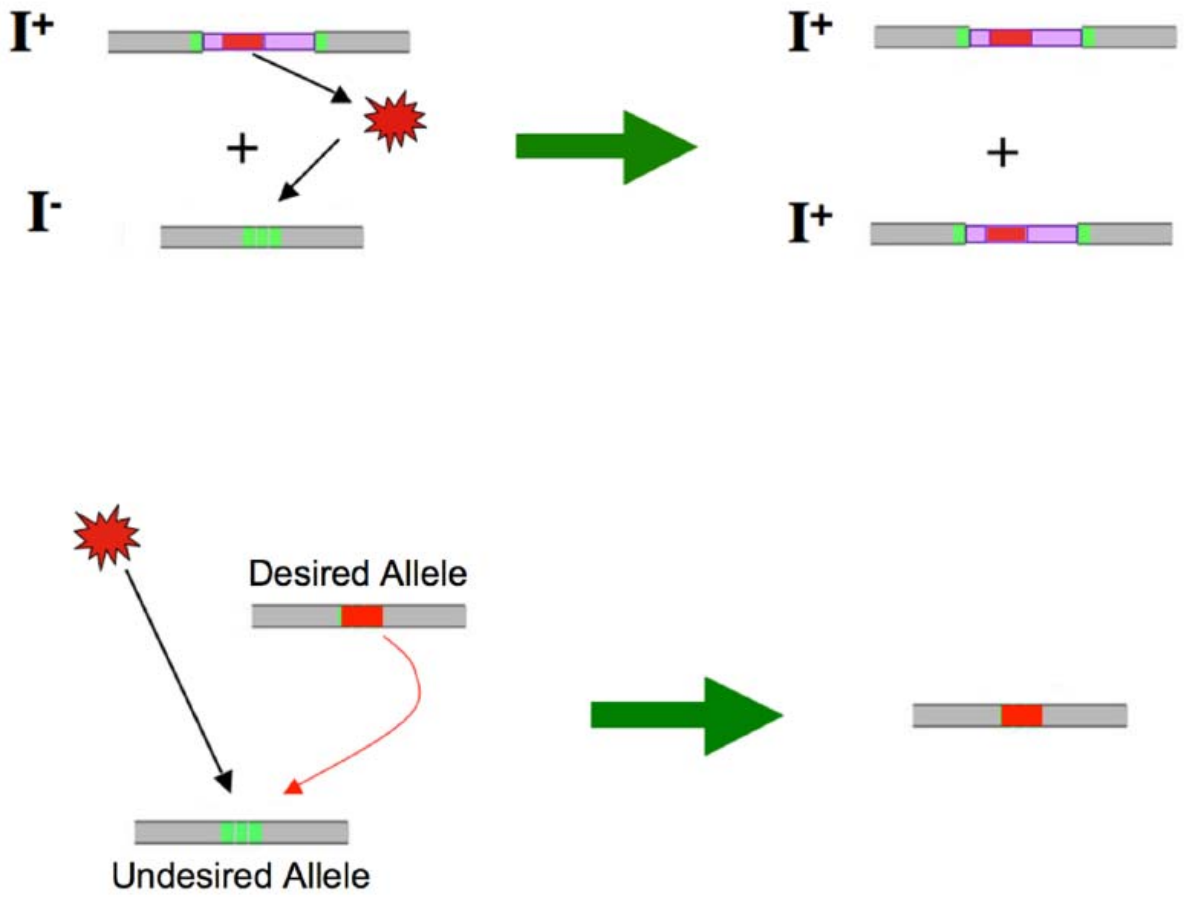
Homing Endonuclease and Genetic Homing



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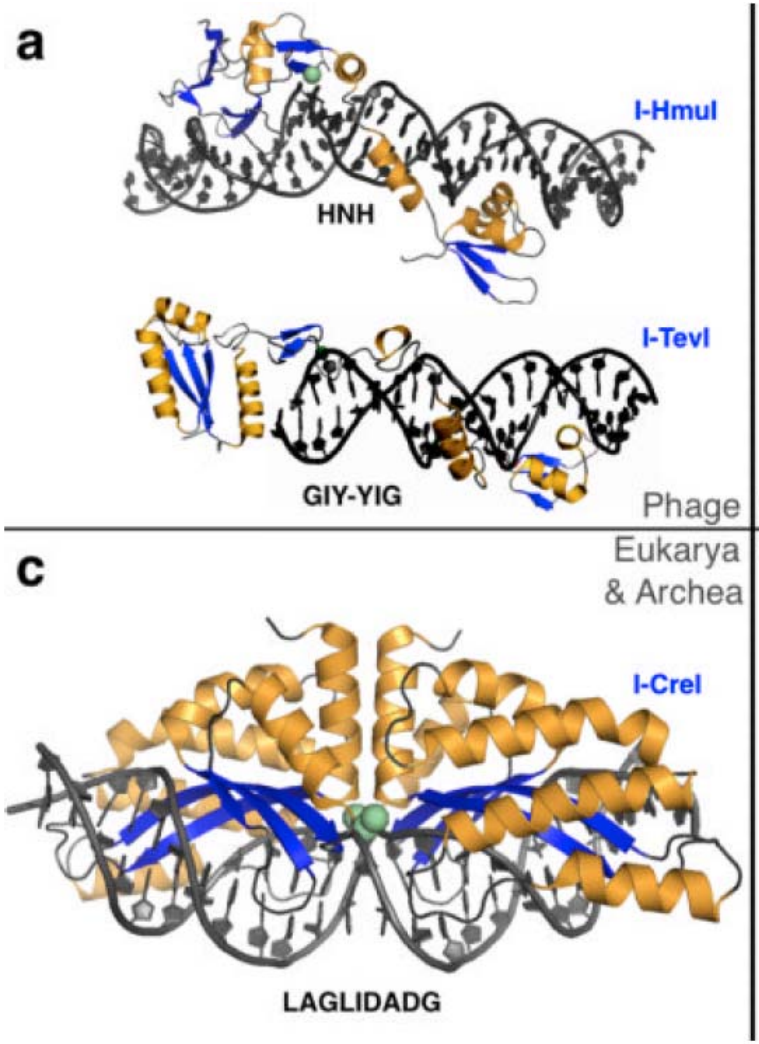
Homing Endonuclease Structural Families



- Less specific,
- More heterogeneous collection of DNA contacts,
- Major and minor groove of their target sites,
- Extended, multi-domain protein structures contact a series of DNA regions.

- Antiparallel β-sheets,
- Major grooves,
- Sequence-specific and non-specific contacts,
- Distributed nonuniformly across multiple consecutive basepairs.

Recognition mechanisms:

- Form highly elongated protein folds with minimal hydrophobic cores.
- Multimerize and thereby double their DNA-contact surface

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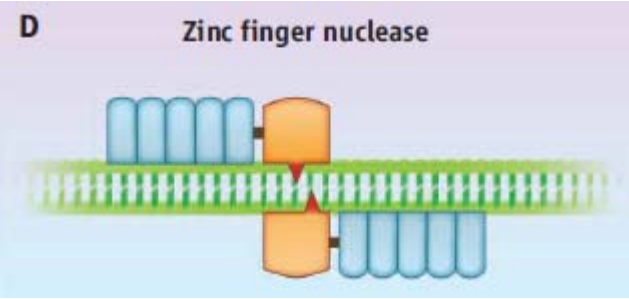
Zinc Finger Nuclease



- Consists of a nuclease fused to a set of zinc finger domains interact with 3 nucleotides.
- Target up to 36 bp.
- Specificity depends on the established triplet domain.
- Modification includes: gene disruption, gene correction, targeted gene addition.

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Structure of Zinc Finger Nucleases

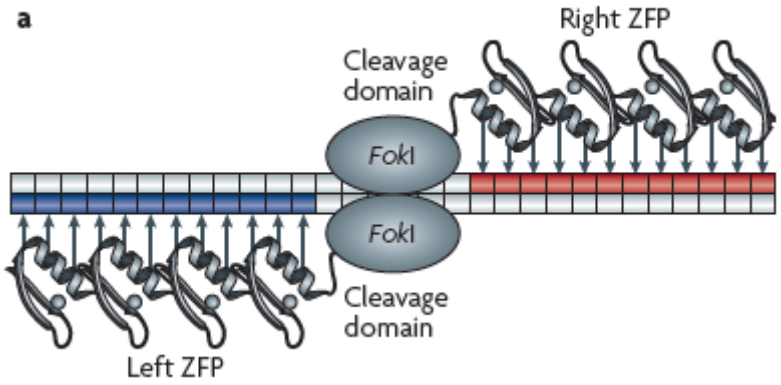

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Table 1 | **Endogenous genes modified by zinc finger nucleases**

Organism	Gene	ZFN development method*	Refs
Gene disruption			
Fruitflies	*yellow*	Modular assembly	2
	rosy, *brown*	Modular assembly	60
CHO cells	*Dhfr*	Two-finger modules	48
	Dhfr, *Glul*	Two-finger modules	50
	Fut8	Two-finger modules	92
	Bax, *Bak1*	Two-finger modules	49
Zebrafish	*kdr*	Bacterial one-hybrid	36
	golden, *no tail*	Two-finger modules	35
	tfr2, *dat*, *telomerase*, *hif1aa*, *gridlock* (also known as *hey2*)	OPEN	37
	cxcr4a	Modular assembly	93
Human T cells	*CCR5*	Two-finger modules‡	76
Hek293 cells	*CCR5*	Modular assembly	17
Rats	*Rab38*, *IgM*	Two-finger modules	38
	Il2rg	Two-finger modules	39
SupT1 cells	*CXCR4*	Two-finger modules	94
K562 cells, HeLa cells	*PPP1R12C* (the AAVS1 locus), *TP73*, *MAP3K14*, *EP300*, *BTK*, *CARM1*, *GNAI2*, *TSC2*, *RIPK1*, *KDR*, *NR3C1*	Two-finger modules	47
Gene correction			
Fruitflies	*yellow*	Modular assembly	3
	rosy	Modular assembly	60
	coilin, *pask*	Modular assembly	34
K562 cells, human T cells	*IL2RG*	Two-finger modules‡	61
K562 cells	*IL2RG*, *VEGF*, *HOXB13*, *CFTR*	OPEN	62
Tobacco	*SuRA*, *SuRB* (acetolactate synthase genes)	OPEN	63
Arabidopsis thaliana	*ABI4*, *KU80*	Modular assembly	42
	ADH1, *TT4*	OPEN	41
Mouse ES cells	*H3f3b*	Two-finger modules	67
Gene addition			
K562 cells	*IL2RG*	Two-finger modules‡	66
Human ES cells	*IL2RG*, *CCR5*	Two-finger modules‡	68
	PIGA	OPEN	70
	OCT4 (also known as *POU5F1*), *PPP1R12C* (AAVS1 locus), *PITX3*	Two-finger modules	71
Tobacco	Chitinase	Two-finger modules	74
Maize	*Ipk1*, *Zein protein 15*	Two-finger modules‡	75
Human tissue culture cells	*PPP1R12C* (AAVS1 locus)	Two-finger modules‡	72
Mouse ES cells	*H3f3b*	Two-finger modules	67

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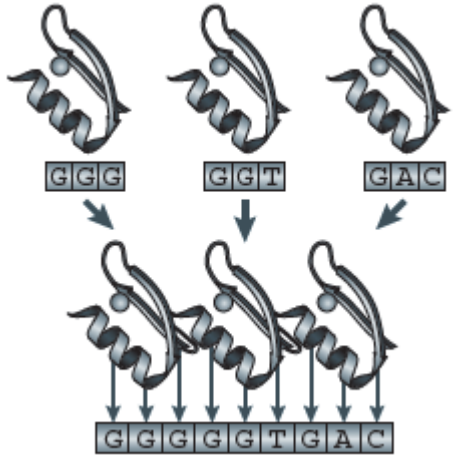
1 Modular Assembly



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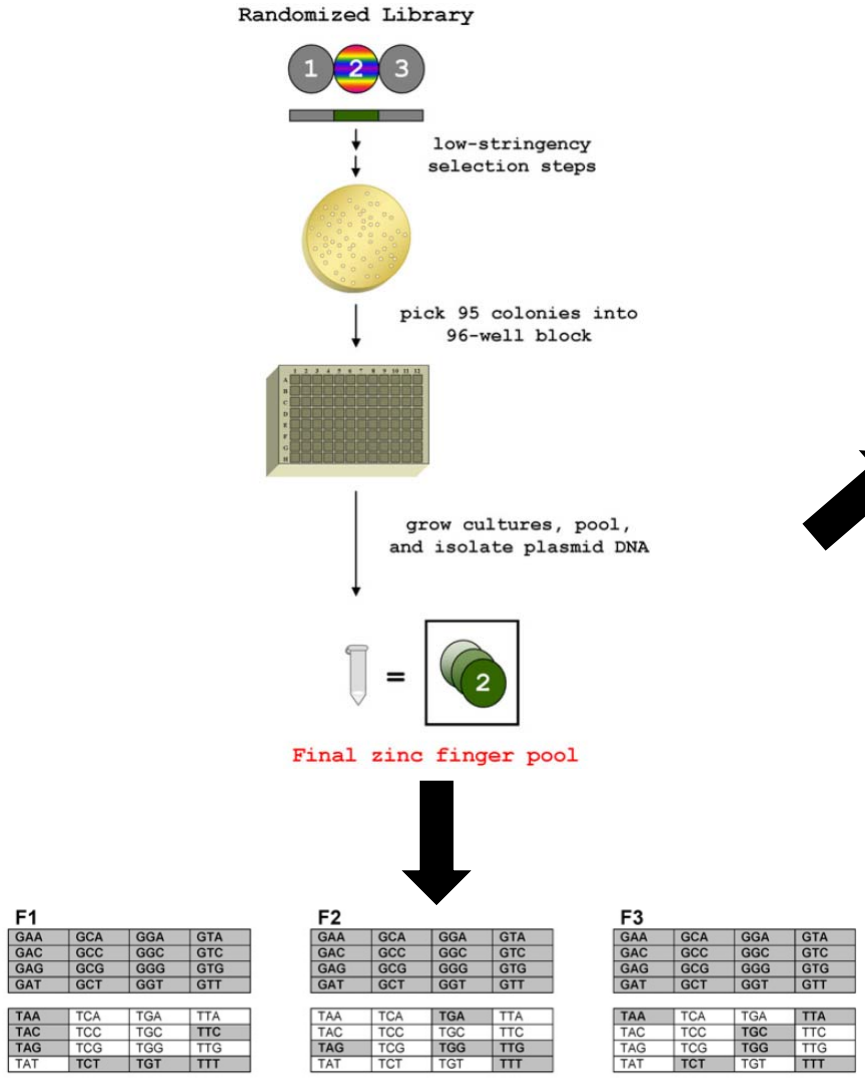
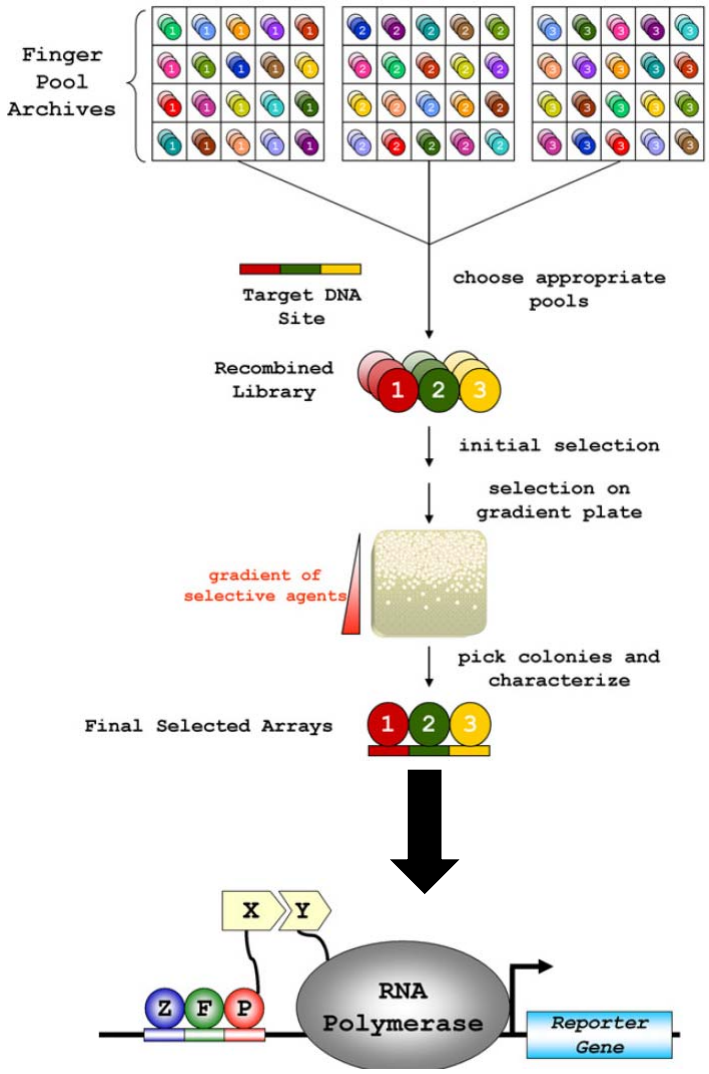
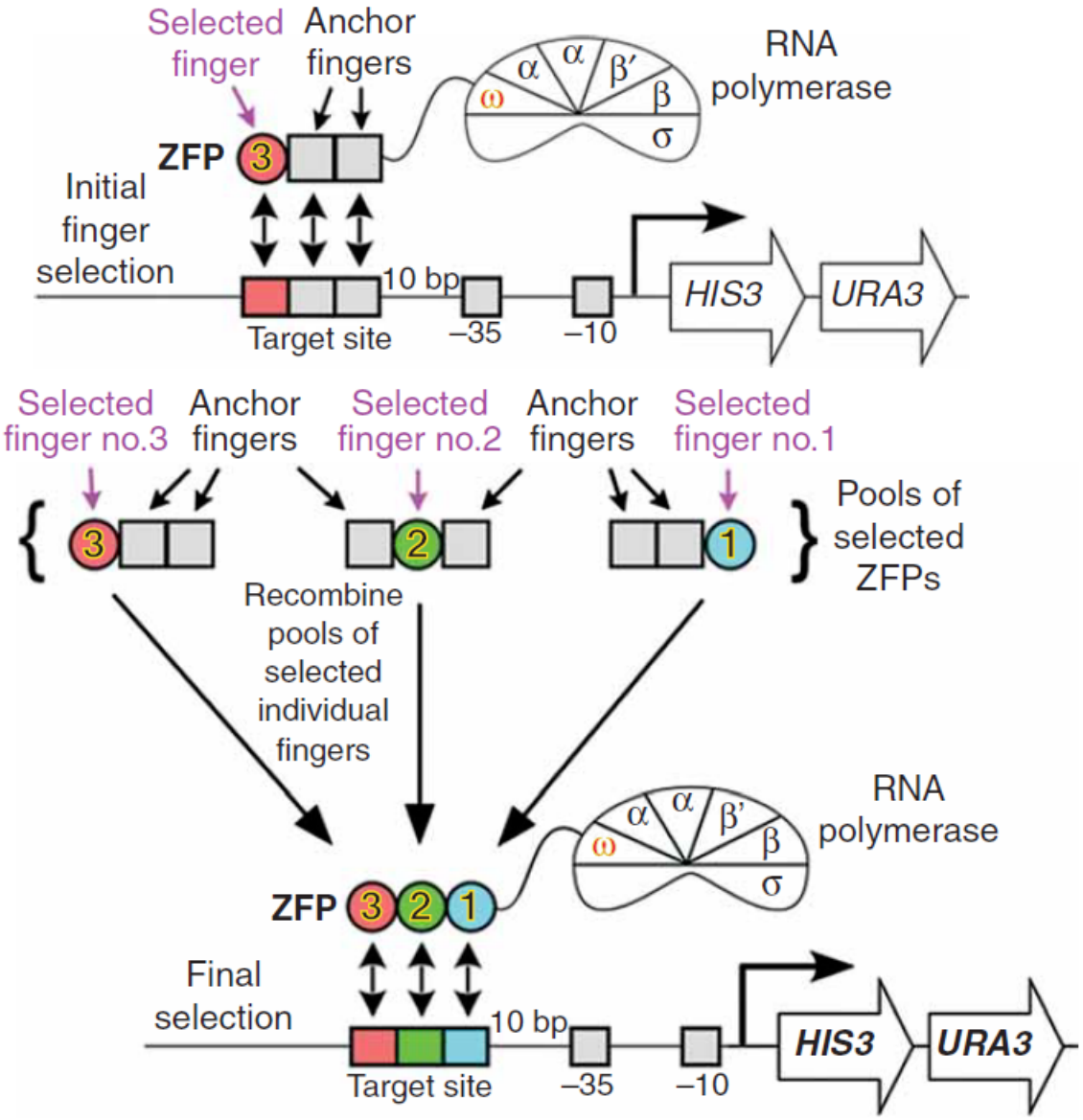
2 OPEN Method for Engineering Zinc-Finger Arrays





Morgan L.M. *et al.*

3 Bacterial One-hybrid System



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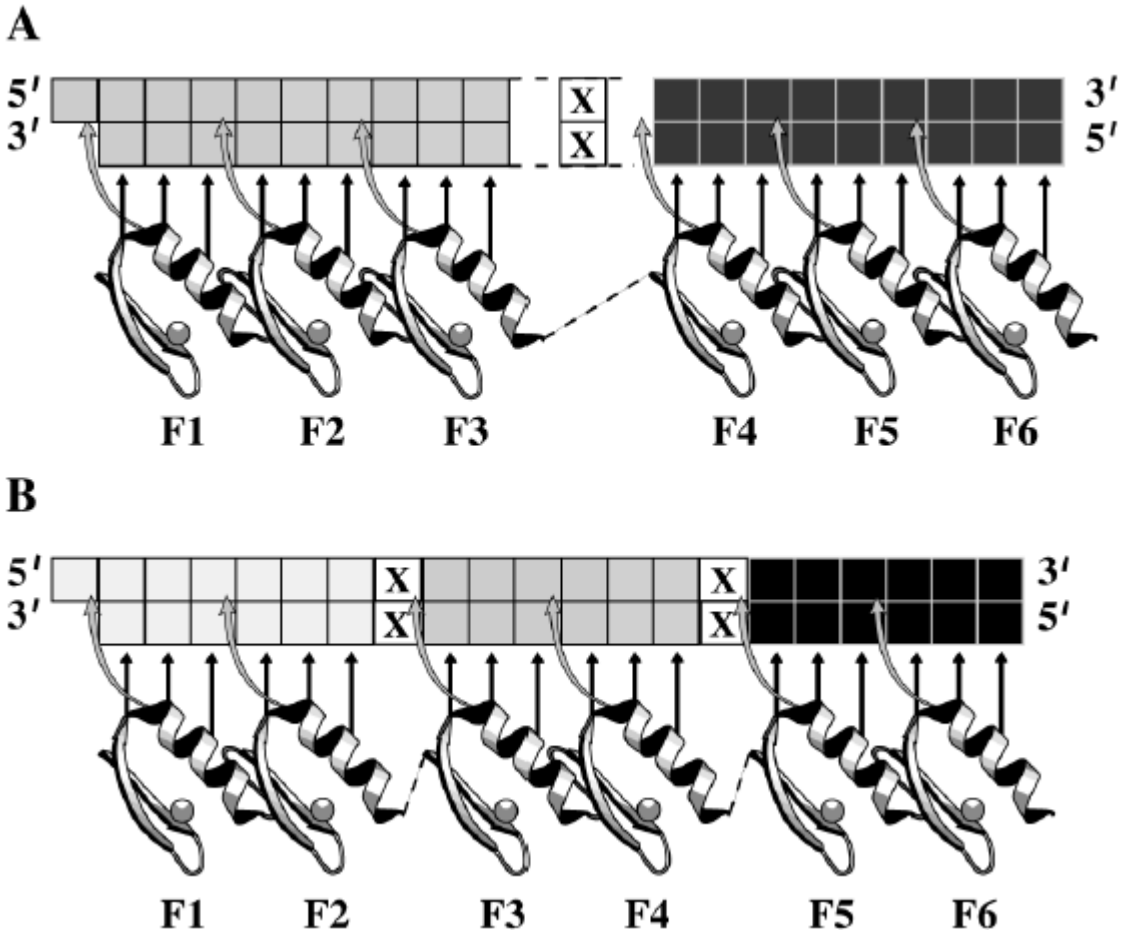
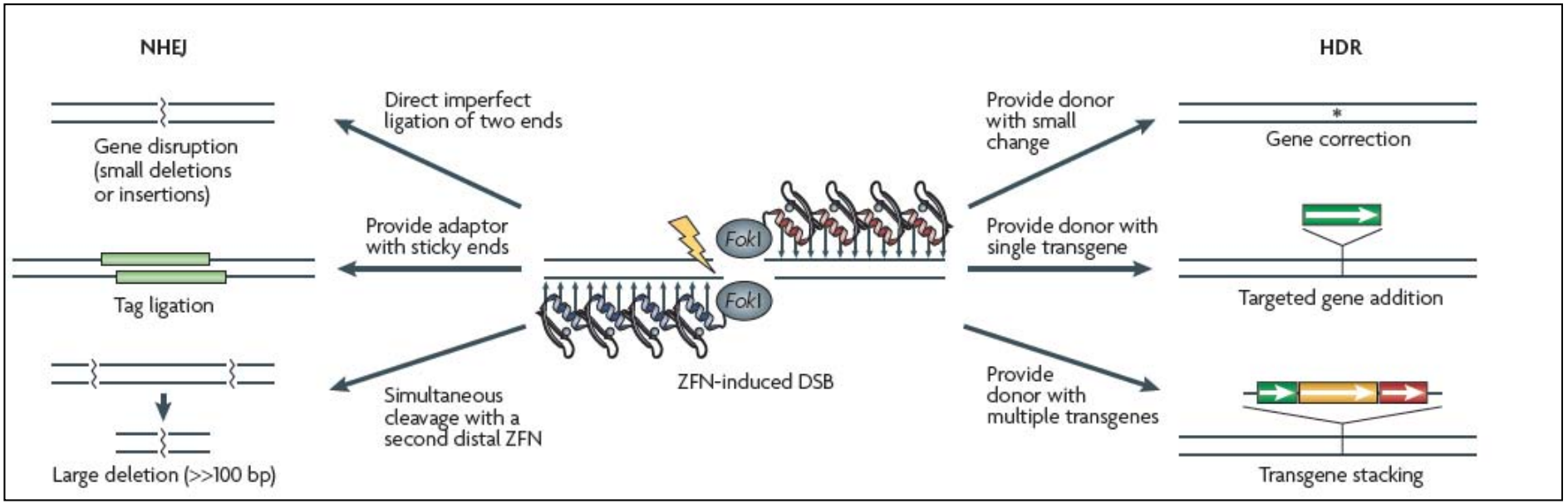
4 Two-finger Modules



Michael M. *et al.*

Types of genome editing made possible using zinc finger nucleases



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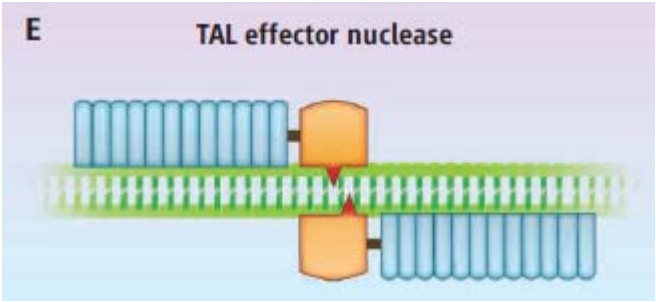
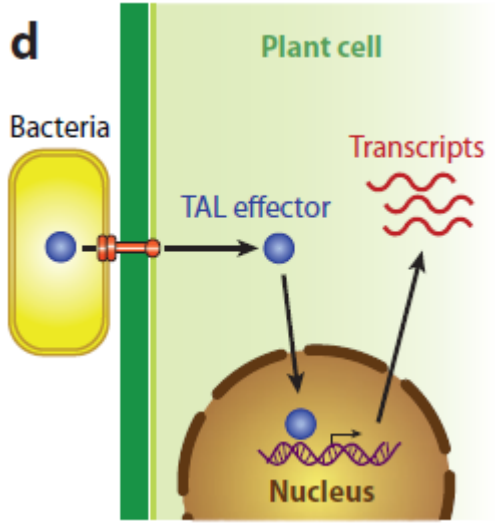
Transcription Activator-like Effector Nuclease (TALEN)





- Found in plant pathogenic bacteria , injected into plant cells via bacterial type III secretion system, imported into the plant cell nucleus, targeted to effector-specific gene promoters.
- A nuclease fused to a protein consisting 12-26 domains, each interact with a single base.
- Comprising tandem, polymorphic amino acid repeats.

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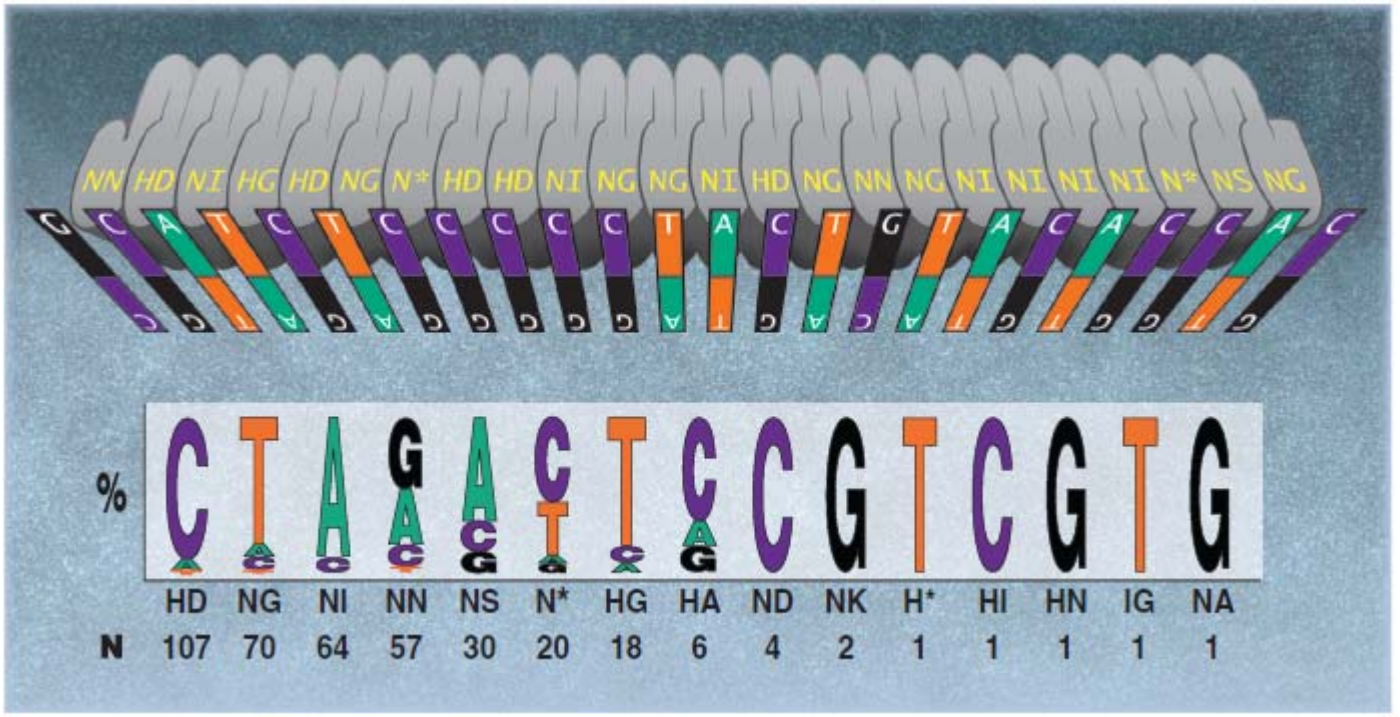
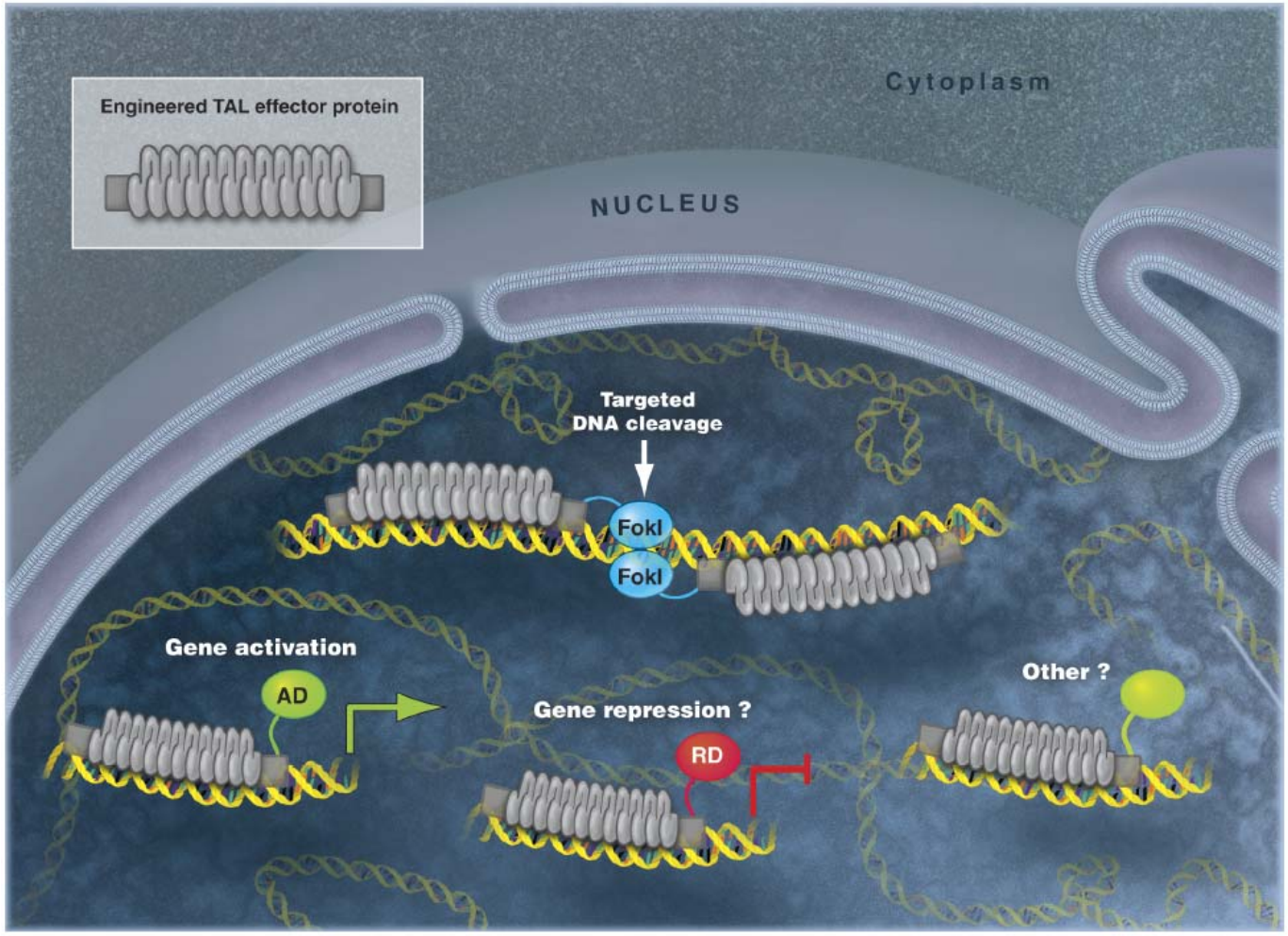
TAL Effector DNA Recognition



- Specificity depends on a variable number of imperfect, typically 34, amino acid repeats.
- Polymorphism is primarily at repeat positions 12 and 13,whichwe call the repeat-variable di-residue (RVD).
- One RVD to one nucleotide, with some degeneracy and no apparent context dependence.

Adam J. B. *et al.*

Genomic Control Enabled by Engineered TAL Effector Proteins



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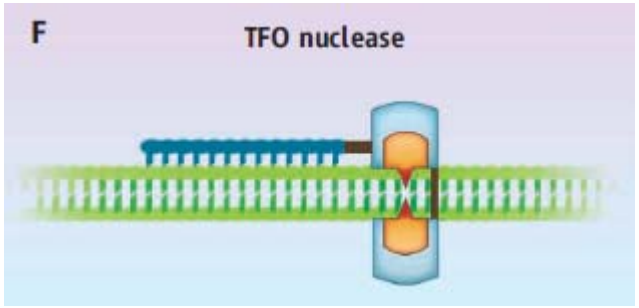
Triplex-helix-forming Oligonucleotides (TFO) with A Single-chain Nuclease



- Use oligonucleotides as a recognition module.
- Triple helix forming oligonucleotide conjugated to a non-specific nucleases or ‘chemical’ nucleases or type II Rease.
- DNA fragments with strands composed of either purines or pyrimidines.
- Bipartite recognition: site of the REase and the DNA sequence matching to the TFO.

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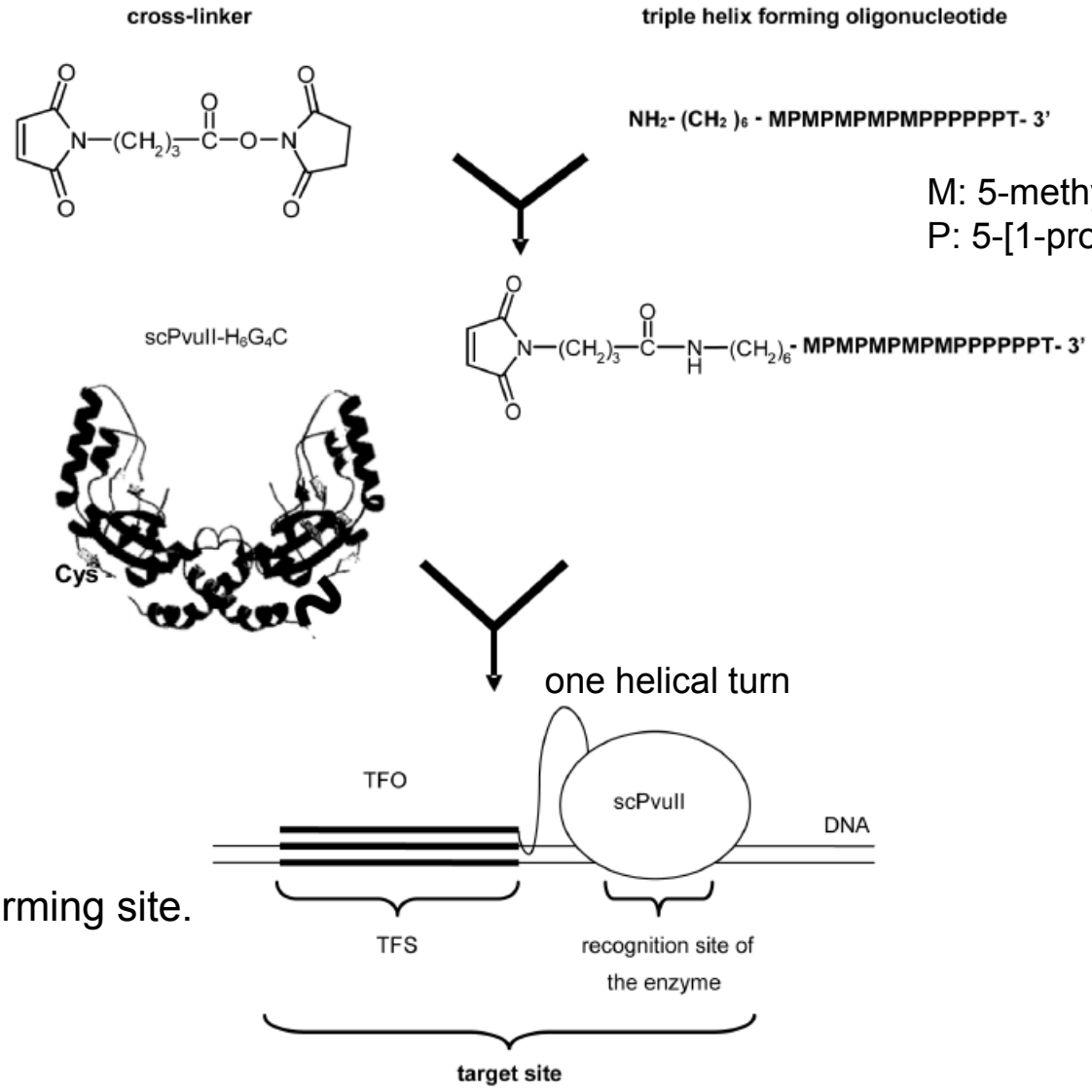
Generate a Programmed Restriction Enzyme



TFS:triple-helix forming site.

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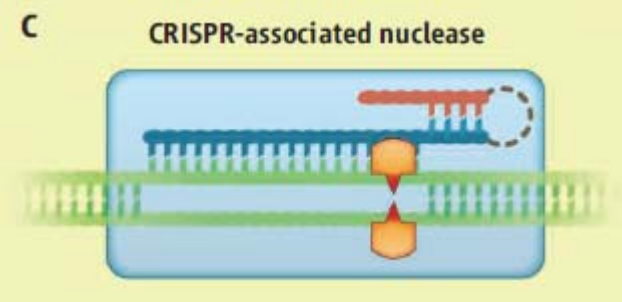
Clustered Regularly interspaced Short Palindromic Repeats (CRISPR)



JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0

Vol. 169, No. 12

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

Department of Experimental Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

John van der Oost., and Michael P. T. *et al.*

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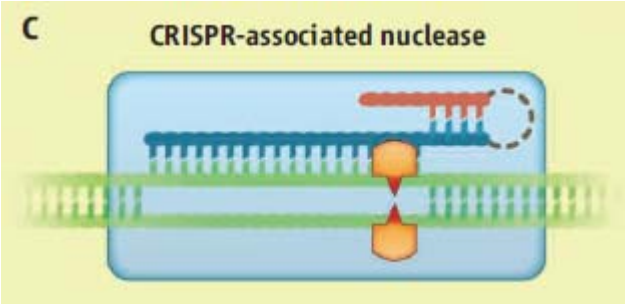

Mol Microbiol. 1993 Dec;10(5):1057-65.

Nature of DNA polymorphism in the direct repeat cluster of Mycobacterium tuberculosis; application for strain differentiation by a novel typing method.

Groenen PM, Bunschoten AE, van Soolingen D, van Embden JD.

Unit of Molecular Microbiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Mol Microbiol. 1995 Jul;17(1):85-93.

Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning.

Mojica FJ, Ferrer C, Juez G, Rodríguez-Valera F.

Departamento de Genética y Microbiología, Universidad de Alicante, Spain.

Biochim Biophys Acta. 1996 Jun 3;1307(1):26-30.

Long tandemly repeated repetitive (LTRR) sequences in the filamentous cyanobacterium Anabaena sp. PCC 7120.

Masepohl B, Görlitz K, Böhme H.

Botanisches Institut, Universität Bonn, Germany.

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Rapid molecular genetic subtyping of serotype M1 group A Streptococcus strains.

Hoe N, Nakashima K, Grigsby D, Pan X, Dou SJ, Naidich S, Garcia M, Kahn E, Bergmire-Sweat D, Musser JM.

Baylor College of Medicine, Houston, Texas 77030, USA.

J Bacteriol. 2000 May;182(9):2393-401.

Genetic variation and evolutionary origin of the direct repeat locus of Mycobacterium tuberculosis complex bacteria.

van Embden JD, van Gorkom T, Kremer K, Jansen R, van Der Zeijst BA, Schouls LM.

Department of Bacteriology of the Research Laboratory for Infectious Disease, National Institute of Public Health and the Environment, 3720 BA Bilthoven, The Netherlands. JDA.van.Embden@rivm.nl

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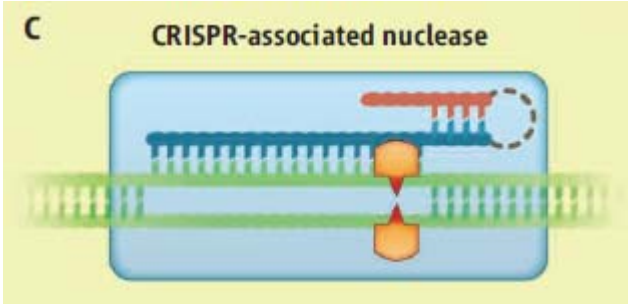
Clustered Regularly interspaced Short Palindromic Repeats (CRISPR)



Mol Microbiol. 2002 Mar;43(6):1565-75.

Identification of genes that are associated with DNA repeats in prokaryotes.

Jansen R, Embden JD, Gaastra W, Schouls LM.

Department of Infectious Diseases and Immunology, Bacteriology Division, Veterinary Faculty, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands. R.jansen@vet.uu.nl

OMICS. 2002;6(1):23-33.

Identification of a novel family of sequence repeats among prokaryotes.

Jansen R, van Embden JD, Gaastra W, Schouls LM.

Department of Infectious Diseases and Immunology,Veterinary Faculty, Utrecht University, The Netherlands. R.jansen@vet.uu.nl

BMC Bioinformatics. 2007 May 23;8:172.

The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats.

Grissa I, Vergnaud G, Pourcel C.

Univ Paris-Sud, Institut de Génétique et Microbiologie, UMR 8621, Orsay, France. ibtissem.grissa@igmors.u-psud.fr <ibtissem.grissa@igmors.u-psud.fr>

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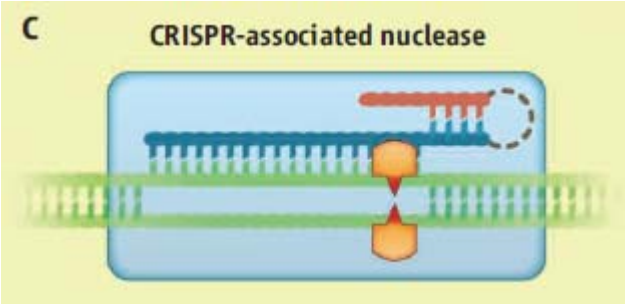
Clustered Regularly interspaced Short Palindromic Repeats (CRISPR)



PLoS Comput Biol. 2005 Nov;1(6):e60. Epub 2005 Nov 11.

A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes.

Haft DH, Selengut J, Mongodin EF, Nelson KE.

The Institute for Genomic Research, Rockville, Maryland, USA. haft@tigr.org

Biol Direct. 2006 Mar 16;1:7.

A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action.

Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV.

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA. makarova@ncbi.nlm.nih.gov

Nat Rev Microbiol. 2011 Jun;9(6):467-77. doi: 10.1038/nrmicro2577. Epub 2011 May 9.

Evolution and classification of the CRISPR-Cas systems.

Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF, van der Oost J, Koonin EV.

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 8600 Rockville Pike, Bethesda, Maryland 20894, USA.

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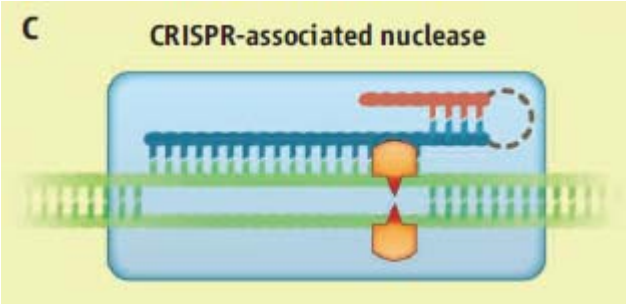

Microbiology. 2005 Aug;151(Pt 8):2551-61.

Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin.

Bolotin A, Quinquis B, Sorokin A, Ehrlich SD.

Génétique Microbienne, Institut National de la Recherche Agronomique, Jouy en Josas, France. bolotine@jouy.inra.fr

J Mol Evol. 2005 Feb;60(2):174-82.

Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements.

Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E.

Divisón de Microbiología, Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Campus de San Vicente, E-03080, Spain. fmojica@ua.es

Mol Microbiol. 2010 Sep;77(6):1367-79. doi: 10.1111/j.1365-2958.2010.07265.x.

Transcription, processing and function of CRISPR cassettes in Escherichia coli.

Pougach K, Semenova E, Bogdanova E, Datsenko KA, Djordjevic M, Wanner BL, Severinov K.

Institutes of Molecular Genetics and Gene Biology, Russian Academy of Sciences, Moscow, Russia.

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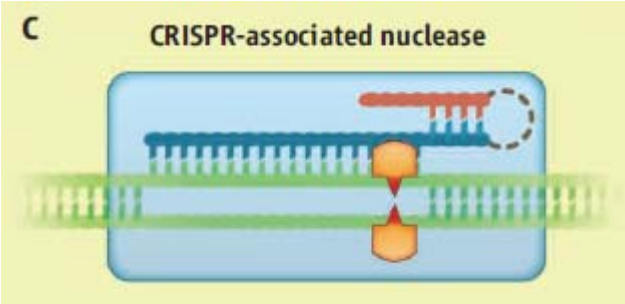


Archaea. 2006 Aug;2(1):59-72.

A putative viral defence mechanism in archaeal cells.

Lillestøl RK, Redder P, Garrett RA, Brügger K.

Institute of Molecular Biology, University of Copenhagen, Sølvgade 83H, DK 1307 Copenhagen K, Denmark.

Proc Natl Acad Sci U S A. 2002 May 28;99(11):7536-41.

Identification of 86 candidates for small non-messenger RNAs from the archaeon Archaeoglobus fulgidus.

Tang TH, Bachellerie JP, Rozhdestvensky T, Bortolin ML, Huber H, Drungowski M, Elge T, Brosius J, Hüttenhofer A.

Institute of Experimental Pathology, Von-Esmarch-Strasse 56, 48149 Münster, Germany.

Mol Microbiol. 2005 Jan;55(2):469-81.

Identification of novel non-coding RNAs as potential antisense regulators in the archaeon Sulfolobus solfataricus.

Tang TH, Polacek N, Zywicki M, Huber H, Brugger K, Garrett R, Bachellerie JP, Hüttenhofer A.

Institute for Research in Molecular Medicine, University Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelatan, Malaysia.

Biol Direct. 2006 Mar 16;1:7.

A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action.

Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV.

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA. makarova@ncbi.nlm.nih.gov

Science. 2007 Mar 23;315(5819):1709-12.

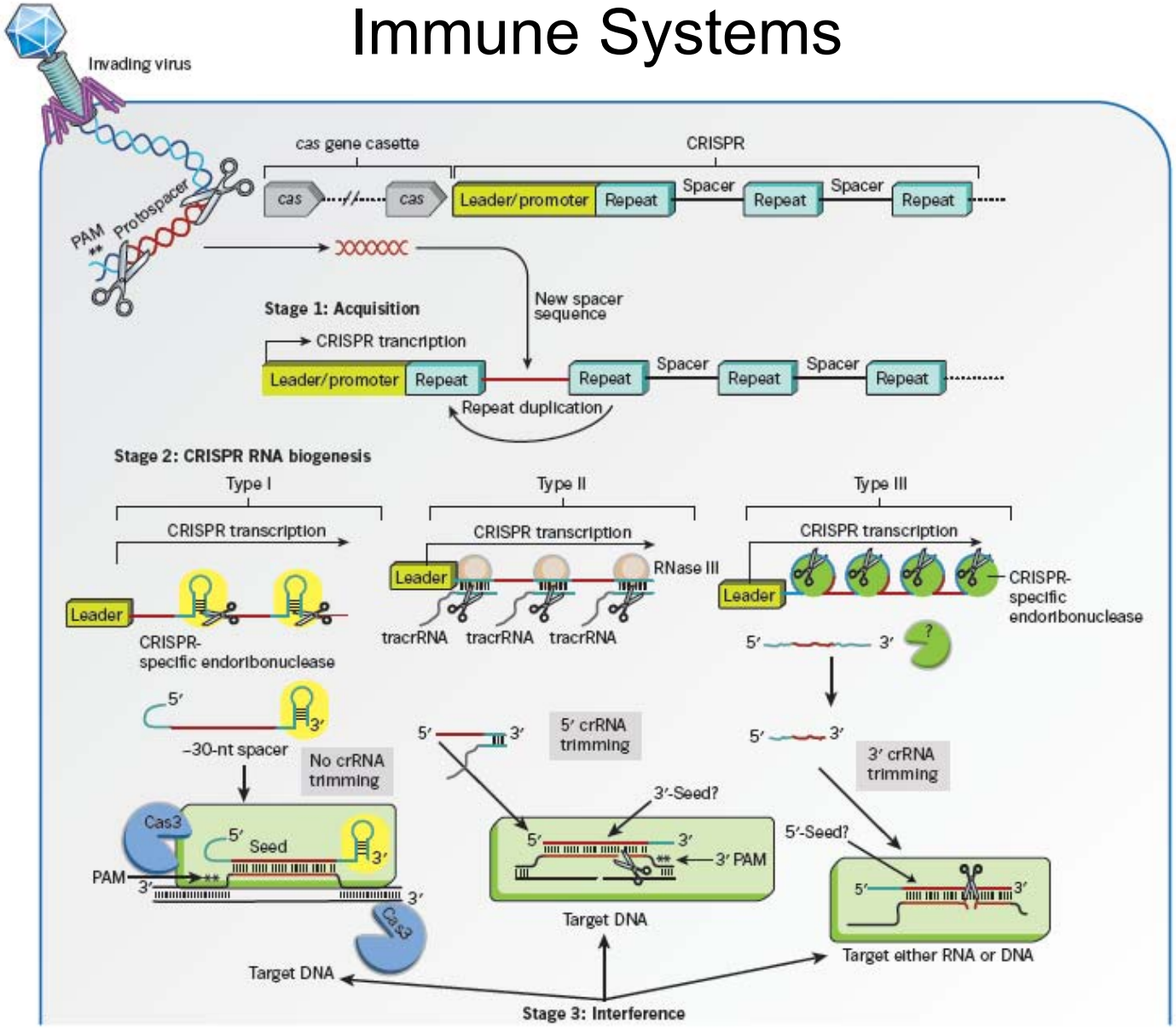
CRISPR provides acquired resistance against viruses in prokaryotes.

Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P.

Danisco USA Inc., 3329 Agriculture Drive, Madison, WI 53716, USA.

John van der Oost

Diversity of CRISPR-mediated Adaptive Immune Systems



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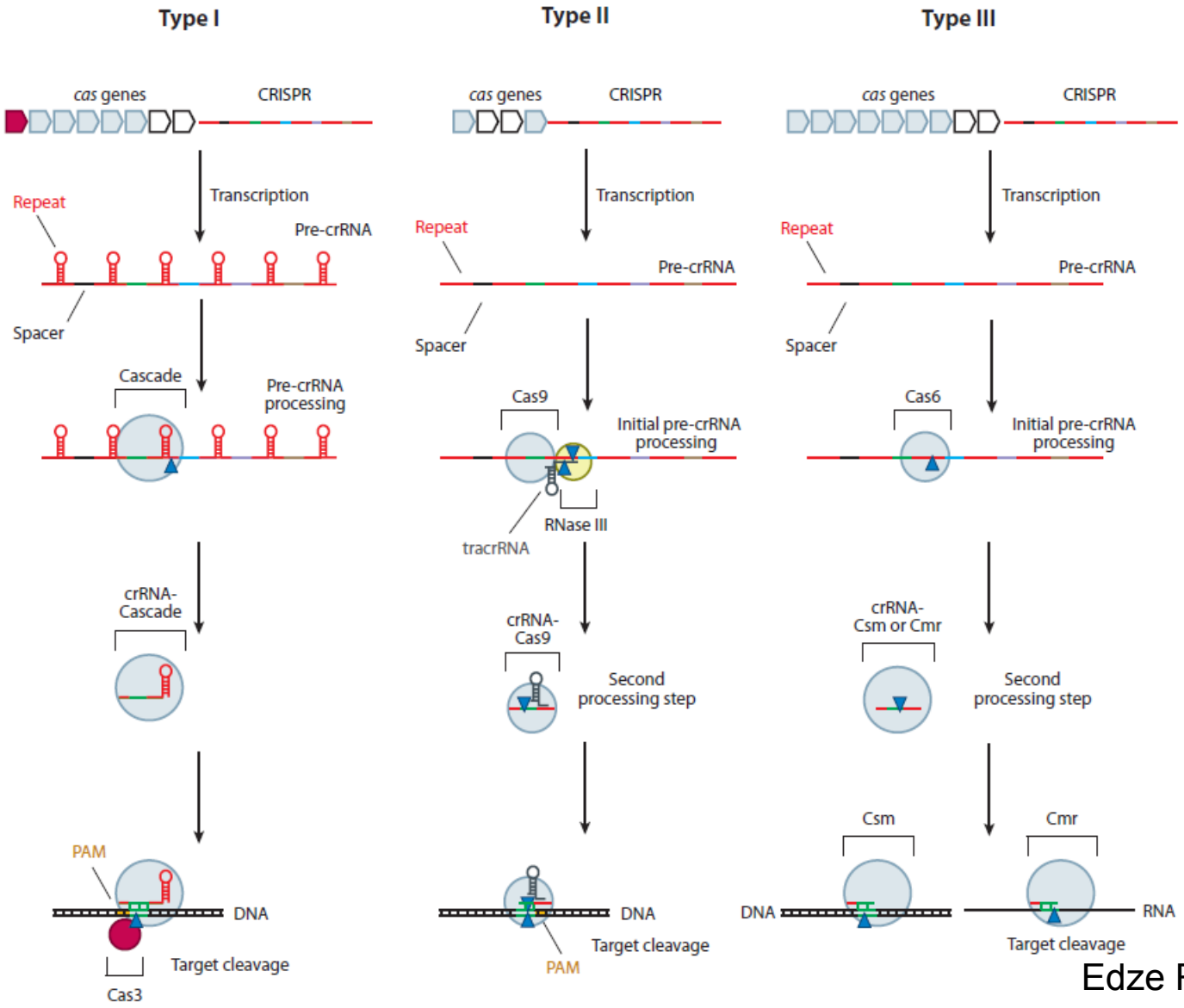
Type I, II AND III CRPSPR Expression and Interference Stages



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Protospacer-adjacent Motif (PAM) Sequences Identified for CRISPR/Cas Subtypes

Table 1 Protospacer-adjacent motif (PAM) sequences identified for CRISPR/Cas subtypes

Type	Species	References	PAM (5′–3′)[a]	Typical repeat	CRISPR cluster	
I-A	*Sulfolobus solfataricus* P2	(62, 94, 111)	Protospacer-NGG	GATAATCTCTTA TAGAATTGAAAG[b]	7	
	Metallosphaera sedula DSM5348	(111)	Protospacer-NGG	GTTAATCTTCTAT AGAGTTGAAAG	7	
			Unknown		11	
I-B	*Methanothermobacter thermautotrophicus* ΔH	(111)	Protospacer-NGG	GTTAAAATCAGA CCAAAATGGGA TTGAAAT	1	
	Listeria monocytogenes	(111)	Protospacer-NGG	GTTTTAACTACTT ATTATGAAATCT AAAT	1	
			Unknown		6,9	
I-C	*Streptococcus pyogenes*	(111)	Protospacer-GAA	GTCTCACCCTTC ATGGGTGAGTG GATTGAAAT	3	
	Xanthomonas oryzae	(111)	Protospacer-GAA	GTCGCGTCCTCA CGGGCGCGTGG ATTGAAAC	3	PAM downstream of protospacer
I-D			Unknown		Unknown	
I-E	*Escherichia coli* K12	(111, 139, 155, 169)	Protospacer-CTT Protospacer-CAT Protospacer-CCT Protospacer-CTC	GWGTTCCCCGCG CCAGCGGGGAT AAACCG[b]	2	
	Pseudomonas aeruginosa 2192	(111)	Protospacer-CTT	GTGTTCCCCACA TGCGTGGGGAT GAACCG	2	
I-F	*P. aeruginosa* PA14	(27a, 111)	Protospacer-GG	GTTCACTGCCGT GTAGGCAGCTA AGAAA[b]	4	
	Shewanella spp.	(111)	Protospacer-GG	GTTCACCGCCGC ACAGGCGGCTT AGAAA	4	
II-A	*Streptococcus thermophilus*	(77)	WTTCTNN - protospacer	GTTTTTGTACTCT CAAGATTTAAGT AACTGTACAAC	10	PAM upstream of protospacer
	S. thermophilus	(20)	TTTYRNNN - protospacer	GTTTTTGTACTCT CAAGATTTAAGT AACTGTACAAC	10	
II-B	*S. thermophilus*	(77)	CNCCN - protospacer	GTTTTAGAGCTG TGTTGTTTCGAA TGGTTCCAAAAC	10	
	S. pyogenes	(111)	CCN - protospacer	GTTTTAGAGCTA TGCTGTTTTGAA TGGTCCCAAAAC[b]	10	

Type	Species	References	PAM (5′–3′)[a]	Typical repeat	CRISPR cluster	
	L. monocytogenes	(111)	CCN - protospacer	GTTTTAGAGCTA TGTTATTTTGAA TGCTACCAAAAC	10	
III-A	*Staphylococcus epidermidis*	(105)	No PAM	GATCGATACCCA CCCCGAAGAAA AGGGGACGAGAAC[b]	8	No PAM
III-B	*Pyrococcus furiosus*	(65)	No PAM	GTTCCAATAAGA CTAAAATAGAA TTGAAAG[b]	6	
	S. solfataricus	(179)	No PAM	GATTAATCCCAA AAGGAATTGAA AG[b]	7	

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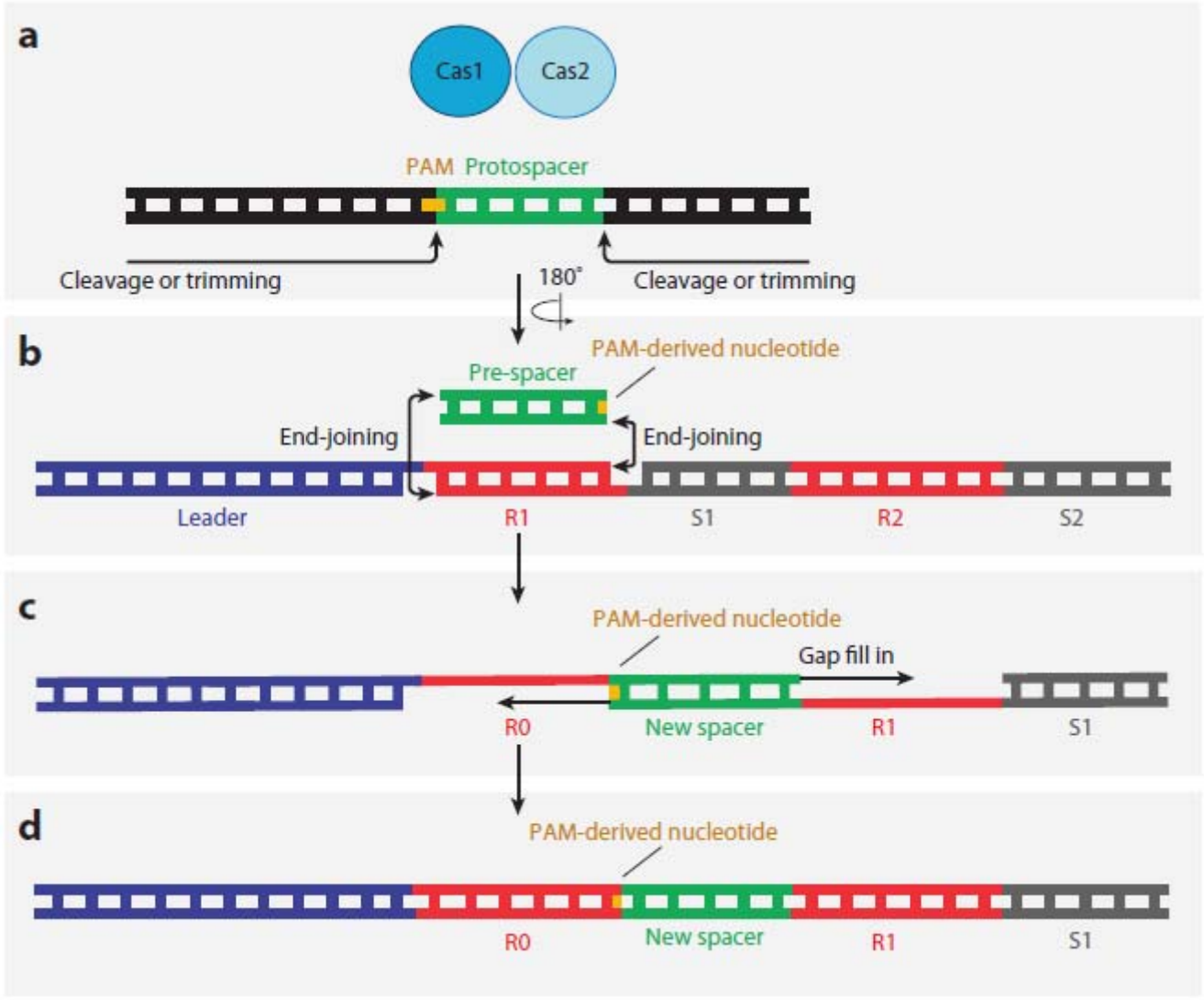
Hypothetical Mechanism of CRISPR Adaptation



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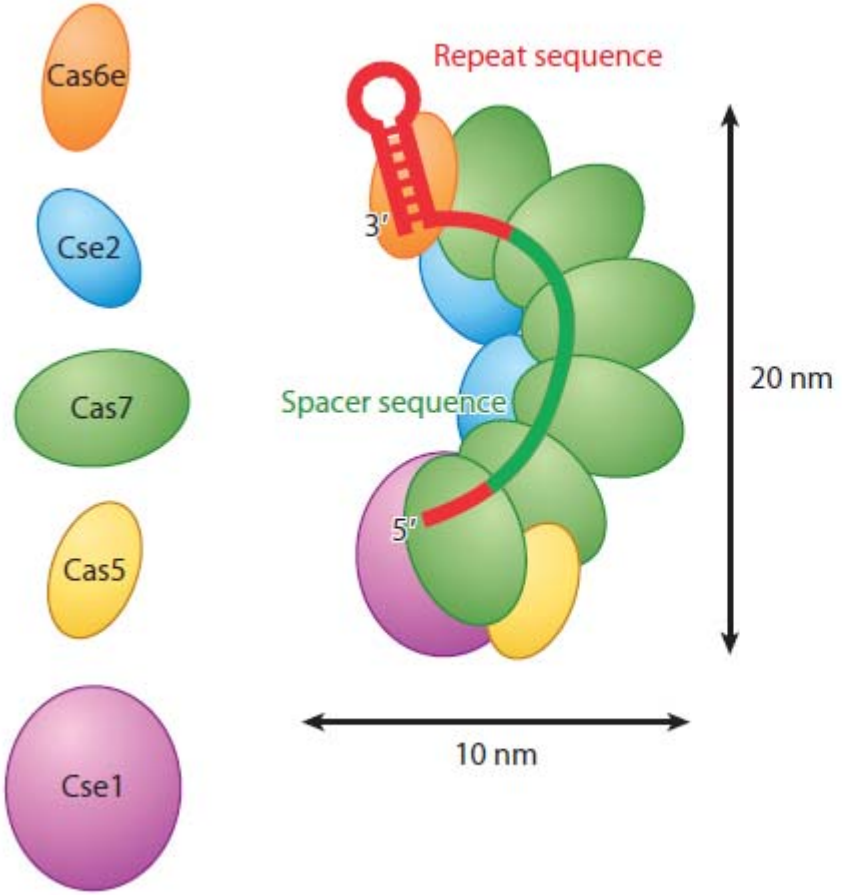
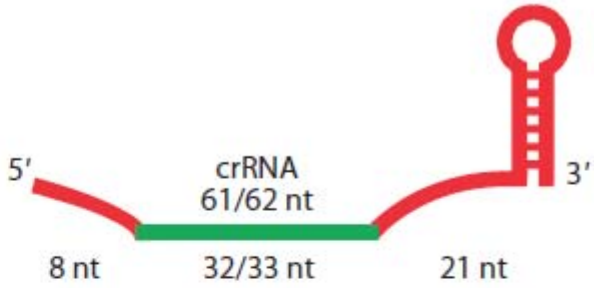
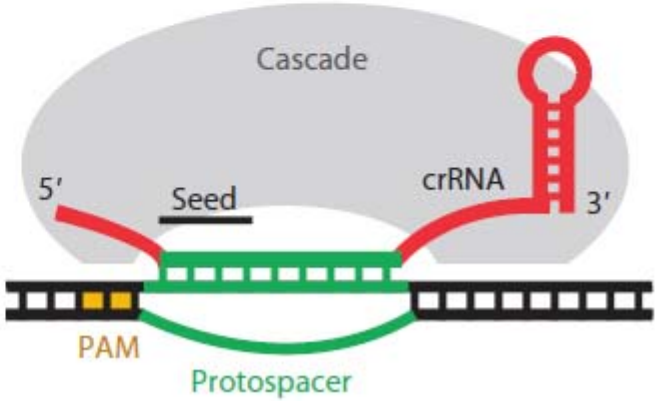
Cascade and Cascade-mediated R-loop Formation



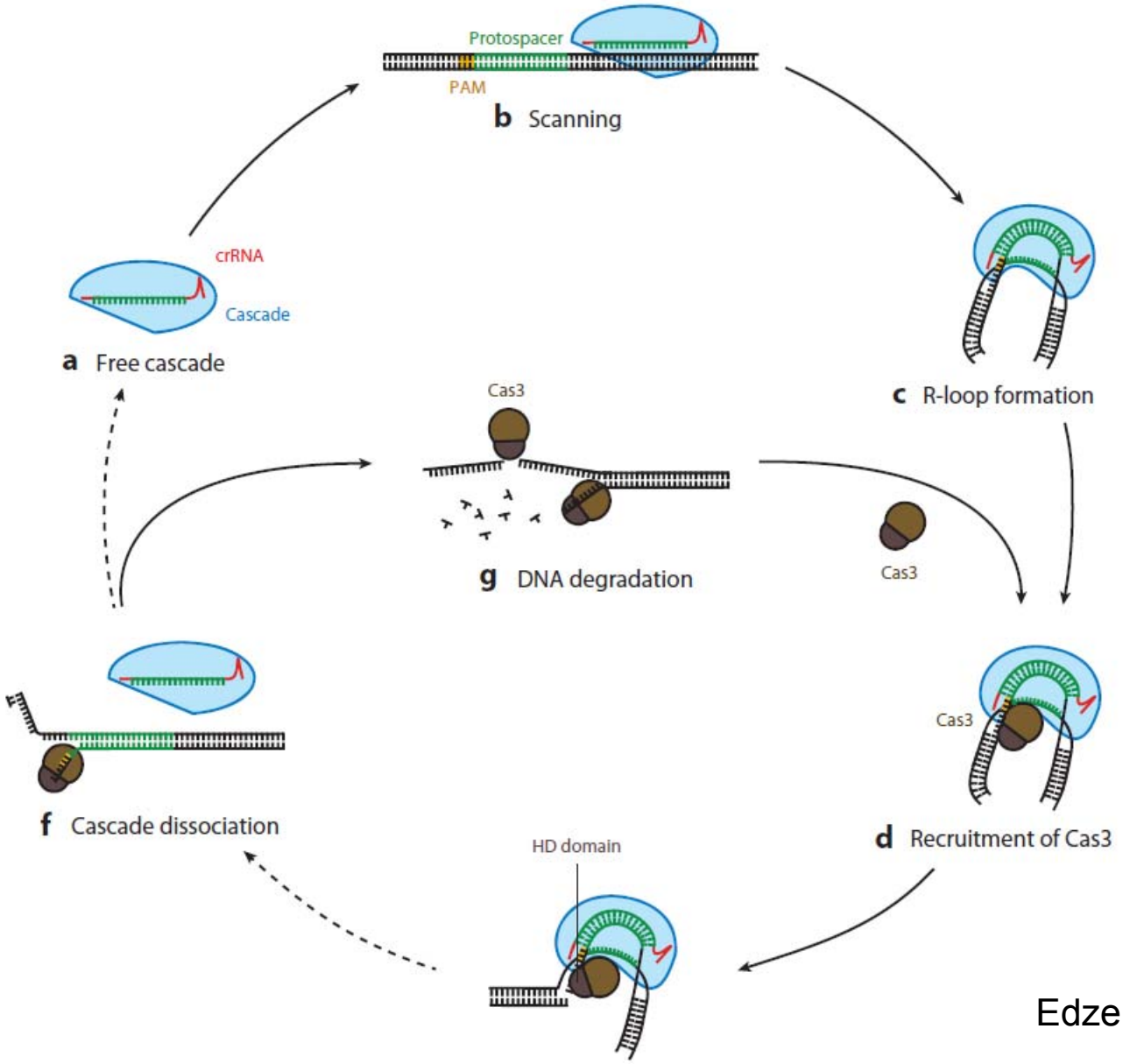




Cascade: CRISPR-associated complex for antiviral defence

Edze R.W. *et al.*

CRISPR Interference by Type I-E System



Edze R.W. *et al.*

ARTICLE

doi:10.1038/nature09886

CRISPR RNA maturation by *trans*-encoded small RNA and host factor RNase III

Elitza Deltcheva[1,2], Krzysztof Chylinski[1,2]*, Cynthia M. Sharma[3]*, Karine Gonzales[2], Yanjie Chao[3,4], Zaid A. Pirzada[2], Maria R. Eckert[2], Jörg Vogel[3,4] & Emmanuelle Charpentier[1,2]

- In *Streptococcus pyogenes,* identify tracrRNA
- tracrRNA directs the maturation of crRNAs by the activities of the widely conserved endogenous RNase III and the CRISPR-associated Csn1 protein.

Science
AAAS

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity
Martin Jinek *et al.*
Science **337**, 816 (2012);
DOI: 10.1126/science.1225829

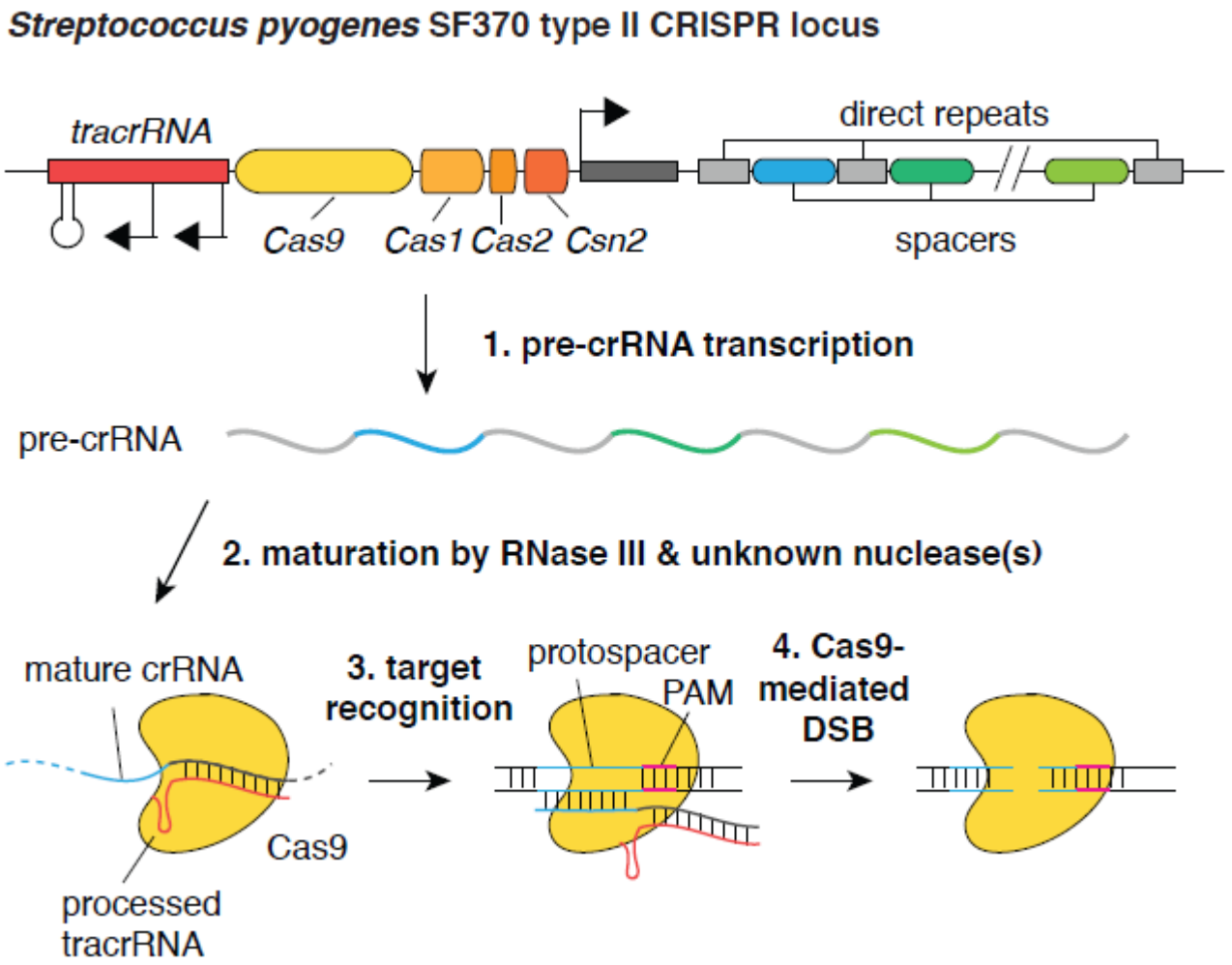
- crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introducedouble-stranded (ds) breaks in target DNA.
- The Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand.
- The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage.

Multiplex Genome Engineering Using CRISPR/Cas Systems

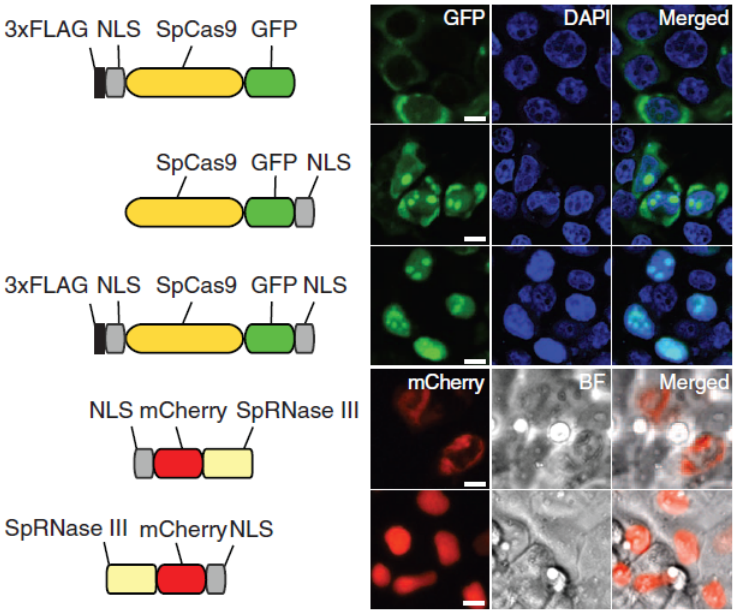
Le Cong,[1,2]* F. Ann Ran,[1,4]* David Cox,[1,3] Shuailiang Lin,[1,5] Robert Barretto,[6] Naomi Habib,[1] Patrick D. Hsu,[1,4] Xuebing Wu,[7] Wenyan Jiang,[8] Luciano A. Marraffini,[8] Feng Zhang[1]†

[1]Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA, and McGovern Institute for Brain Research, Department of Brain and Cognitive Sciences, Department of Biological Engineering, Massachusetts Institute of Technology (MIT), Cambridge, MA 02139, USA. [2]Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, MA 02115, USA. [3]Harvard-MIT Health Sciences and Technology, Harvard Medical School, Boston, MA 02115, USA. [4]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. [5]School of Life Sciences, Tsinghua University, Beijing 100084, China. [6]Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA. [7]Computational and Systems Biology Graduate Program and Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [8]Laboratory of Bacteriology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

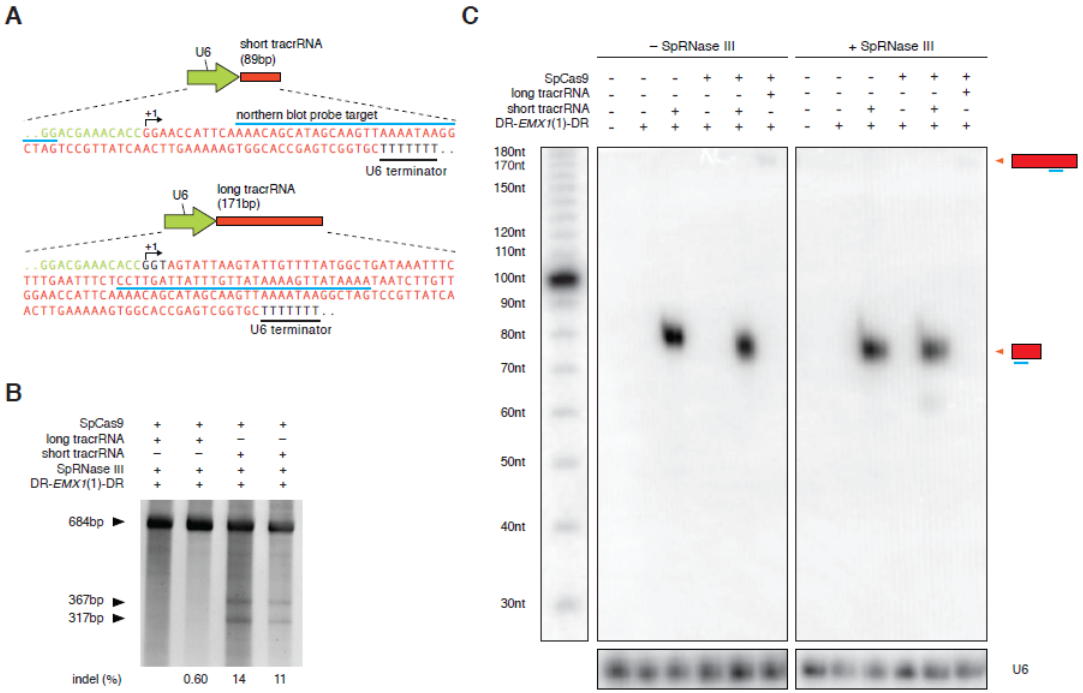
Type II CRISPR-mediated DNA Double-strand Break



Optimization: *S. pyogenes* **Cas9** (SpCas9), **RNase III** and **tracrRNA**

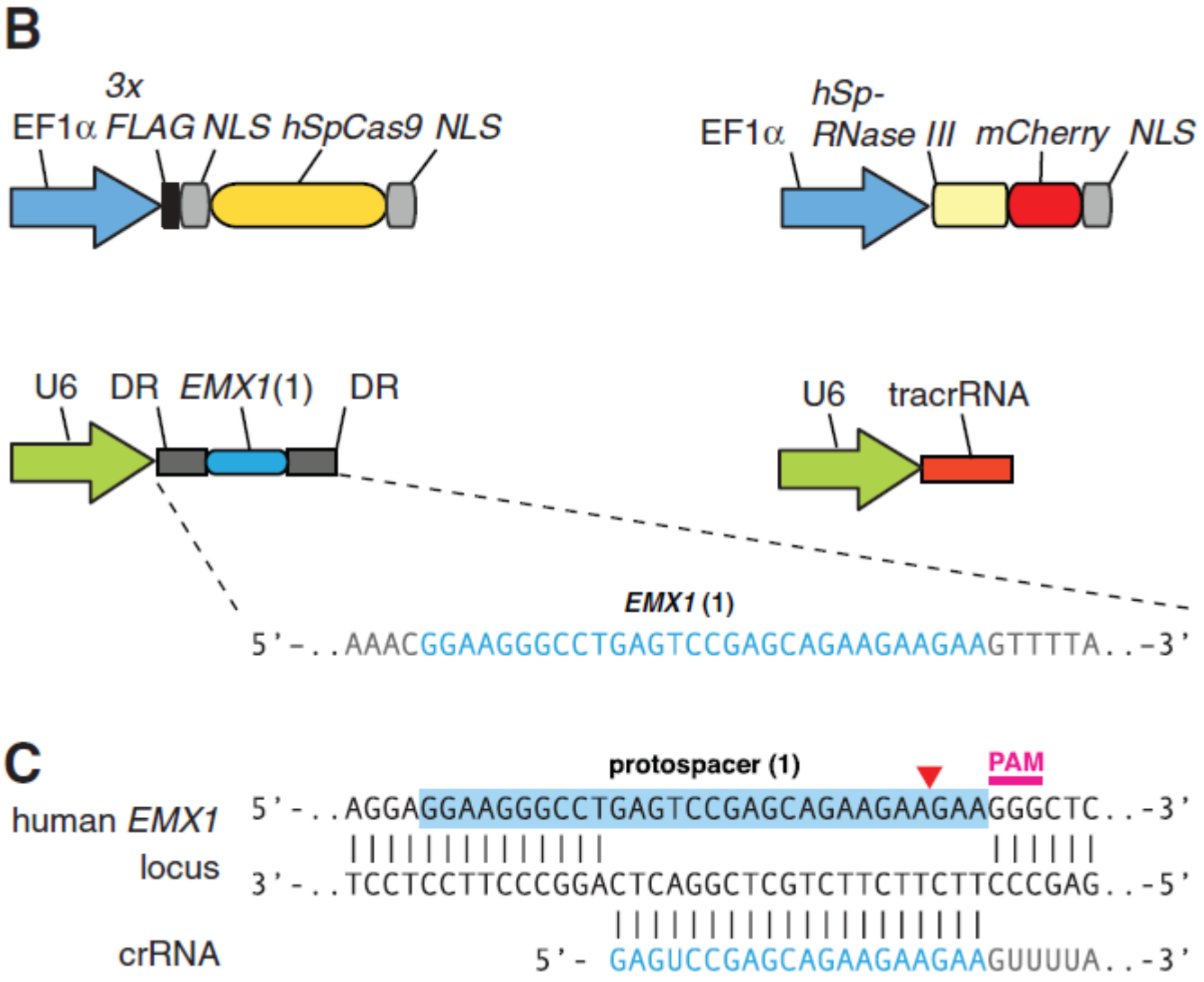


SpCas9 and SpRNase III with NLSs enables import into the mammalian nucleus



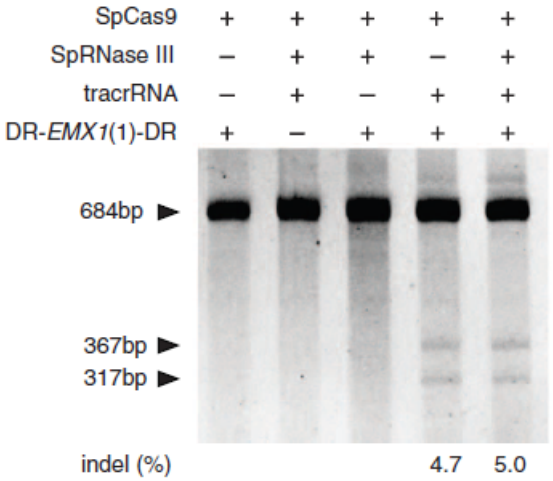
Comparison of different tracrRNA transcripts for Cas9-mediated gene targeting

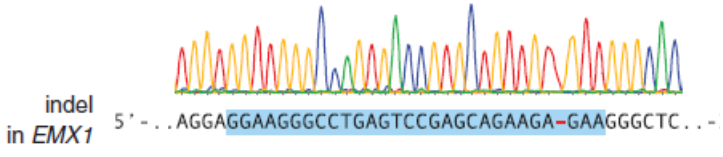
Schematic Representation of Base Pairing between Target Locus and EMX1- targeting crRNA

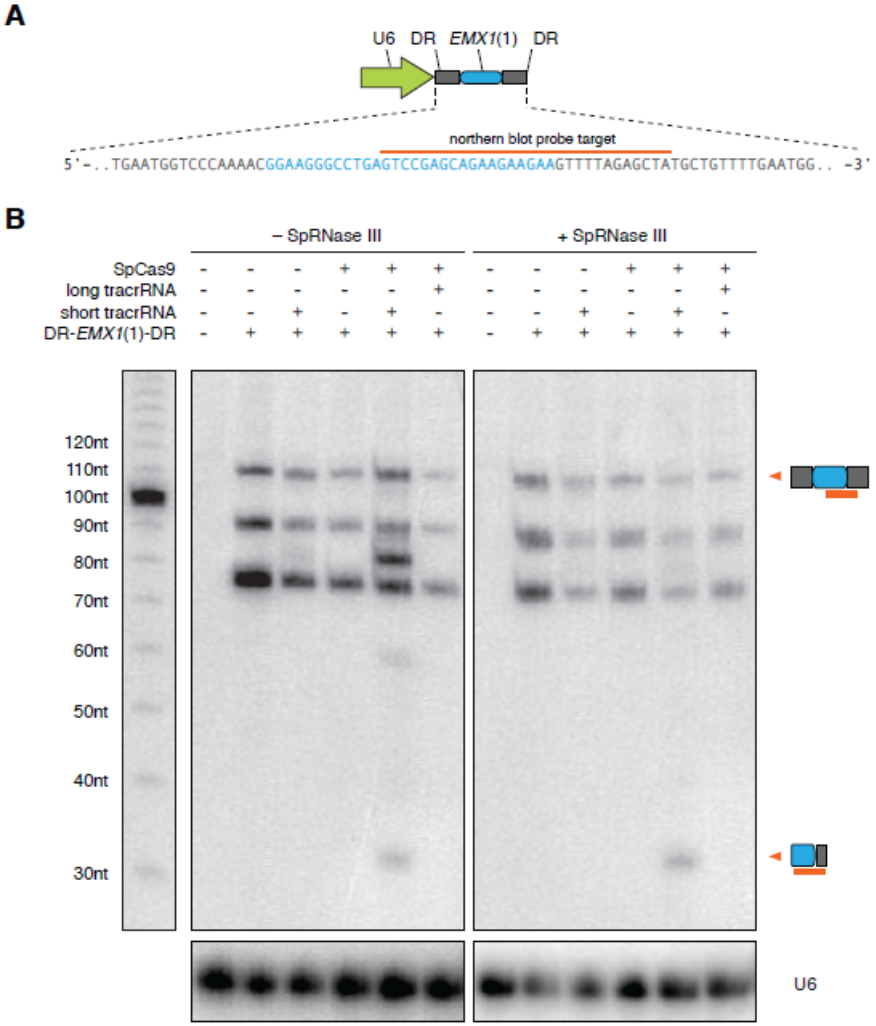


Define a Minimal Three-component System for Efficient RNA-guided Genome Modification in Mammalian Cells

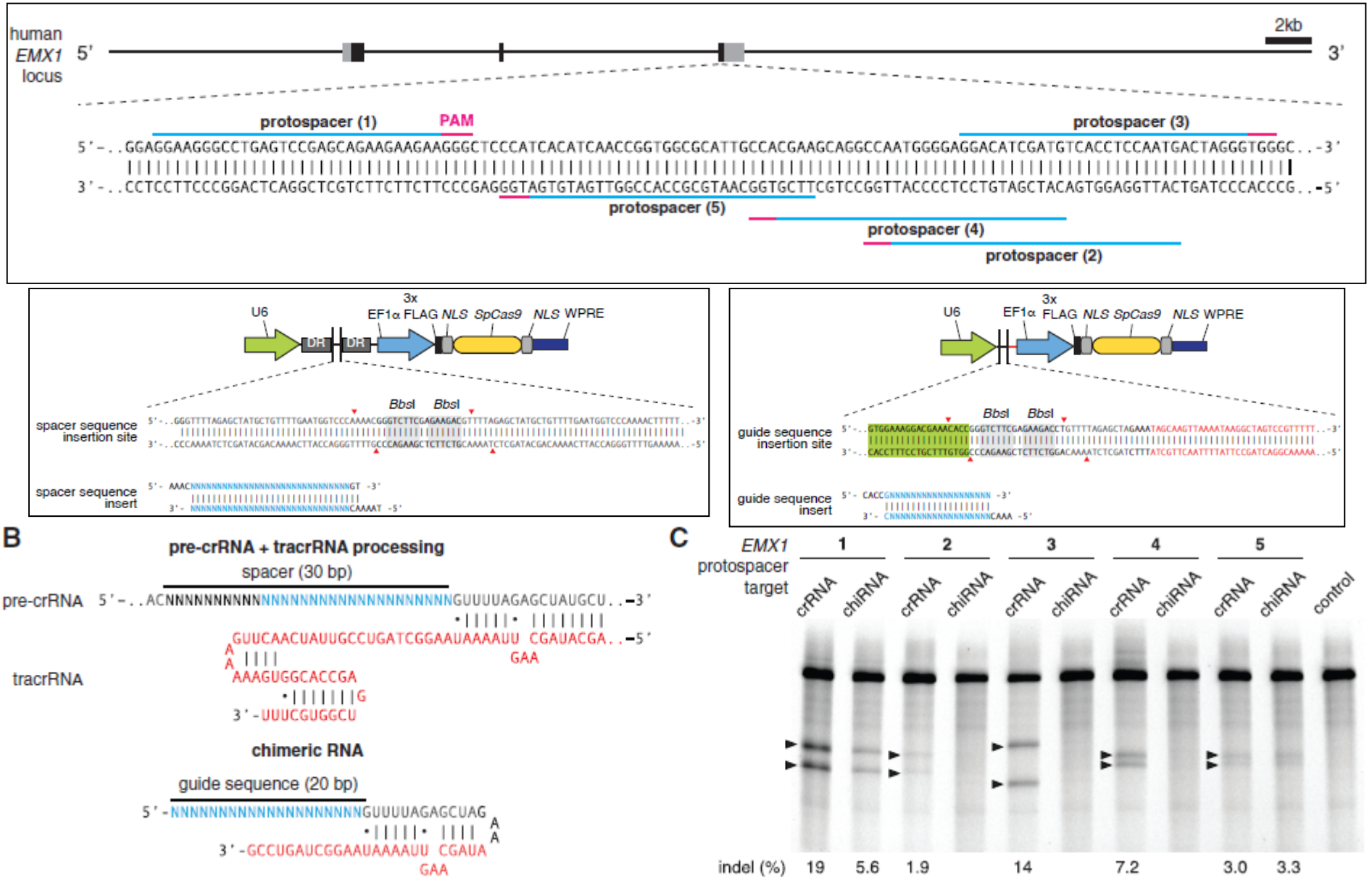
293FT cells



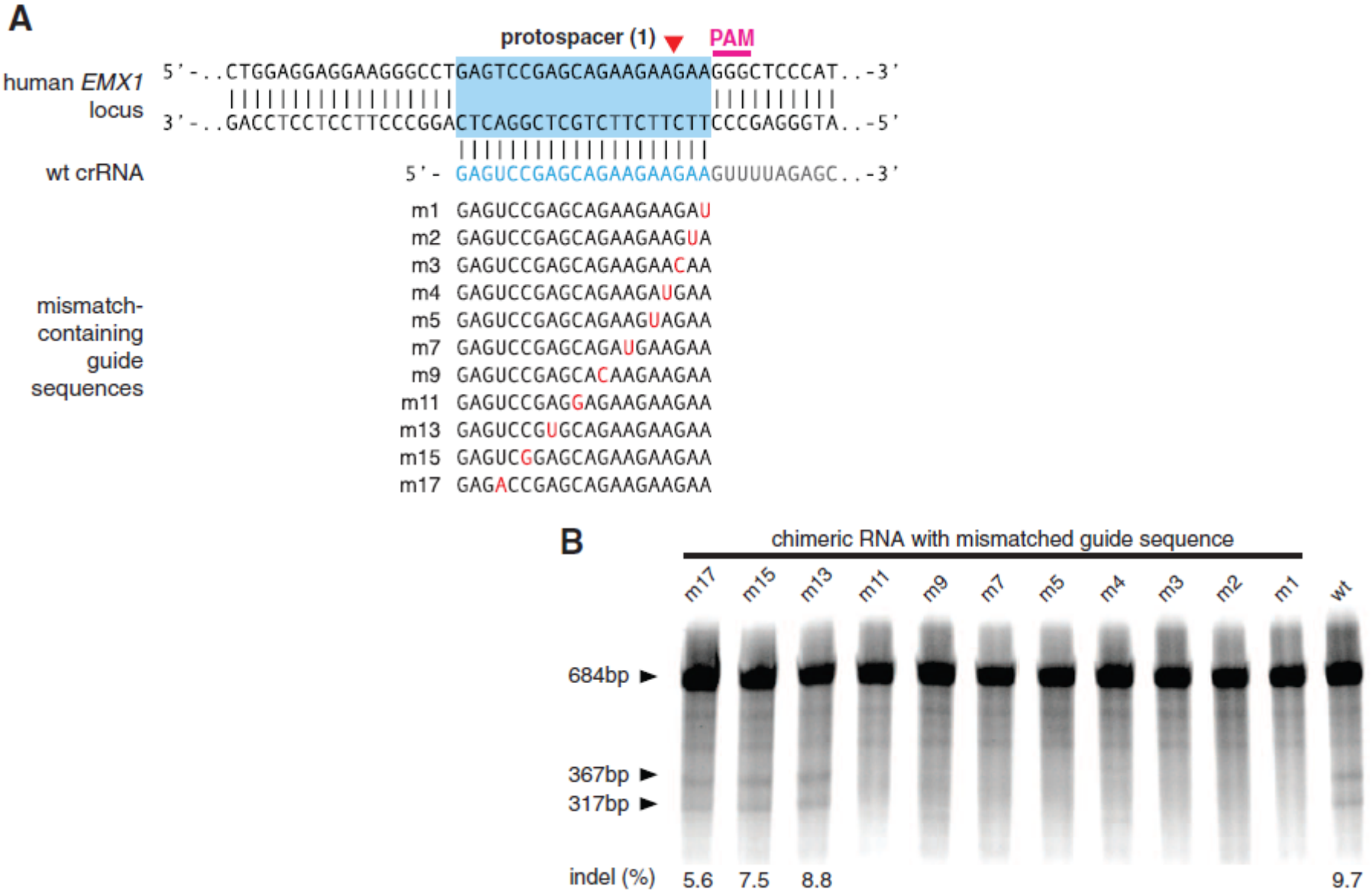




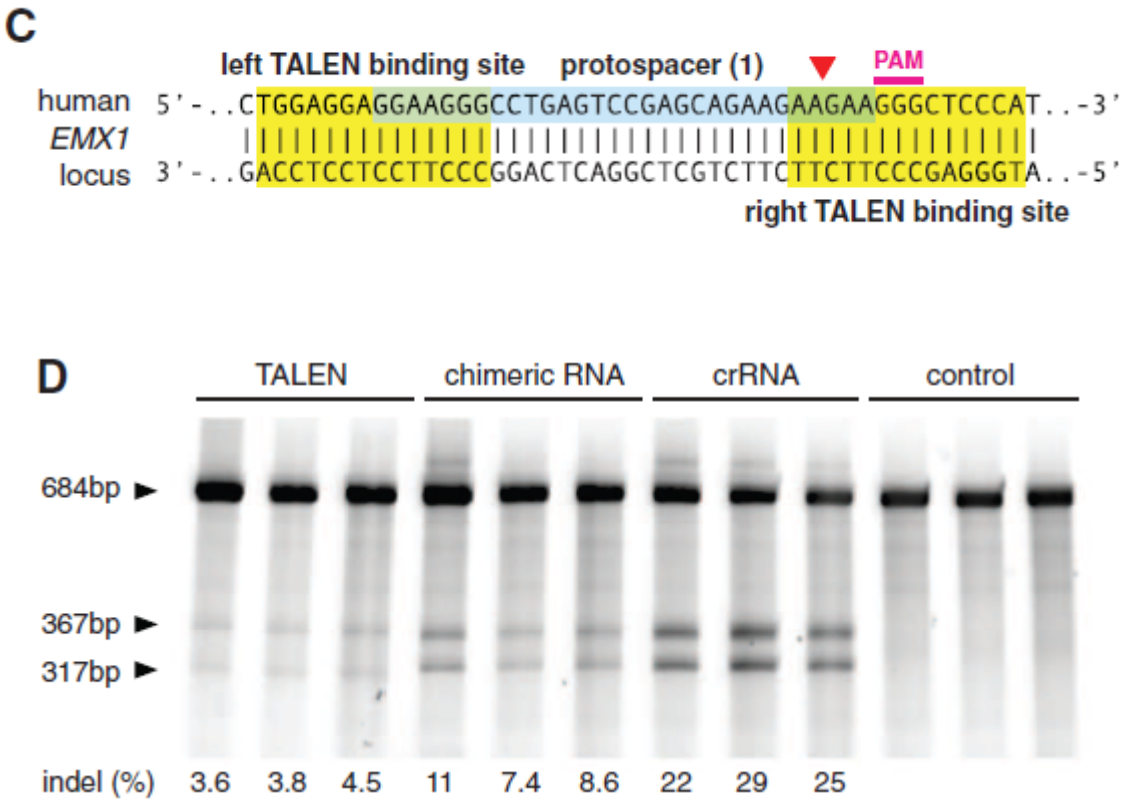
SpCas9 Can Be Reprogrammed to Target Multiple Genomic Loci in Mammalian Cells



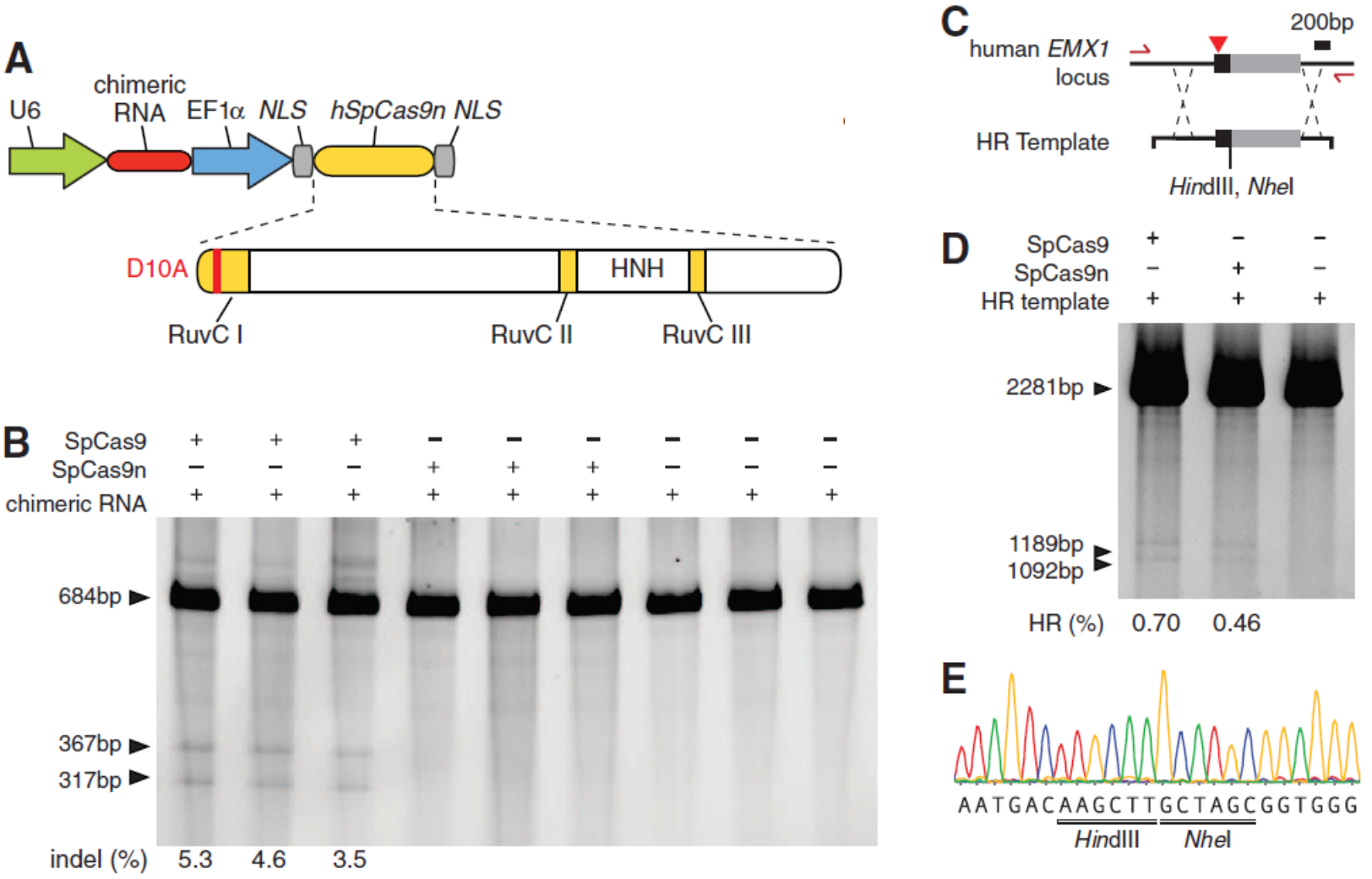
Effects of Spacer-protospacer Mismatches



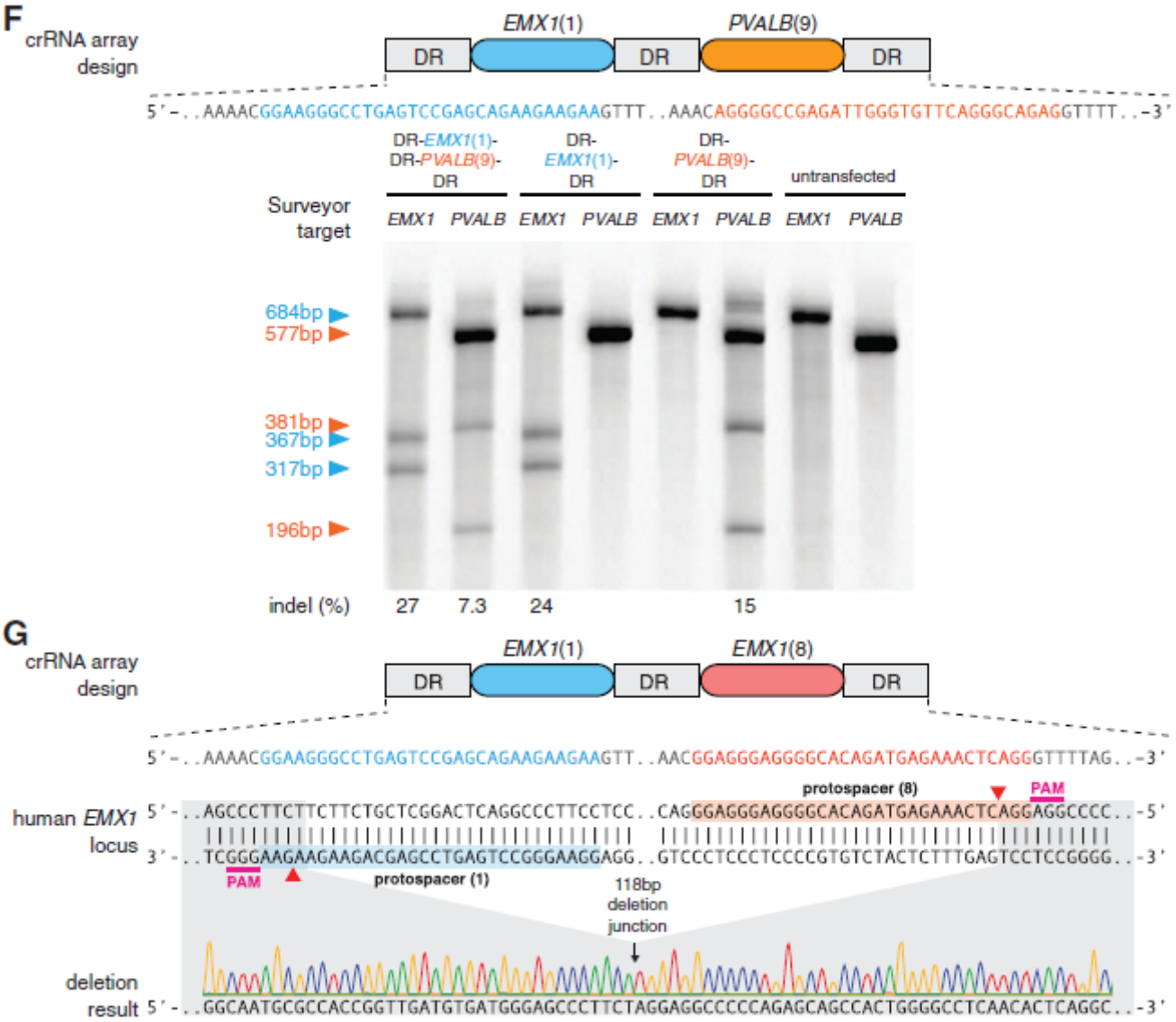
Comparison of The Efficiency of TALEN and SpCas9



Cas9 for Homologous Recombination



Multiplex Genome Engineering



Protospacer Sequences and Modification Efficiencies of Mammalian Genomic Targets

Cas9	target species	gene	protospacer ID	protospacer sequence (5' to 3')	PAM	strand	cell line tested	% indel (pre-crRNA + tracrRNA)	% indel (chimeric RNA)
S. pyogenes SF370 type II CRISPR	*Homo sapiens*	*EMX1*	1	GGAAGGGCCTGAGTCCGAGCAGAAGAAGAA	GGG	+	293FT	20 ± 1.8	6.7 ± 0.62
		EMX1	2	CATTGGAGGTGACATCGATGTCCTCCCCAT	TGG	–	293FT	2.1 ± 0.31	N.D.
		EMX1	3	GGACATCGATGTCACCTCCAATGACTAGGG	TGG	+	293FT	14 ± 1.1	N.D.
		EMX1	4	CATCGATGTCCTCCCCATTGGCCTGCTTCG	TGG	–	293FT	11 ± 1.7	N.D.
		EMX1	5	TTCGTGGCAATGCGCCACCGGTTGATGTGA	TGG	–	293FT	4.3 ± 0.46	2.1 ± 0.51
		EMX1	6	TCGTGGCAATGCGCCACCGGTTGATGTGAT	GGG	–	293FT	4.0 ± 0.66	0.41 ± 0.25
		EMX1	7	TCCAGCTTCTGCCGTTTGTACTTTGTCCTC	CGG	–	293FT	1.5 ± 0.12	N.D.
		EMX1	8	GGAGGGAGGGGCACAGATGAGAAACTCAGG	AGG	–	293FT	7.8 ± 0.83	2.3 ± 1.2
	Homo sapiens	*PVALB*	9	AGGGGCCGAGATTGGGTGTTCAGGGCAGAG	AGG	+	293FT	21 ± 2.6	6.5 ± 0.32
		PVALB	10	ATGCAGGAGGGTGGCGAGAGGGGCCGAGAT	TGG	+	293FT	N.D.	N.D.
		PVALB	11	GGTGGCGAGAGGGGCCGAGATTGGGTGTTC	AGG	+	293FT	N.D.	N.D.
	Mus musculus	*Th*	12	CAAGCACTGAGTGCCATTAGCTAAATGCAT	AGG	–	Neuro2A	27 ± 4.3	4.1 ± 2.2
		Th	13	AATGCATAGGGTACCACCCACAGGTGCCAG	GGG	–	Neuro2A	4.8 ± 1.2	N.D.
		Th	14	ACACACATGGGAAAGCCTCTGGGCCAGGAA	AGG	+	Neuro2A	11.3 ± 1.3	N.D.
S. thermophilus LMD-9 CRISPR1	*Homo sapiens*	*EMX1*	15	GGAGGAGGTAGTATACAGAAACACAGAGAA	GTAGAAT	–	293FT	14 ± 0.88	N.T.
		EMX1	16	AGAATGTAGAGGAGTCACAGAAACTCAGCA	CTAGAAA	–	293FT	7.8 ± 0.77	N.T.

Summary

- CRISPR system can be heterologously reconstituted in mammalian cells to facilitate efficient genome editing.
- Use RNA to program sequences pecific DNA cleavage defines a new class of genome engineering tools.
- Multiplex genome editing in mammalian cells enables powerful applications .
- Efficiency and versatility could be further improved.
- Potential constraints posed by PAM, crRNA secondary structure or genomic accessibility resulting from chromatin and DNA methylation states.

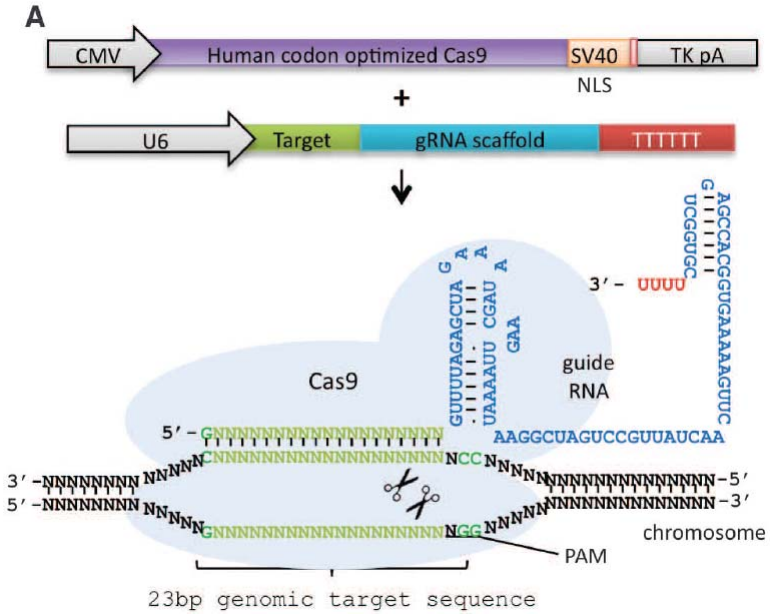
RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,[1]* Luhan Yang,[1,3]* Kevin M. Esvelt,[2] John Aach,[1] Marc Guell,[1] James E. DiCarlo,[4] Julie E. Norville,[1] George M. Church[1,2]†

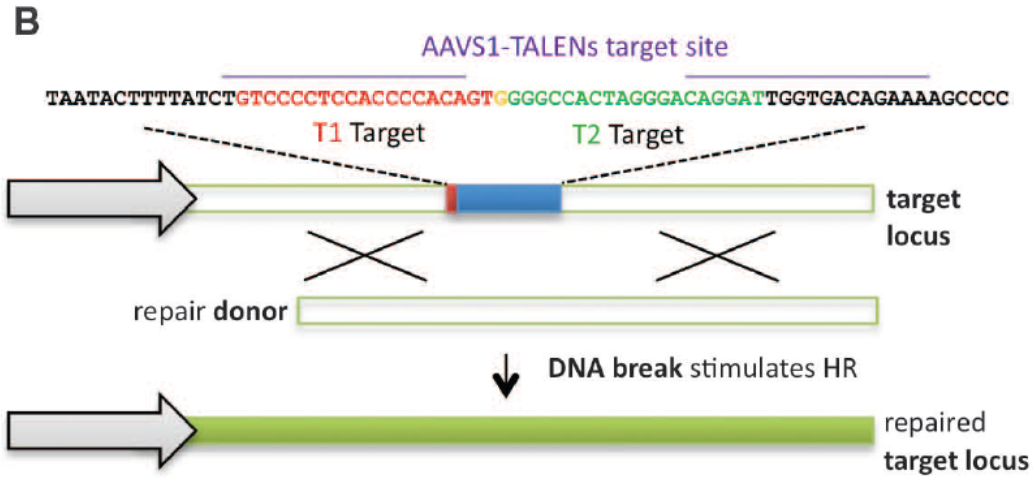
[1]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. [2]Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA 02138, USA. [3]Biological and Biomedical Sciences Program, Harvard Medical School, Boston, MA 02115, USA. [4]Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA.

Engineer Type II CRISPR System in Human Cells

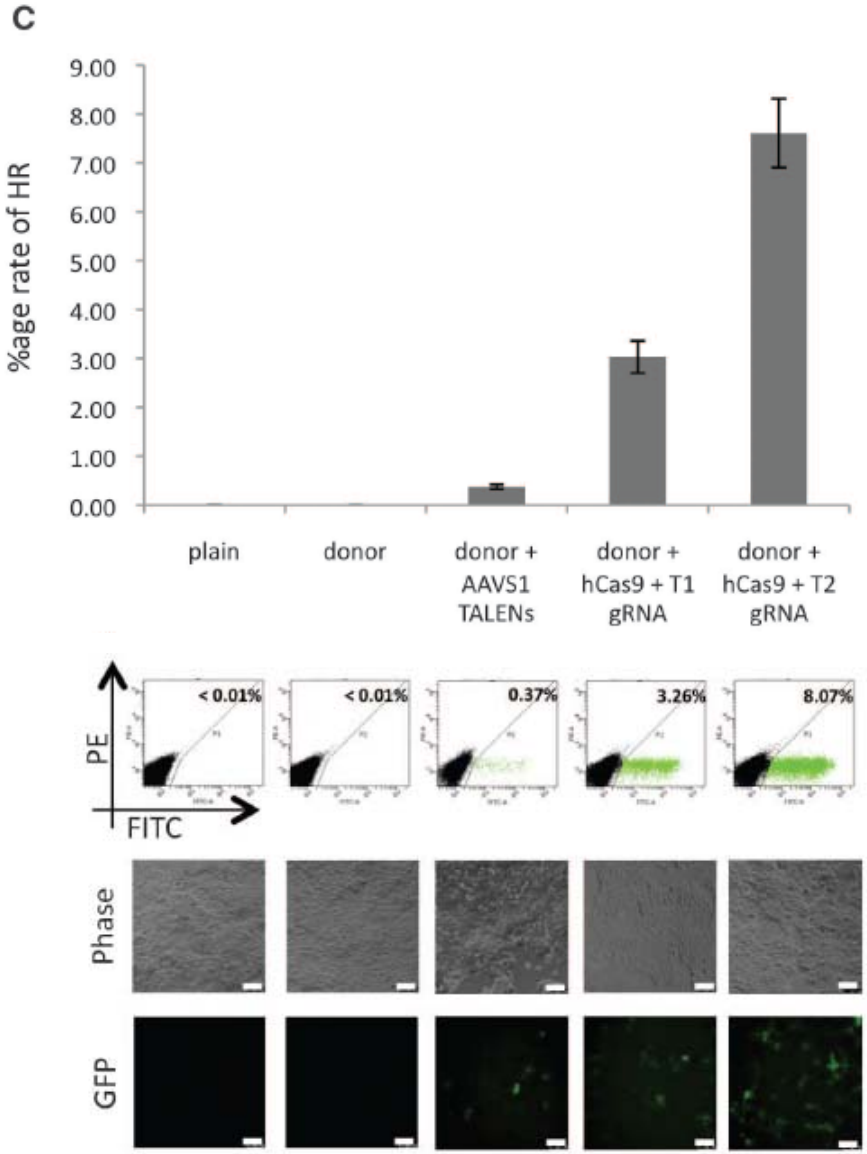
Codon-optimization



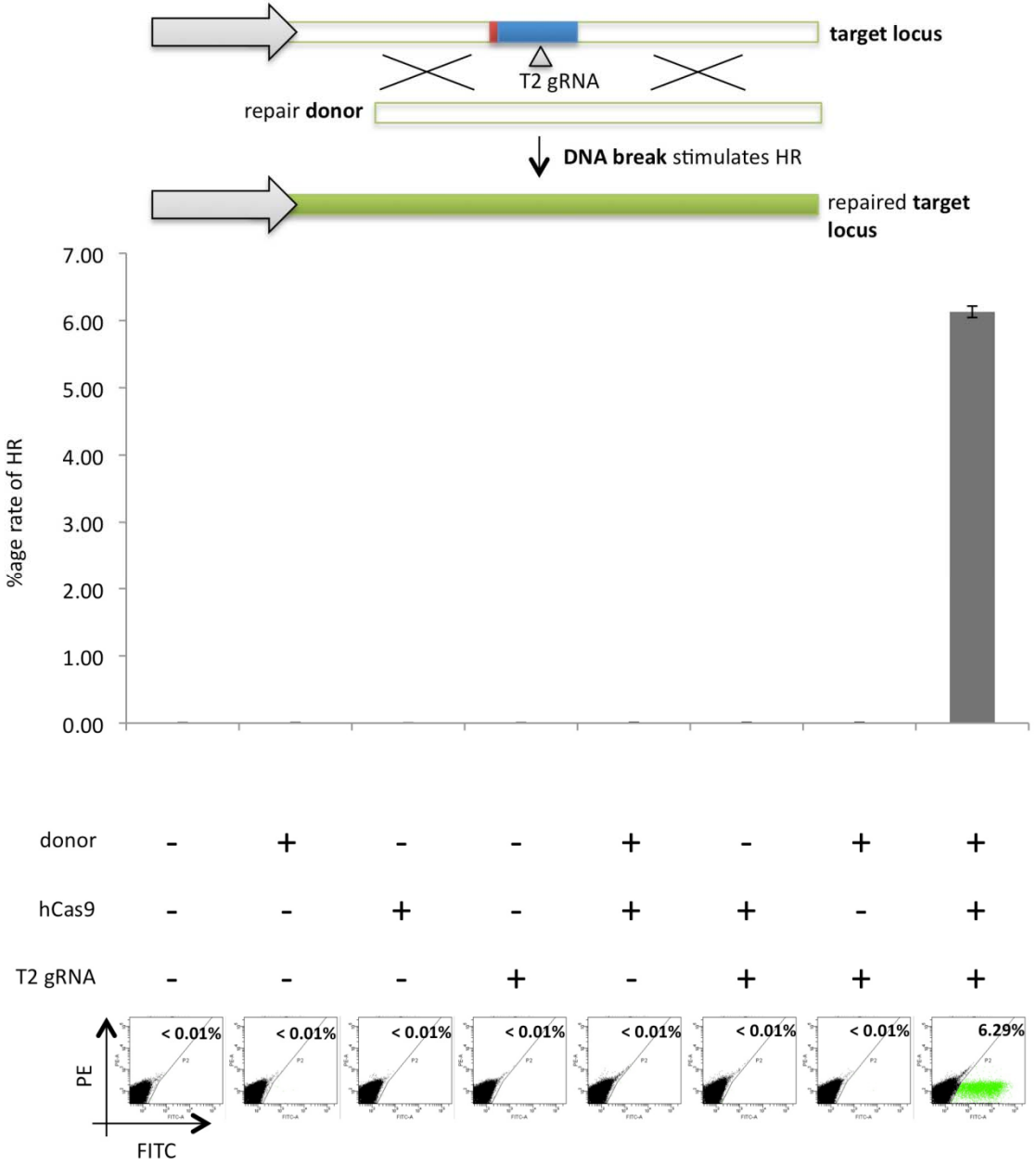
GFP reporter assay



Test The Functionality of CRISPR System by GFP Reporter Assay

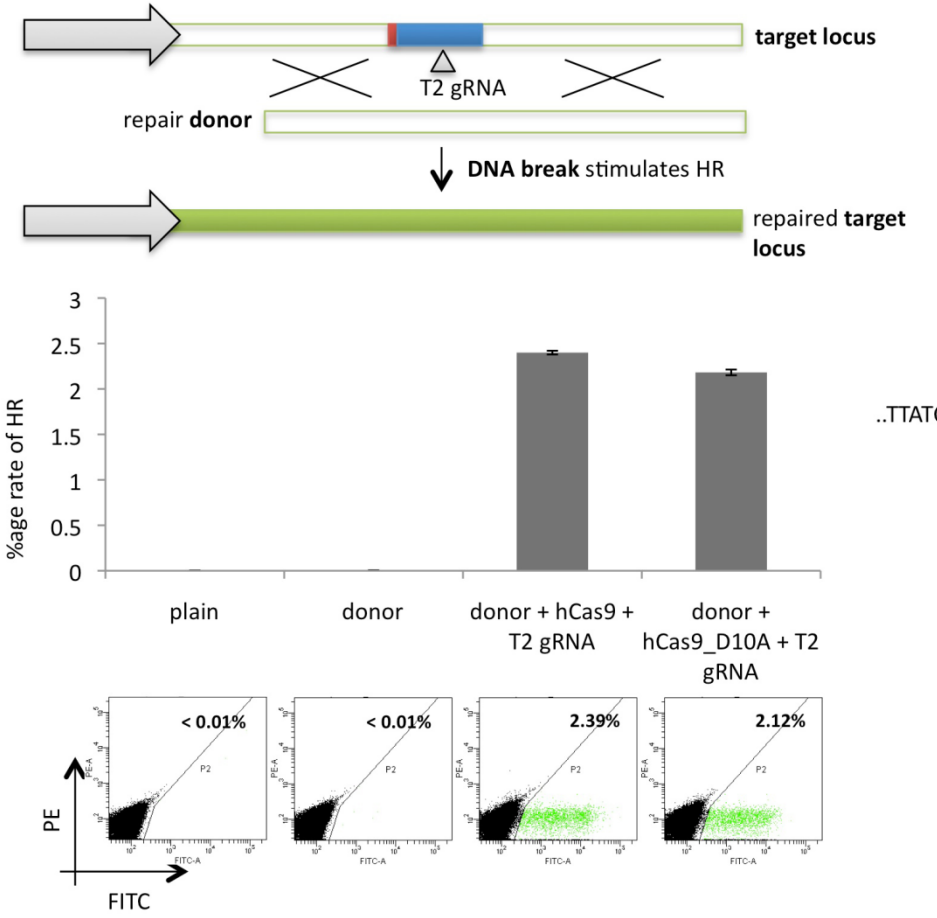


Components Required for Genome Editing



Effects of Cas9D10A Mutant Nickase on NHEJ

A



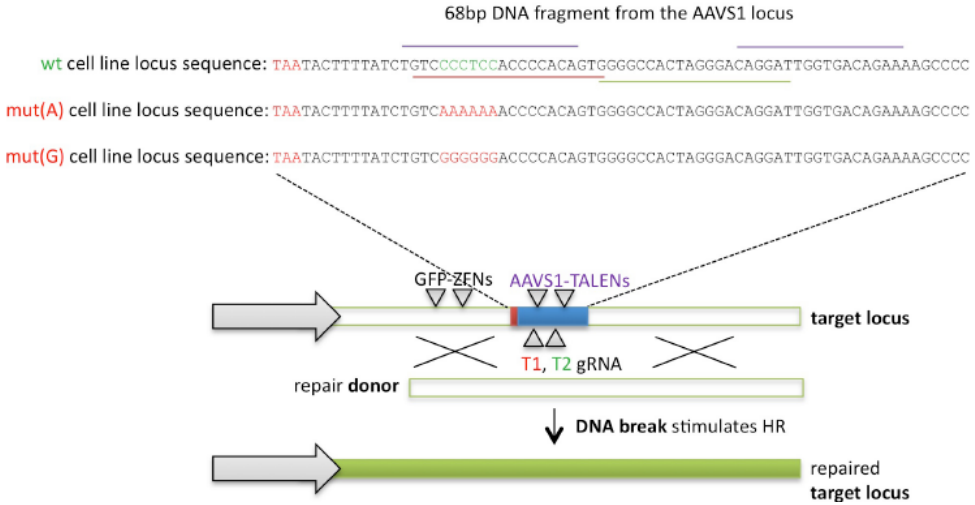
endogenous 'native' hAAVS1 locus sequence (**293T**)

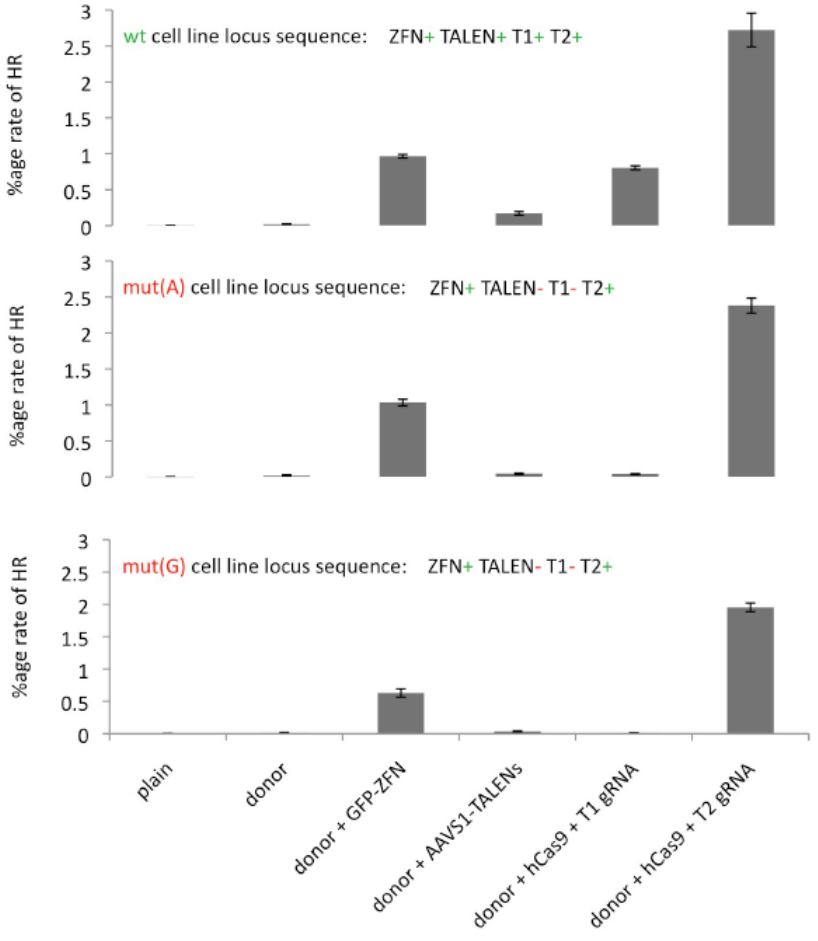
..TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGA..

T2 target

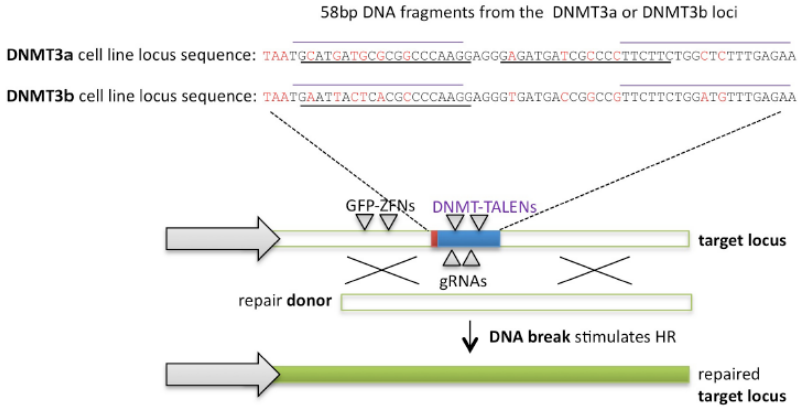
NHEJ rates evaluated using NGS of targeted AAVS1 locus

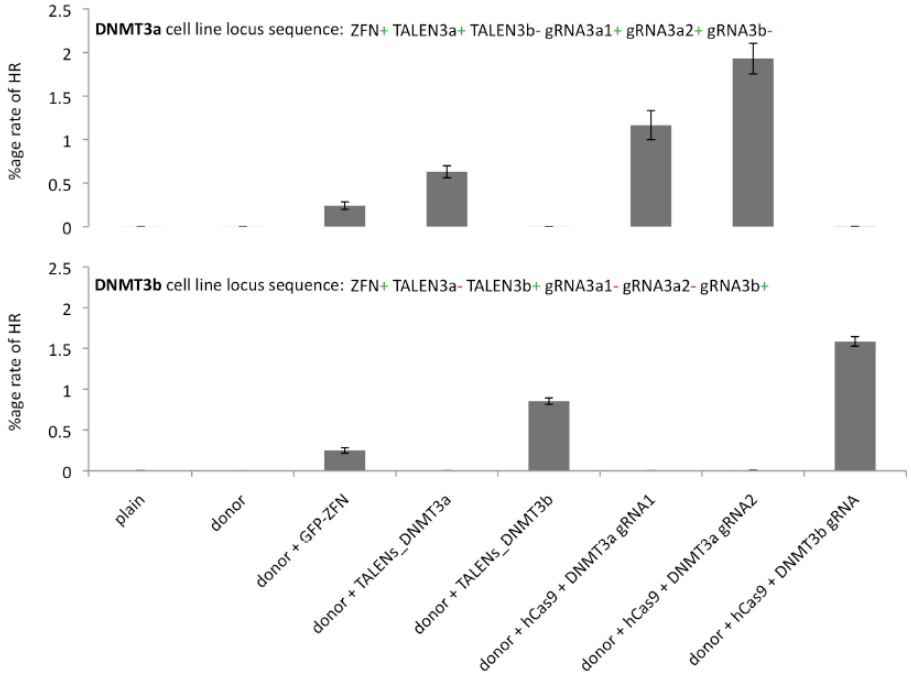
CRISPR Mediated
Genome Editing is Sequence-specific





DNMT3a and DNMT3b Genes Targeting in Human Cells





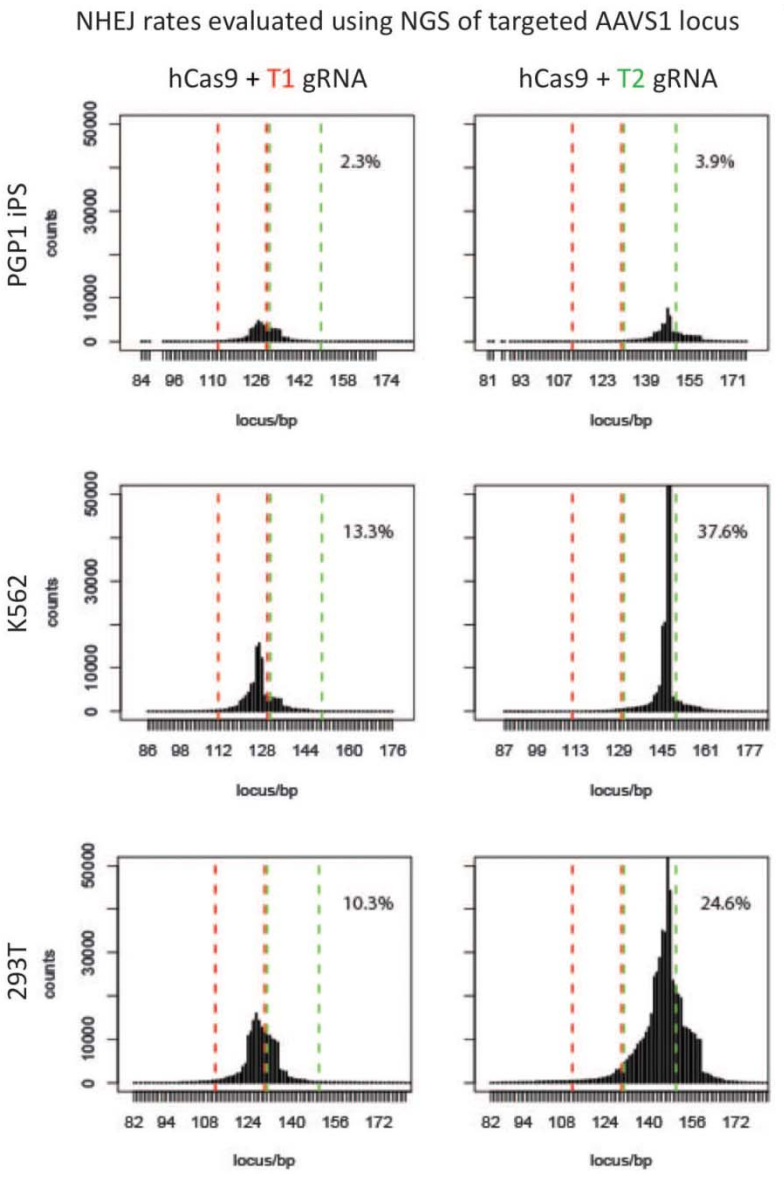
Target theAAVS1 Locus in 293Ts, K562 cells, and PGP1 Human (iPS) Cells

A

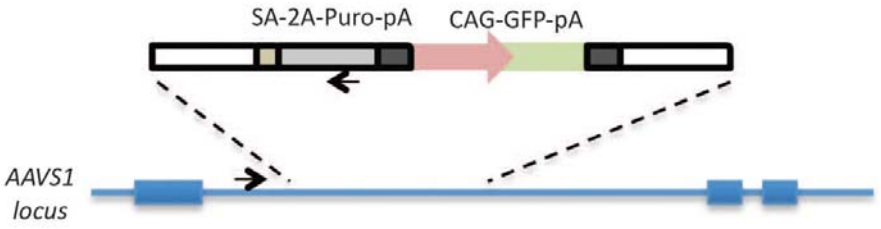
endogenous 'native' hAAVS1 locus sequence

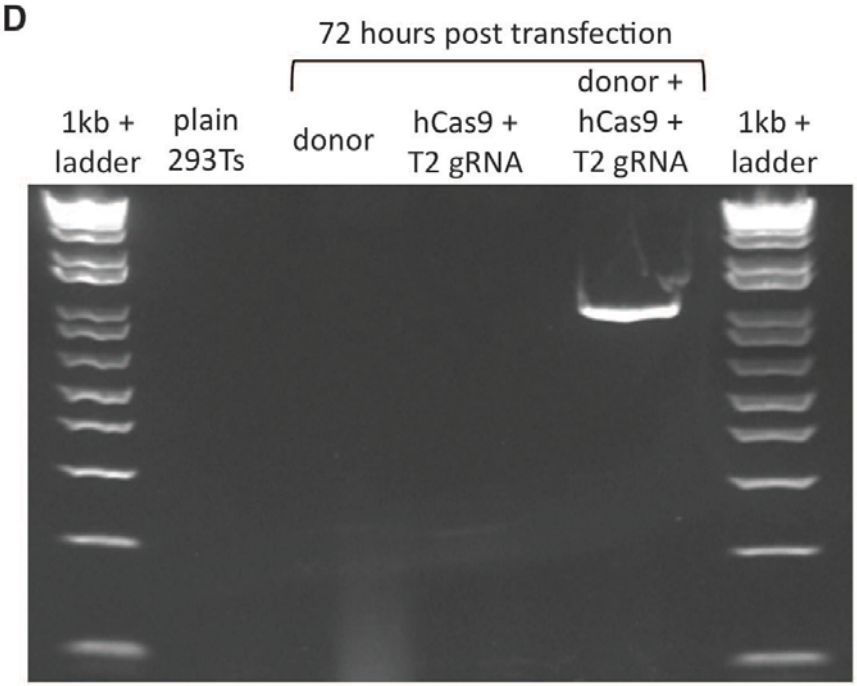
..TTATCTGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGA..

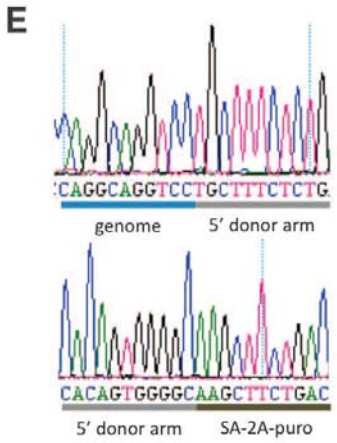
T1 target T2 target

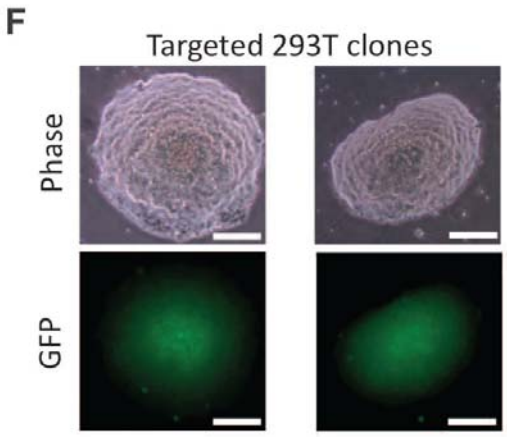


HR mediated integration

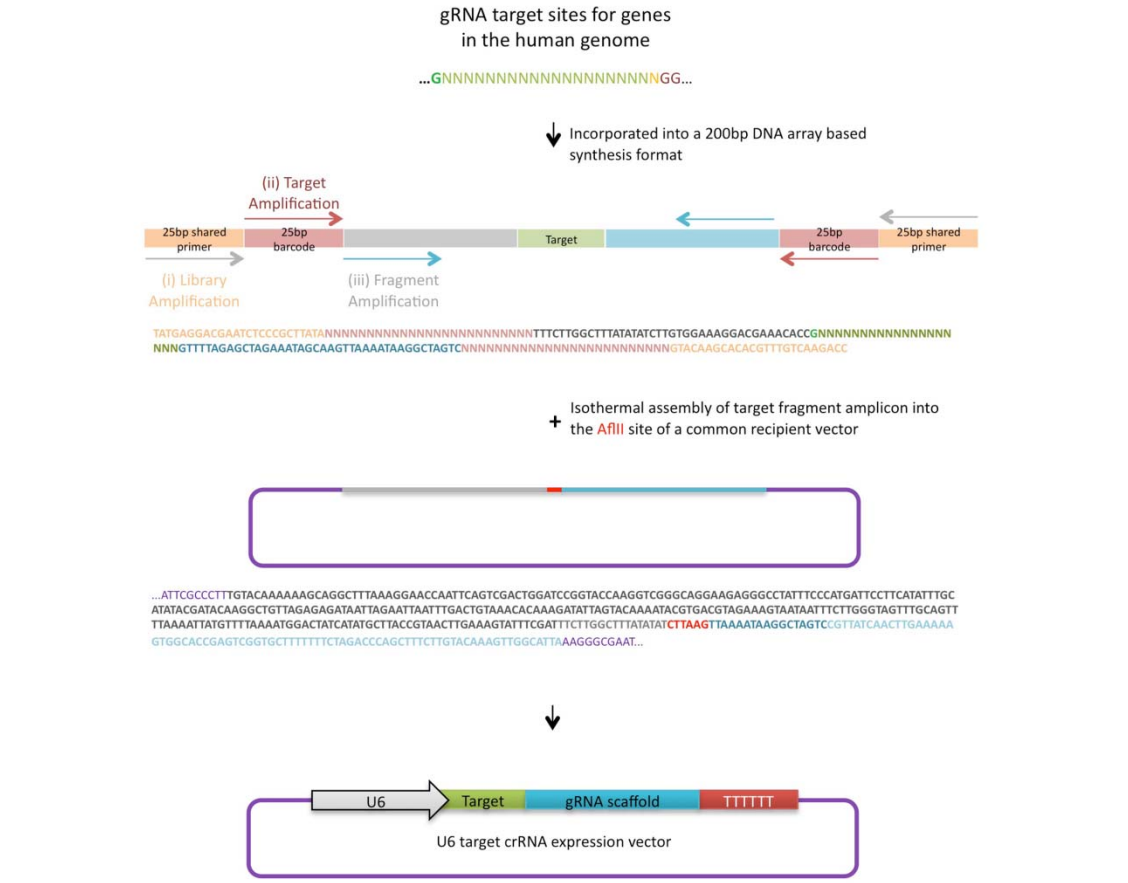








Multiplex Synthesis, Retrieval and U6 Expression Vector Cloning of Guide RNAs Targeting Genes in The Human Genome



Summary

- CRISPR-mediated gene targeting for RNAguided, robust, and multiplexable mammalian genome engineering.
- Expand the range of CRISPR-targetable sequences through the use of homologs with different PAM requirements or by directed evolution.
- Inactivating one of the Cas9 nuclease domains increases the ratio of HR to NHEJ and may reduce toxicity.
- Target locus's underlying chromatin structure and epigenetic state will also affect the efficiency of genome editing in eukaryotic cells.
- Evaluating Cas9 homologs identified through bioinformatics and directed evolution of these nucleases toward higher specificity.

Resource

Cell

Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression

Lei S. Qi,[1,2,8,*] Matthew H. Larson,[2,3,8] Luke A. Gilbert,[2,3,8] Jennifer A. Doudna,[4,5,6,8,9] Jonathan S. Weissman,[2,3,8] Adam P. Arkin,[7,8,9] and Wendell A. Lim[1,2,3,8]

[1]UCSF Center for Systems and Synthetic Biology
[2]Department of Cellular and Molecular Pharmacology
[3]Howard Hughes Medical Institute
University of California, San Francisco, San Francisco, CA 94158, USA
[4]Department of Molecular and Cellular Biology
[5]Department of Chemistry
[6]Howard Hughes Medical Institute
[7]Department of Bioengineering
University of California, Berkeley, Berkeley, CA 94720, USA
[8]California Institute for Quantitative Biomedical Research, San Francisco, CA 94158, USA
[9]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

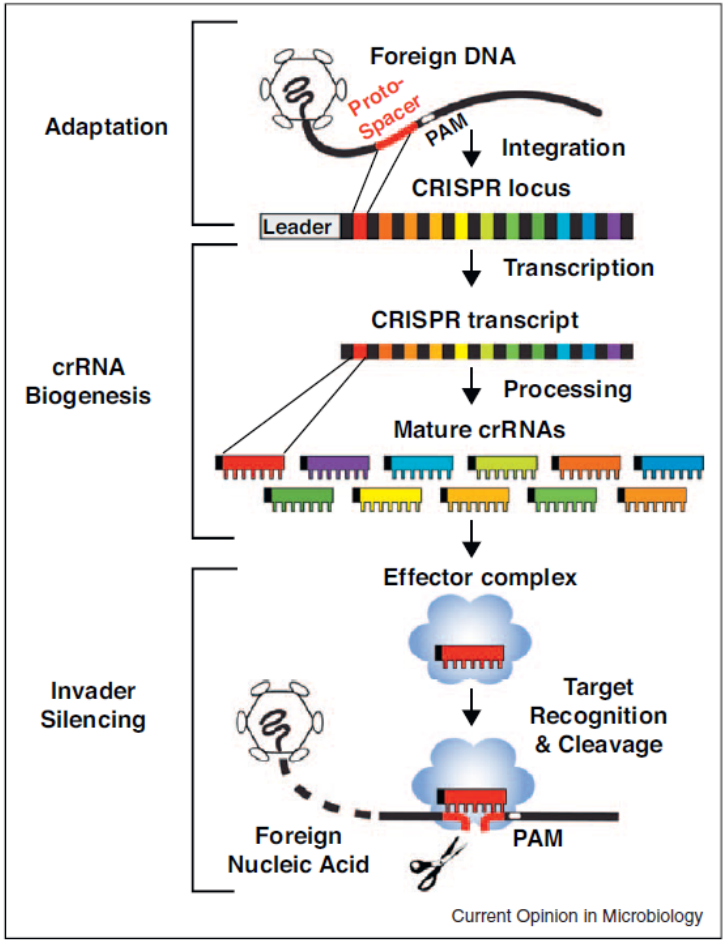
LETTER

doi:10.1038/nature11927

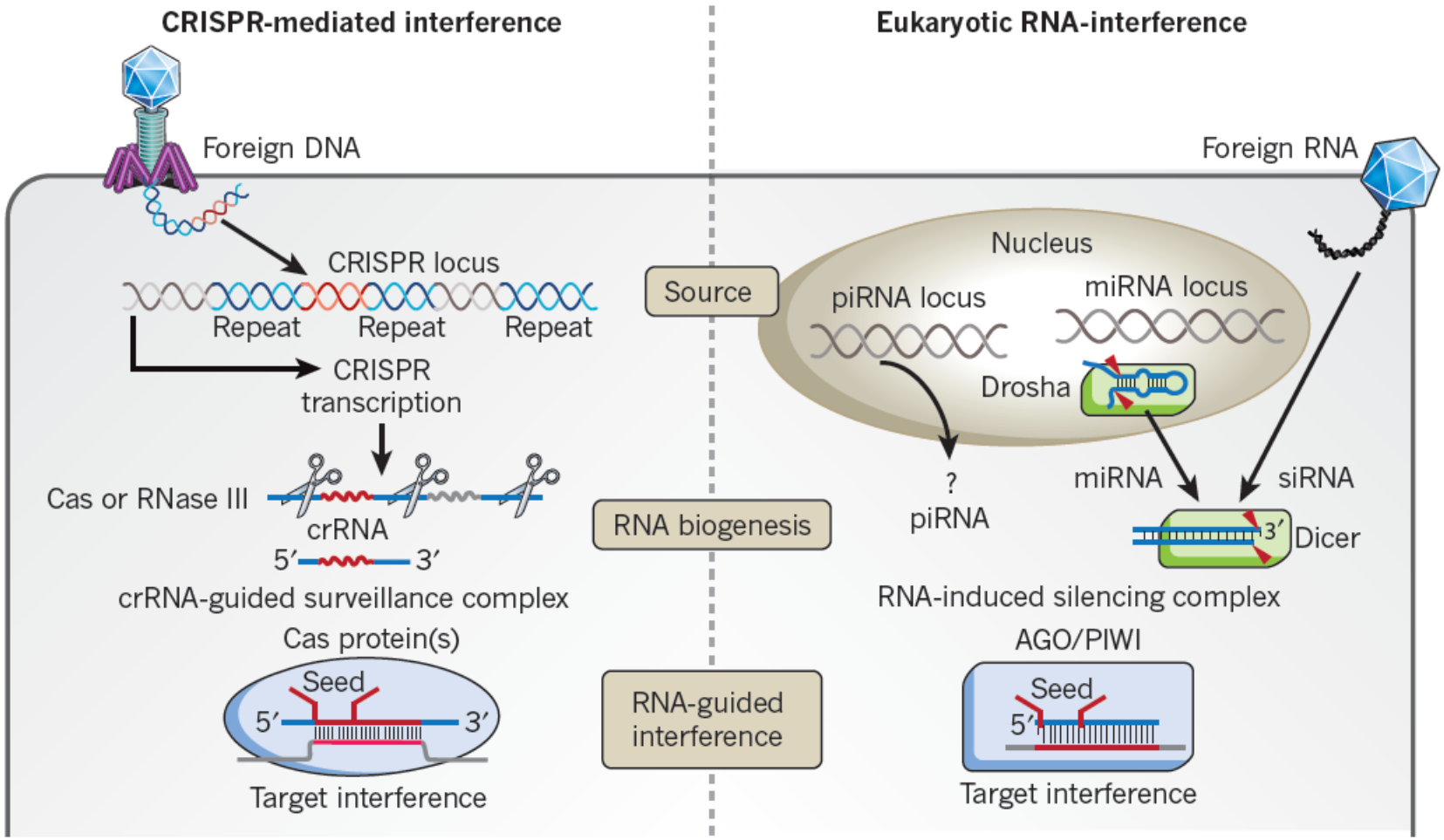
A bacteriophage encodes its own CRISPR/Cas adaptive response to evade host innate immunity

Kimberley D. Seed[1], David W. Lazinski[1], Stephen B. Calderwood[2,3] & Andrew Camilli[1]

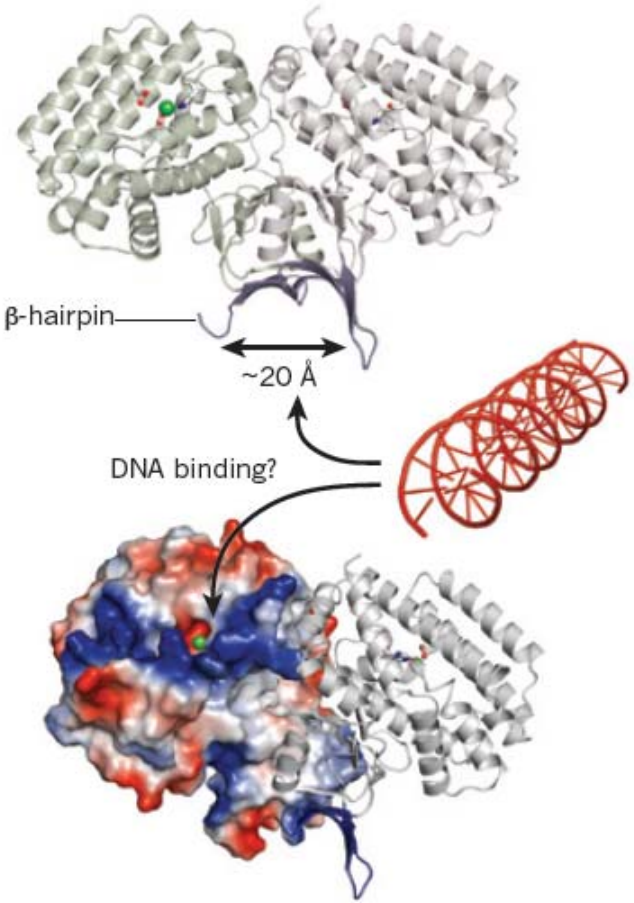

Thank You!

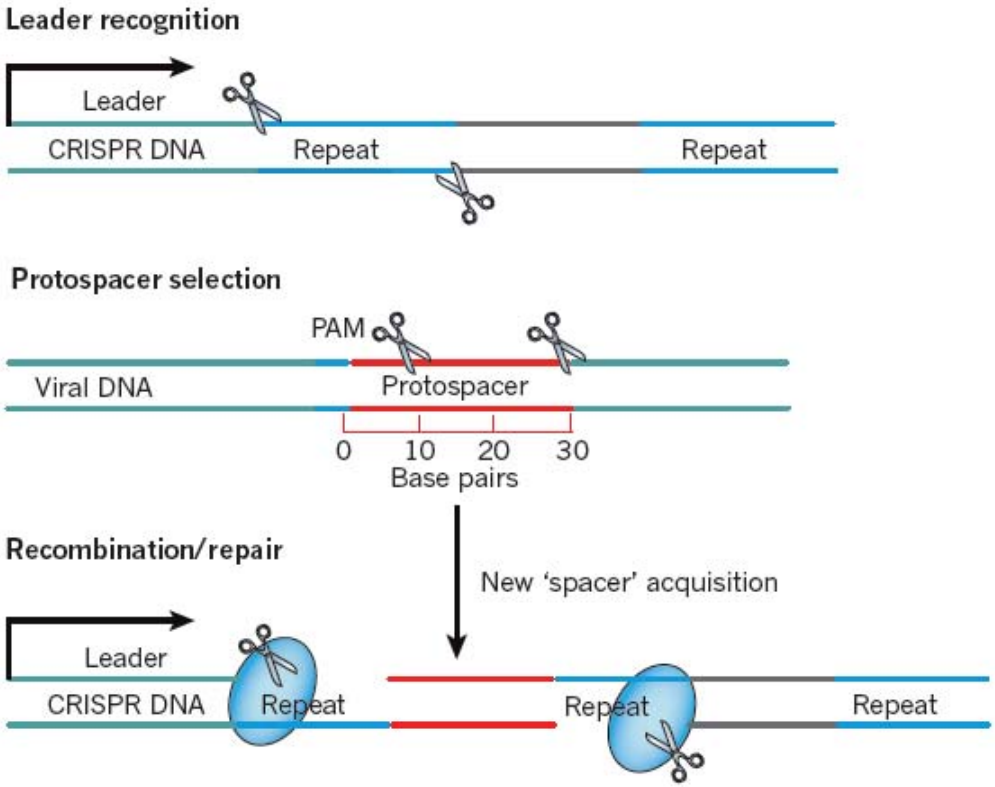

Adaptation
Foreign DNA
Proto-
Spacer
PAM
Integration
CRISPR locus
Leader
Transcription
crRNA
Biogenesis
CRISPR transcript
Processing
Mature crRNAs
Effector complex
Invader
Silencing
Target
Recognition
& Cleavage
Foreign
Nucleic Acid
PAM
Current Opinion in Microbiology

Parallels and distinctions between CRISPR RNA-guided silencing systems and RNAi

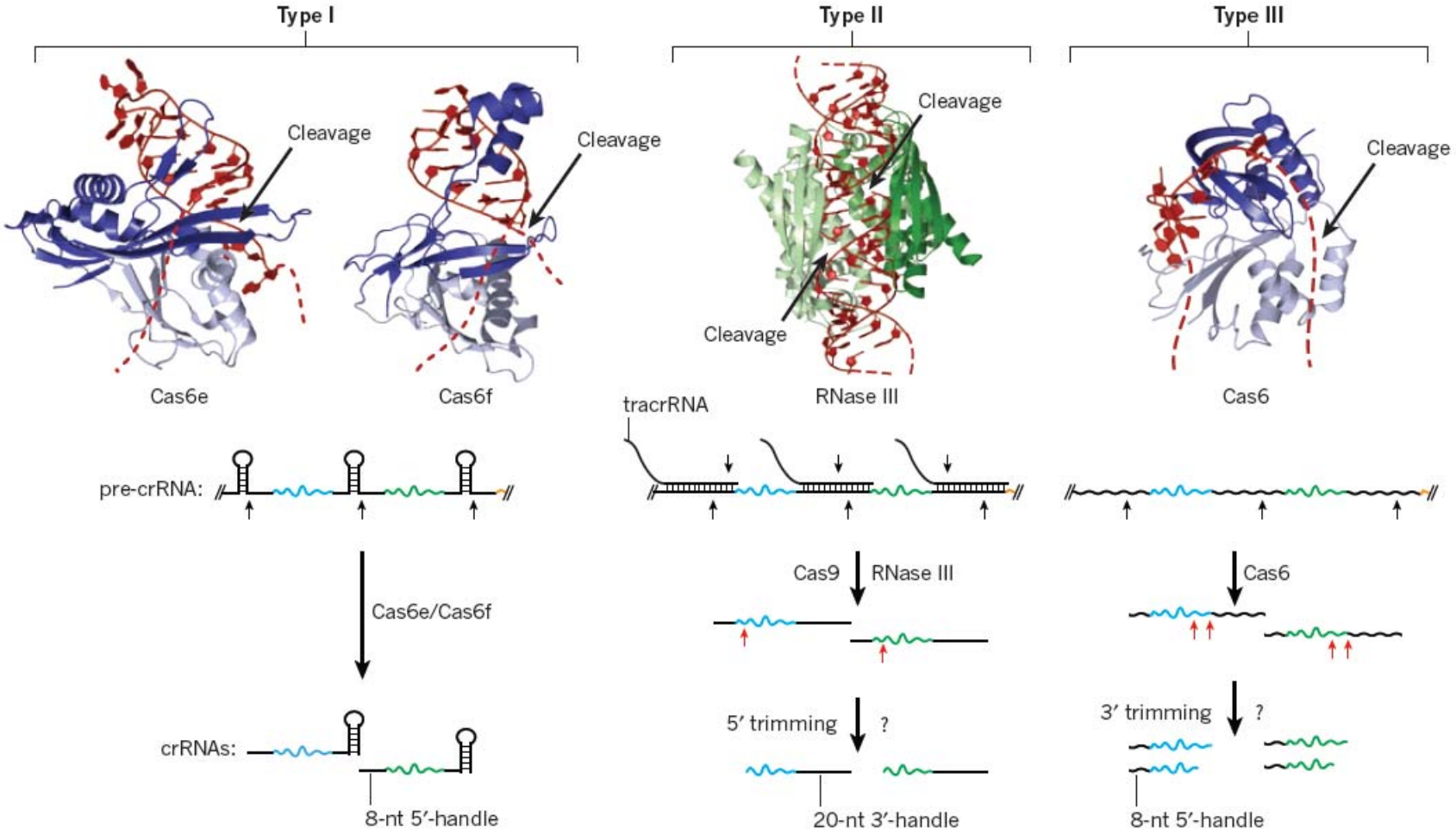


Steps leading to new spacer integration

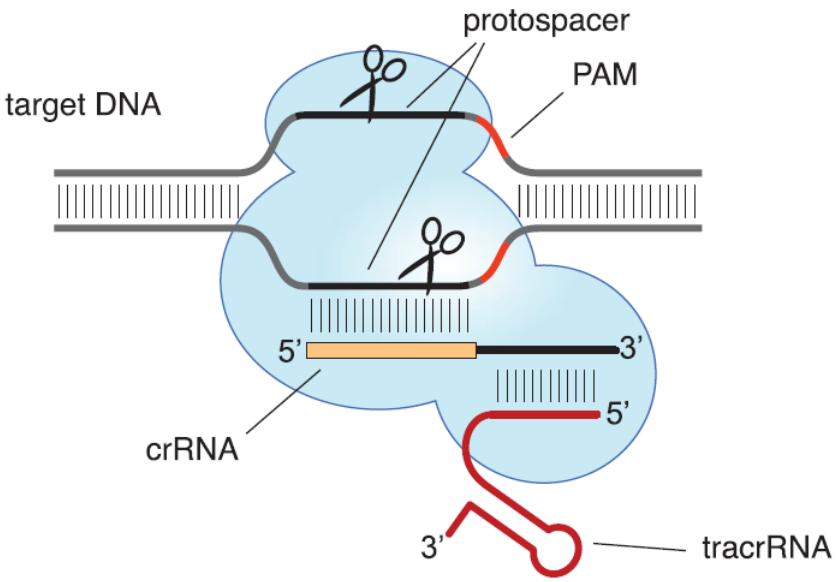




Diverse mechanisms of CRISPR RNA biogenesis



Cas9 programmed by crRNA:tracrRNA duplex



Cas9 programmed by single chimeric RNA