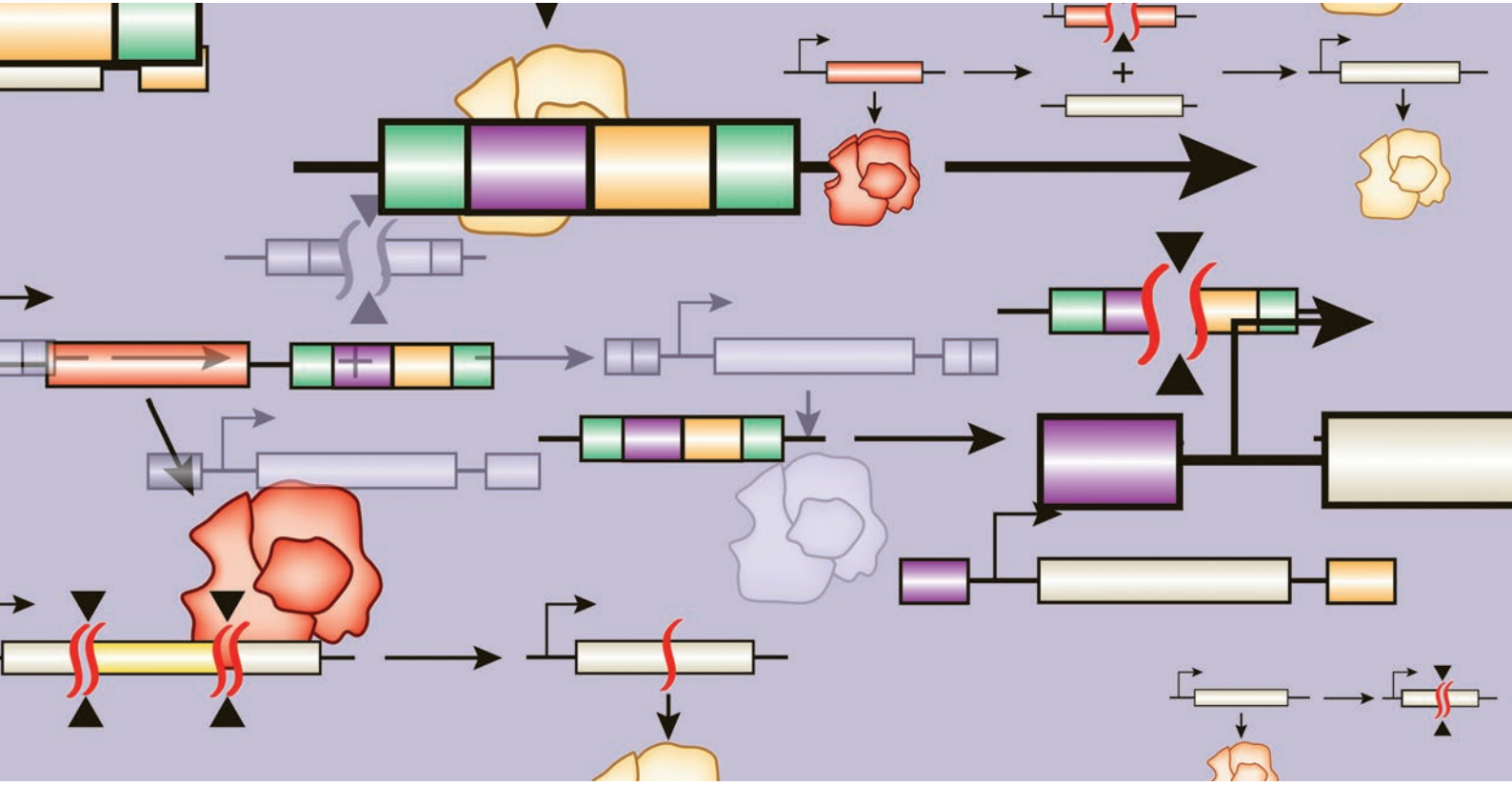
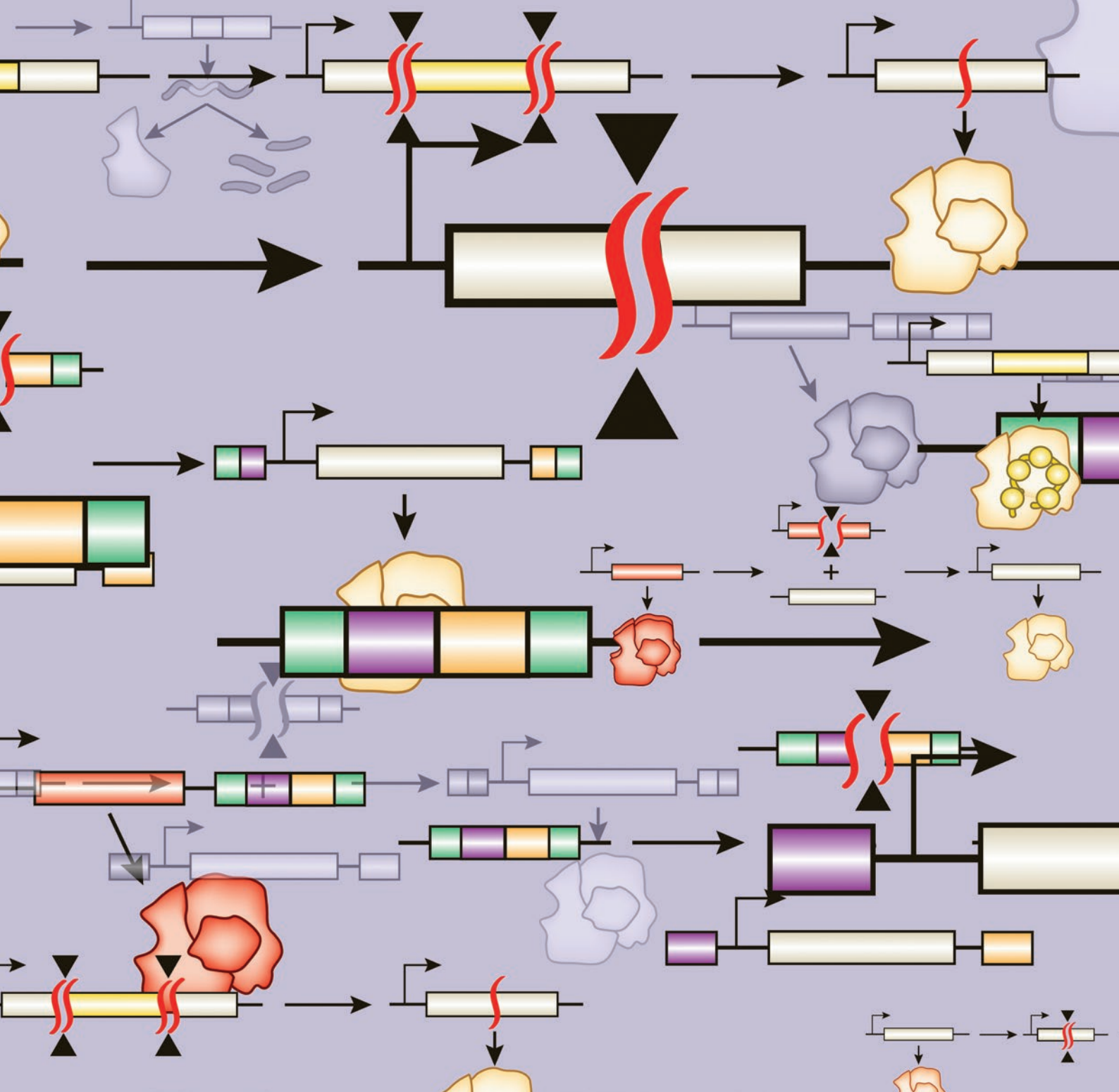
nature protocols
Recipes for Researchers
www.nature.com/nprot

2016 calendar

CRISPR/Cas





nature protocols
Recipes for Researchers

The *Nature Protocols* calendar for 2016 highlights the technical aspects of CRISPR/Cas, a ground-breaking gene-editing technology that has captivated the scientific community. Following on from the 2015 CRISPR/Cas calendar from *Nature Reviews Microbiology,* this collaborative project—which includes images from *Nature Protocols, Nature Methods* and *Nature Medicine*—has a particular focus on the role of CRISPR/Cas in genome editing of disease-affected cells and tissues. At the end of the calendar, we have included a brief summary of each figure, along with details of the original papers and a list of some of the key scientific meetings scheduled for 2016. The calendar is freely available online at http://www.nature.com/nprot/calendars/2016/index.html thanks to support from Origene. As always, Nature Publishing Group retains sole responsibility for all editorial content.

nature methods

nature medicine

Nature Protocols:
http://www.nature.com/nprot/index.html

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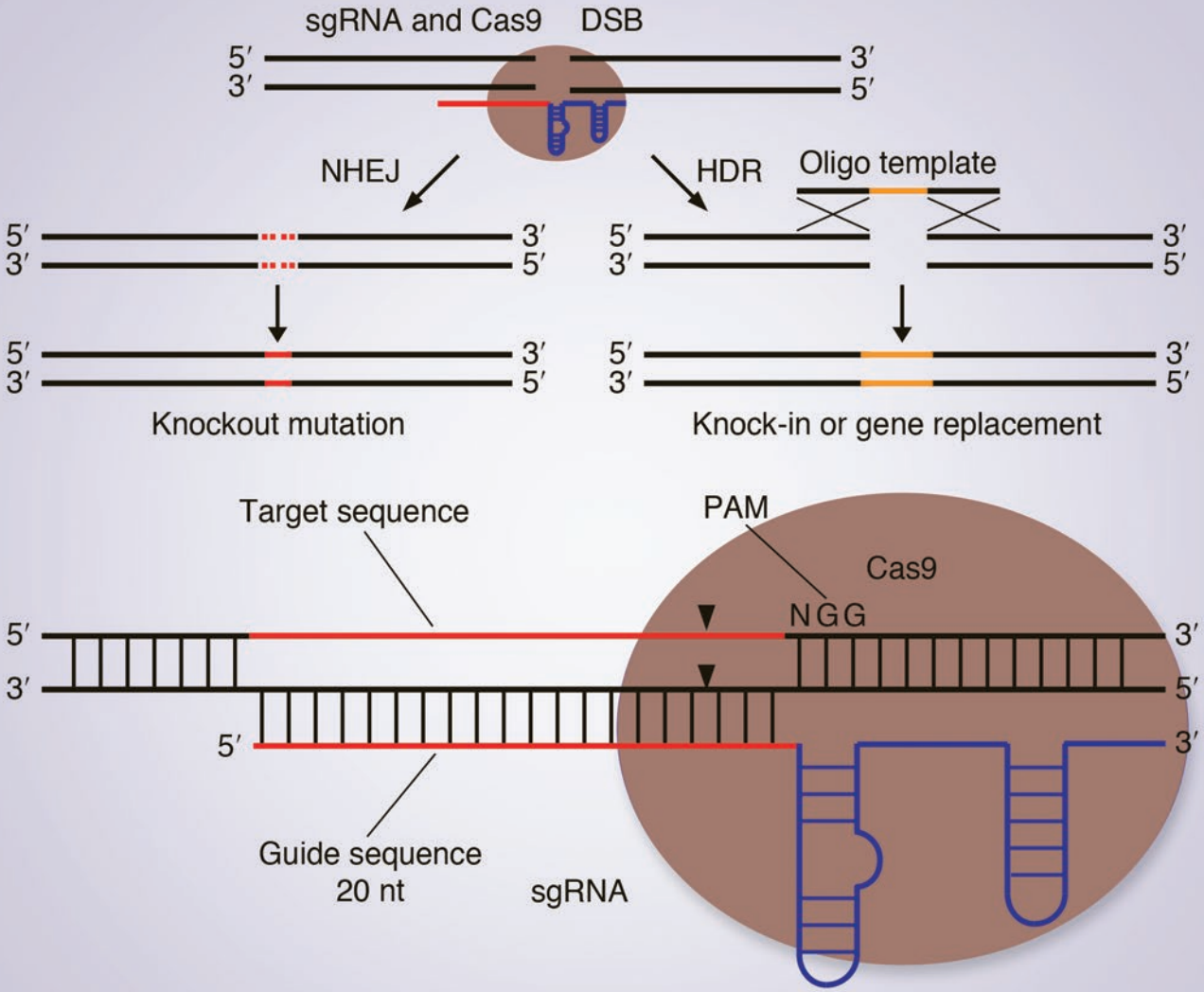
Calendar compiled and edited by Melanie Clyne
Copyedited by Rebecca Barr
Designed by Erin Dewalt

LIST OF ABBREVIATIONS USED IN THE CALENDAR:

bp, base pair; d.p.c., days *post coitum*; DSB, double-strand break; GOI, gene of interest; HCG, human chorionic gonadotropin; HDR, homology-directed repair; HR, homologous recombination; indel, insertion or deletion; NHEJ, nonhomologous end-joining; nt, nucleotide; PAM; protospacer-adjacent motif; sgRNA, single guide RNA; ssDNA, single-stranded DNA; ssODN, single-stranded oligodeoxynucleotide.



RNA-guided genome editing using the CRISPR/Cas system. DSBs induced by Cas9 trigger NHEJ (causes imprecise gene knockouts) and HDR (targeted gene knock-in or replacement). The HNH and RuvC-like domains of Cas9 (brown) each cleave one strand of the sequence targeted by the sgRNA (red and blue), provided that the correct PAM sequence is present at the 3′ end.

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JANUARY 2016

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
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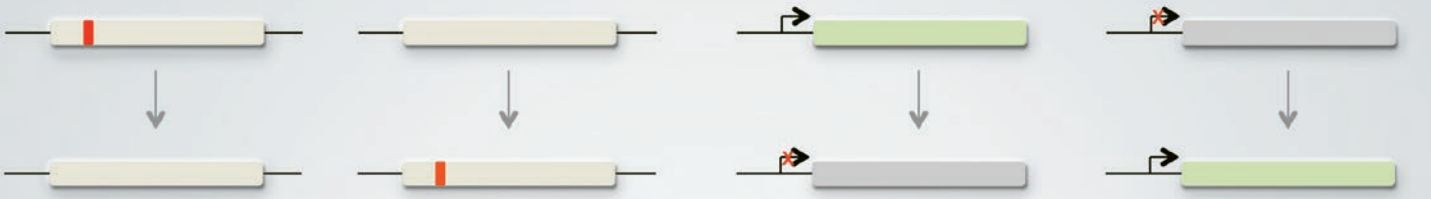

Genome repair:
e.g., genetic disorders,
genetic risk factors

Genome disruption:
e.g., invading pathogen genomes,
dominant negative mutations

Genome repression:
e.g., oncogenes,
host viral receptors

Genome activation
e.g., tumor suppressors,
globins

Key genome-engineering objectives	Potential approaches
Minimize off-target events	Cas9 evolution; cooperativity in effector functions
Maximize HR versus NHEJ in mitotic and postmitotic cells	Coordinate DSBs and DNA editing, e.g., Cas9 recombinases and transposases
Avoid immunogenicity	'Humanize' Cas9 peptide fragments; disrupt trafficking of major histocompatibility complex
Minimize delivery size	Employ small Cas9 orthologs; truncate Cas9

Cas9 therapeutics. Potential Cas9-mediated therapeutic approaches include targeted genome editing to correct genetic disorders and targeted genome regulation to modify endogenous protein levels.

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Recipes for Researchers

FEBRUARY 2016

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
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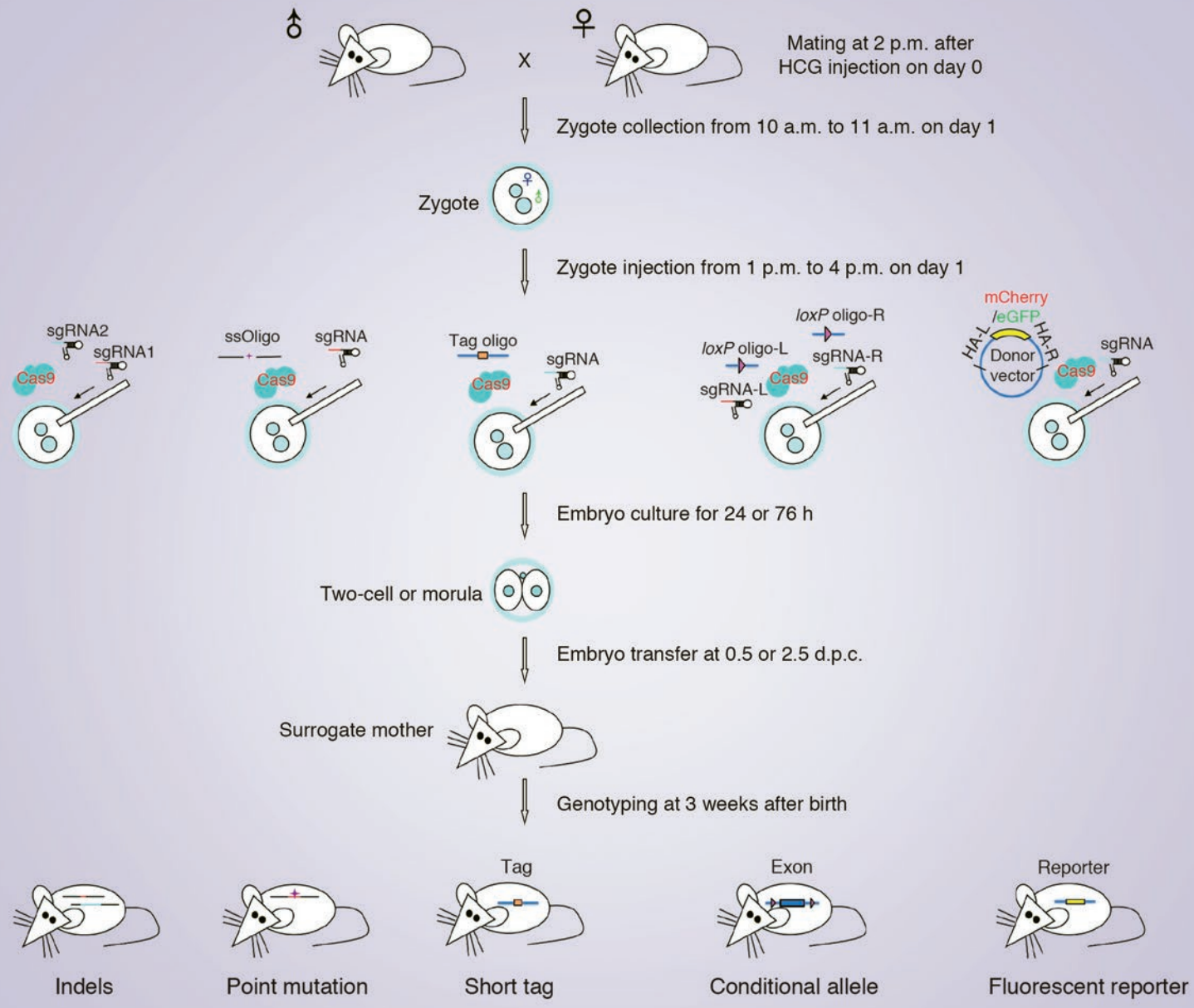

Procedure for the generation of gene-modified mice by CRISPR/Cas. By injection of Cas9 mRNA along with sgRNAs targeting specific genes—independently or with ssDNA harboring point mutations, tags, *loxP* sites or plasmid DNA (shown left to right)—gene-modified mice can be generated with indels, precise mutations, tags, conditional alleles or fluorescent reporters, respectively.

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Recipes for Researchers

MARCH 2016

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
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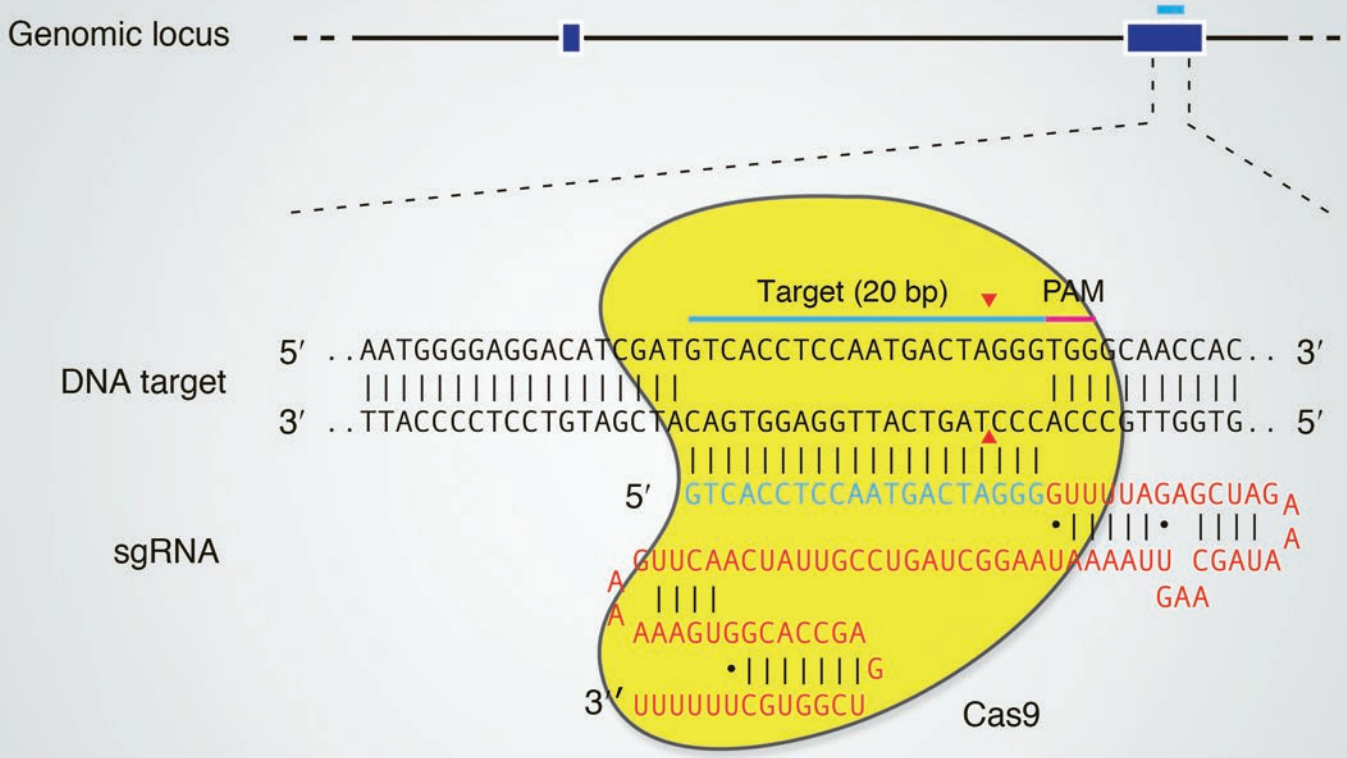

The RNA-guided Cas9 nuclease. The Cas9 nuclease from *Streptococcus pyogenes* is targeted to genomic DNA by an sgRNA consisting of a 20-nt guide sequence (blue) and a scaffold (red). The guide sequence pairs with the DNA target (blue bar on top strand), directly upstream of a requisite 5′-NGG adjacent motif (PAM; pink). Cas9 mediates a DSB ~3 bp upstream of the PAM (red triangle).

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Recipes for Researchers

APRIL 2016

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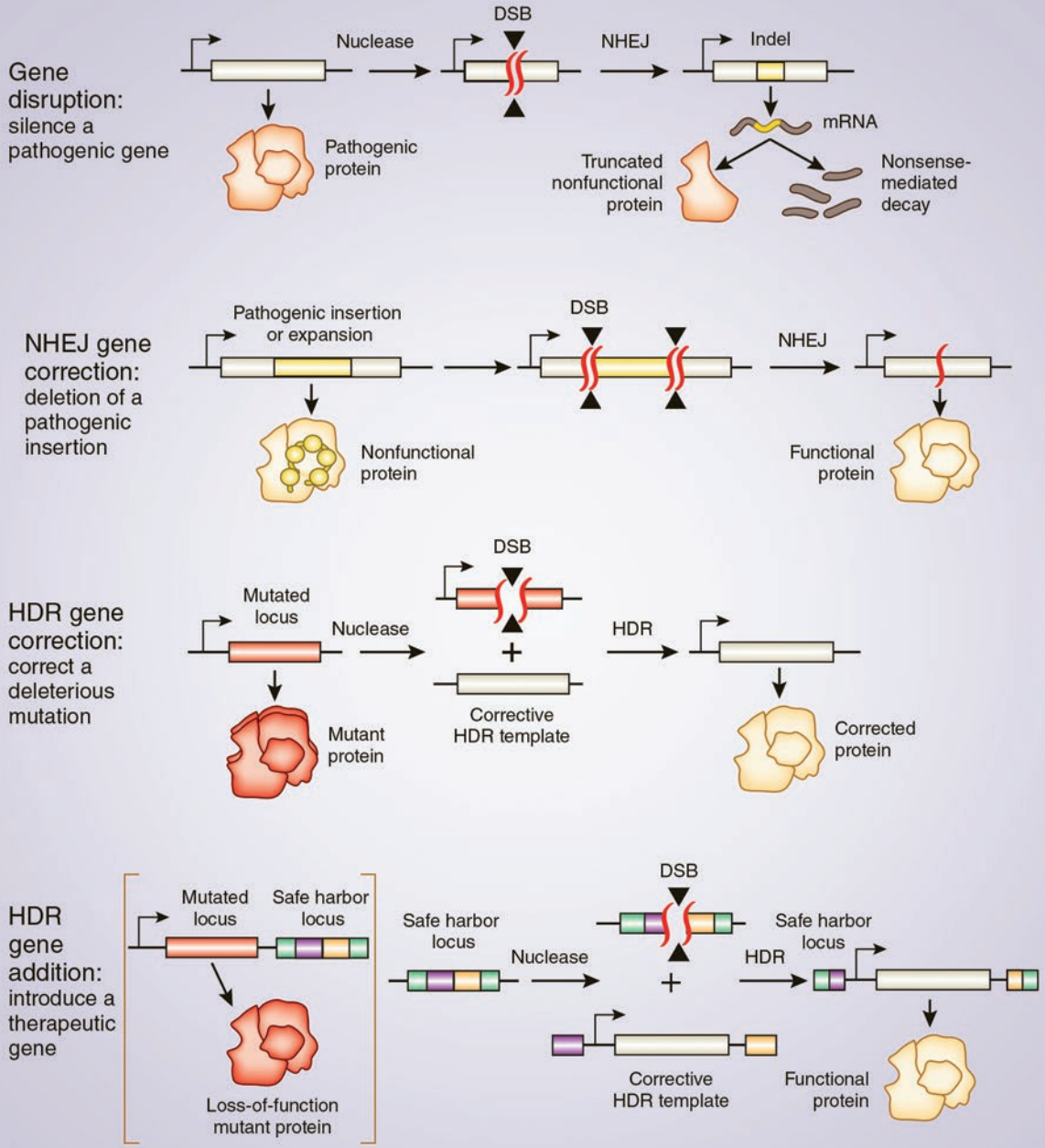

Types of therapeutic genome modifications. Genome editing can be used to disrupt or replace disease-causing genes, or to insert a new gene affording a therapeutic benefit. The particular gene-editing approach selected for each therapeutic indication will therefore depend on the exact genetic etiology of the disease.

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Recipes for Researchers

MAY 2016

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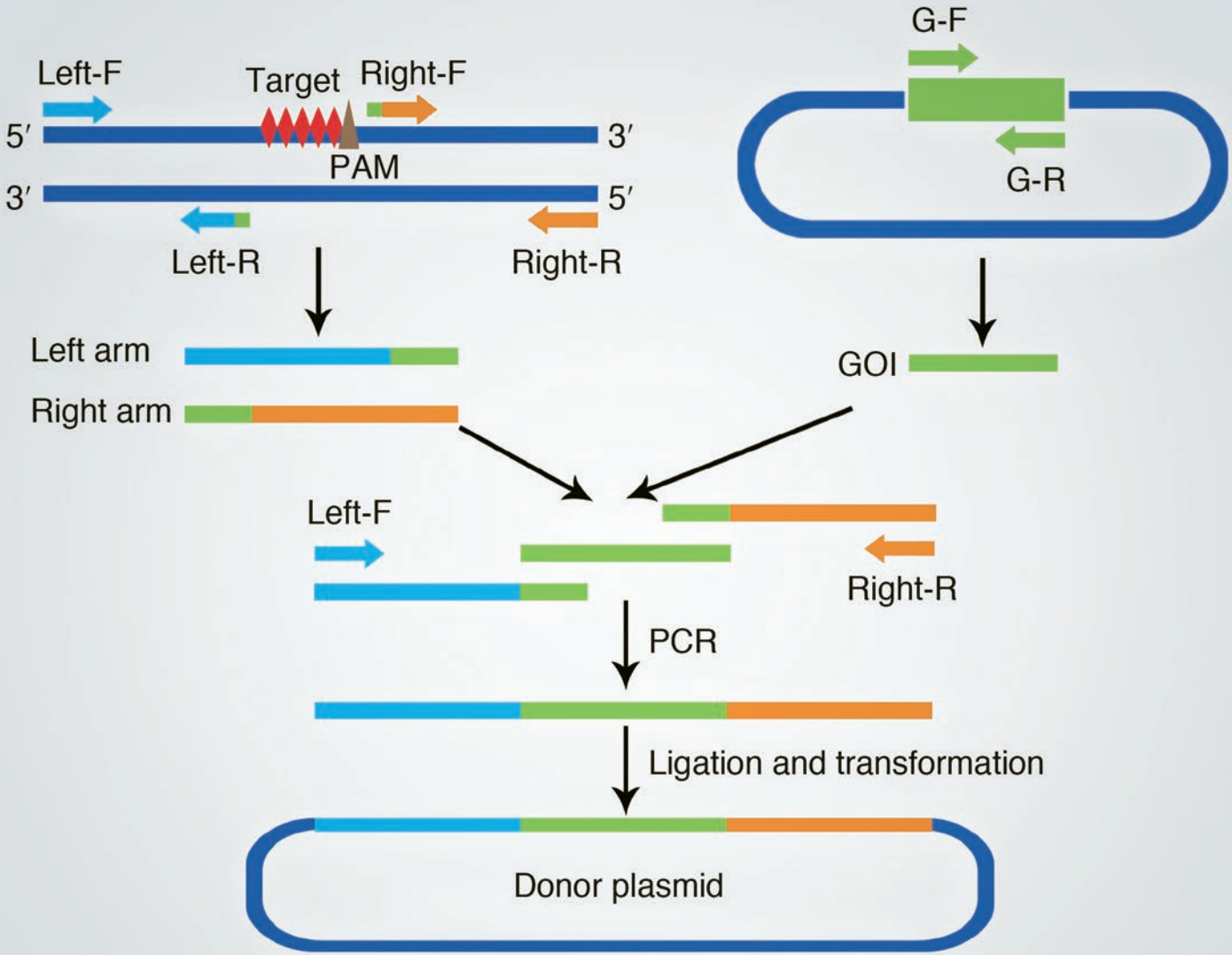

Construction of a rat gene-targeting vector through overlapping PCR. The left and right homology arms are amplified with chimeric primers containing overlapping sequence with the gene of interest (GOI). The GOI is amplified using G-F (GOI-forward) and G-R (GOI-reverse) primers. The donor DNA template is amplified using the left-forward (left-F) and right-reverse (right-R) primers in the presence of the left arm, the right arm and the GOI fragments as templates. The donor DNA template is then inserted into the chosen vector to create the circular donor plasmid.

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Recipes for Researchers

JUNE 2016

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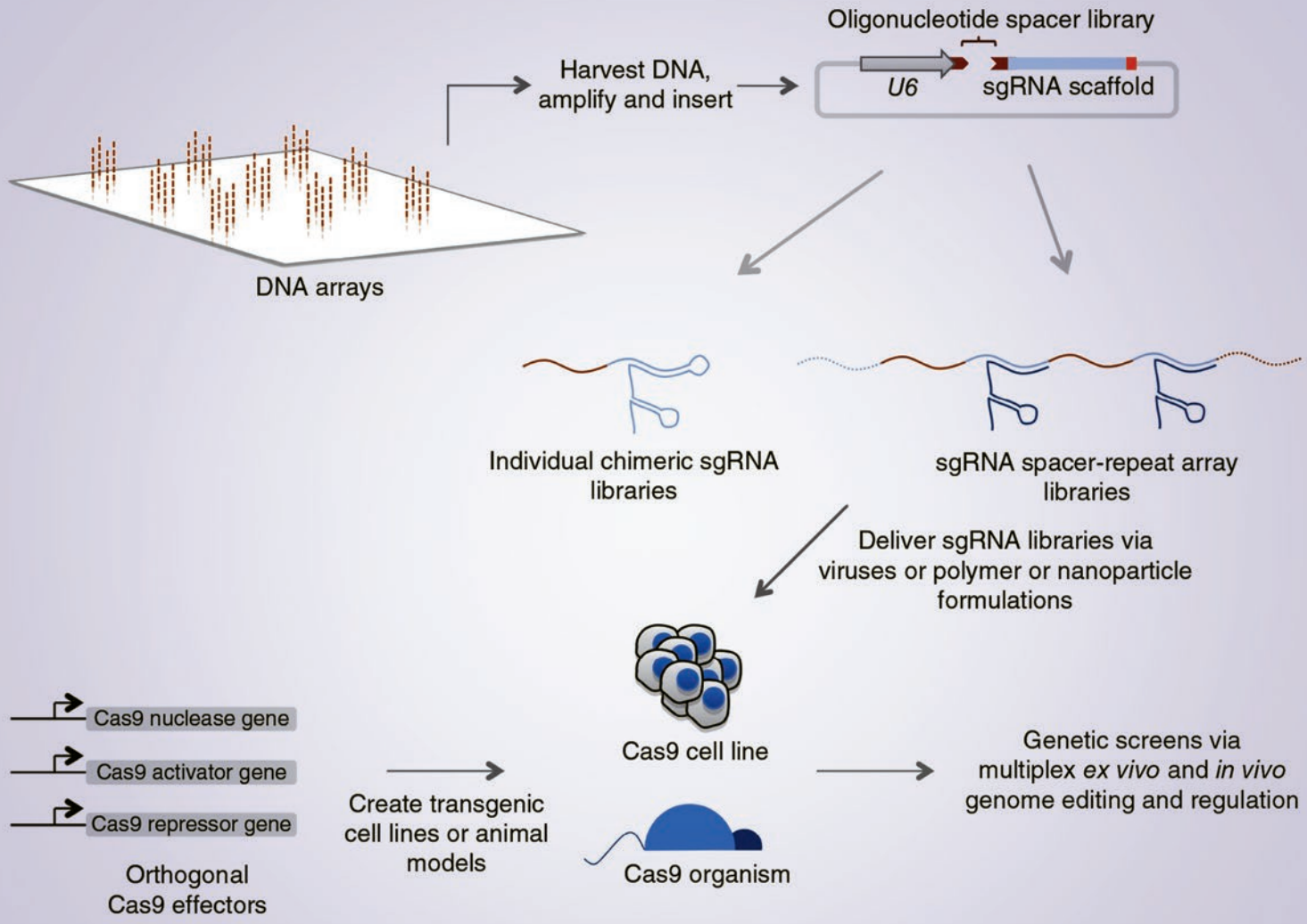

Platform for multiplex biological screens. To modulate multiple genomic sites, sgRNA libraries can be generated and delivered into target cells that also express orthogonal Cas9 effectors. This format enables multiplex *ex vivo* and *in vivo* genetic screens.

nature protocols
Recipes for Researchers

JULY 2016

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
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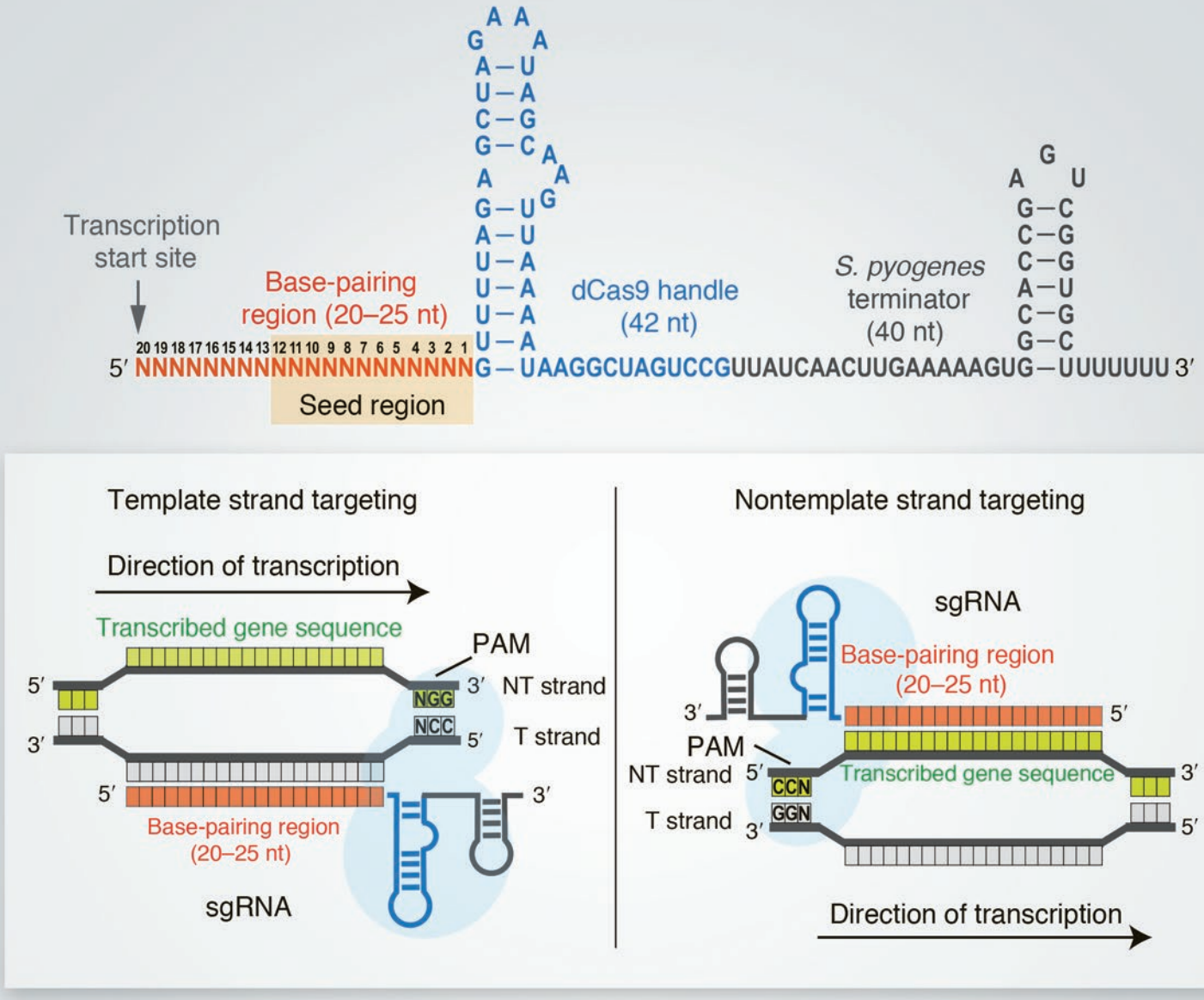

Creating customized sgRNAs for transcriptional repression of any gene.
The customized sgRNA consists of three regions: one for DNA binding (containing a 12-nt seed region), one for Cas9 binding and a transcription terminator hairpin. These sgRNAs can target the template or nontemplate DNA strands.

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Recipes for Researchers

AUGUST 2016

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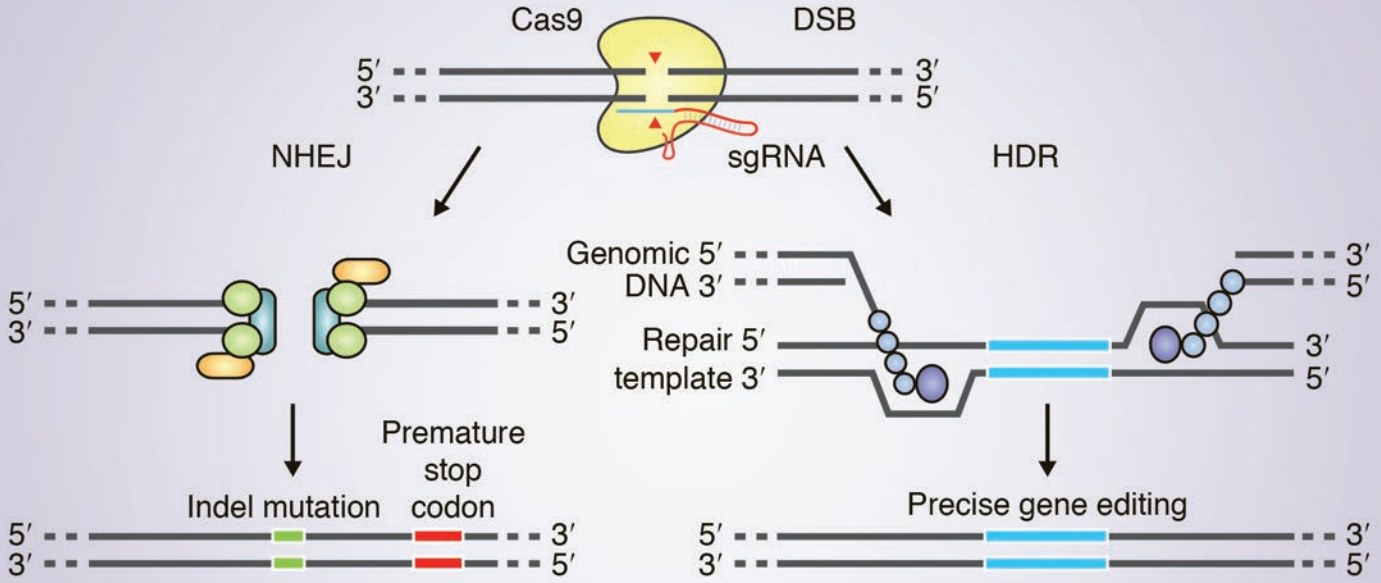

DSB repair promotes gene editing. DSBs induced by Cas9 can be repaired in one of two ways. In the error-prone NHEJ pathway, rejoining of the DSB ends results in random indel mutations. If these occur within the coding region of a gene, frameshifts and the creation of a premature stop codon lead to gene knockout. Alternatively, if a repair template (plasmid or ssODN) is available, the HDR pathway allows high fidelity and precise editing.

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Recipes for Researchers

SEPTEMBER 2016

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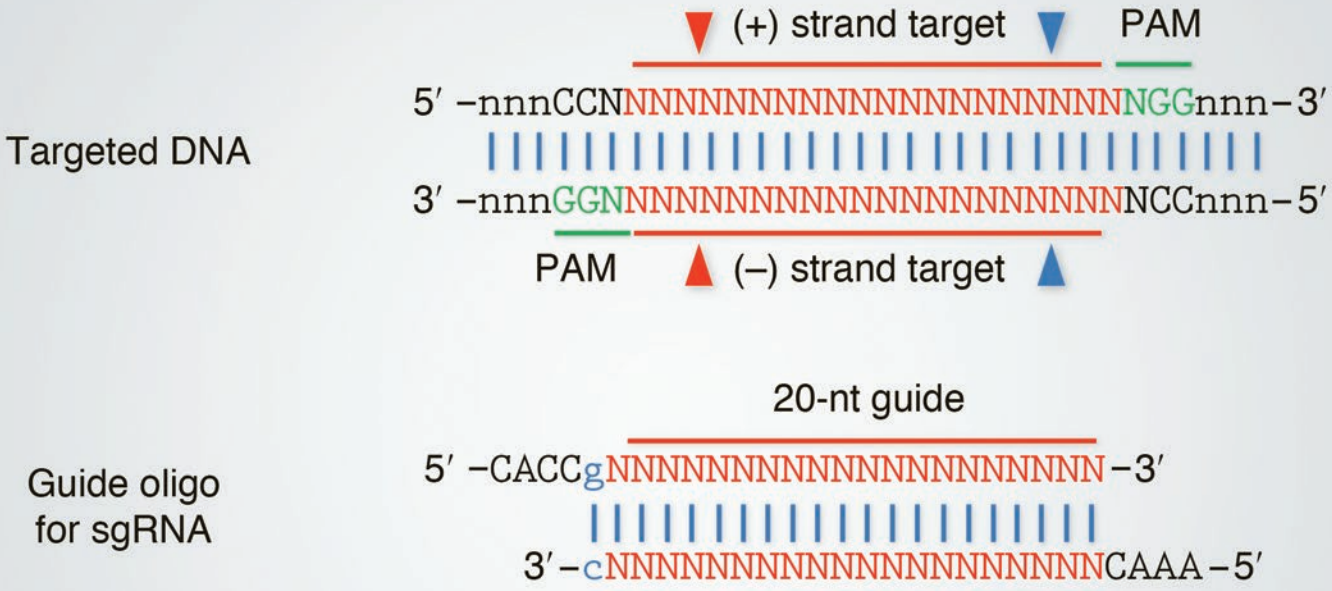

sgRNA construction. Targeted DNA sequence consists of the DNA target (red bar) directly upstream of a requisite 5′-NGG PAM motif (green). Cas9 mediates a DSB ~3 bp upstream of the PAM for the (+) strand (blue triangle) or (–) strand (red triangle). The guide oligonucleotides contain overhangs for ligation into an expression plasmid containing the rest of the sgRNA scaffold and Cas9. A G-C base pair (blue) is added at the 5′ end of the 20-bp guide sequence for T7 transcription.

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Recipes for Researchers

OCTOBER 2016

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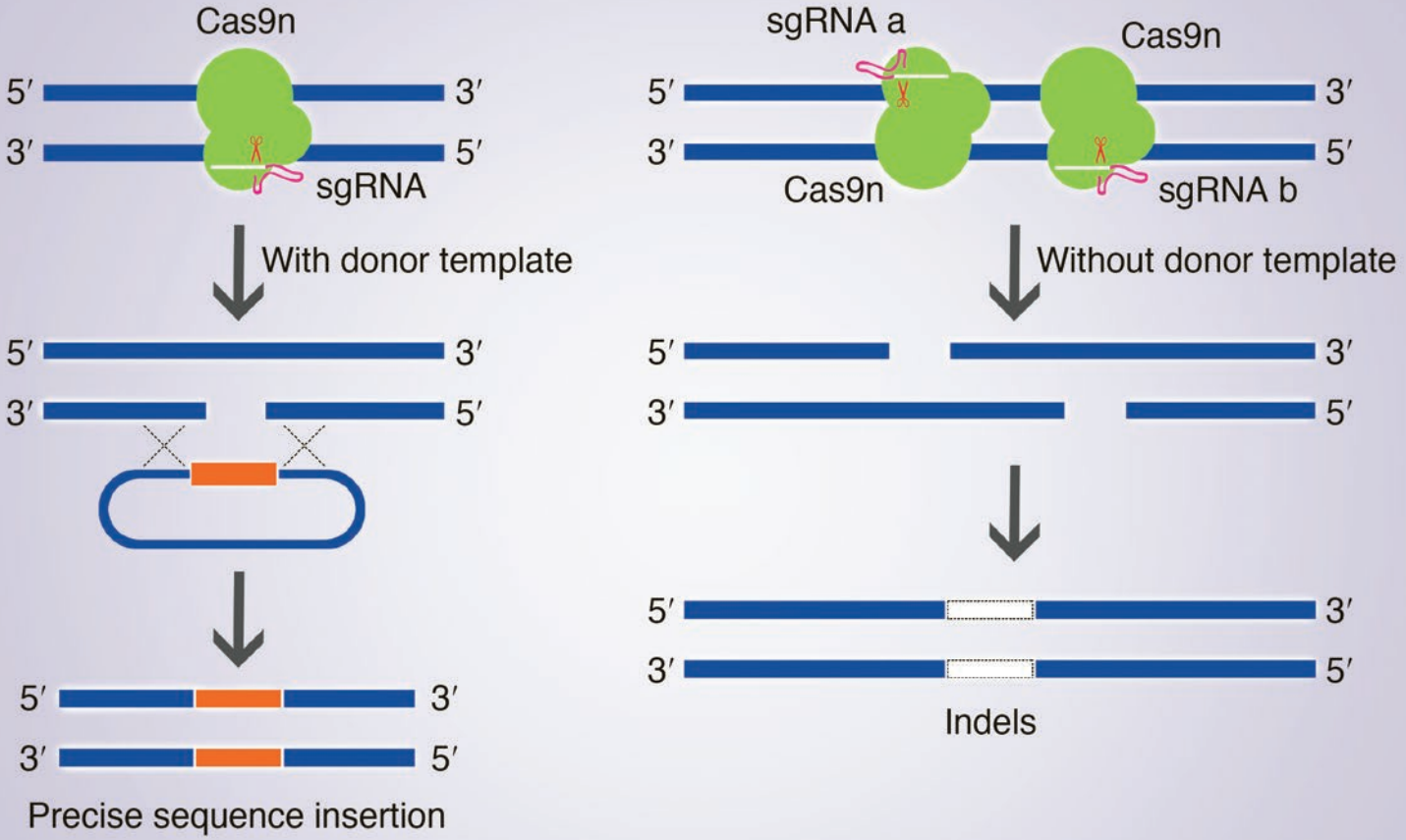

Highly specific gene editing by a Cas9 nickase (Cas9n). Strand-specific nicking (induced by Cas9n and guided by an sgRNA) stimulates HR in the presence of homologous donor templates, leading to precise sequence insertion. If no donor template is present, Cas9ns can induce a DSB with sticky ends, which is repaired by error-prone NHEJ to produce indels.

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Recipes for Researchers

NOVEMBER 2016

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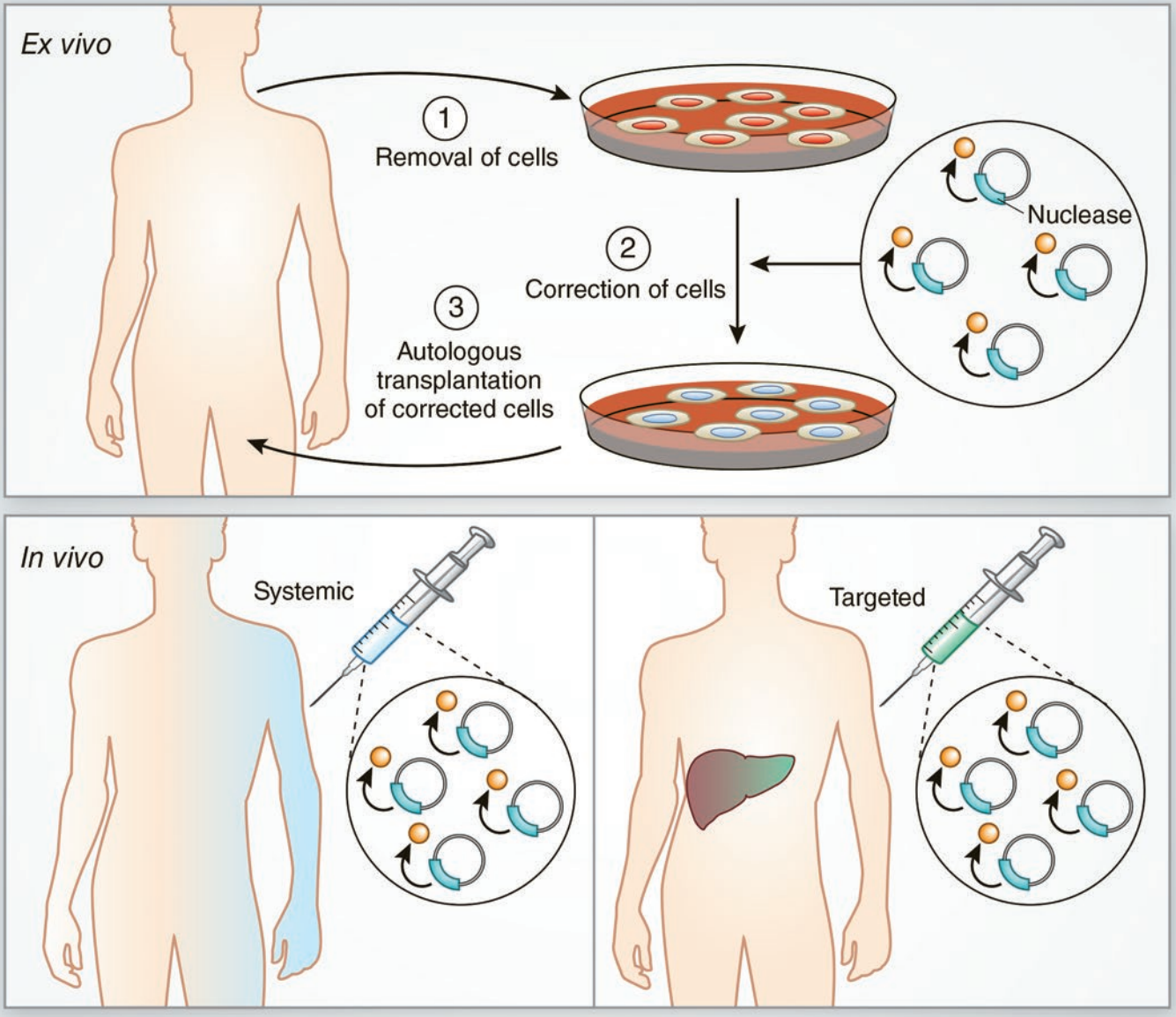

***Ex vivo* versus *in vivo* editing therapy.** Gene-editing therapy can in theory be administered in cells *ex vivo,* or directly *in situ*. The cellular and anatomical nature of the disease will influence the choice of delivery method. For example, *ex vivo* gene editing will be most useful in diseases where patient cells can survive outside the body and, upon re-injection, home to and functionally engraft within target tissues.

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DECEMBER 2016

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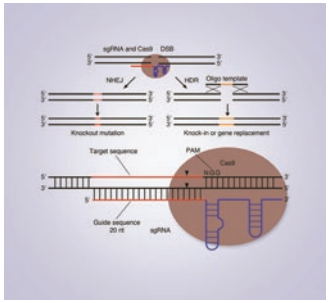
IMAGE DETAILS

JANUARY

RNA-guided genome editing using the CRISPR/Cas system

Adapted from Shan, Q., Wang, Y., Li, J. & Gao, C. Genome editing in rice and wheat using the CRISPR/Cas system. *Nat. Protoc.* **9**, 2395–2410 (2014)

DSBs induced by Cas9 trigger NHEJ (causes imprecise gene knockouts) and HDR (targeted gene knock-in or replacement). The HNH and RuvC-like domains of Cas9 (brown) each cleave one strand of the sequence targeted by the sgRNA (red and blue), provided that the correct PAM sequence is present at the 3′ end.

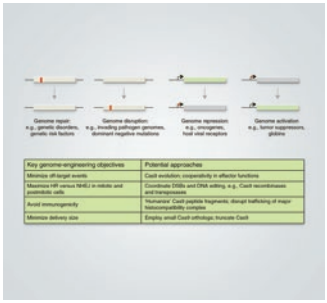


FEBRUARY

Cas9 therapeutics

Adapted from Mali, P., Esvelt, K.M. & Church, G.M. Cas9 as a versatile tool for engineering biology. *Nat. Methods* **10**, 957–963 (2013)

Potential Cas9-mediated therapeutic approaches include targeted genome editing to correct genetic disorders and targeted genome regulation to modify endogenous protein levels.

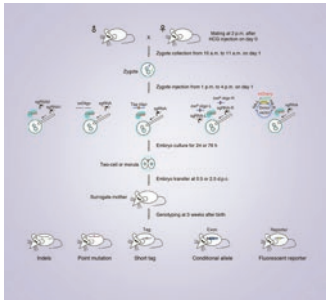


MARCH

Procedure for the generation of gene-modified mice by CRISPR/Cas

Adapted from Yang, H., Wang, H. & Jaenisch, R. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nat. Protoc.* **9**, 1956–1968 (2014)

By injection of Cas9 mRNA along with sgRNAs targeting specific genes—independently or with ssDNA harboring point mutations, tags, *loxP* sites or plasmid DNA (shown left to right)—gene-modified mice can be generated with indels, precise mutations, tags, conditional alleles or fluorescent reporters, respectively.



APRIL

The RNA-guided Cas9 nuclease

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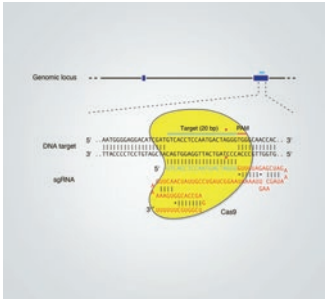
The Cas9 nuclease from *Streptococcus pyogenes* is targeted to genomic DNA by an sgRNA consisting of a 20-nt guide sequence (blue) and a scaffold (red). The guide sequence pairs with the DNA target (blue bar on top strand), directly upstream of a requisite 5′-NGG adjacent motif (PAM; pink). Cas9 mediates a DSB ~3 bp upstream of the PAM (red triangle).

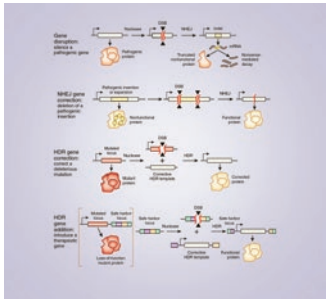


MAY

Types of therapeutic genome modifications

Adapted from Cox, D.B.T., Platt, R.J. & Zhang, F. Therapeutic genome editing: prospects and challenges. *Nat. Med.* **21**, 121–131 (2015)

Genome editing can be used to disrupt or replace disease-causing genes, or to insert a new gene affording a therapeutic benefit. The particular gene-editing approach selected for each therapeutic indication will therefore depend on the exact genetic etiology of the disease.



JUNE

Construction of a rat gene-targeting vector through overlapping PCR

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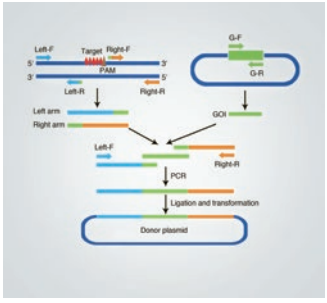
The left and right homology arms are amplified with chimeric primers containing overlapping sequence with the gene of interest (GOI). The GOI is amplified using G-F (GOI-forward) and G-R (GOI-reverse) primers. The donor DNA template is amplified using the left-forward (left-F) and right-reverse (right-R) primers in the presence of the left arm, the right arm and the GOI fragments as templates. The donor DNA template is then inserted into the chosen vector to create the circular donor plasmid.



JULY

Platform for multiplex biological screens

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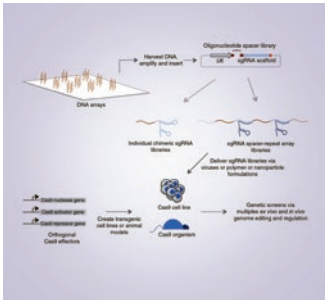
To modulate multiple genomic sites, sgRNA libraries can be generated and delivered into target cells that also express orthogonal Cas9 effectors. This format enables multiplex *ex vivo* and *in vivo* genetic screens.

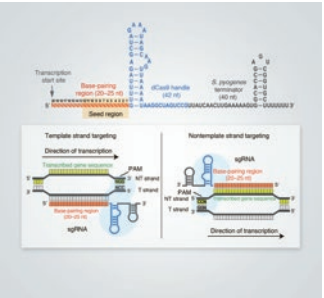


AUGUST

Creating customized sgRNAs for transcriptional repression of any gene

Adapted from Larson, M.H. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nat. Protoc.* **8**, 2180–2196 (2013)

The customized sgRNA consists of three regions: one for DNA binding (containing a 12-nt seed region), one for Cas9 binding and a transcription terminator hairpin. These sgRNAs can target the template or nontemplate DNA strands.



SEPTEMBER

DSB repair promotes gene editing

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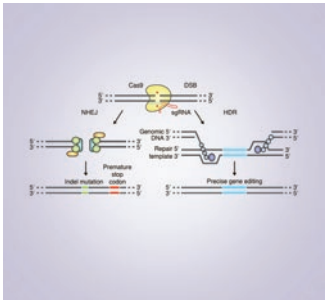
DSBs induced by Cas9 can be repaired in one of two ways. In the error-prone NHEJ pathway, rejoining of the DSB ends results in random indel mutations. If these occur within the coding region of a gene, frameshifts and the creation of a premature stop codon lead to gene knockout. Alternatively, if a repair template (plasmid or ssODN) is available, the HDR pathway allows high fidelity and precise editing.

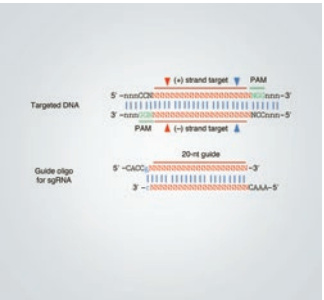


OCTOBER

sgRNA construction

Yang, H., Wang, H. & Jaenisch, R. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nat. Protoc.* **9**, 1956-1968 (2014)

Targeted DNA sequence consists of the DNA target (red bar) directly upstream of a requisite 5′-NGG PAM motif (green). Cas9 mediates a DSB ~3 bp upstream of the PAM for the (+) strand (blue triangle) or (–) strand (red triangle). The guide oligonucleotides contain overhangs for ligation into the restriction enzyme sites, a G-C base pair (blue) added at the 5′ end of the guide sequence for T7 transcription and the 20-bp sequence preceding 5′-NGG in genomic DNA.

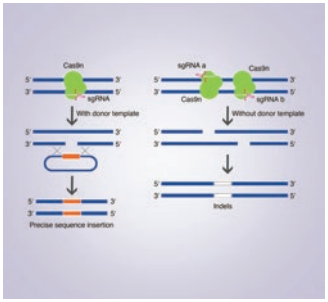


NOVEMBER

Highly specific gene editing by a Cas9 nickase (Cas9n)

Adapted from Shao, Y. *et al.* CRISPR/Cas-mediated genome editing in the rat via direct injection of one-cell embryos. *Nat. Protoc.* **9**, 2493–2512 (2014)

Strand-specific nicking (induced by Cas9n and guided by an sgRNA) stimulates HR in the presence of homologous donor templates, leading to precise sequence insertion. If no donor template is present, Cas9ns can induce a DSB with sticky ends, which is repaired by error-prone NHEJ to produce indels.



DECEMBER

Ex vivo versus *in vivo* editing therapy

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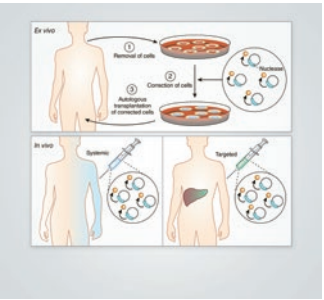

CALENDAR OF EVENTS 2016

JANUARY

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18 Creating a CRISPR Community: The Sanger Institute–AstraZeneca Conference 2016, Wellcome Trust Sanger Institute, Cambridge, UK					23
24	25	26	27	28 Genome Engineering & Synthetic Biology: Tools & Technologies, Ghent, Belgium		
31						

FEBRUARY

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18 Genome Editing & Engineering Congress, San Diego, USA		
21	22	23 CRISPR Precision Gene Editing Congress, Boston, USA			26	27
28	29					

MARCH

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

APRIL

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

MAY

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	2	3	4 American Society of Gene & Cell Therapy annual meeting, Washington, DC, USA			
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

JUNE

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2	3	4
5 Genome Engineering: Cutting-Edge Research and Applications, Lisbon, Portugal				9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26 *Nature Protocols,* 10-year anniversary		28	29	30		

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
JULY						1	2
	3	4	5	6	7	8	9
	10	11	12	13	14	15	16
	17	18	19	20	21	22	23
	24	25	26	27	28	29	30
	31						
AUGUST		1	2	3	4	5	6
	7	8	9	10	11	12	13
	14	15	16	17 Genome Engineering: The CRISPR/Cas Revolution, Cold Spring Harbor, USA			20
	21	22	23	24	25	26	27
	28	29	30	31			
SEPTEMBER					1	2	3
	4	5	6	7	8	9	10
	11	12	13	14	15	16	17
	18	19	20	21	22	23	24
	25	26	27	28	29	30	
OCTOBER							1
	2	3	4	5	6	7	8
	9	10	11	12	13	14	15
	16	17	18	19	20	21	22
	23	24	25	26	27	28	29
	30	31					
NOVEMBER			1	2	3	4	5
	6	7	8	9	10	11	12
	13	14	15	16	17	18	19
	20	21	22	23	24	25	26
	27	28	29	30			
DECEMBER					1	2	3 American Society for
	Cell Biology annual meeting, San Francisco, USA		6	7	8	9	10
	11	12	13	14	15	16	17
	18	19	20	21	22	23	24
	25	26	27	28	29	30	31

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