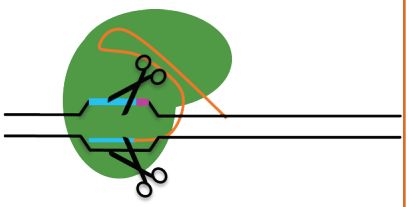
addgene

A better way to share plasmids



Getting Started with CRISPR

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) genome editing is a popular technology that uses a short RNA (gRNA) to recruit a nuclease (typically Cas9) to target a DNA sequence. Addgene has assembled a collection of CRISPR resources and plasmids from top published papers and labs.

Get started planning your CRISPR experiment:

1. Choose a CRISPR system:

- Addgene's CRISPR collection includes Cas9 expression plasmids for:
  - ▶ Knocking-out a gene
  - ▶ Making site-specific mutations
  - ▶ And more!
- Addgene's collection is curated by model organism and function.

2. Design a gRNA for your genome target:

- Genome target should be ~20 nucleotides in length and followed (or preceded) by the appropriate Protospacer Adjacent Motif (PAM) sequence. The PAM will vary based on the bacterial species the Cas9 was derived from.
- gRNA considerations:
  Your gRNA should not contain the PAM sequence, can be on either strand of the genomic DNA, and should be designed using bioinformatics software to minimize off-target effects. Check out www.addgene.org/crispr/ to browse validated gRNAs, software tools to help you choose/design target sequences, and more.

3. Clone the gRNA into a plasmid:

- *Note:* If you are using one of our validated gRNA plasmids, you can skip this step.
- Depositor plasmids may have specific cloning guides in their protocols.
  Visit Addgene's molecular cloning guide for a general overview of cloning: www.addgene.org/plasmid-reference/
  After cloning, sequence verify your final plasmid product.

4. Deliver your CRISPR components:

- Each model system has its own best practices for delivery of CRISPR components. The table to the right provides an overview of common mammalian DNA delivery methods.

5. Evaluate the outcome:

- If CRISPR is being used for genome modification, the modification has to be evaluated after delivery of CRISPR components
- Design PCR primers and amplify the genomic region containing the modification
  There are software tools available on the web to help you design your PCR primers. More details can be found on www.addgene.org/crispr/

Delivery Method:	Transformed Cell Lines (HeLa, HEK 293)	Stem Cells (hES, iPS)	Primary Cells (fibroblasts, epithelial cells)
Transfection • lipid-mediated • cationic polymers • calcium phosphate	+		
Electroporation • nucleofection	+	+	
Viral delivery • lentivirus • retrovirus • adenovirus	+		+

* Promotion code expires on April 30, 2015 may only be used once, and cannot be combined with any other promotion codes
Plasmids are $65USD each, promotion value equal to $20USD

www.addgene.org/CRISPR

Addgene is a nonprofit plasmid repository that stores, archives, and distributes plasmids to academic scientists around the world

For questions about plasmids, shipping, or ordering, please contact help@addgene.org.

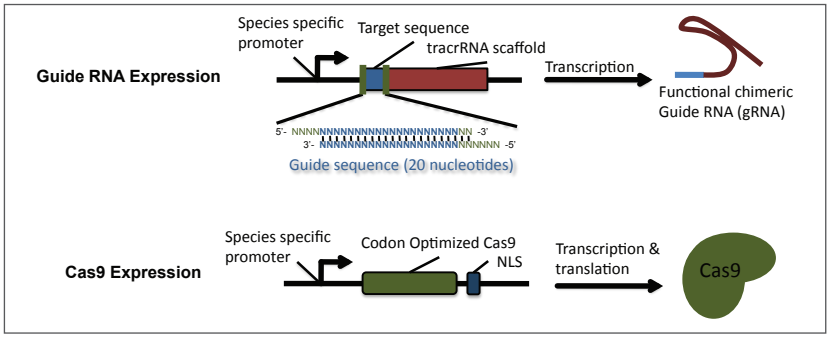
Addgene
75 Sidney St, Suite 550A
Cambridge, MA 02139
USA
Tel: +1.617.225.9000

Addgene's CRISPR Collection

Function	Vector Type	Sample Plasmids	Gene/Insert	Promoter	Depositing Scientist
Cut	Mammalian	pMJ920 (cat# 42234)	Cas9 (Synthetic)	CMV	Jennifer Doudna
		lentiCas9-Blast (cat# 52962)	Cas9 (Synthetic), Blasticidin resistance	EFS-NS	Feng Zhang
	Bacteria	pCas9 (cat# 42876)	tracr/Cas9		Luciano Marraffini
	Drosophila	pAc-sgRNA-Cas9 (cat# 49330)	Cas9 (Synthetic), dU6-sgRNA (D. melanogaster)	Actin-5c, Drosophila U6	Ji-Long Liu
	C. elegans	pDD162 (Peft-3::Cas9 + Empty sgRNA) (cat# 47549)	Cas9 (Synthetic), Empty sgRNA	eft-3, U6	Bob Goldstein
	Plant	pK7WGF2::hCas9 (cat# 46965)	hCas9 (Synthetic)	35S	Sophien Kamoun
	Zebrafish	MLM3613 (cat# 42251)	Streptococcus pyogenes Cas9	CMV	Keith Joung
Nick	Mammalian	hCas9_D10A (cat# 41816)	Cas9_D10A	CMV	George Church
	Drosophila	pDCC5 (cat# 59984)	Cas9-D10A (Synthetic)	hsp70Bb	Peter Duchek
	Plant	pBUN501 (cat# 50582)	zCas9D10A (Synthetic), gRNA scaffold (Synthetic)	Ubi1p, AtU6-26p	Qi-Jun Chen
Interfere	Mammalian	pdCas9-humanized (cat# 44246)	dead Cas9 with 3X NLS (Homo sapiens)	MSCV LTR promoter	Stanley Qi
	Bacteria	pdCas9 (cat# 46569)	Bacterial Expression, CRISPR		Luciano Marraffini
	Yeast	pTDH3-dCas9 (cat# 46920)	dCas9	TDH3	Qi-Jun Chen
Activate	Mammalian	pAC154-dual-dCas9VP160-sgExpression (cat# 48240)	dCas9 (Synthetic)	MSCV LTR promoter	Rudolf Jaenisch
	Bacteria	pWJ66 (cat# 46570)	tracrRNA, dcas9-w, CRISPR array		Luciano Marraffini
	Yeast	pTPGI_dCas9_VP64 (cat# 49013)	dCas9_VP64 (codon-optimized for S. cerevisiae)	pTPGI (galactose+aTc inducible)	Timothy Lu
Label Genomic Loci	Mammalian	pHR-SFFV-dCas9-BFP (cat# 46910)	dCas9-BFP fusion (Homo sapiens)	SFFV	Jonathan Weissman
DNA Purification	Mammalian	3xFLAG-dCas9/pCMV-7.1 (cat# 47948)	3xFLAG-dCas9	CMV	Hodaka Fujii
dCas9-FokI Double-strand Break (Cut)		pSQT1601 (cat# 53369)	hCsy4-T2A-NLS-hFokI-dCas9-NLS	CAG	Keith Joung
Empty gRNA Expression Vectors		gRNA_Cloning Vector (cat# 41824)	hCsy4-T2A-NLS-hFokI-dCas9-NLS	hU6	George Church
Validated gRNA Plasmids		PU6::unc-119_sgRNA (cat# 46169)	unc-119 targeting sgRNA (Synthetic)		John Calarco

CRISPR Libraries Available Through Addgene	Description	Depositing Scientist(s)
SAM Activation Library	The synergistic activation mediator (SAM) library consists of 3 unique sgRNAs targeting each human RefSeq coding isoform (human SAM library includes library, dCas9-VP64 & MS2-P65-HSF1 plasmids)	Feng Zhang
GeCKO Lentiviral CRISPR Toolbox	Mouse and Human libraries available in a 1 vector (lentiCRISPRv2) or 2 vector (lenticas9-blast and lentiGuide-Puro) format.	Feng Zhang
Human Lentiviral sgRNA Libraries	A total of 73,151 sgRNA plasmids that cover a total of 7,114 human genes and include 100 non-targeting controls. The collection has been separated into 6 enriched sub-pools.	David Sabatini, Eric Lander
Genome-wide Mouse Lentiviral CRISPR gRNA Library	A total of 87,897 gRNA plasmids targeting 19,150 mouse protein coding regions designed for lentiviral expression in mouse cells.	Kosuke Yusa

The gRNA will direct the Cas9 to the target sequence in the genome and Cas9 will cut the DNA or perform another activity, depending on your selection of Cas9.



To view Addgene's entire CRISPR Collection, visit **www.addgene.org/CRISPR**

For questions about plasmids, shipping, or ordering, please contact help@addgene.org.

Addgene is a nonprofit plasmid repository that stores, archives, and distributes plasmids to academic scientists around the world.