Gene editing with CRISPR

What is CRISPR and why is it important?

Background

Practical example (DIY CRISPR kit)

CHARLOTTESVILLE
Open Bio Labs

Charlottesville Open Bio Labs - Charlottesville, VA

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Charlottesville, VA
Founded Nov 2, 2014

About us...

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Help support your Meetup

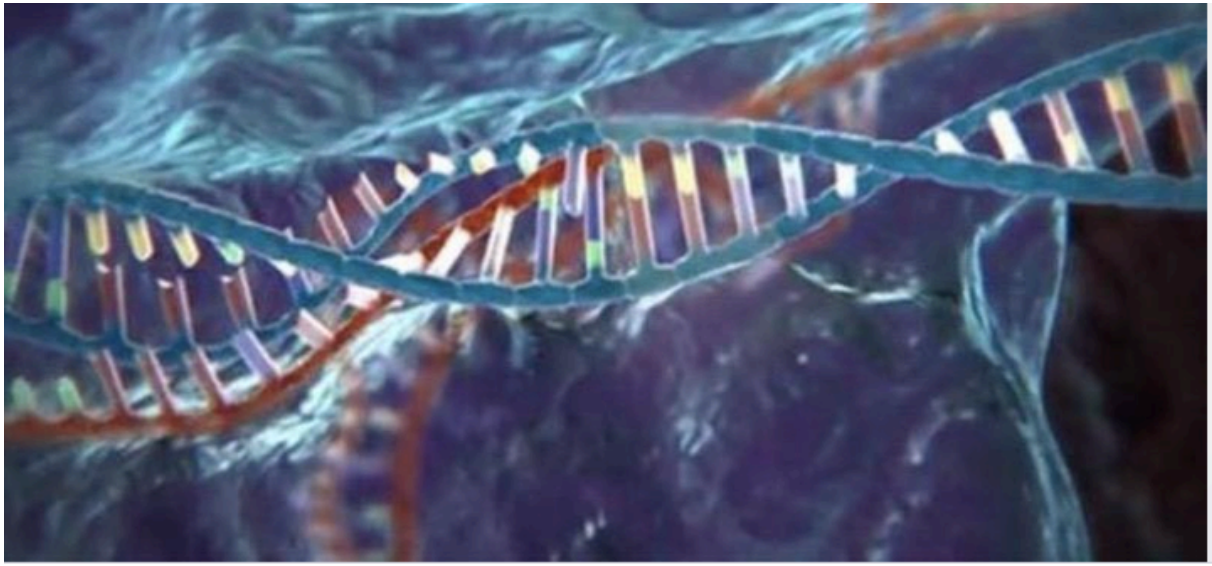
Chip in

CRISPR/Cas9 Experimentation!

May 31 · 6:30 PM
Open Bio Labs

This meetup we will be experimenting with our CRISPR/Cas9 kit! Stop by if you want to see this amazing technology in action. Feel free to work on a different project if you are so inclined. We have made huge progress this week with Kombucha paper, and have begun producing it on a large scale. We are also in the midst of other projects including our "exohatchlings", moss graffiti, and green wall. Come by and check it out!

If you want to join our slack.com group, enter you information in this form!



JUN
26

Genome Editing with CRISPR-Cas9

by Genspace NYC

$400

TICKETS

DESCRIPTION

Four-Session Class:

June 26,27,28 and July 5 - 7:00 PM TO 10:00 PM

Want to learn how to do hands-on genome editing? This is an intensive laboratory class with limited space. Class size is limited to ten students.

DATE AND TIME

Mon, June 26, 2017
7:00 PM – 10:00 PM EDT
Add to Calendar

LOCATION

Genspace
33 Flatbush Avenue
Brooklyn, NY 11217
View Map

RTP AgBio + AgTech

Home Members Sponsors Photos Pages Discussions More

Durham, NC
Founded Jan 31, 2017

About us...

Invite friends

Members	175
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Our calendar	

Organizers:

Angel Turner, Bettina Darveaux, Steve Screen

Design in Agriculture - Transformational Technologies

Print ticket Export Tell a friend Share

Tuesday, June 20, 2017
4:00 PM to 5:15 PM

The Frontier
800 Park Office Drive, RTP, NC (map)

This meeting will be held in **'The Classroom' at the Frontier**. Lots of space, bring your friends. The event will highlight two transformational technologies that have the potential to transform Plant and Animal Agriculture, Food production and Manufacturing. **Gene-editing - "the impact of the CRISPR craze on AgBio" will be presented by Rodolphe Barrangou** and **3D printing - "Patterning and Actuating Functional Materials" will be presented by Michael Dickey** - both are elite scientists from NC State.

CRISPR

CRISPR = **C**lustered **R**egularly-**I**nterspaced **S**hort **P**alindromic **R**epeats

Bacterial defense mechanism against bacteriophages
Bacteriophages: viruses that infect bacteria
Adaptive/acquired immunity in bacteria

Can be used for *targeted gene editing* in many eukaryotic species

Main elements: Cas9 (CRISPR associated protein from *Streptococcus pyogenes*) is an endonuclease that makes double stranded cuts to DNA

gRNA: guide RNA = sequence used by Cas9 for targeting (tracrRNA + crRNA)

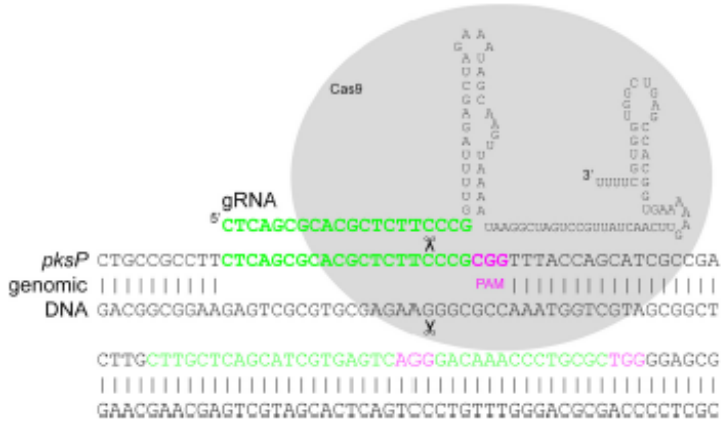
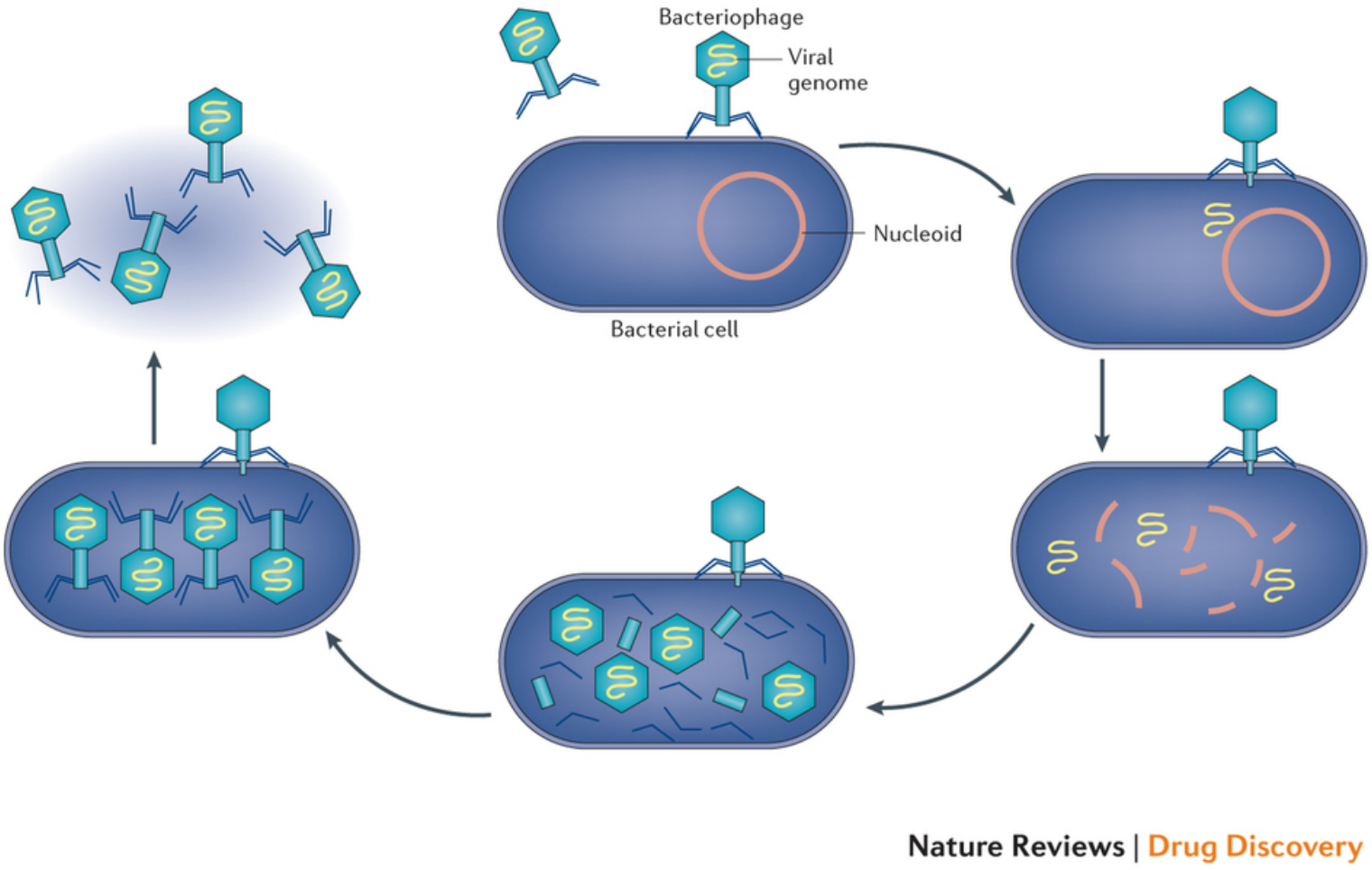


FIG 1 Diagram of the *pksP*-targeting Cas9/gRNA complex. The illustration shows the *pksP*-specific guide RNA (gRNA) used in this study associating with the Cas9 endonuclease. The locations of the protospacer-adjacent motif (PAM), Cas9 double-strand cut site (3 to 4 nucleotides upstream of the PAM site), and other candidate gRNAs within the first exon are shown (green).

Lytic bacteriophage life cycle



Nature Reviews Drug Discovery 14, 515–516 (2015)

Restriction Enzymes

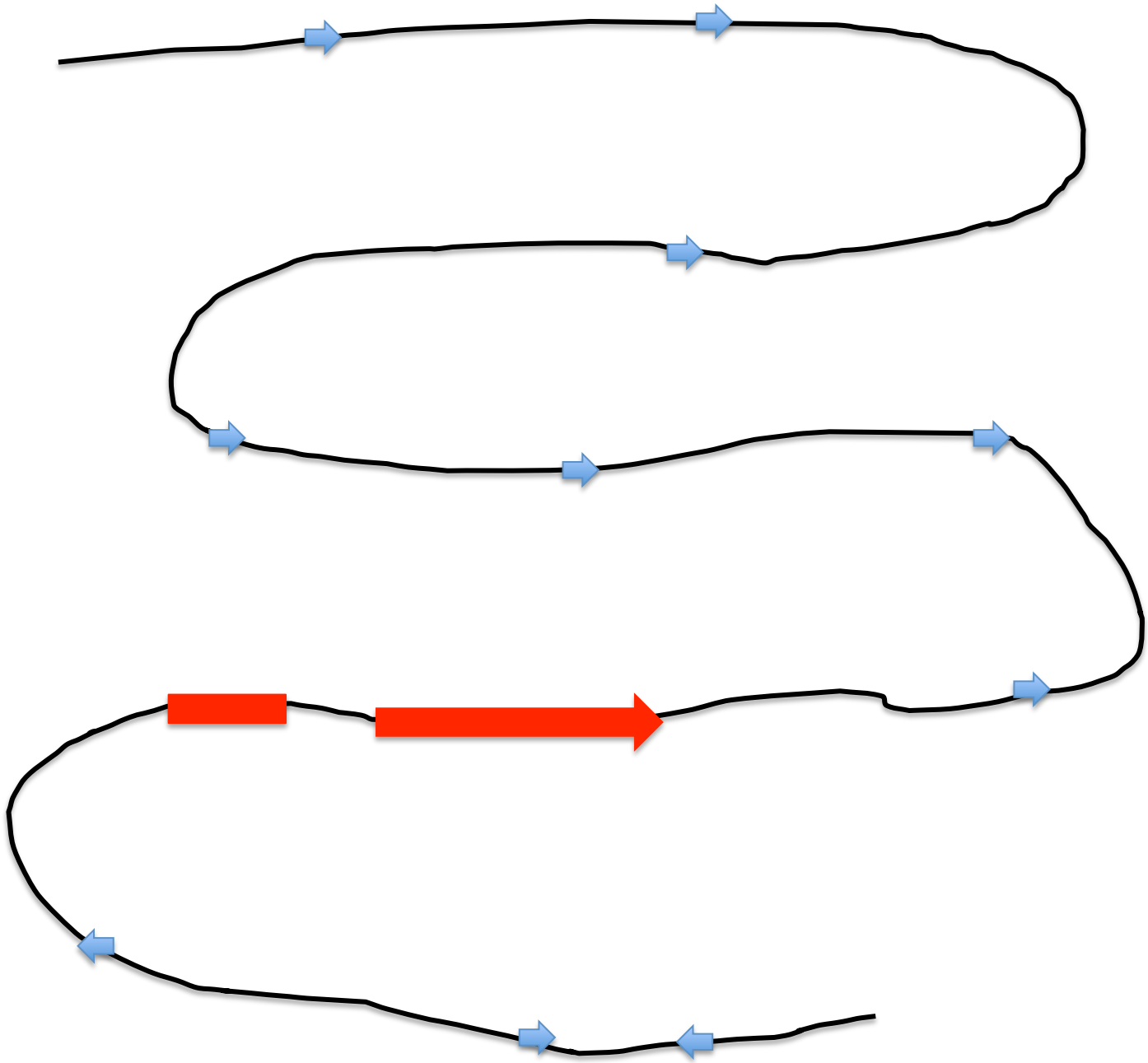
Basis for gene cloning/splicing for the last 40 yrs

Also a bacterial defense mechanism against bacteriophage

Endonucleases – cut double stranded DNA (dsDNA)

Specific recognition sequences

Below – *Eco*RI (***E****scherichia* ***co****li* **R**estriction enzyme **I**)



The Nobel Prize in Physiology or Medicine 2007

Mario R. Capecchi, Sir Martin J. Evans, Oliver Smithies

Oliver Smithies - Facts

Targeted gene knockouts in mice

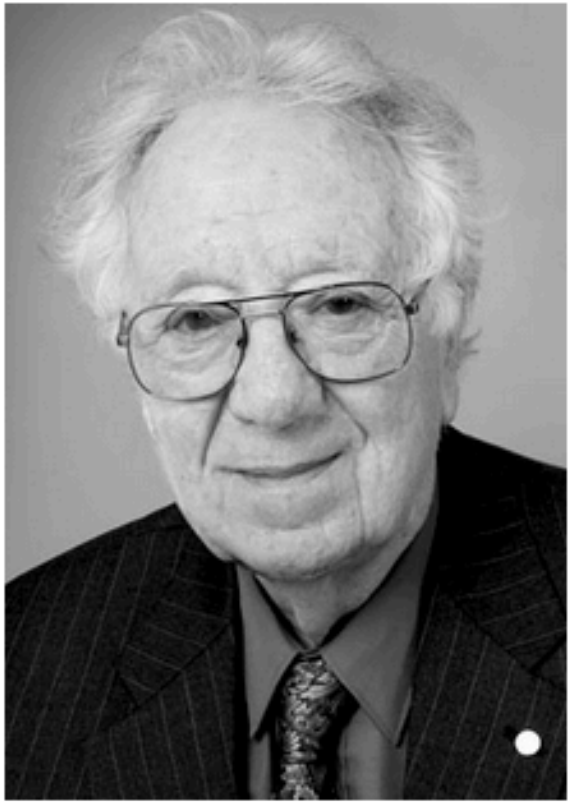


Photo: U. Montan

Oliver Smithies

Born: 23 June 1925, Halifax, United Kingdom

Died: 10 January 2017, Chapel Hill, NC, USA

Affiliation at the time of the award: University of North Carolina, Chapel Hill, NC, USA

Prize motivation: "for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells"

Field: genetics

Prize share: 1/3

https://www.nobelprize.org/nobel_prizes/medicine/laureates/2007/smithies-facts.html

CRISPR

CRISPR = **C**lustered **R**egularly-**I**nterspaced **S**hort **P**alindromic **R**epeats

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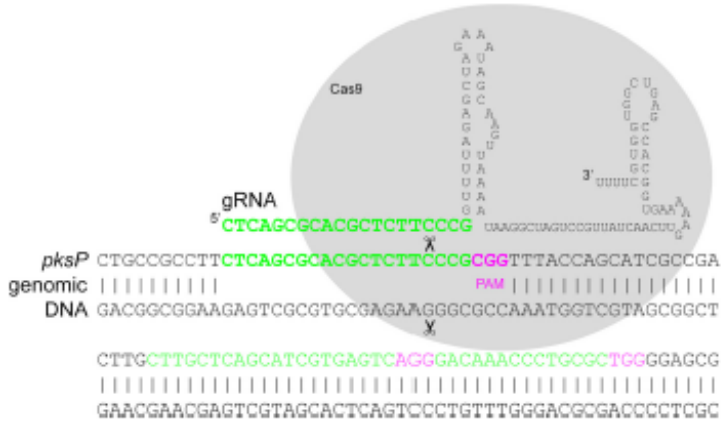

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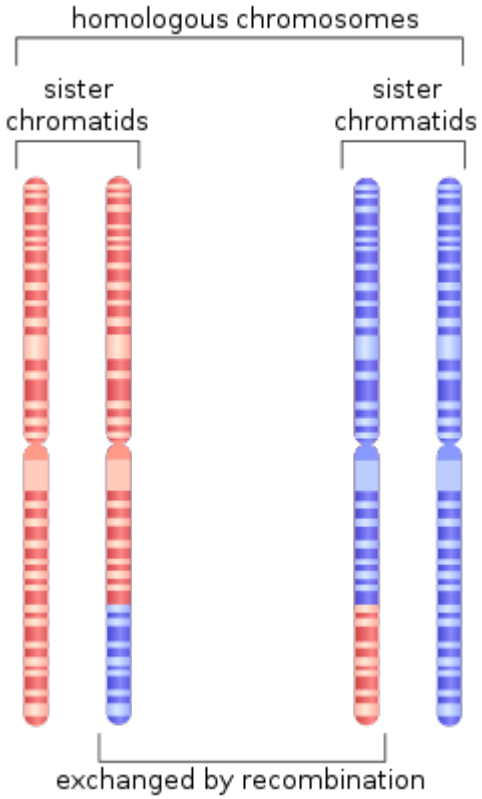
Homologous recombination

Reciprocal exchange of DNA between identical/near identical DNA sequences
Critically important in meiosis
Meiosis *IS* the fundamentally central event in sexual reproduction in *ALL* eukaryotes
yeast, birds, bees, humans
Generates diverse combinations of genes in all progeny



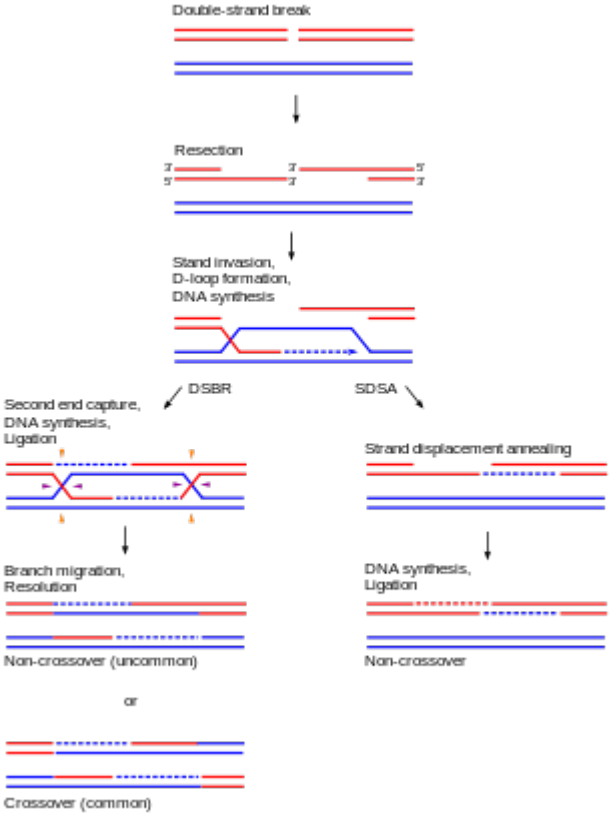
Also involved in DNA repair (good)
Genome re-arrangements (bad)

Very tightly regulated

Eukaryotes are full of repetitive sequence
Non gene coding sequence
Junk DNA – or not

Sequences that are repeated a lot
Humans are >50% repetitive
Many plants are >70 %

Homologous recombination



Single crossover

AGCTGTTGTACGGTACGGTCAGATCGATCTCGTCCCTAAA
TCGACAACATGCCATGCCAGTCTAGCTAGAGCAGGGATTT

X

AGCTGTCGTACGGTACGGTCAGATCGATCTCGTCACTAAA
TCGACAGCATGCCATGCCAGTCTAGCTAGAGCAGTGATTT

AGCTGTTGTACGGTACGGTCAGATCGATCTCGTCACTAAA
TCGACAACATGCCATGCCAGTCTAGCTAGAGCAGTGATTT

AGCTGTCGTACGGTACGGTCAGATCGATCTCGTCCCTAAA
TCGACAGCATGCCATGCCAGTCTAGCTAGAGCAGGGATTT

Homologous recombination

Single crossover

AGCTGTTGTACGGTACGGTCAGATCGATCTCGTCCCTAAA
TCGACAACATGCCATGCCAGTCTAGCTAGAGCAGGGATTT

X

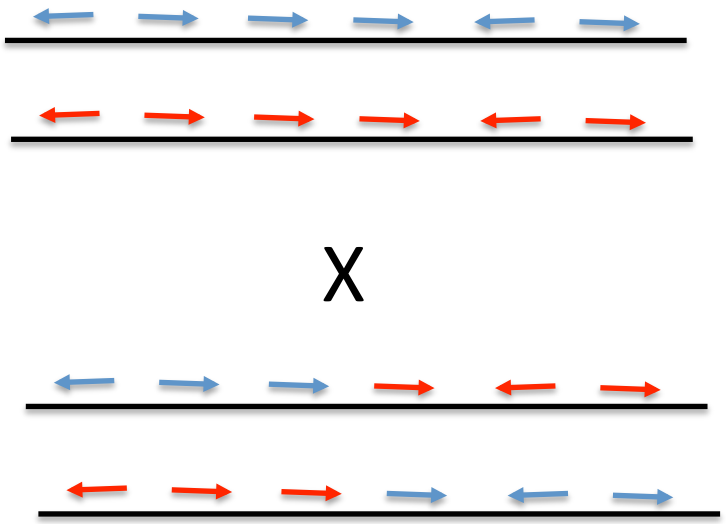
AGCTGTCGTACGGTACGGTCAGATCGATCTCGTCACTAAA
TCGACAGCATGCCATGCCAGTCTAGCTAGAGCAGTGATTT

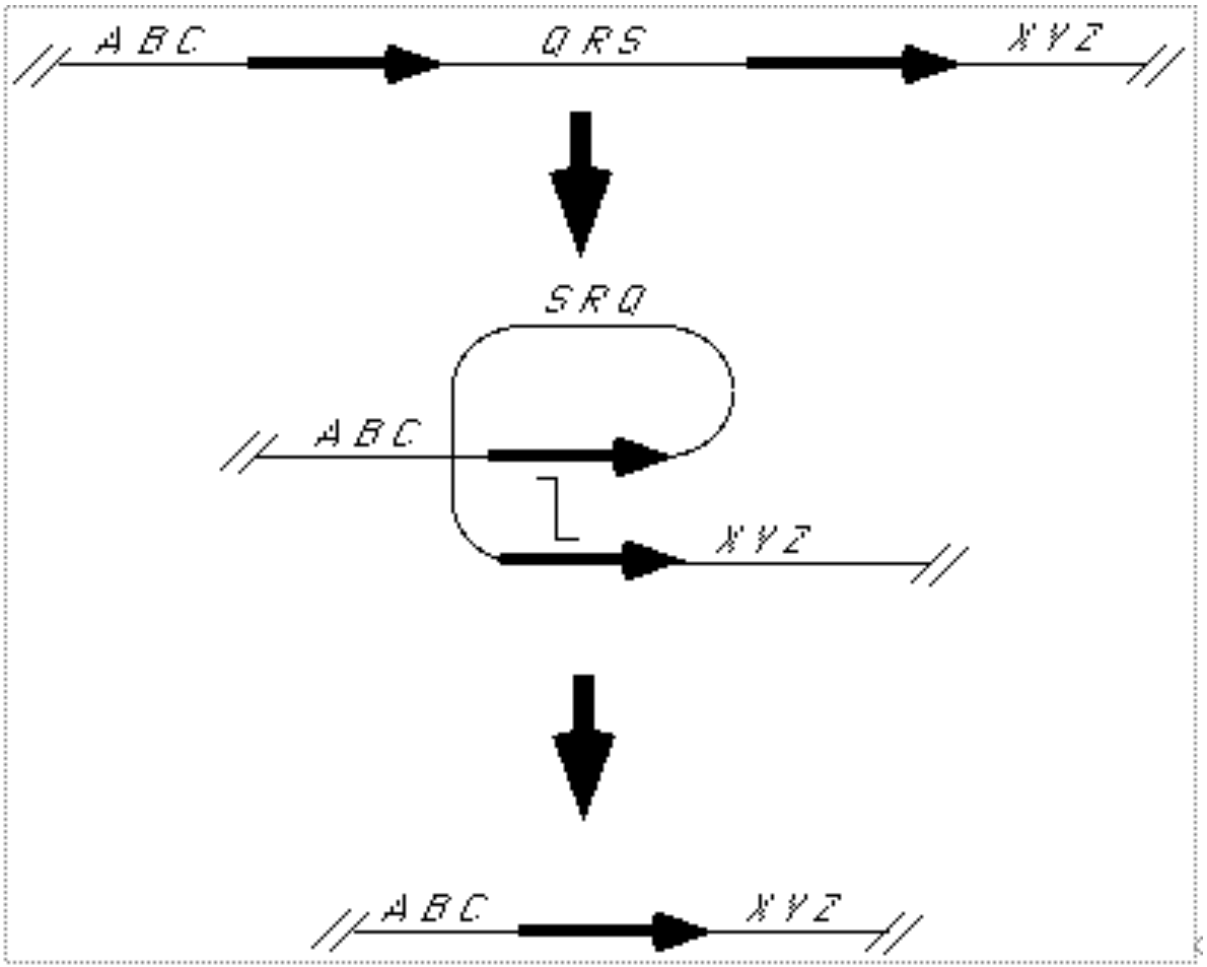
↓

AGCTGTTGTACGGTACGGTCAGATCGATCTCGTCACTAAA
TCGACAACATGCCATGCCAGTCTAGCTAGAGCAGTGATTT

AGCTGTCGTACGGTACGGTCAGATCGATCTCGTCCCTAAA
TCGACAGCATGCCATGCCAGTCTAGCTAGAGCAGGGATTT

Single crossover




ABC
QRS
XYZ
SRQ
ABC
XYZ
ABC
XYZ

REVIEW | OPEN ACCESS

Diversity of CRISPR-Cas immune systems and molecular machines

Rodolphe Barrangou

Genome Biology 2015 16:247 | DOI: 10.1186/s13059-015-0816-9

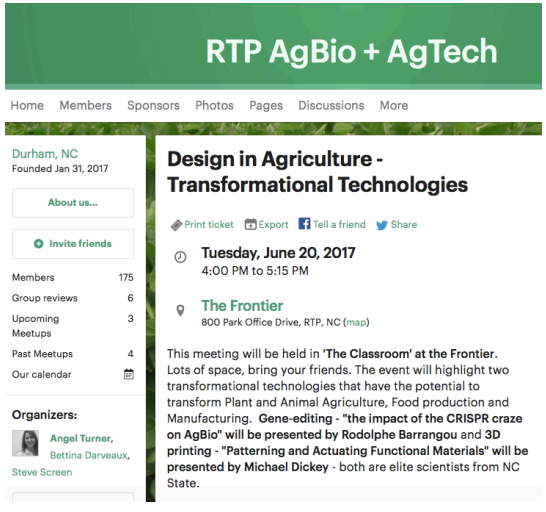
Published: 9 November 2015

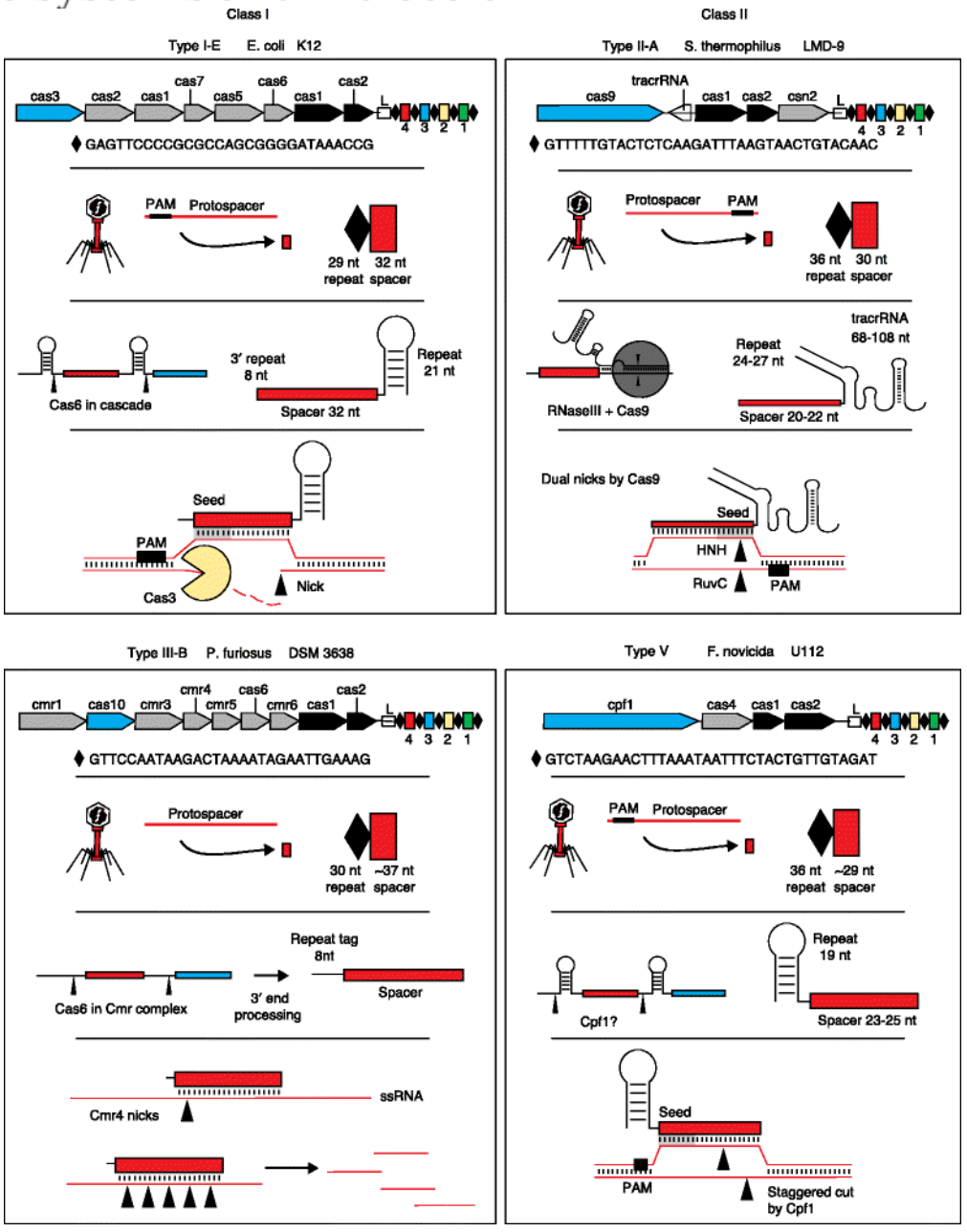
Acknowledgments

The author is supported by start-up funds from North Carolina State University, and the North Carolina Biotechnology Center.

Radio In Vivo talk by Barrangou:

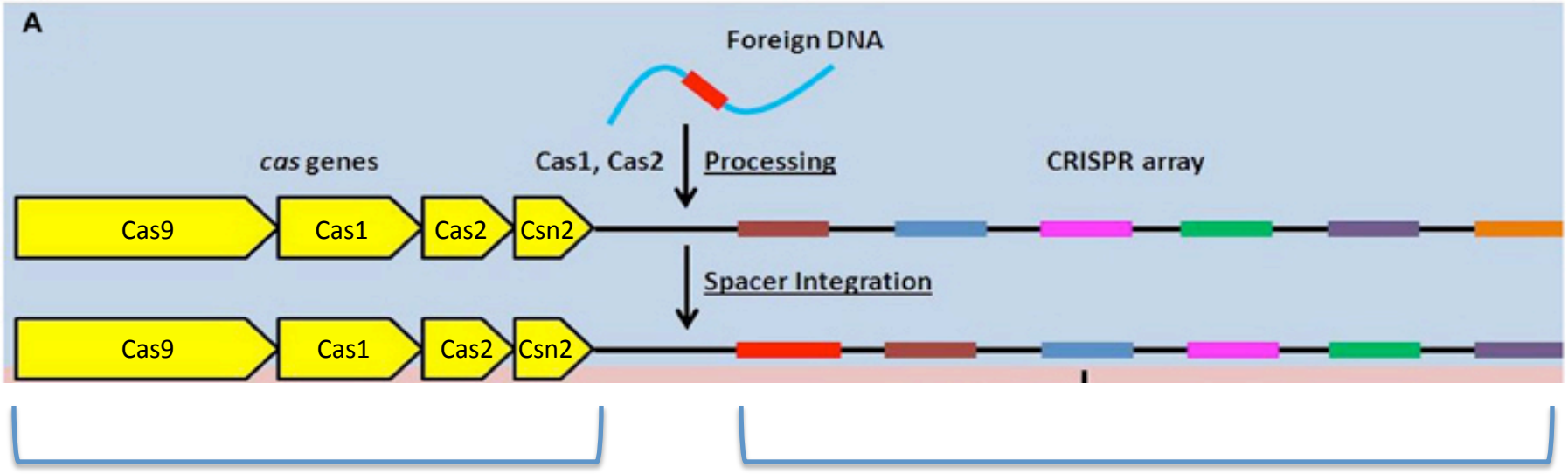
https://radioinvivo.org/2016/05/





Class 2 CRISPR array

Streptococcus pyogenes



CRISPR associated genes

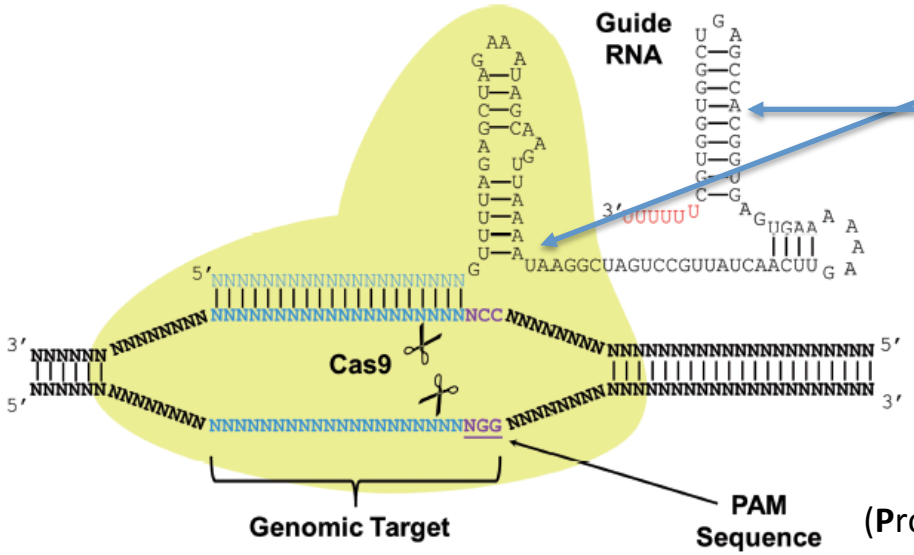
CRISPR Array
repeats – black lines
DNA as memory

Both repeats and spacers are 30-60 bp

CAS9 – endonuclease cuts dsDNA
CAS1, CAS2, CSN2 – recognize/process foreign DNA

Foreign DNA recognized as it is replicating faster, more nicks/breaks present

Figure adapted from Sampson and Weiss, Cell. Infect. Microbiol. 4:1



Guide RNA composed of
crRNA (from CRISPR array)
tracrRNA (trans-acting crRNA)

4336–4343 Nucleic Acids Research, 2013, Vol. 41, No. 7 Published online 4 March 2013
doi:10.1093/nar/gkt135

Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems

James E. DiCarlo[1,2], Julie E. Norville[2], Prashant Mali[2], Xavier Rios[2], John Aach[2] and George M. Church[2,*]

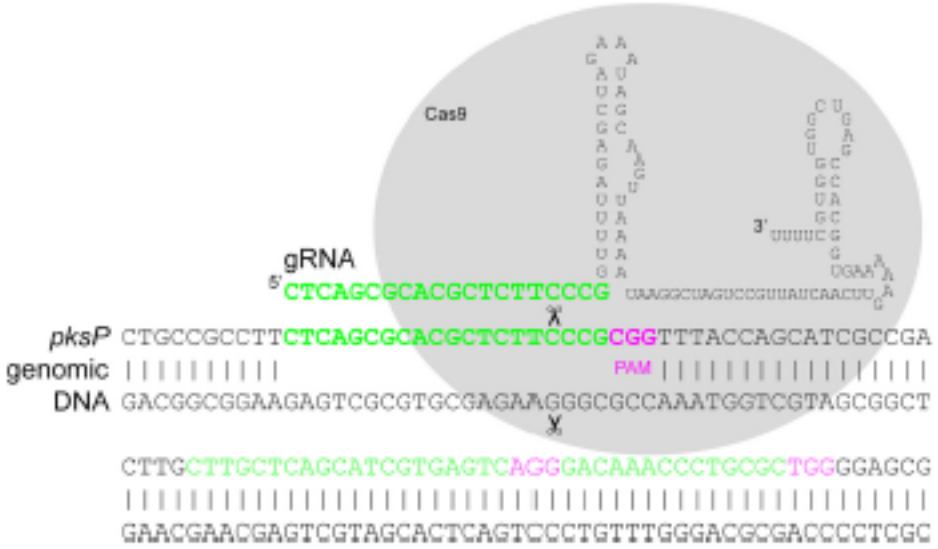
(**P**rotospacer **A**djacent **M**otif = NGG)

nature biotechnology

RNA-guided editing of bacterial genomes using CRISPR-Cas systems

Wenyan Jiang[1,4], David Bikard[1,4], David Cox[2,3], Feng Zhang[2,3] & Luciano A Marraffini[1]

Nature Biotechnology 31: 234



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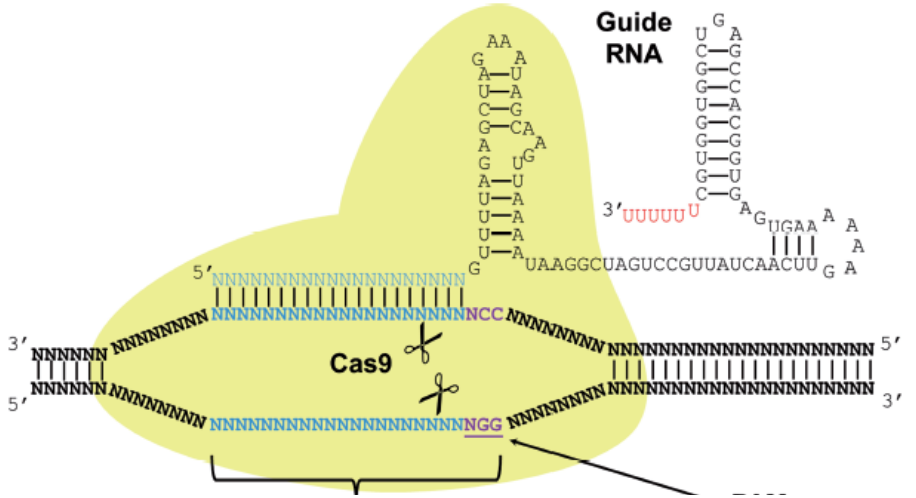
ds DNA break generated
triggers DNA repair machinery
no repair template
messy repair (NHEJ)



D

gRNA CAN1.Y Genomic Target (PAM)

	5′ → 3′
Reference	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGG--AGGATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
CAN1.Y Cas9 Population	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGG---GGATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGATACGTTCTCT---------ATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGGAA-GGATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGGAAAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGATACGTTCTCT---------ATGGCATAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGAT----------TAGGTGATGAAGATGAAGGAGAAGTAC
	CCGACGAGAGTAAATGGCGAGGATACGTTCTCTATGG-AAGGATGGCATAGGTGATGAAGATGAAGGAGAAGTAC

gRNA CAN1.Z Genomic Target

	5′ → 3′
Reference	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATG--AGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
CAN1.Z Cas9 Population	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATG--AGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATG--A-CCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAAT---AGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAAAGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAAAGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGT----------ACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAATG--AGCCGGTCACAACCCTCTTTCACGACGTTGAAGCT
	AGAAGACGCCGACATAGAGGAGAAGCATATGTACAAT------CCTCTTTCACGACGTTGAAGCT

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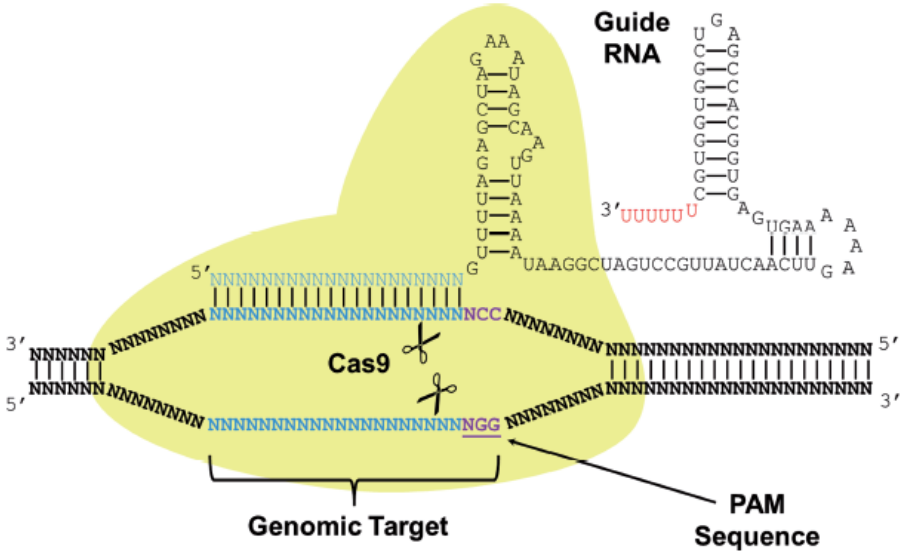
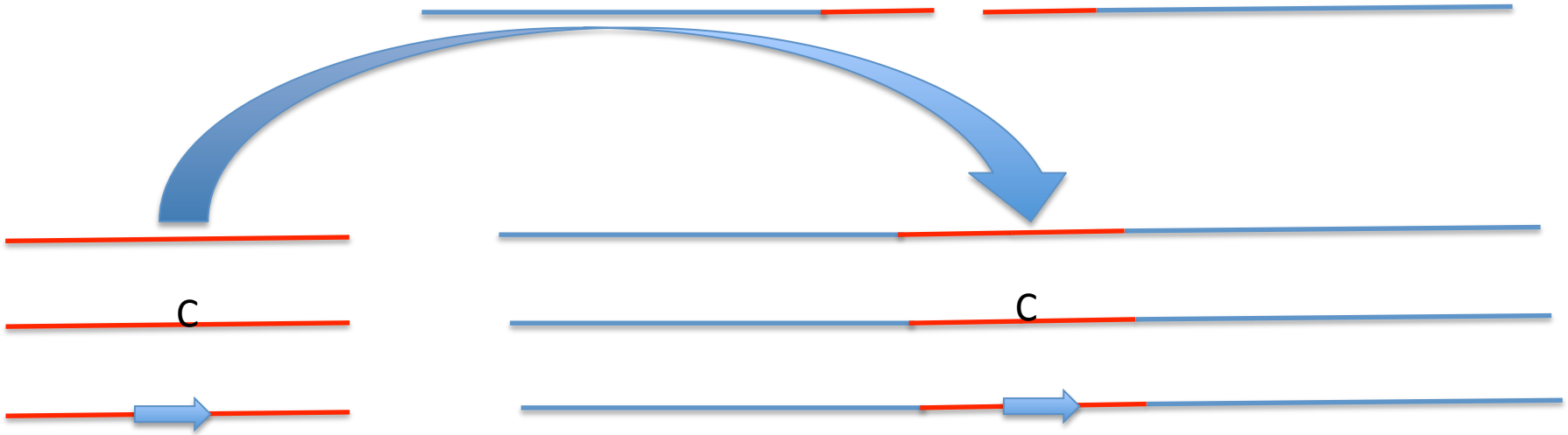
If you supply an exogenous homologous template then a clean homologous repair pathway will become involved clean(er) repair





Off target effects of CRISPR

GAATTC – occurs once every 4^6 = 4096 base pairs

E. coli genome ~ 4.3 Mb (4,300,000) bp

GAATTC occurs ~1050 times

20 bp sequence occurs once every 4^{20} = 1,000,000,000 bp (1 Gb)

For mammals, this is an issue human genome is 3 Gb

Also, there is mismatch tolerance in a homology search

TGTGACTGATATCGTCAGAT vs TGTGACTGA**A**ATCGTCAGAT

Options:
Higher fidelity enzymes – from different species or re-engineering of existing enzymes
More specific gRNAs

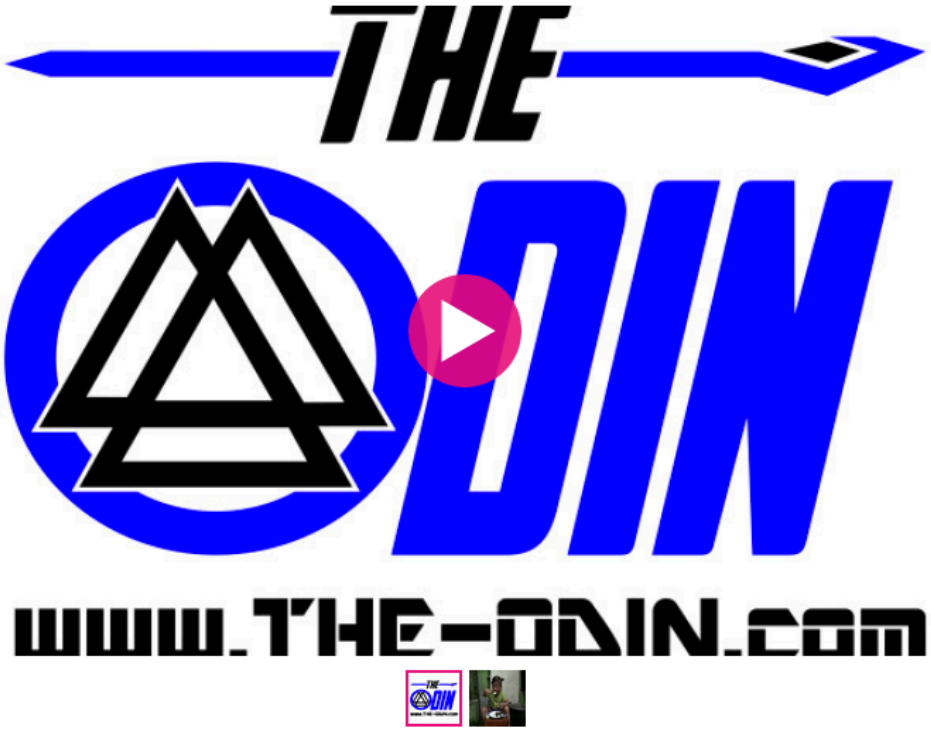
CRISPR/gRNA Design tools

https://en.wikipedia.org/wiki/CRISPR/Cas_Tools

List of CRISPR/Cas tools

Tool Name	Provider	Searches whole genome for targets	Returns all targets of genome	Seed span and location can be defined	Maximum number of mismatches supported	Predicts gRNA activity	Available Protospacer adjacent motif (PAM) sequences	Annotation is reported	gRNA suggestion or scoring	External Link	References
Benchling CRISPR gRNA Design	Benchling	Yes	Yes	Yes	4	Yes	User customizable	Yes	Yes	Webserver	-
Breaking-Cas	Spanish National Center for Biotechnology	Yes	Yes	Yes (by weights)	4	No	User customizable	Yes	Yes	Webserver	[3]
Cas-OFFinder	Seoul National University	Yes	Yes	No	0-10	No	NGG, NRG, NNAGAAW, NNNNGMTT	No	Yes	Webserver Source code	[4]
CCTop	University of Heidelberg	Yes	Yes	Partial	5 (0-5)	No	NGG, NRG, NNGRRT, NNNNGATT, NNAGAAW, NAAAAC	Yes	Yes	Webserver	[5]
CHOPCHOP	Harvard University	Yes	Yes	Partial	0, 2	No	NGG, NNAGAA, NNNNGANN	No	Yes	Webserver	[6]
CHOPCHOP v2	University of Bergen	Yes	Yes	Yes	3 (0-3)	Yes	User customizable	Yes	Yes	Webserver	[7]
COD	Dayong Guo	No	No	No	0, 3, 5, 8	No	NGG and NAG	No	Yes	Webserver	-
CRISPR Configurator & Specificity Tool	Dharmacon, Inc.	Yes (over 30 species)	Yes	Yes	8 (gaps or mismatches)	Internally	NGG and NAG	mRNA exons, Links to UCSC genome browser annotations	No	Webserver Specificity Tool	-

INDIEGOGO Explore How it works START A CAMPAIGN Sign U



Closed

DIY CRISPR Kits, Learn Modern Science By Doing

If you had access to modern synthetic biology tools, what would you create?

Josiah Zayner
San Francisco, United States
About

$71,036 USD total funds raised
333% funded on December 8, 2015

$130 USD + Shipping

Bacteria DIY CRISPR Kit

Want to really know what this whole CRISPR thing is about? Why it could revolutionize genetic engineering. This kit includes everything you need to make precision genome edits in bacteria at home including Cas9, gRNA and Donor DNA template for an example experiment. Item contents include but are not limited to: a laboratory grade pipette, media and plates, bacteria and DNA. Shipping might not be available to all locations, please check with local laws before ordering.

52 out of 50 claimed
Ships Worldwide

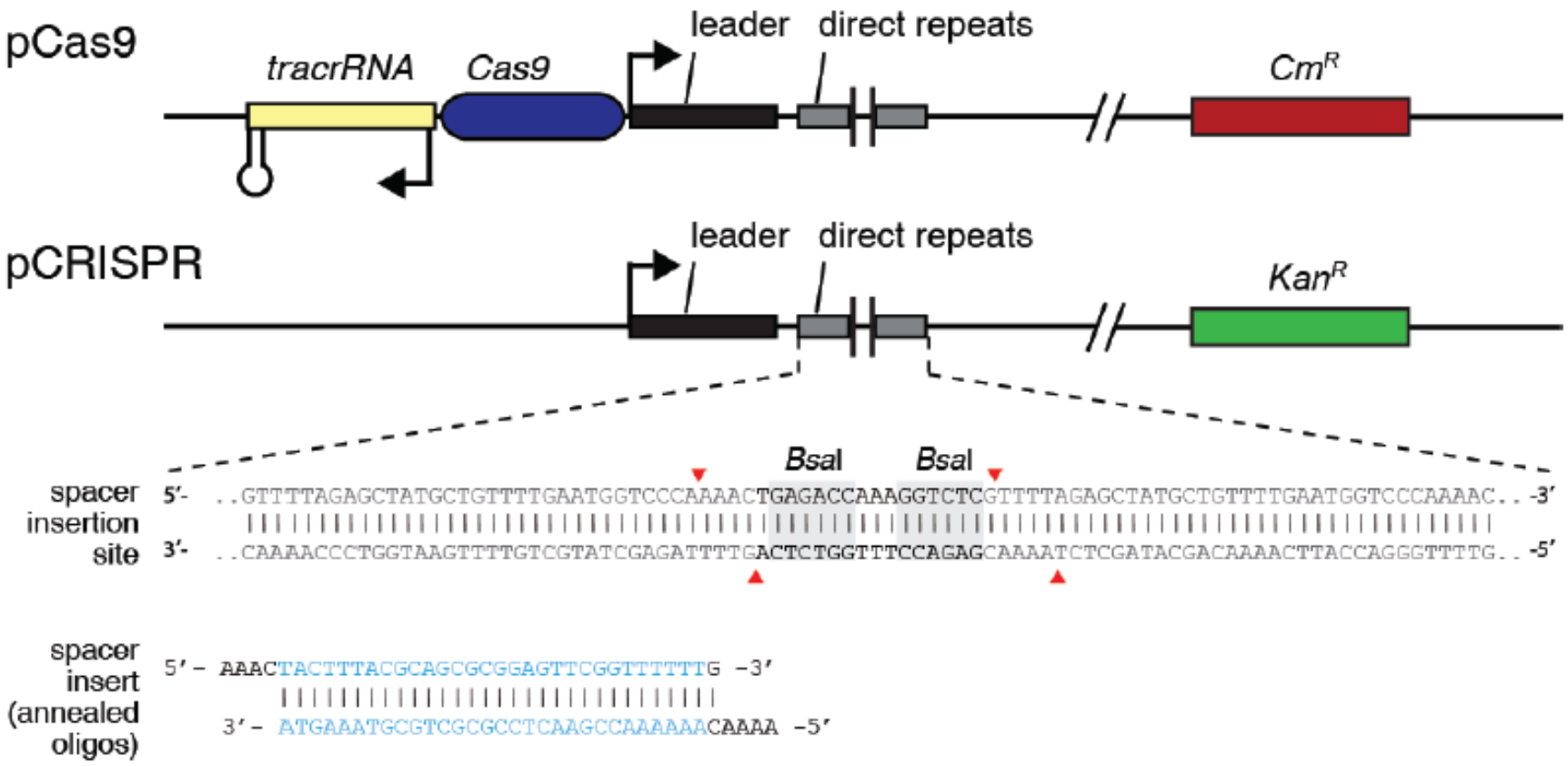
ESTIMATED MARCH 2016

nature biotechnology

RNA-guided editing of bacterial genomes using CRISPR-Cas systems

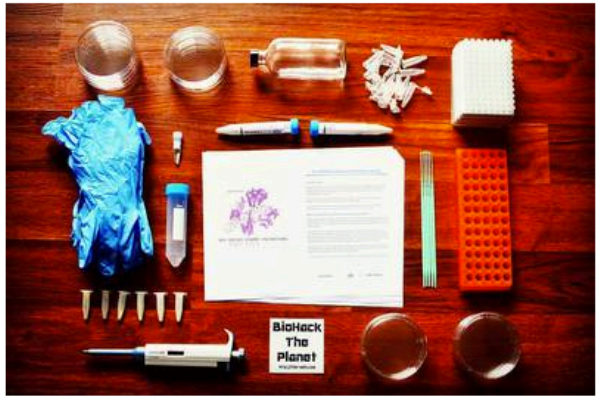
Wenyan Jiang[1,4], David Bikard[1,4], David Cox[2,3], Feng Zhang[2,3] & Luciano A Marraffini[1]

Nature Biotechnology 31: 234 (2013)



Supplementary Fig. 9. The essential elements of the *S. pyogenes* CRISPR locus 1 are reconstituted in *E. coli* using pCas9. The plasmid contains tracrRNA, Cas9, as well as a leader sequence driving the crRNA array. The pCRISPR plasmids contains the leader and the array only. Spacers can be inserted into the crRNA array between *Bsa*I sites using annealed oligonucleotides. Oligonucleotide design is shown at bottom. pCas9 carries chloramphenicol resistance (CmR) and is based on the low-copy pACYC184 plasmid backbone. pCRISPR is based on the high-copy number pZE21 plasmid. Two plasmids are required because a pCRISPR plasmid containing a spacer targeting the *E. coli* chromosome cannot be constructed using this organism as a cloning host if Cas9 is also present (it will kill the host).

DIY Bacterial Gene Engineering CRISPR Kit



Roll over to magnify and click to enlarge

$140.00

Shipping: Calculated at checkout

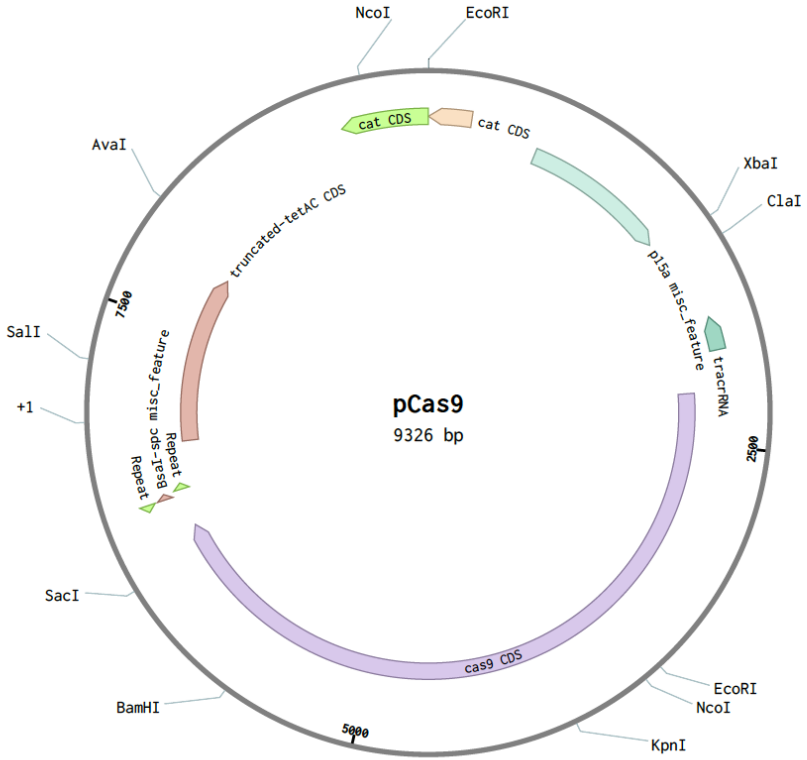
2 product reviews

Quantity:

Kit contains enough materials for around 5 experiments or more

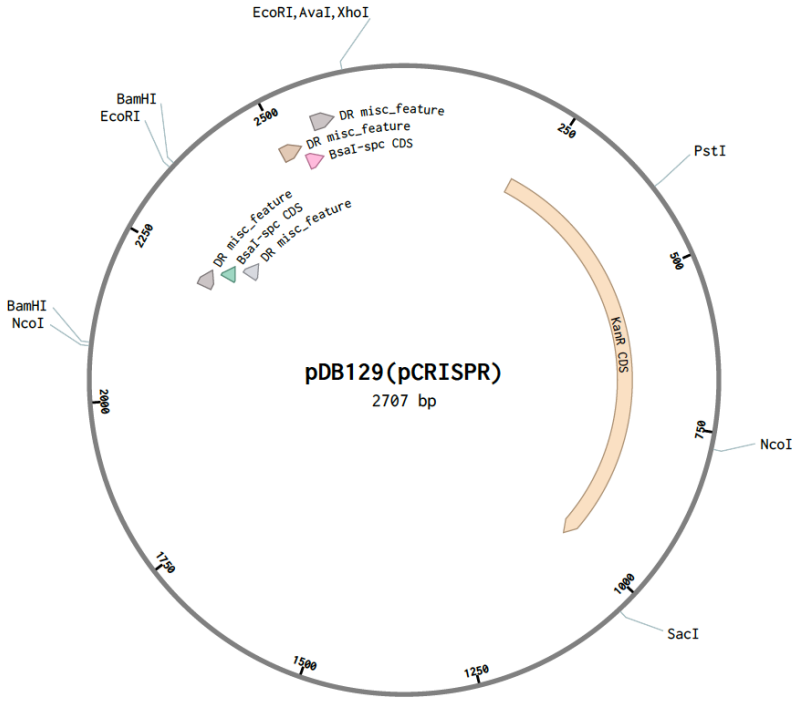
- LB Agar
- LB Strep/Kan Agar
- Glass bottle for pouring plates
- E. coli HME63 strain
- Inoculation Loops/Plate Spreader
- 10-100uL variable volume adjustable pipette(1uL increments)
- Box of 96 Pipette Tips
- 14 Petri Plates
- Microcentrifuge tube rack
- Nitrile Gloves
- Microcentrifuge tubes
- 50mL Tube for measuring
- Bacterial transformation buffer 25mM CaCl2, 10% PEG 8000 5% DMSO
- LB Media for transformation recovery
- Cas9 and tracrRNA plasmid
- crRNA plasmid
- Template DNA

pCAS9



Tube 1: Cas9 and tracrRNA Plasmid, Cm^r

pCRISPR::rpsL



Tube 2: Bacterial crRNA Plasmid, Km^r

E. coli HME63 *galK tyr145UAG ΔlacU169 mutS<>amp [l cI857. Δ(cro-bioA)]*

cI; the λ repressor. cI857, is temperature sensitive (the protein is active at 32 degrees centigrade but inactivated at 39 degrees centigrade)

mutS – mismatch repair defective (increases recombination)

A>C at bp 127 in rpsL (ribosomal protein subunit S12) causes a K43T (Lysine > Tryptophan) substitution resulting in resistance to Streptomycin

>rpsL_Ecoli_MG1655

atgGCAACAGTTAACCAGCTGGTACGCAAACCACGTGCTCGCAAAGTTGCGAAAAGCAACG
TGCCTGCGCTGGAAGCATGCCCGCAAAAACGTGGCGTATGTACTCGTGTATATACTACCAC
TCCTAAAAAACCGAACTCCGCGCTGCGTAAAGTATGCCGTGTTCGTCTGACTAACGGTTTC
GAAGTGACTTCCTACATCGGTGGTGAAGGTCACAACCTGCAGGAGCACTCCGTGATCCTGA
TCCGTGGCGGTCGTGTTAAAGACCTCCCGGGTGTTCGTTACCACACCGTACGTGGTGCGCT
TGACTGCTCCGGCGTTAAAGACCGTAAGCAGGCTCGTTCCAAGTATGGCGTGAAGCGTCCT
AAGGCTtaa

>W542_rpsL_A>C

ATACTTTACGCAGCGCGGAGTTCGGTTTTgTAGGAGTGGTAGTATATACACGAGTACAT

Streptomycin + kanamycin plates

pCAS9 only

pCAS9 + pCRISPR + W542-oligo

CAS9

Matsu-ura *et al. Fungal Biol Biotechnol (2015) 2:4*
DOI 10.1186/s40694-015-0015-1

FUNGAL BIOLOGY
AND BIOTECHNOLOGY

SHORT REPORT Open Access

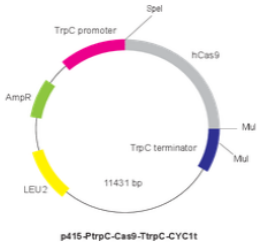
Efficient gene editing in *Neurospora crassa* with CRISPR technology

Toru Matsu-ura[1], Mokryun Baek[1], Jungin Kwon[1] and Christian Hong[1,2*]

p415-PtrpC-Cas9-TtrpC-CYC1t

(Plasmid #68059)

Print



p415-PtrpC-Cas9-TtrpC-CYC1t

Enlarge | Download

PURPOSE

Express humanized Cas9 under the control of trpC promoter and terminator from A. nidulans.

DEPOSITING LAB

Christian Hong

PUBLICATION

Matsu-ura et al Fungal Biology and Biotechnology 2015, 2:4

PRICE

$65 (USD)

FORMAT

Shipped as bacteria in an agar stab at ambient temperature

Add to Cart

Available to Academic and Nonprofits Only

BACKBONE

Vector backbone: pRS415
(Search Vector Database)

Total vector size (bp): 11431

Vector type: Yeast Expression, CRISPR ; N. crassa, Fungi

Selectable markers: LEU2

GROWTH IN BACTERIA

Bacterial Resistance(s): Ampicillin

Growth Temperature: 37°C

Growth Strain(s): Stbl3

Copy number: Low Copy

SEQUENCE INFORMATION

Depositor Sequences: Full (1)

Addgene Sequences: Partial (7)

GENE/INSERT

Gene/Insert name: humanized Cas9

Species: H. sapiens (human); N. crassa, S.pyogenes, other fungi

Insert Size (bp): 4140

RESOURCE INFORMATION

Supplemental documents:

- p415-PtrpC-Cas9-TtrpC-CYC1t.docx

A portion of this plasmid was derived from a plasmid made by:
Addgene Plasmid #43804

Terms and Licenses:

- UBMTA

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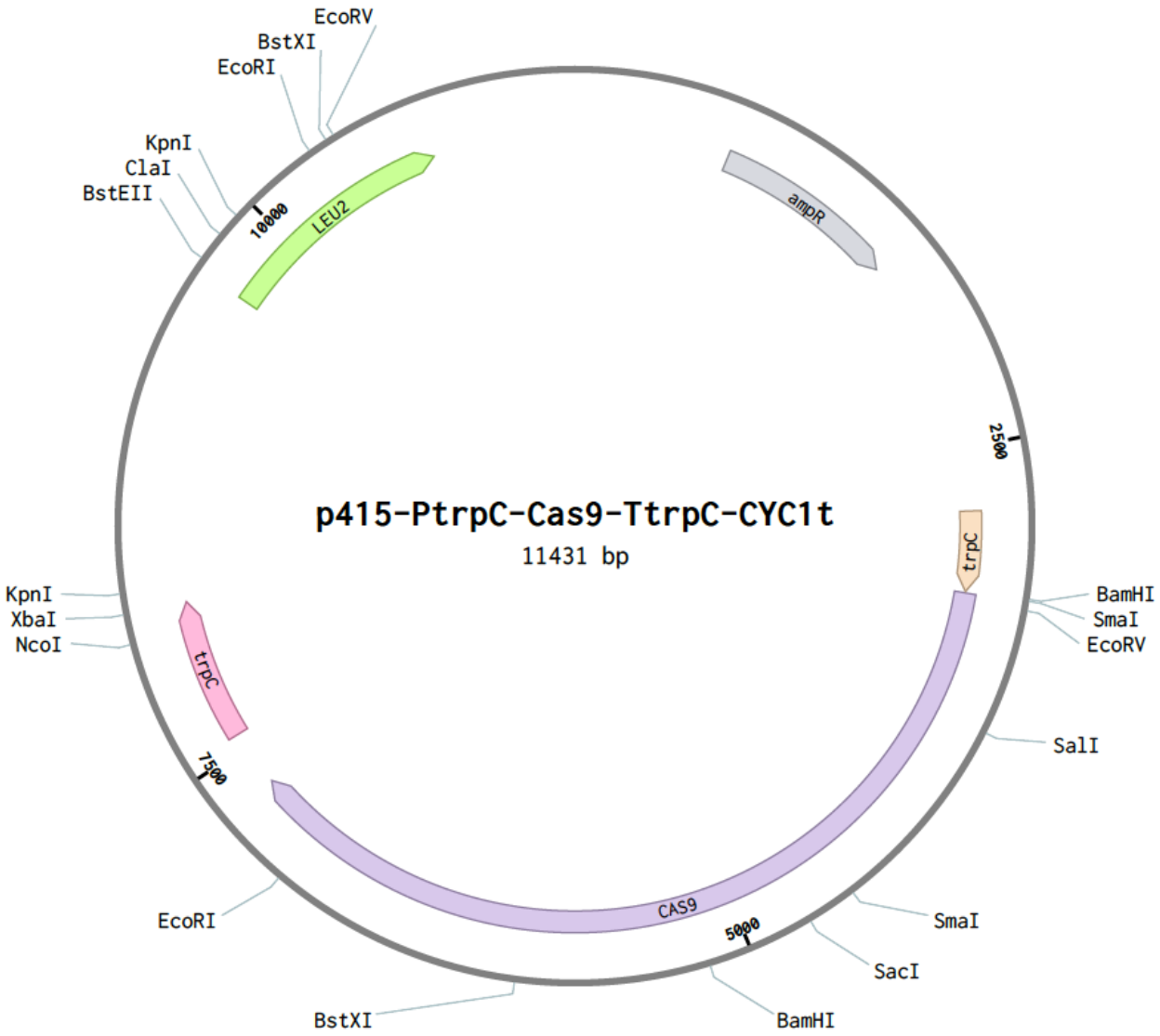
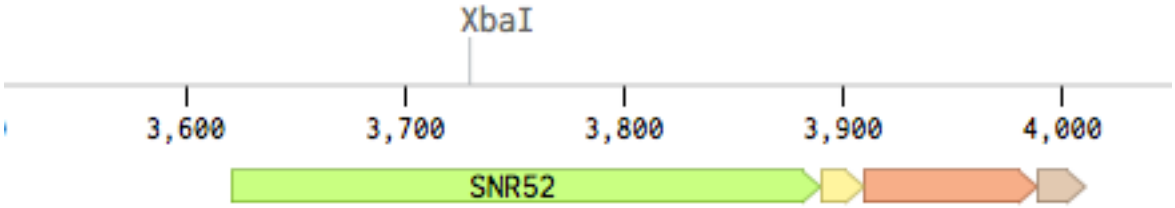
CRISPR/Cas plasmids

p415-PtrpC-Cas9-TtrpC-CYC1t (11431 bp)



p426-SNR52p-gRNA.clr-2.Y-SUP4t (6274 bp)



4336–4343 Nucleic Acids Research, 2013, Vol. 41, No. 7 Published online 4 March 2013
doi:10.1093/nar/gkt135

Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems

James E. DiCarlo[1,2], Julie E. Norville[2], Prashant Mali[2], Xavier Rios[2], John Aach[2] and George M. Church[2,*]

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390 bp