CRISPR-Cas9 Mediated Mouse Model Creation and Transcription Regulation

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Precision Medicine

rgaaggggataggaccataggggtcttgaccaagcctgat GCCCTGCCTGGCAGATGATACCTAGAGACTGACTGAGG<u>AGCTCACCTCCCA</u> AAGAAA GGAAGC CTGAGATGACCCAGCACCTGAAAGCCTACTA GACGGAATP GAAGGG TTCAGCTTTTACAGGACACCAGTAAG GGAGG ACCTTGCCAAATTCTCCGATTAACCAGGCTAGCTATGTGGCT TCCTGG GTGCAAGTC TGAGGAGATGAGGAGAGAGGAGC Disease GAA G TAACAA TCAGTCC ATGATGTTGG AATCAGC GAACCTCACAAGTAGTTCTTAGGTGAGTTTC TGTCAGTGTTAC TATC GC CTGTTTGTGAGGT CAGGGAAACATT TTCATGT CAGCAAGACATCAT1 CAAAACA сттесаатааааттеттестаастеастесте GGAAGAGCTCTGTGCTGGAAGCACTGTCTGGAGT GGT GAG CCTATGATGACATTGAAGTGGAGC CAGCAATGTGGACATTGCTAC CAGAGGTGCTGAAGGCAAGGTCTTGGATGTGATGCGGGAACCTGGTGTA GACTGAGGCTTATCAGACAGTGCAAGTCTTCTTCAAGGATCACTCATACTTCAGCATTCTTCTGGA TAACCAGGCTAGCTATGTGGCTTTCCTGGTCGCTGTGCA PAGC TTGTAAACTCTGAGGAGATGAGGAGAGAGGAGCTATGGAGAGTCATTCTACTTCAGCAGG **GCTGAGCACC** CAGAA CCTTTCCACAGGCAGAAACCAAGAATGAATCAGCAGCTGAAGTTCAATCTTGATGAA

Genome Editing

ZFN-induced DSB

- Zinc-finger Nuclease (ZFN)
- Transcription Activator–Like Effector Nuclease (TALEN)

Direct imperfect

Provide adaptor with sticky ends

Simultaneous

cleavage with a

second distal ZFN

ligation o'f two ends

CRISPR-Cas9

NHEJ

Gene disruption

(small deletions or insertions)

Tag ligation

Large deletion (>>100 bp)



Urnov et al., Nat Rev Genet 2011

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,^{1,2}* Krzysztof Chylinski,^{3,4}* Ines Fonfara,⁴ Michael Hauer,²† Jennifer A. Doudna,^{1,2,5,6}‡ Emmanuelle Charpentier⁴‡



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Cas9 programmed by single chimeric RNA





chimera A



chimera B

Jinek et al., 2012

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 Zhang¹†

RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,^{1,5} Luhan Yang,^{1,3,5} Kevin M. Esvelt,² John Aach,¹ Marc Guell,¹ James E. DiCarlo,⁴ Julie E. Norville,¹ George M. Church^{1,2*}

CRISPR mediated mouse model generation



Wang, Yang and Shivalila et al., Cell 2013

Phenotype in Founder Animals

Tet3 Mutants with Neonatal Lethality



Wang, Yang and Shivalila et al. 2013

CRISPR mediated mouse model generation



Yang, Wang and Shivalila et al., Cell 2013

CRISPR-mediated Genome Editing: Faster Timeline, Lower Cost, More Flexibility

| | | Conventional Gene Targeting | CRISPR-based Gene Knockout | CRISPR-based Gene Knock in |
|--|--|--------------------------------|-------------------------------|-------------------------------|
| Timeline | | 40 weeks | 6 weeks | 8 weeks |
| Cost – Model Creation | | \$15,000 | \$3,000 | \$7,000 |
| Cost - Breeding to GLT/NEO Excision | | \$20,000 | \$4,000 | \$4,000 |
| Flexibility | | 129 | any strain | any strain |
| | | C57BL/6 | | |
| Cost – Strain Change | | \$13,000 | \$0,0 | \$0.0 |



Zygote Electroporation of Nuclease (ZEN)

- Electroporation parameters
- Electroporation solution
- CRISPR concentration

ECM 830 Square Wave Electroporation System (with 1 mm electroporation cuvette, Model 610, P/N 45-0124)





FEATURES

- A wide range of voltages from 5 to 3000 V
- □ Finer voltage discrimination
- \Box Pulse durations from 10 µsec to 10 sec
- Arc Quenching
- Digital display of actual voltage and pulse length delivered

ZEN Improvement

Aicda KI using Cas9 protein



Wang et al. , 2016

CRISPR-mediated Genome Editing in Mice, High Throughput.



Qin et al., Genetics 2015

Generating mouse models in high throughput

- Quickly screen candidate genes in founder animals
- Generate human genetic variant in mouse ortholog

Different Flavors of Cas9



WT

CRISPR-on

dCas9VP160 + sgRNAs



(Cheng et al, 2013)

dCas9VP160 + sgRNAs

CRISPR-on



(Cheng, 2013)

sgRNA-dCas9 fusions Cross-react

• We cannot use the multiple sgRNA to direct different dCas9 fusions to different loci





Casilio = <u>Cas</u>9 + Pum<u>ilio</u>



Casilios are orthogonal without significant cross-reaction



Casilios activate endogenous genes more efficiently than dCas9 direct fusion





Casilios simultaneously activate and repress separate endogenous genes





Casilios recruit HAT to enhancers activating gene expression





Casilio isotypes can achieve labeling using cognate sgRNA-PBS





Labeling of Centromeres





Simultaneous labeling of Centromeres and Telomeres







Improving Non-repeat labeling?



Preliminary data: Casilio allows labeling of non-repeat region with 7 sgRNA-15xPBS32 targeting MUC4 locus. Representative confocal microscopy image of the MUC4 labeling. Need IF-FISH to confirm



Casilio applications



Multiplexing

Complex formation

Polymerization

Gene Editing as Therapeutics

- Identify a list of actionable diseases and genes
- Could we developed a relatively cost-effective model for the therapeutic development for rare diseases
- How do we evaluate the risk/benefit

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