

TALENs技术实现定向基因打靶在斑马鱼中的应用

Targeted Gene Disruption in Zebrafish Using Engineered TALENs

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报告人：伍应丹

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研究背景

- 斑马鱼作为模式生物，具有以下几个优点：
 - 体积小，生长周期短，易于大规模饲养。
 - 产卵量大，并且其产卵过程可以人为控制。
 - 体外受精，胚胎透明，其发育过程可以进行连续观察。
 - 多种器官、系统的发育与人类相似。
- 遗传学的研究有两种方法：
 - 正向遗传学：人工诱变，大规模筛选，获得突变体，费时费力。
 - 反向遗传学：定向突变目的基因，快速获得突变体。
- 在以斑马鱼为模式生物的研究中，应用TALEN技术进行定向基因打靶，具有重要的意义。

References about TALE and TALEN

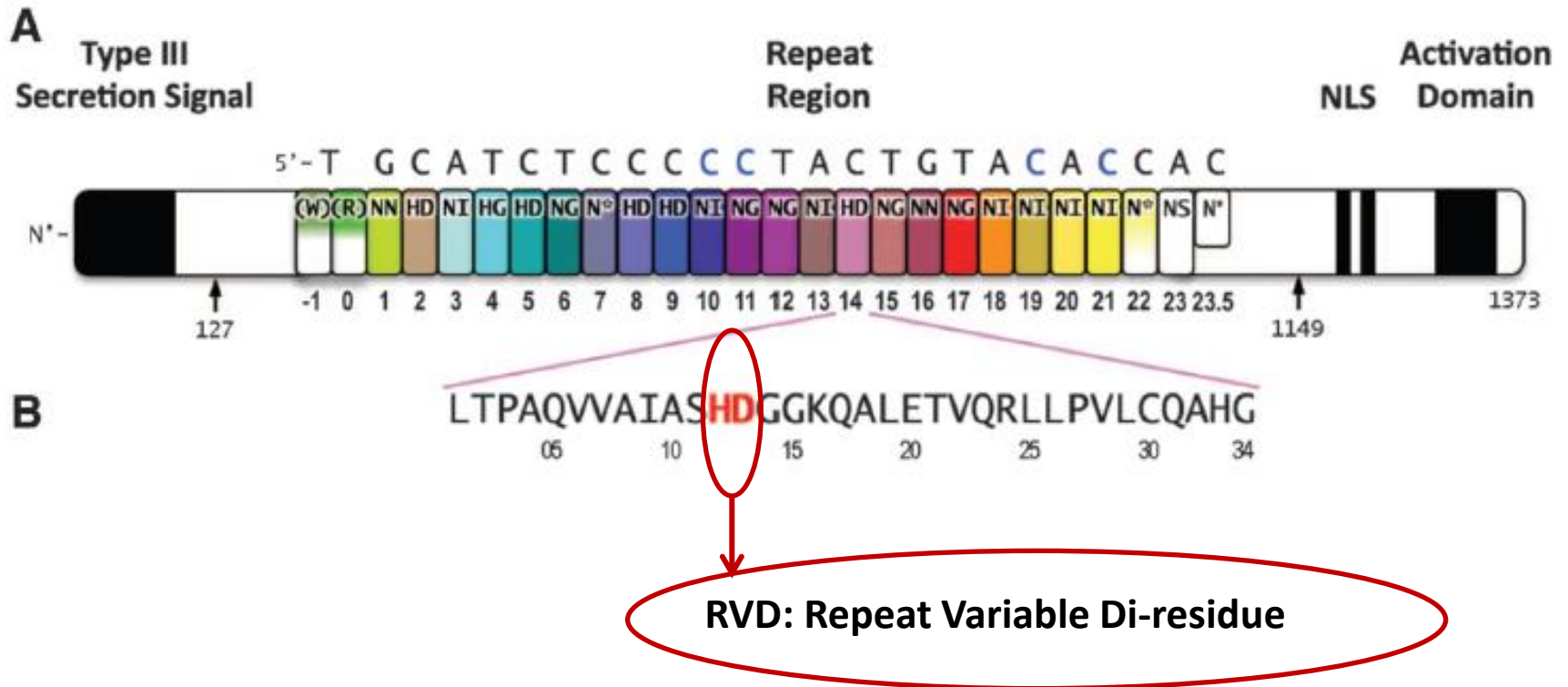
#	A	B	C	D
		PMID	Ref.	Title
1				
2	TALE	15283671	Alfano JR. et al., Annu Rev Phytopathol. 2004, Collmer A.	Type III secretion system effector proteins: double agents in bacterial disease
3	TALE	15553245	Bing Yang et al., Mol Plant Microbe Interact. 2004, Frank F. White	Diverse members of the AvrBs3/PthA family of type III effectors are major virulence
4	TALE	16388329	Gürlebeck D. et al., J Plant Physiol. 2006, Bonas U.	Type III effector proteins from the plant pathogen Xanthomonas and their role
5	TALE	16570663	Fujikawa T. et al., Mol Plant Microbe Interact. 2006, Tsuyumu S.	Suppression of defense response in plants by the avrBs3/pthA gene family of X
6	TALE	17563377	Sugio A, Proc Natl Acad Sci U S A. 2007, White FF.	Two type III effector genes of Xanthomonas oryzae pv. oryzae control the induc
7	TALE	17962565	Sabine Kay et al., Science, 2007, Eric Marois.	A Bacterial Effector Acts as a Plant Transcription Factor and Induces a Cell
8	TALE	19168386	Kay S. et al., Curr Opin Microbiol. 2009, Bonas U.	How Xanthomonas type III effectors manipulate the host plant.
9	TALE	19236567	Gürlebeck D. et al., Mol Plant Pathol. 2009, Bonas U.	Visualization of novel virulence activities of the Xanthomonas type III effec
10	TALE	19400638	Jens Boch and Ulla Bonas et al., Annu. Rev. Phytopathol. 2010, Jens Boch and Ulla Bonas.	Xanthomonas AvrBs3 Family-Type III Effectors: Discovery and Function.
11	TALE	19849788	Gu K et al., Mol Plant Pathol. 2009, Yin Z	Transcription activator-like type III effector AvrXa27 depends on OsTFIIAgamma
12	TALE	21965534	Fonfara I et al., Nucleic Acids Res. 2011, Wende W, Medizinische Hochschule Hannover	Creating highly specific nucleases by fusion of active restriction endonuclea
13	TALE	19910532	Römer P, Proc Natl Acad Sci U S A. 2009, Lahaye T.	A single plant resistance gene promoter engineered to recognize multiple TAL
14	Engineered TALE (Milestone)	19933106	Moscov MJ et al., Science. 2009, Bogdanove AJ	A simple cipher governs DNA recognition by TAL effectors.
15	Engineered TALE (Milestone)	19933107	Boch J et al., Science. 2009, Jens Boch	Breaking the code of DNA binding specificity of TAL-type III effectors.
16	TALE	20345643	Römer P et al., New Phytol. 2010, Lahaye T.	Promoter elements of rice susceptibility genes are bound and activated by spe
17	TALE	20521952	Song C et al., Mol Plant Microbe Interact. 2010, Yang B.	Mutagenesis of 18 type III effectors reveals virulence function of XopZ (PXO99
18	TALE	20570209	Bogdanove AJ et al., Curr Opin Plant Biol. 2010, Lahaye T.	TAL effectors: finding plant genes for disease and defense.
19				
20	TALEN - in vitro/yeast	20680643	Christian M et al., Genetics, Jul 2010, Voytas DF.	Targeting DNA double-strand breaks with TAL effector nucleases.
21	TALEN - in vitro/yeast	20699274	Li T. and Huang s. et al., Nucleic Acids Res, 2011 (Jul 2010), Yang B, Iowa State U.	TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA
22	TALE	21098734	Antony G et al., Plant Cell. 2010, Yang B.	Rice xal3 recessive resistance to bacterial blight is defeated by induction o
23	TALE	21106758	Morbitz R. et al., Proc Natl Acad Sci USA. 2010 Nov, Lahaye T, Ludwig-Maximilians-U. Munich	Regulation of selected genome loci using de novo-engineered transcription act
24	TALEN - in vitro	21262818	Magdy M. Mahfouz et al., Proc Natl Acad Sci U S A. 2010 Dec, Zhu JK.	De novo-engineered transcription activator-like effector (TALE) hybrid nuclea
25	TALE	21178484	Heidi Scholze et al., Virulence. 2010, Jens Boch	TAL effector-DNA specificity.
26	TALEN - K562 cell	21179091	Miller JC et al., Nat Biotechnol. Feb 2011 (Dec 2010), Rebar EJ, Sangamo Inc.	A TALE nuclease architecture for efficient genome editing.
27	TALE	21208999	Yuan T et al., Mol Plant. 2011, Wang S.	Characterization of Xanthomonas oryzae-responsive cis-acting element in the p
28	TALE	21215685	Scholze H et al., Curr Opin Microbiol. 2011, Boch J.	TAL effectors are remote controls for gene activation.
29	TALE/Engineered TALE	21248753	Zhang F et al., Nat Biotechnol. 2011, Arlotto P.	Efficient construction of sequence-specific TAL effectors for modulating mamm
30	TALE	21478322	Li YR et al., Appl Environ Microbiol. 2011, Chen GY.	Hpa2 required by HrpF to translocate Xanthomonas oryzae transcriptional activ
31	TALE/Engineered TALE	21421566	Robert Morbitzer et al., Nucleic Acids Research, Mar 2011, Thomas Lahaye.	Assembly of custom TALE-type DNA binding domains by modular cloning.
32	TALEN - yeast	21459844	Li T. and Huang s. et al., Nucleic Acids Res. 2011, Yang Binz.	Modularly assembled designer TAL effector nucleases for targeted gene knockou
33	TALEN- yeast/human/Arabidopsis	21493687	Cermak T et al., Nucleic Acids Res. 2011, Adam J. Bogdanove and Voytas DF.	Efficient design and assembly of custom TALEN and other TAL effector-based co
34	TALE	21515727	Triplett LR et al., Appl Environ Microbiol. 2011, Leach JE.	Genomic analysis of Xanthomonas oryzae isolates from rice grown in the United
35	TALE	21615204	Li YR et al., Mol Plant Microbe Interact. 2011, Chen GY.	A novel regulatory role of HrpD6 in regulating hrp-hrc-hpa genes in Xanthomon
36	Engineered TALE - tobacco	21625552	Weber E et al., PLoS One. 2011, Marillionet S.	Assembly of designer TAL effectors by Golden Gate cloning.
37	Engineered TALE-Hek293 cells/tobacco	21625585	Geissler R et al., PLoS One. 2011, Boch J.	Transcriptional activators of human genes with programmable DNA-specificity
38	TALE	21679014	Yu Y et al., Mol Plant Microbe Interact. 2011, Boris Szurek	Colonization of rice leaf blades by an African strain of Xanthomonas oryzae p
39	TALEN - worm	21700836	Andrew J. Wood et al., Science, 2011, Barbara J. Meyer.	Targeted Genome Editing Across Species Using ZFNs and TALENs.
40	TALEN - hPS	21738127	Hockemeyer D et al., Nat Biotechnol. 2011, Jaenisch R.	Genetic engineering of human pluripotent cells using TALE nucleases.
41	TALEN - Hek293	21813459	Claudio Mussolino et al., Nucleic Acids Research, 2011, Toni Cathomen.	A novel TALE nuclease scaffold enables high genome editing activity in combin
42	TALEN - rat	21822240	Laurent Tesson et al., Nat Biotechnol. 2011, Ignacio Anegón and Gregory J Cost.	Knockout rats generated by embryomicroinjection of TALENs.
43	TALEN - zebrafish	21822241	Jeffrey D Sander et al., Nat Biotechnol. 2011, J Keith Joung and Jing-Ruey J Yeh.	Targeted gene disruption in somatic zebrafish cells using engineered TALENs.
44	TALEN - zebrafish	21822242	Huang Peng et al., Nat Biotechnol. 2011, Shuo Lin and Bo Zhang.	Heritable gene targeting in zebrafish using customized TALENs.
45	Comment	21822235	Nat Biotechnol. 2011	Move over ZFNs.
46	Review	21865862	Mahfouz MM et al., GM Crop. 2011, Mahfouz MM.	TALE nucleases and next generation GM crops.
47	Review	21929364	Karl J. Clark et al., Zebrafish, 2011, (Voytas DF) Ekker SC, Mayo Clinic	A TALE of Two Nucleases: Gene Targeting for the Masses?
48	Review	21960622	Adam J. Bogdanove et al., Science 2011, (Voytas DF) Bogdanove AJ, Iowa State U.	TAL effectors: customizable proteins for DNA targeting.
49	Review	22072577	Julie C. Kiefer et al., Dev Dyn. 2011, Kiefer JC.	Primer and interviews: Advances in targeted gene modification.

研究背景

- **TALEN**（Transcription activator-like effector nucleases, TALEN, 转录激活子样效应因子核酸酶）：一种**人工核酸酶**，是用于**定向基因打靶**的一种**新技术**，由**转录激活子样效应因子（TALE）**和**核酸酶**构成。
- **核酸酶（nucleases）**：主要是**Fok I**，一种**II S型的核酸内切酶**，具有特异的识别位点结构域（**突变**）和**切割位点结构域**，**二聚化**后才具有**切割活性**。

研究背景

- TALE的结构:

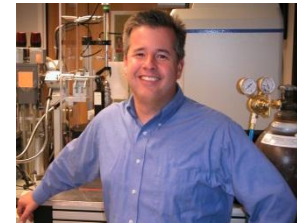


The three-dimensional structure of TALE

- **The Crystal Structure of TAL Effector PthXo1 Bound to Its DNA Target**

Mak AN *et al.*, *Science*, Jan 2012

Stoddard BL, Fred Hutchinson Cancer Research Center



Stoddard BL

Fred Hutchinson Cancer Research Center

Link to: [Lab](#)

- **Structural Basis for Sequence-Specific Recognition of DNA by TAL Effectors**

Deng D *et al.*, *Science*, Jan 2012

Shi YG, Yan N, Tsinghua University



Shi YG

Tsinghua University

Link to: [Lab](#)

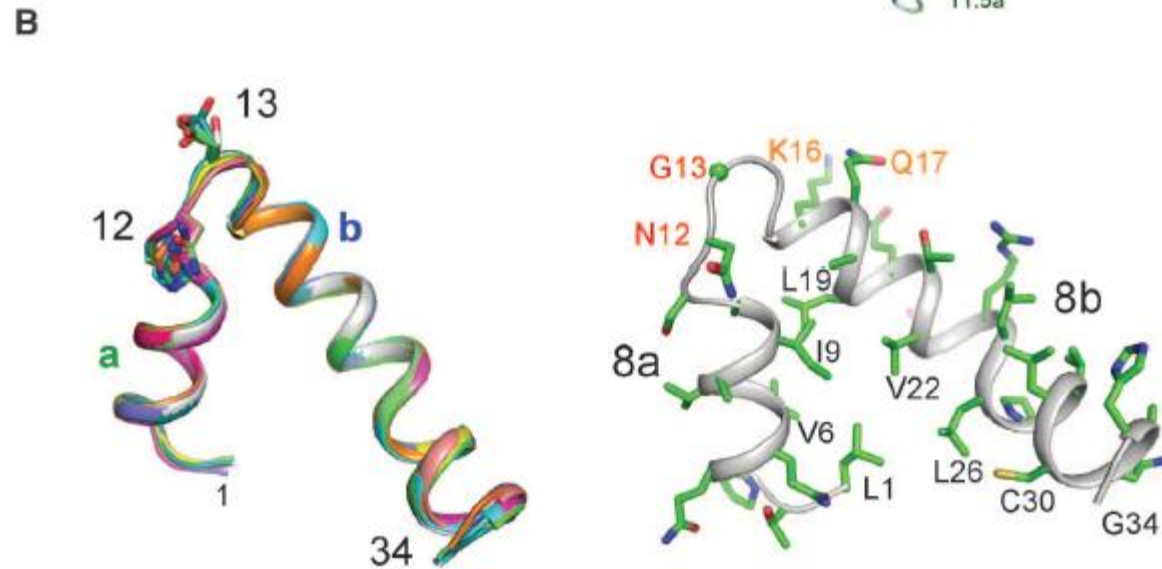
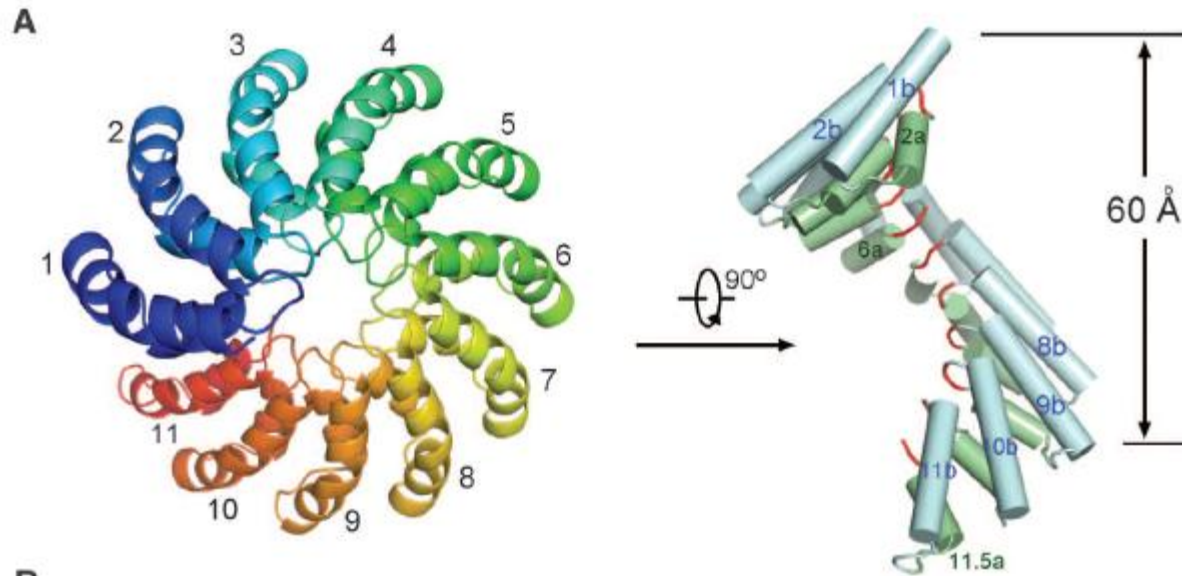


Yan N

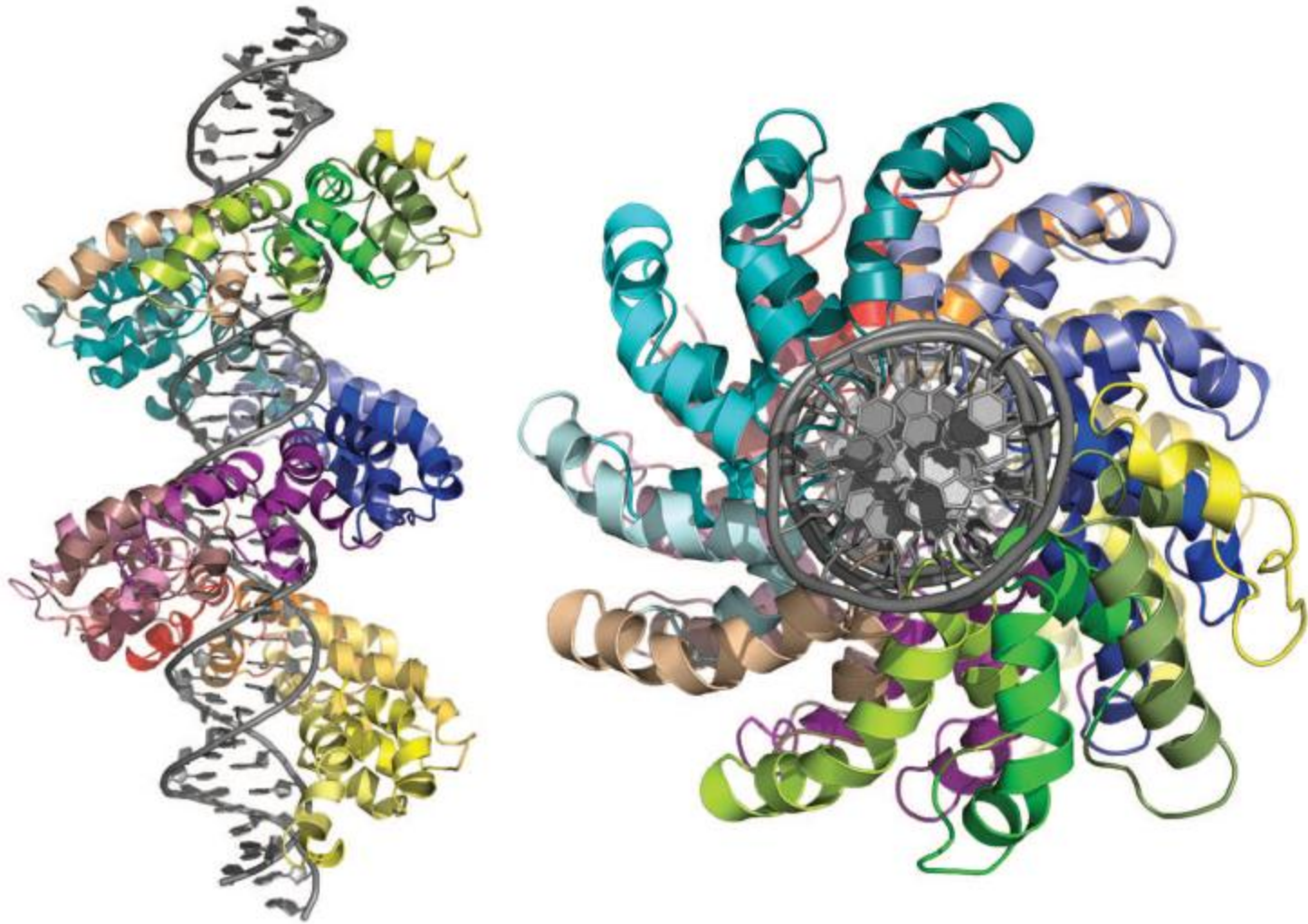
Tsinghua University

Link to: [Lab](#)

Structure of the TALE repeats in DNA-free



Structure of the TALE repeats in DNA binding region



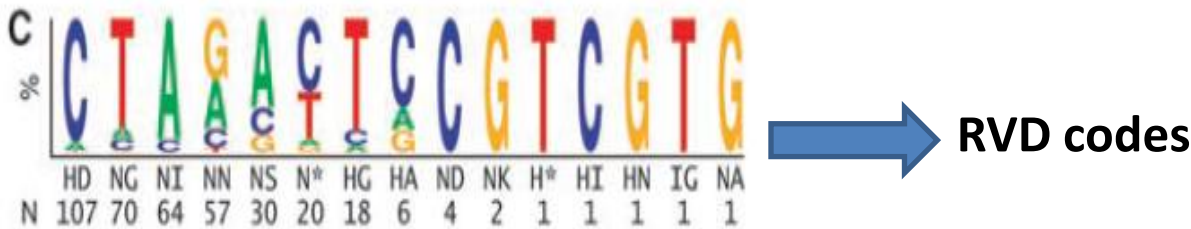
Milestones in the development of TALE engineering

- **A simple cipher governs DNA recognition by TAL effectors**

Moscou MJ *et al.*, *Science*, Sep 2009
 Bogdanove AJ, Iowa State University



Bogdanove AJ
 Iowa State University
 Link to: [Lab](#)

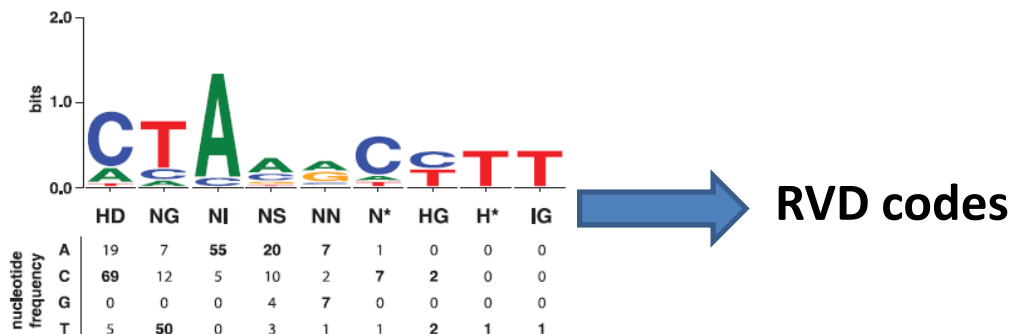


- **Breaking the code of DNA binding specificity of TAL-type III effectors**

Boch J *et al.*, *Science*, Sep 2009
 Boch J, Martin-Luther-University Halle-Wittenberg

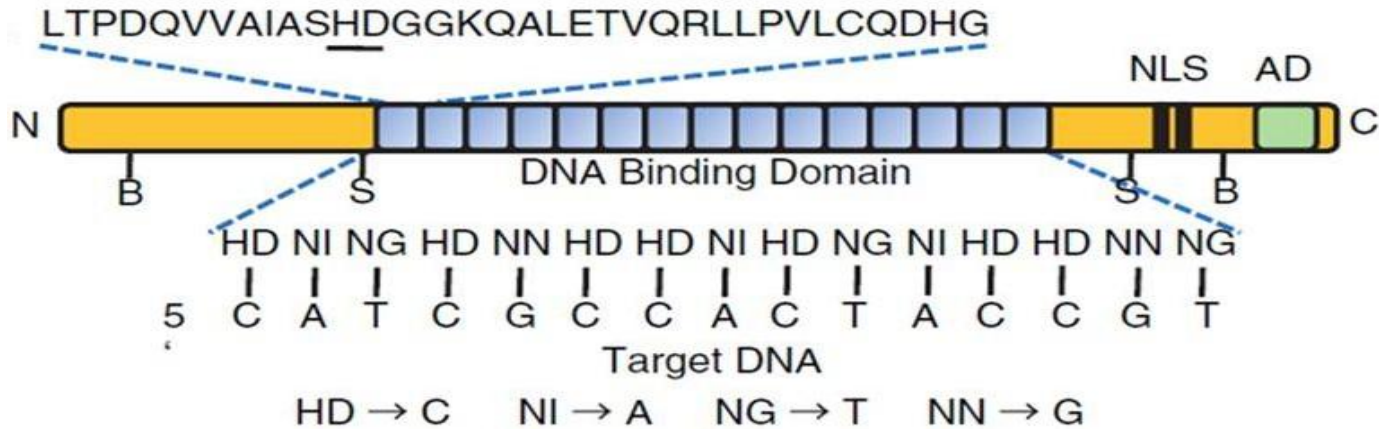


Boch J
 Martin-Luther-University Halle-Wittenberg
 Link to: [Lab](#)

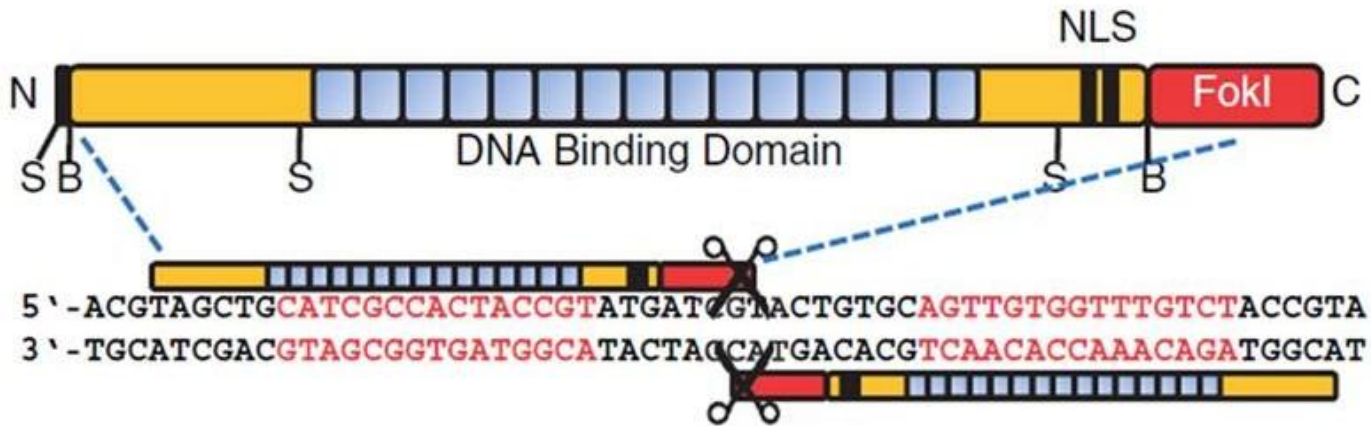


研究背景

- **TALE的结构:**



- **TALEN的作用原理:**



实验背景

- 目的基因：
 - *mlip*主要在斑马鱼肌肉和心脏中表达，目前对于它的研究很少，其在斑马鱼发育过程中的具体功能未知。本课题旨在使用TALEN技术对该基因进行打靶，获得此基因的突变体，为后续进一步研究它的功能奠定基础。

实验设计流程

选择目的基因



TALEN靶点设计



靶点检测



Unit assembly合成
TALEN



TALEN效率检测

目的基因的选择



Research

General Information

ZIRC

[Home](#) [Genes / Markers / Clones](#) [BLAST](#) [GBrowse](#) [Expression](#) [Antibodies](#) [Mutants / Morphants / Tg](#) [Anatomy](#) [Publications](#) [Maps](#)

ZFIN ID: ZDB-GENE-041111-277

Gene Name: *muscular LMNA-interacting protein*

Gene Symbol: *mlip*

Previous Names: im:7151231

[Nomenclature History](#)

GENE EXPRESSION i

Directly Submitted Expression Data: [4 figures \(4 images\)](#) from [Thisse *et al.*, 2004](#) [IMAGE:7151231]

Wild-type Stages, Structures: [Segmentation:20-25 somites \(19.0h-22.0h\)](#) to [Hatching:Long-pec \(48.0h-60.0h\)](#)
[heart](#) , [heart tube](#) , [myotome](#)

MUTANTS AND TARGETED KNOCKDOWNS [No data available](#)

PHENOTYPE i [No data available](#)

GENE ONTOLOGY

Ontology i

	GO Term
Biological Process	biological_process <input type="checkbox"/> (more)
Cellular Component	cellular_component <input type="checkbox"/> (more)
Molecular Function	molecular_function <input type="checkbox"/> (more)

[GO Terms \(all 3\)](#)

PROTEIN FAMILIES, DOMAINS AND SITES [No links to external sites available](#)

TRANSCRIPTS [No data available](#)

目的基因的选择



Research

General Information

ZIRC

[Home](#) [Genes / Markers / Clones](#) [BLAST](#) [GBrowse](#) [Expression](#) [Antibodies](#) [Mutants / Morphants / Tg](#) [Anatomy](#) [Publications](#) [Maps](#)

ZFIN ID: ZDB-GENE-041111-277

ANTIBODIES No data available

CONSTRUCTS WITH SEQUENCES FROM *mlip* No data available

SEGMENT (CLONE AND PROBE) RELATIONSHIPS

mlip Contained in: [BAC] [CH73-344J23](#) ([order this](#))

mlip Encodes: [EST] [IMAGE:7151231](#) (1) ([order this](#))

SEQUENCE INFORMATION

Type	Accession #	Length (bp/aa)	Analysis i
RNA	GenBank:CK688193	672 bp	Select Tool
Genomic	GenBank:CU571255 (1)	80442 bp	Select Tool
Sequence Clusters	UniGene:135375 (1)		

OTHER *mlip* GENE PAGES

[Gene:492702](#) (1)

MAPPING INFORMATION

Chr: 13 [Details](#)

ORTHOLOGY

Species	Symbol	Chromosome (Position)	Accession #	Evidence i	
				AA	CL
Zebrafish	<i>mlip</i>	13			
Human	<i>MLIP</i>	6 (6p12.1)	OMIM:614106 Gene:90523		
Mouse	<i>2310046A06Rik</i>	9 (42.67 cM)	MGI:1916892 Gene:69642		

[Orthology Details](#)

[CITATIONS](#) (4)

目的基因的选择

The image shows the Ensembl genome browser interface. At the top, the Ensembl logo is followed by navigation links: BLAST/BLAT, BioMart, Tools, Downloads, Help & Documentation, Blog, and Mirrors. A search bar on the right contains the text "Search all species...".

On the left side, there is a sidebar menu with the following items: Resequencing, Linkage Data, Markers, Other genome browsers (UCSC, NCBI, Vega), Configure this page, Manage your data, Export data (highlighted with a red circle), and Bookmark this page.

The main content area displays a genomic track for Danio rerio (Zv9) on Chromosome 13, spanning from 1,712,312 to 2,212,311. A 500.00 Kb region is highlighted with a red box, centered around the gene < CU57 1255.6 >. The track shows various gene models, including COL21A1 (1 of 2), GFRAL, hcrtr2, fam83b, MLIP, lrrc1, CABZ01066734.1, hth31, and cclc. A legend below the track identifies red bars as "protein coding" and yellow bars as "merged Ensembl/Havana".

Below the track, there is a search bar with "Location: 13:1930015-1994608" and a "Go" button, and another search bar for "Gene:" with a "Go" button. Navigation arrows are visible to the right of the search bars.

The bottom section shows a detailed view of the selected region (64.59 Kb). It includes tracks for 5-way GERP elements, Vertebrate cDNAs, EST cluster (Unigene), Contigs (CU10476.1.7 and < CU57 1255.6 >), Genes (Merged Ensembl) (CU571255.1-201 protein coding), EST cluster (Unigene), Vertebrate cDNAs, Zebrafish cDNAs, and %GC. The tracks show the gene structure with exons and introns, and the %GC track shows the GC content across the region. The reverse strand is indicated at the bottom.

目的基因的选择

Export data

Export Configuration - Feature List

Location to export:

chromosome:Zv9:13:1930015:1994608:1

Output:

FASTA sequence *

Select location:

13 * 1930015 * 1994608 * 1

5' Flanking sequence (upstream):

0 * (Maximum of 1000000)

3' Flanking sequence (downstream):

0 * (Maximum of 1000000)

Fields marked * are required

Options for FASTA sequence

Genomic:

Unmasked *

Fields marked * are required

Export data

Export Configuration - Output Format

Please choose the output format for your export

- [HTML](#)
- [Text](#)
- [Compressed text \(.gz\)](#)

TALEN靶点设计

Published online 14 April 2011

*Nucleic Acids Research, 2011, Vol. 39, No. 12 e82
doi:10.1093/nar/gkr218*

Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting

**Tomas Cermak¹, Erin L. Doyle², Michelle Christian¹, Li Wang², Yong Zhang^{1,3},
Clarice Schmidt², Joshua A. Baller^{1,4}, Nikunj V. Somia¹, Adam J. Bogdanove^{2,*} and
Daniel F. Voytas^{1,*}**

¹Department of Genetics, Cell Biology & Development and Center for Genome Engineering, 321 Church Street SE, University of Minnesota, Minneapolis, MN 55455 ²Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, IA 50011, USA, ³Department of Biotechnology, School of Life Sciences and Technology, University of Electronic Science and Technology of China, Chengdu 610054, China and ⁴Biomedical Informatics and Computational Biology Program, University of Minnesota Rochester, 111 South Broadway, Rochester, MN 55904, USA

Received March 8, 2011; Revised March 23, 2011; Accepted March 24, 2011

TALEN靶点设计

TAL Effector Nucleotide Targeter 2.0

[Tools](#)[About TALE-NT](#)[Resources](#)[Software](#)[FAQs](#)[Tutorials](#)[Contact Us](#)

Tools

Software, protocols, tools, and information for designing, evaluating, and assembling custom TAL effector constructs

Welcome to the new TAL Effector Nucleotide Targeter website! In addition to a new look, we have added more options to our tools to allow you to design custom TAL effectors that work with a variety of TAL effector/TAL effector nuclease architectures.

Choose the TAL Effector-Nucleotide Targeter tool you would like to use. For information and tips on using the tools, check out the Help page:

TALEN Targeter

Design arrays for TAL Effector Nucleases to target your DNA sequence.

NOTICE: Off-target counting is now limited to sequences of 1000 bases or less. See the News section below for more details

TALEN Targeter (old version with design guidelines)

Design arrays for TAL Effector Nucleases to target your DNA sequence.

TAL Effector Targeter

Design a single TAL effector to target your DNA sequence. A newer, updated version of Single TALE Targeter.

Target Finder

Identify the best-scoring sites in a DNA sequence for a specified RVD sequence. This new tool combines the functions of the older TAL Effector Site Finder and Off-target Site Finder into one tool. **Update:** Now incorporates NH-G specificity.

Paired Target Finder

Identify the best-scoring sites in a DNA sequence for a pair of RVD sequences. Use to find potential off-targets for a TALEN. **Update:** Now incorporates NH-G specificity.

TALENT 2.0 has been published in the 2012 Nucleic Acids Research Webserver issue!

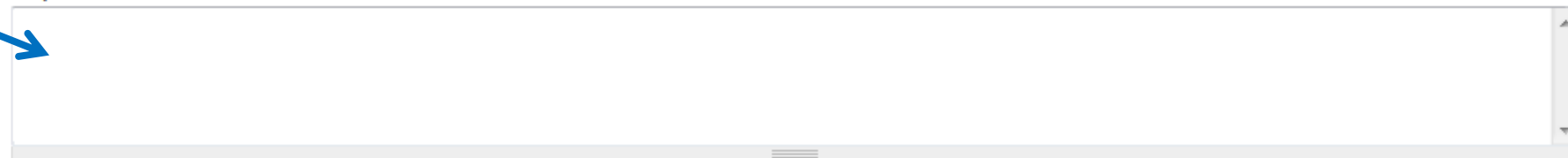
Doyle E.L., Booher, N.J., Standage, D.S., Voytas, D.F., Brendel, V.P., VanDyk, J.K., and Bogdanove, A.J. (2012) TAL Effector-Nucleotide Targeter (TALE-NT) 2.0: tools for

<https://tale-nt.cac.cornell.edu/>

Submit Old TALEN Targeter

Test the tool using sample data

Sequence



Enter up to 5000 total bases. E.g., you may have 1 sequence of 5k bases, 5 sequences with 1k bases, or any such combinations. Sequences should be in FASTA format and include a header line that starts with a '>'. For larger data sets, attach a file instead of pasting text in.

Sequence File

Files must be less than 2 MB.

Allowed file types: fasta.

Use a Preset Architecture Provide Custom Spacer/RVD Lengths

Preset Architecture

- None -
Cermak et al., 2011 (spacer 15-24, RVDs = 15-20)
Miller et al., 2011 (spacer 15-20, RVDs = 15-20)
Mussolino et al., 2011 (spacer 12-15, RVDs = 15-20)
Li et al., 2011 (spacer 16-31, RVDs = 15-20)

Select the base that should precede target sites on the 5' end.

Options

- Require C, G, or T at position 2 (not an A)
- Percent composition
- Do not allow sites to end in G
- Require A, C, or G at position 1 (not a T)
- Require a T at position N

Email

You can, but are not required to, enter an email address here to notify once your results are ready.

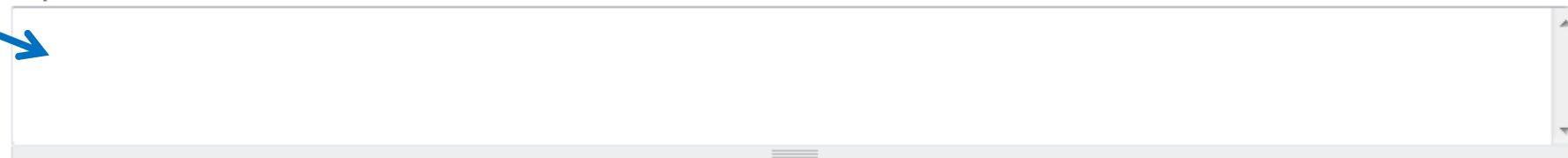
Expires *

How long your results will be available for downloading from the server. After this time, your results will be permanently deleted.

Submit Old TALEN Targeter

Test the tool using sample data

Sequence



Enter up to 5000 total bases. E.g., you may have 1 sequence of 5k bases, 5 sequences with 1k bases, or any such combinations. Sequences should be in FASTA format and include a header line that starts with a '>'. For larger data sets, attach a file instead of pasting text in.

Sequence File

Files must be less than 2 MB.

Allowed file types: fasta.

Use a Preset Architecture **Provide Custom Spacer/RVD Lengths**

Spacer

Minimum Spacer Length

Maximum Spacer Length

Repeat Array

Minimum Repeat Array Length

Maximum Repeat Array Length

Upstream Base *

Select the base that should precede target sites on the 5' end.

Options

- Require C, G, or T at position 2 (not an A)
- Percent composition
- Do not allow sites to end in G
- Require A, C, or G at position 1 (not a T)
- Require a T at position N

Email

You can, but are not required to, enter an email address here to notify once your results are ready.

Expires *

How long your results will be available for downloading from the server. After this time, your results will be permanently deleted.

TALEN靶点设计

Nucleic Acids Research Advance Access published November 29, 2012

Nucleic Acids Research, 2012, 1–8
doi:10.1093/nar/gks1144

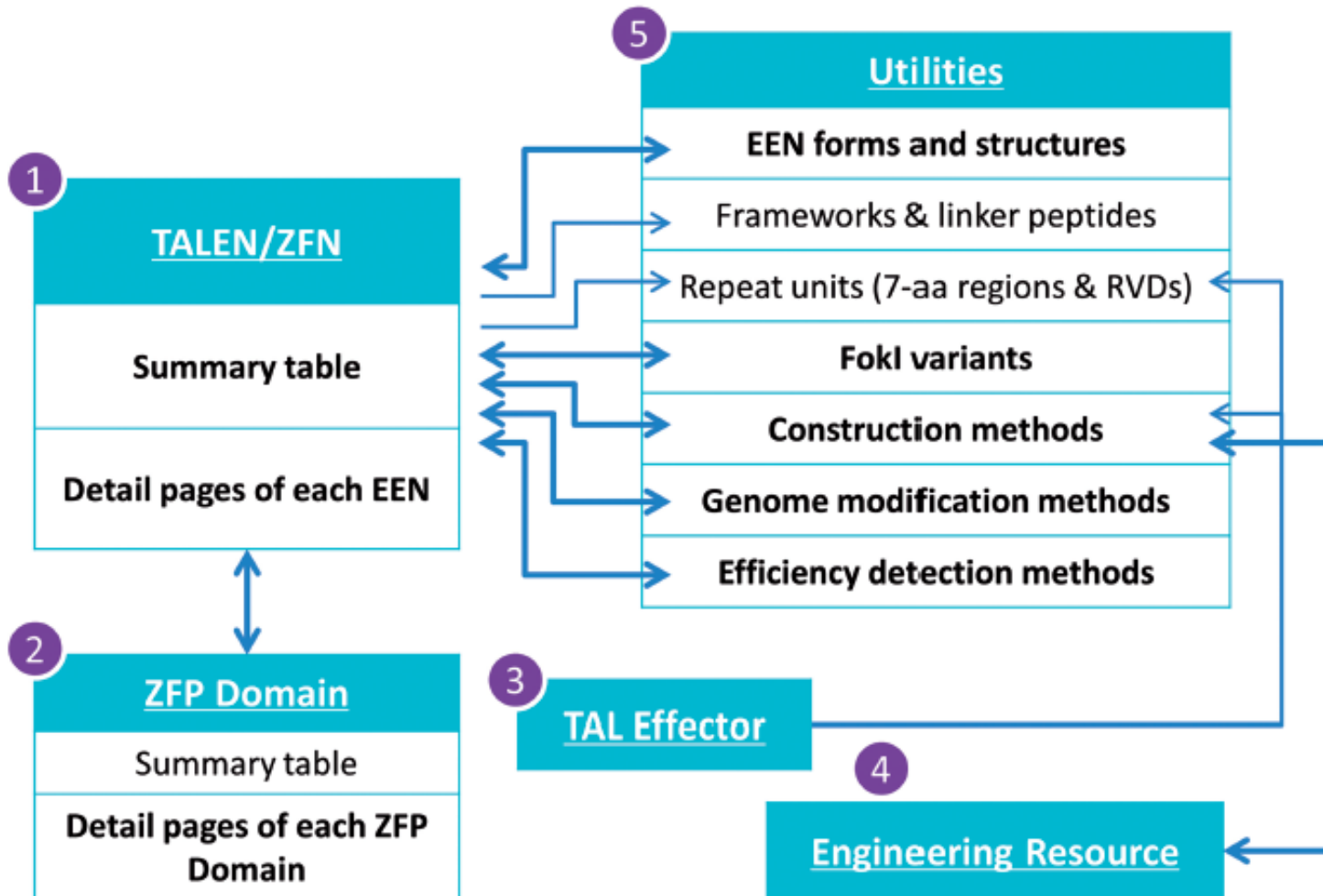
EENdb: a database and knowledge base of ZFNs and TALENs for endonuclease engineering

An Xiao¹, Yingdan Wu¹, Zhipeng Yang¹, Yingying Hu¹, Weiye Wang¹, Yutian Zhang¹, Lei Kong², Ge Gao², Zuoyan Zhu¹, Shuo Lin^{3,*} and Bo Zhang^{1,*}

¹Key Laboratory of Cell Proliferation and Differentiation of the Ministry of Education, ²State Key Laboratory of Protein and Plant Gene Research, Center for Bioinformatics, College of Life Sciences, Peking University, Beijing 100871, China and ³Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA

Received August 15, 2012; Revised October 14, 2012; Accepted October 24, 2012

TALEN靶点设计



TALEN靶点设计

A **EENdb - TALEN/ZFN**

Search TALENs/ZFNs targeting the sequence:
 ->3 nt (recommended >=6 nt), 5'>3', any strand
 - use [...] for alternate nucleotides, N = [ACGT]

Search TALENs/ZFNs targeting the gene(s):
 ->10 symbols or IDs (currently Ensembl ID only)
 - symbols are variable in publications and databases

Search EENs by first reported reference:
 ->10 PubMed IDs or surnames of 1st authors.

List by species

	TALEN+ZFN	TALEN (All 315)	ZFN (All 357)
(artificial)	94	46	48
(GFP)	80	68	12
butterfly (<i>Danone plexippus</i>)	1		1
cattle (<i>Bos taurus</i>)	1		1

C **EENdb - Utilities > FokI variants**

Summary

Click on the variant name to see its sequence

Variants	Mutations	Reference
WT (Wild Type)	(None)	157727 EENs with WT
EL+KK	EL: Q486E, I499L KK: E490K, I538K	17603475 EENs with EL+KK
D+R	D: R487D R: D483R	17603476 EENs with D+R
EA+KV	EA: Q486E, I499A KV: E490K, I538V	17603476 EENs with EA+KV
A+V DA+RV	A: I499A, V: I538V D: R487D, R: D483R	17603476

B

TALEN ID	Whole Site	Species & Locus	RVDs	Framework	FokI	Construc.	Modif.	Ref.	Effic.
TN0013	CAATTCGAGGCTGATTCGctggccatggactctctgAGCATGCGCTTCCGGSa ATAAACTGCGMOTAAAGaaccggtaactgaagaaCTGATAGCGAAAGGCGCT 17+(18)+16	zebrafish, hey2 <small>(hey2 (zebrafish))</small>	(std.)	+231	WT	GG(V&B)		21493687	
TN0033	TCTTCGCTTTTCACATCCaccacatcccaacagaggaAGCGGAAAGCAACATAAa aAAAGCAAAAGTATAAGTggtgaggttctctctgTCCGCTTCGCTGATTT 17+(18)+17	zebrafish, hey2 <small>(others)</small>	(others)	+63	WT	REAL	NHEJ	21822241	
TN0034	TGGCCCAATAgCTTCcagtcacgaacggcctgTAAGTTCTGCTCCTTt aGCAAGTTATCGAAAGctcagtcgctgaggaacCTGMAAAGCGAGMAAt 13+(17)+13	zebrafish, gr1a3e	(std.)	+63	WT	REAL	NHEJ	21822241	
TN0035	TGGCCCAATAgCTTCcagtcacgaacggcctgTAAGTTCTGCTCCTTt CGCCCTGCGCAAGctcagtcgctgaggaacCTGMAAAGCGAGMAAt 13+(17)+13	zebrafish, gr1a3e	(std.)	+63	WT	REAL	NHEJ	21822241	

ZFN ID	Whole Site	Species & Locus	ZFP Domain L	ZFP Domain R	Linker	FokI	Construc.	Modif.	Ref.	Effic.
ZN0014	gCCCGCAGGCTGCaagatGCACAGCAGGTTt CGAGCTGCGACGtgtctaCCTGTGCTCAAC 4F+(6)+4F	zebrafish, slc24e5	ZP10039	ZP10038	LRG5	EL+KK	Sangamo	NHEJ	18500334 <small>Dixon et al., Nat Biotechnol, 2010</small>	
ZN0015	gTTCATCCAGACCGtgtcttGTGAGCGTGGGCT cAAATGAGTCTGCGcaaaacACTGCAACCGSa 4F+(6)+4F	zebrafish, slc24e5	ZP10041	ZP10040, ZP10042	LRG5	EL+KK	Sangamo	NHEJ	18500334	
ZN0016	tCTCGACCTTAATGcaatgTACTCGGTCCTGc aAGCTGAGATTAgtttaaATAGCCAGAAAG 4F+(6)+4F	zebrafish, nr1e	ZP10043	ZP10044, ZP10045	LRG5	WT	Sangamo	NHEJ	18500334	

- A1** Choose search type

A2 List all EEN by species

B1 Links to detail page
- B2** Links to external database and shows the alias used in original article

B3 Links to related utility page
- C1** Links to sequence of FokI

C2 List EENs used these FokI variants

酶切位点查找



NEBcutter V2.0

[\[NEB homepage\]](#)

Local sequence file:

GenBank number:

or paste in your DNA sequence: *(plain or FASTA format)*

```
AGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCC
GCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGC
GAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGAC
```

The sequence is: Linear Circular

Enzymes to use: NEB enzymes
 All commercially available specificities
 All specificities
 All + defined oligonucleotide sequences
 Only defined oligonucleotide sequences
[\[define oligos\]](#)

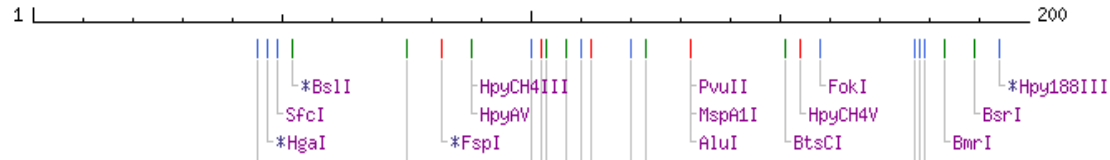
Minimum ORF length to display: a.a.

Name of sequence: *(optional)*

Earlier projects:
[no name](#)

*Note: Your earlier projects will be deleted 2 days after they were last accessed.
You need to have cookies enabled in your browser for this feature to work.*

酶切位点查找



Single cutters

unnamed sequence

Number of cuts = Sort order:

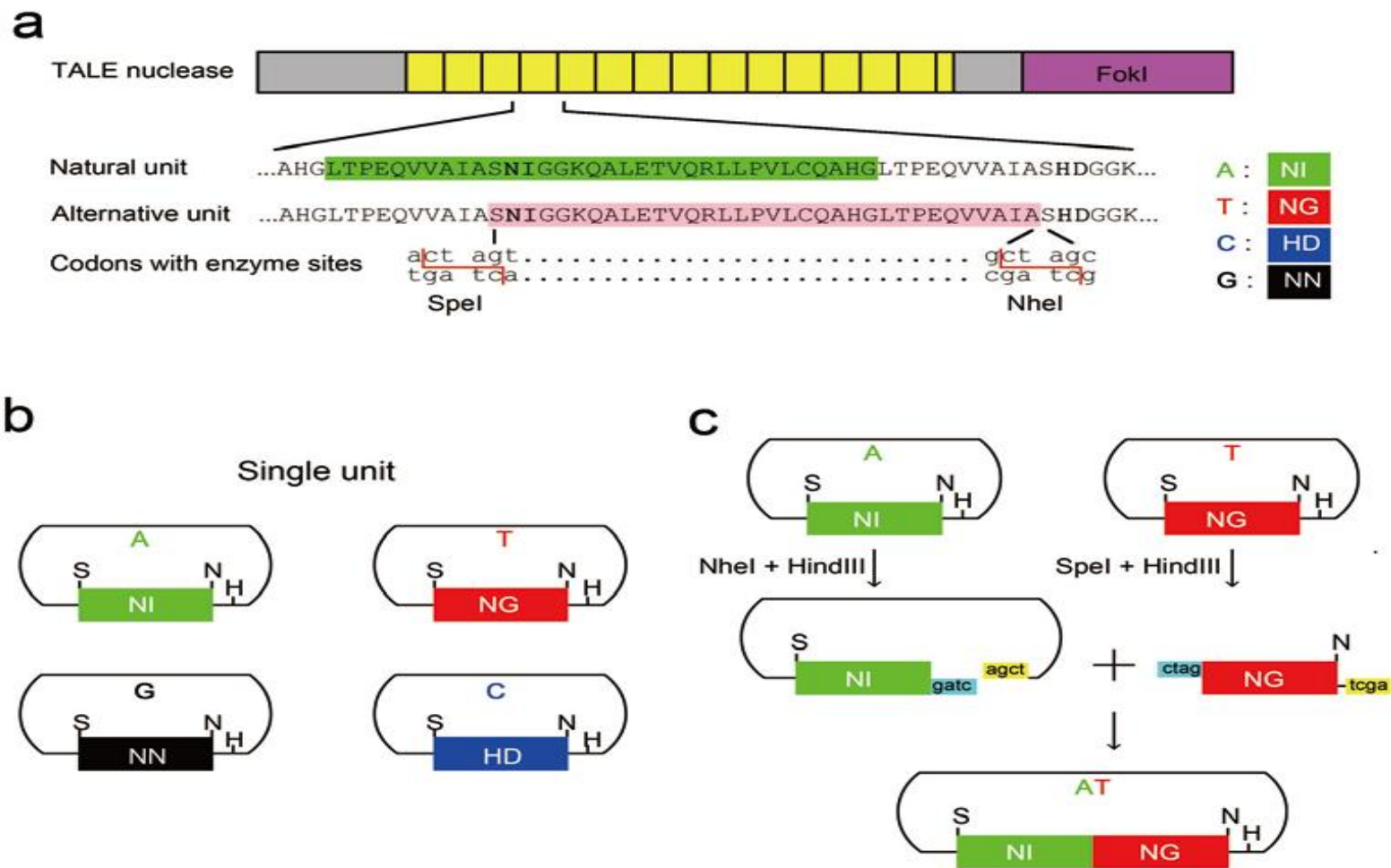
#	Enzyme	Specificity	Sites & flanks	Cut positions (bhunt - 5' ext. - 3' ext.)
1	AluI	AG ∇ CT	list	133
2	BfuCI	∇ GATC $_$	list	*101/105
3	BglI	GCCN $_$ NNN ∇ NGGC	list	*76/73
4	BmrI	ACTGGGNNNN $_$ N ∇	list	184/183
5	BsaJI	C ∇ CNNG $_$ G	list	179/183
6	BsiEI	CG $_$ RY ∇ CG	list	*104/102
7	BsiI	CCNN $_$ NNN ∇ NNGG	list	*53/50
8	BsmFI	GGGAC (N) $_{10}$ ∇ NNNN $_$	list	*46/50
9	BsrI	ACTG $_$ GN ∇	list	190/188
10	BssKI	∇ CCNGG $_$	list	#178/183
11	BstNI	CC ∇ W $_$ GG	list	180/181
12	BtsCI	GGATG $_$ NN ∇	list	152/150
13	DpnI	GA ∇ TC	list	*103
14	DpnII	∇ GATC $_$	list	#101/105
15	EarI	CTCTCN ∇ NNN $_$	list	*121/124

- Main options
- New DNA
- Custom digest
- View sequence
- ORF summary
- Save project
- Print

- List
- 0 cutters
- 1 cutters**
- All sites
- Save all sites
- Flanking enzymes

实验方法

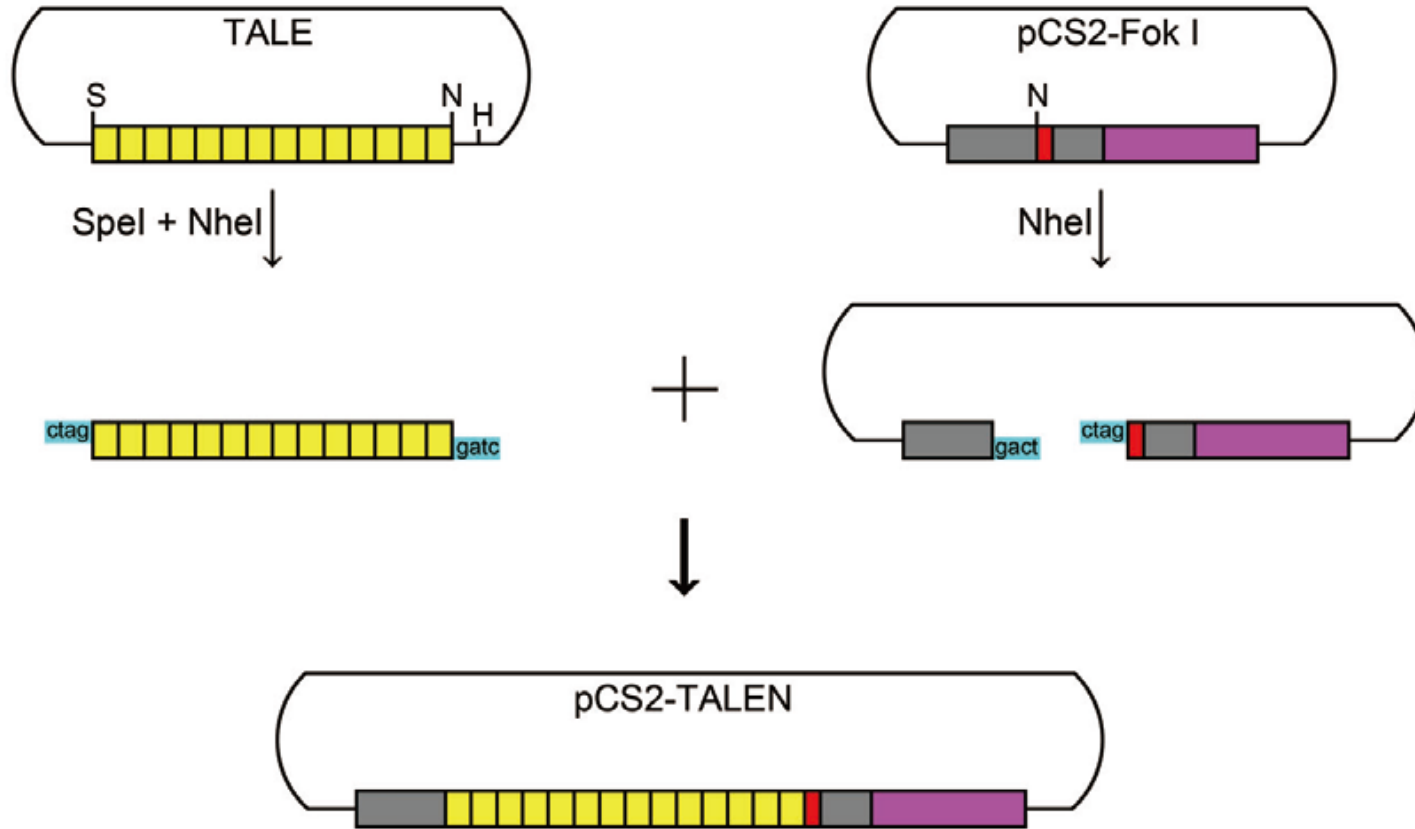
- Unit assembly



实验方法

- Unit assembly

d



质粒序列比对

Vector NTI - [DNA/RNA Molecule from document Clipboard]

File Edit View Primer Design Analyses Cloning Gel Analysis List Window Align Assemble Tools Help

Clipboard

General Description

Standard Fields

Comments

Annotations

Feature Map

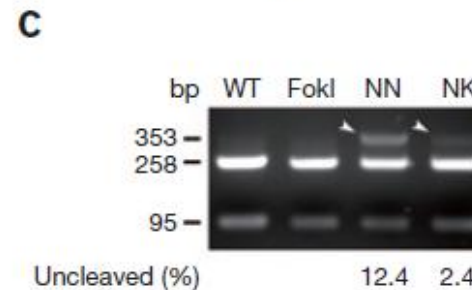
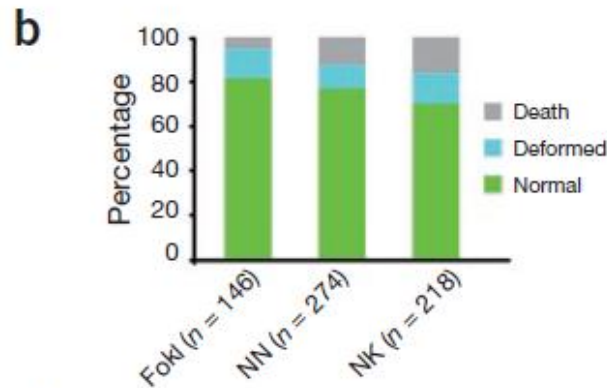
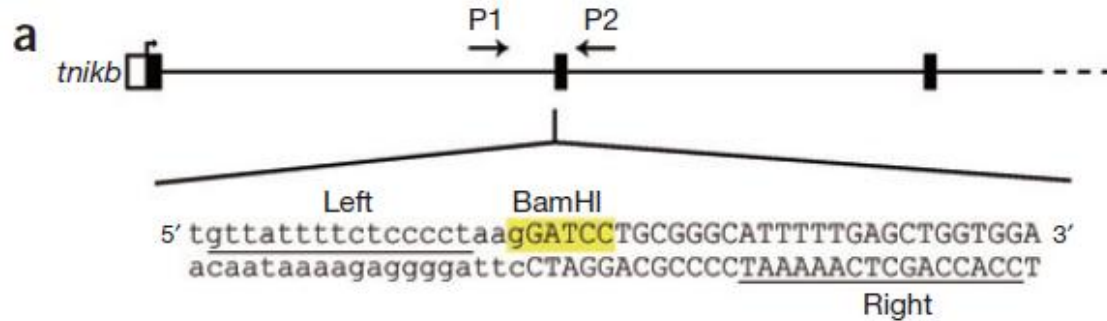
Restriction/Methylation Map

Clipboard
4457 bp

AseI (494) HindIII (776) ApeLI (1025) HindIII (1434) AseI (1447)

1	GCGACATGAC	ATGATTACGA	CTCGTCCTCG	GTTCCCGGCG	ATCCTCTGGA	GATTACTAGT	AATGGGGGTTG	GCAAACAGGC	TCTTGAGACT	GTCAGCGAC
	CGCTGTACTG	TACTAATGCT	GAGCAGGAGC	CAAGGGCCGC	TAGGAGACCT	CTAATGATCA	TTACCCCCAC	CGTTTGTCCG	AGAACTCTGA	CAAGTCGCTG
101	TACTACCAGT	TCTCTGTCAA	GCCCACGGAC	TTACCCCAGA	TCAAGTTGTA	GCGATTGCTA	GTAATATTGG	TGGCAAACAG	GCTCTTGAGA	CGGTCAGCG
	ATGATGGTCA	AGAGACAGTT	CGGGTGCCTG	AATGGGGTCT	AGTTCAACAT	CGCTAACGAT	CATTATAACC	ACCGTTTGTG	CGAGAACTCT	GCCAAGTCGC
201	CCTCCTTCCA	GTTCTCTGTC	AAGCCCACGG	ACTCACCCCA	GATCAAGTTG	TAGCGATTGC	TAGTAATGGG	GGTGGCAAAC	AGGCTCTTGA	AACCGTGCAA
	GGAGGAAGGT	CAAGAGACAG	TTCGGGTGCC	TGAGTGGGGT	CTAGTTCAAC	ATCGCTAACG	ATCATTACCC	CCACCGTTTG	TCCGAGAACT	TTGGCACGTT

TALEN效率检测



d

NK

tep17: ttattttctcccctaagG-----GCATTTTGGAGCTGGTGGGA (-9)

tep37: ttattttctcccctaagGAGAAAAATGCGGGCATT TTTGAGCTGG (-4, +8)

tep40: ttattttctcccc-----CTCC--CGGGCATT TTTGAGCTGGTGGGA (-11, +4)

NN

tep45: ttattttctcccctaagGAT---GCGGGCATT TTTGAGCTGGTGGGA (-3)

tep46: ttattttctcccctaagGATTCGGATGCTGCGGGCATT TTTGAGC (+7)

tep54: ttattttctcccctaagGATC-----ATTTTGGAGCTGGTGGGA (-8)

感谢：

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Thank you for your patience !