CRISPR/Cas在肿瘤研究中的应用

2017-09 杨兴林

yxl@oobio.com.cn

主要内容

OBiO® 和元上海

- CRISPR/Cas基本知识
- CRISPR/Cas与基因功能研究
- 基于CRISPR/Cas的高通量功能筛选
- CRISPR/Cas与动物肿瘤模型
- CRISPR/Cas与肿瘤治疗

CRISPR/Cas基本知识

CRISPR/Cas是一类基因组编辑工具的统称

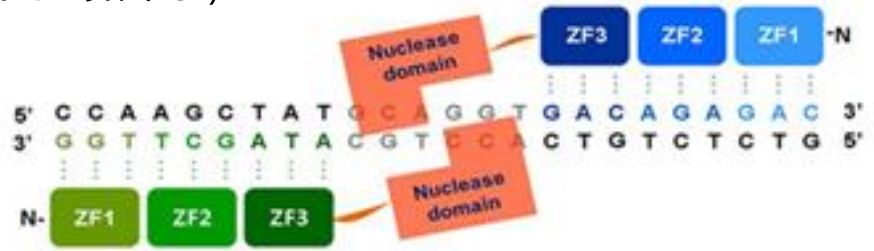
基因组编辑(Genome Editing)是一种可以按照人为设计永久性改变生物体内源基因组DNA编码的遗传学技术。可实现成体细胞基因组的遗传密码的永久性改变。

基因组编辑的历史

- ZFN(Zinc-Finger Nucleases锌指蛋白酶技术) 2002

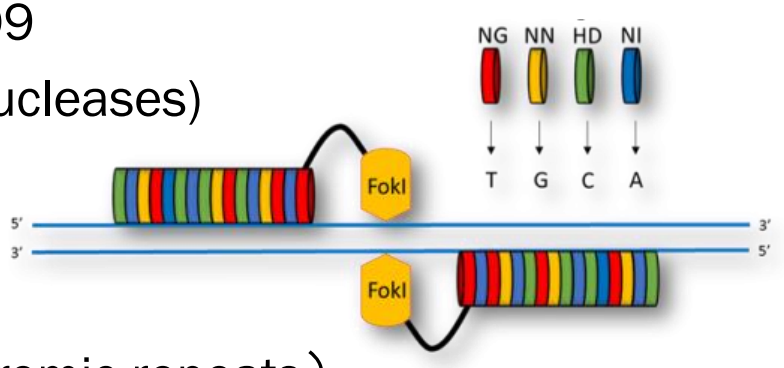
  Sangamo公司

  ZFN的开源方式(J. Keith Joung)



- TALEN(转录激活因子样效应物核酸酶) 2009

  (Transcription Activator-Like (TAL) Effector Nucleases)



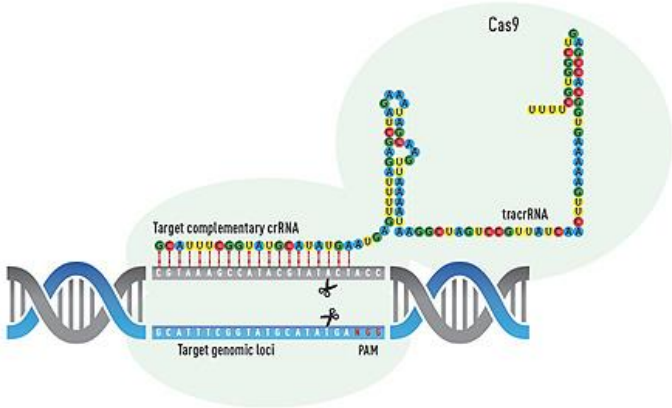
- CRIPSR/Cas 2011

  （Clustered regularly interspaced short palindromic repeats）

Jennifer Doudna

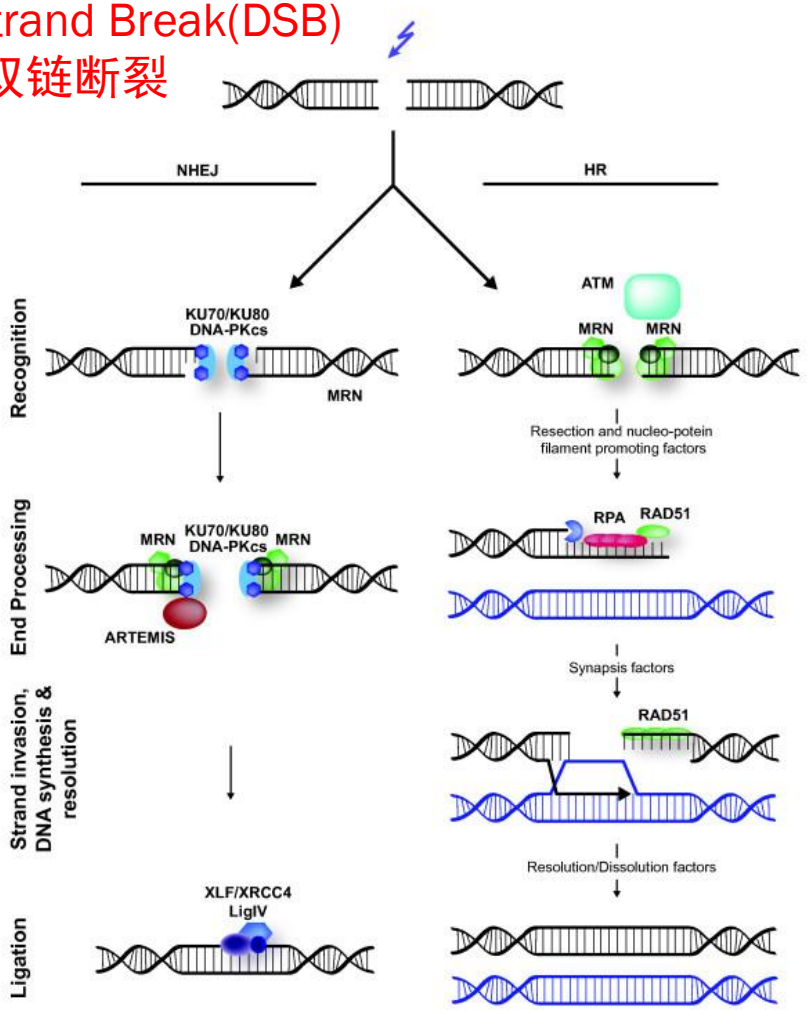
Emmanuelle Charpentier

Feng Zhang



基因组编辑的原理

Double-Strand Break(DSB)
双链断裂

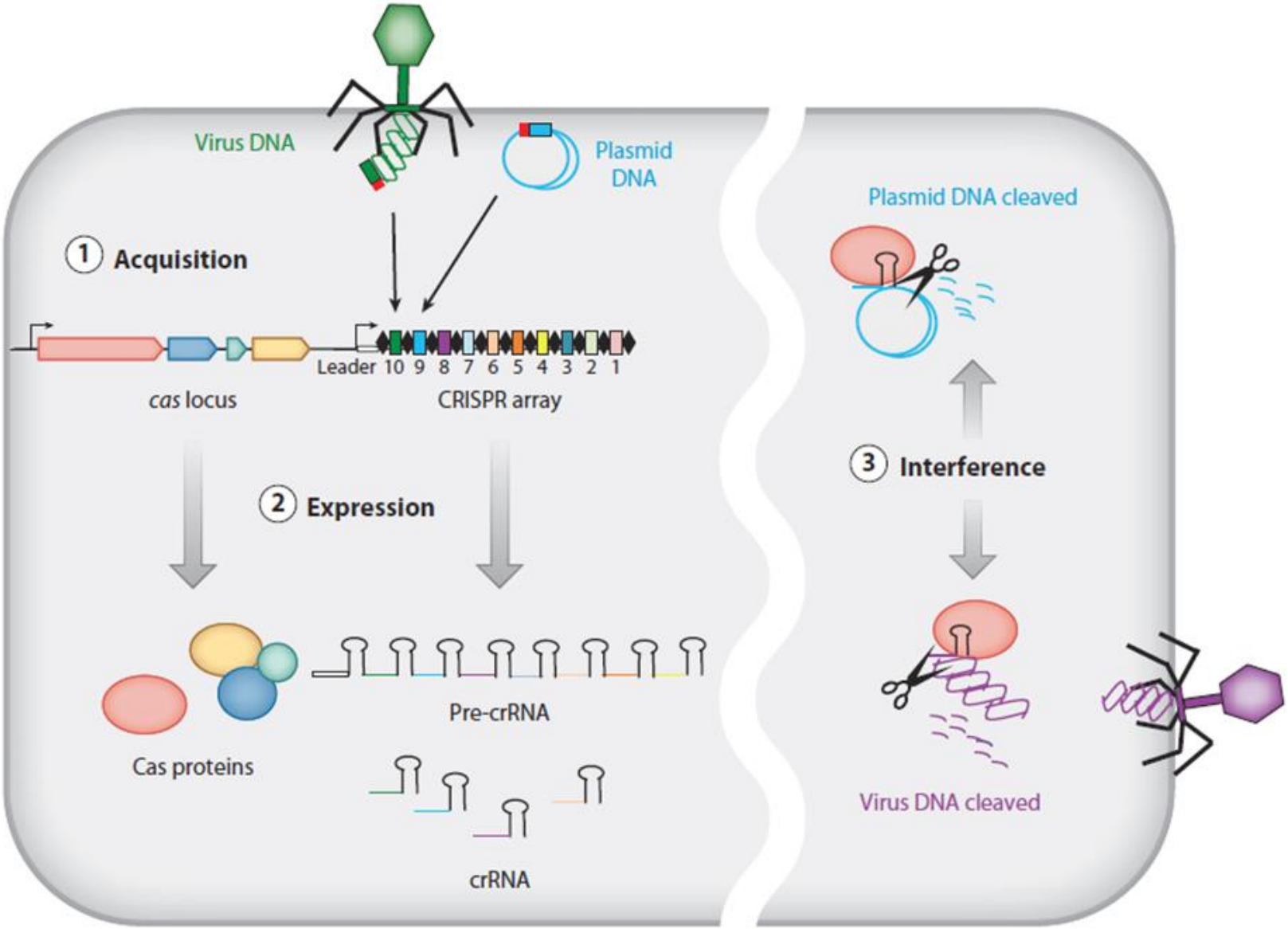


Non-Homologous End-Joining (NHEJ)
非同源重组末端连接

Homologous Recombination(HR)
同源重组

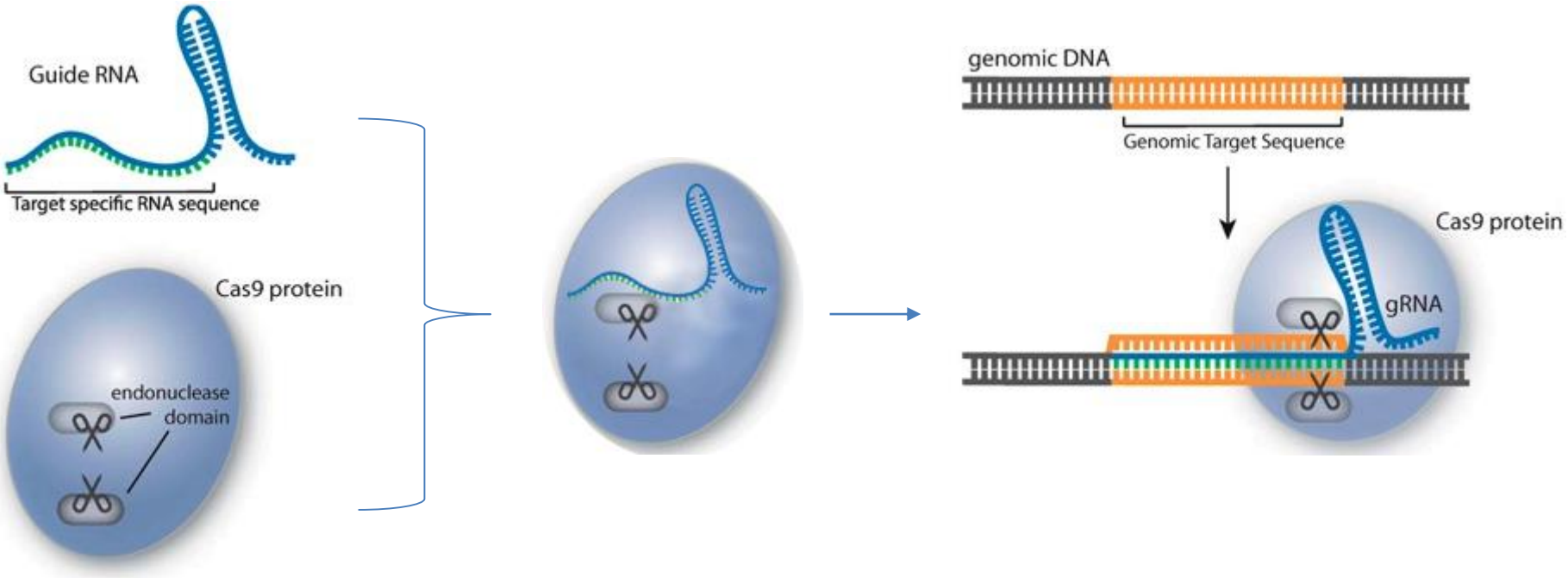
当前基因组编辑技术基于同源重组及非同源末端连接

CRISPR/Cas系统的起源



CRISPR–Cas system

CRISPR/Cas系统



两部分组成:

Cas 蛋白　　gRNA

最终目的:

实现对基因组特定序列的定点切割

双链断裂(DSB)

CRISPR/Cas切割原理

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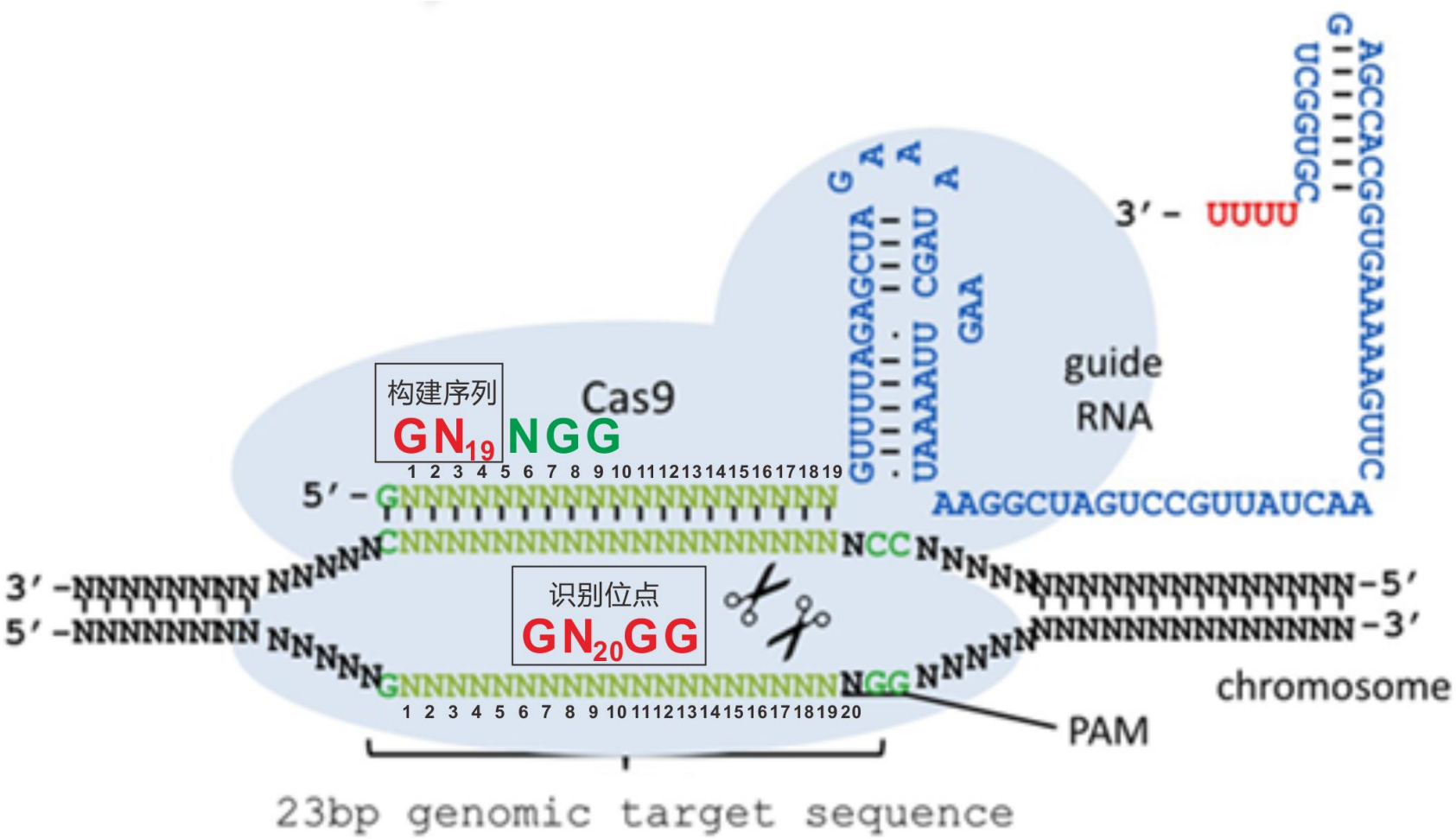
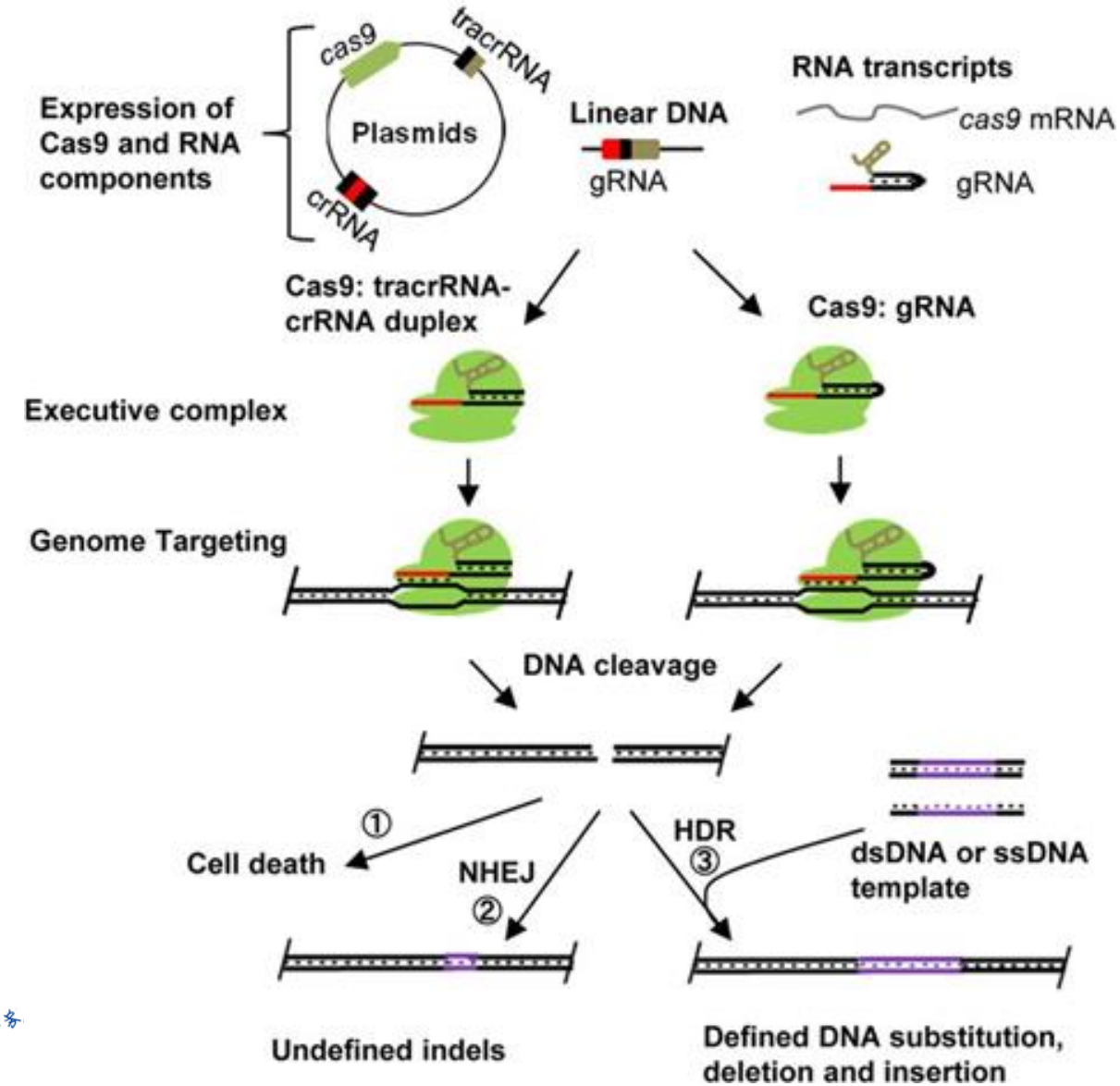


CRISPR/Cas基本原理



常见的CRISPR/Cas系统

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- spCas9, spyCas9 (Staphylococcus pyogenes,酿脓葡萄球菌)
  第一个Cas9, 基因~4.2kb长,识别靶点:$GN_{19}NGG$
- saCas9 ,sauCas9 (Staphylococcus aureus,金黄色葡萄球菌)
  基因~3.3kb长,识别靶点: $(G)N_{21}$ NNGRRT(N)
- Cpf1, AsCpf1, LbCpf1 (Francisella novicida,弗朗西丝菌)
  基因~4.0kb识别靶点: $TTNN_{24}$
- CjCas9 (Campylobacter jejuni ,空肠弯曲杆菌)
  基因~3kb长,识别靶点: $GN_{22}NNNNACAC$

常见的CRISPR/Cas系统活性比较

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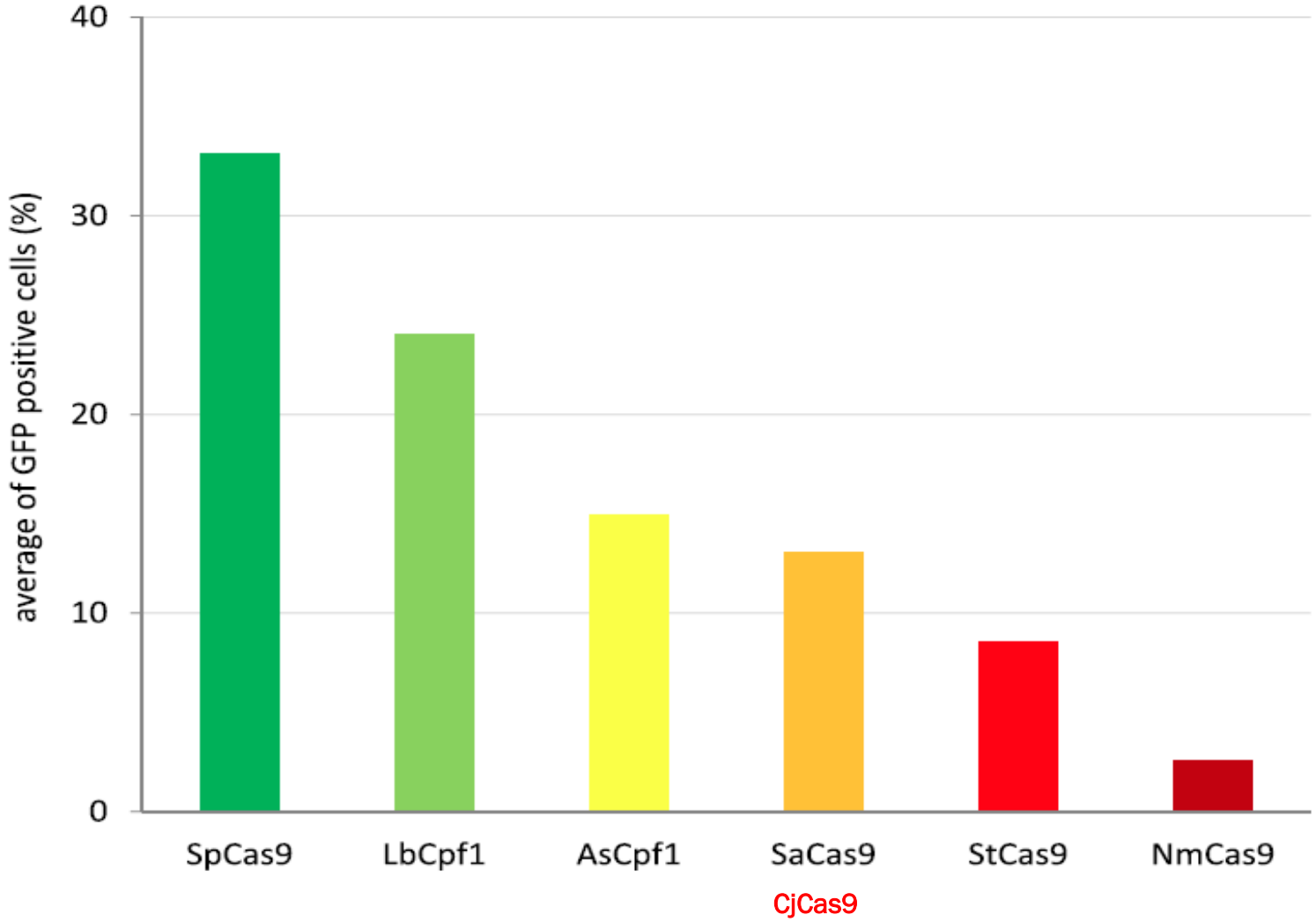
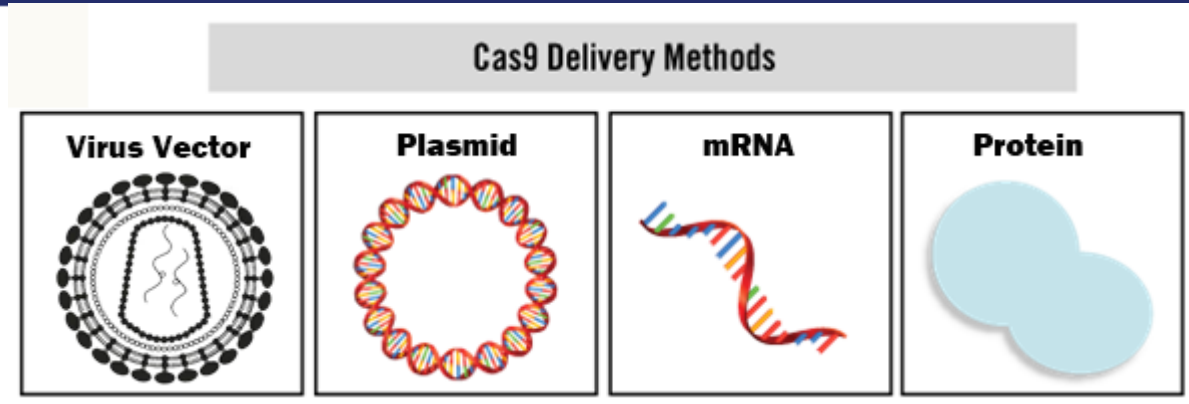
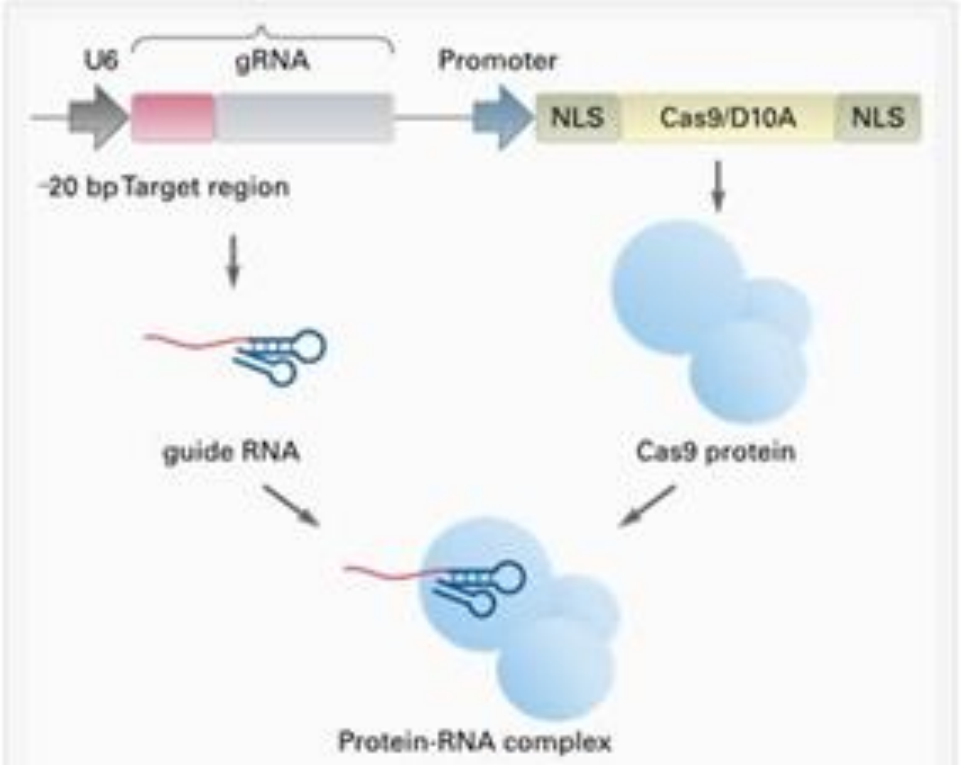
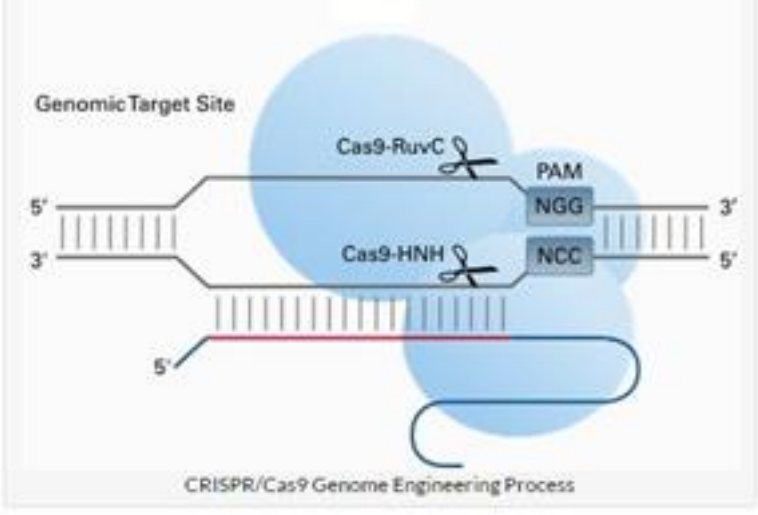


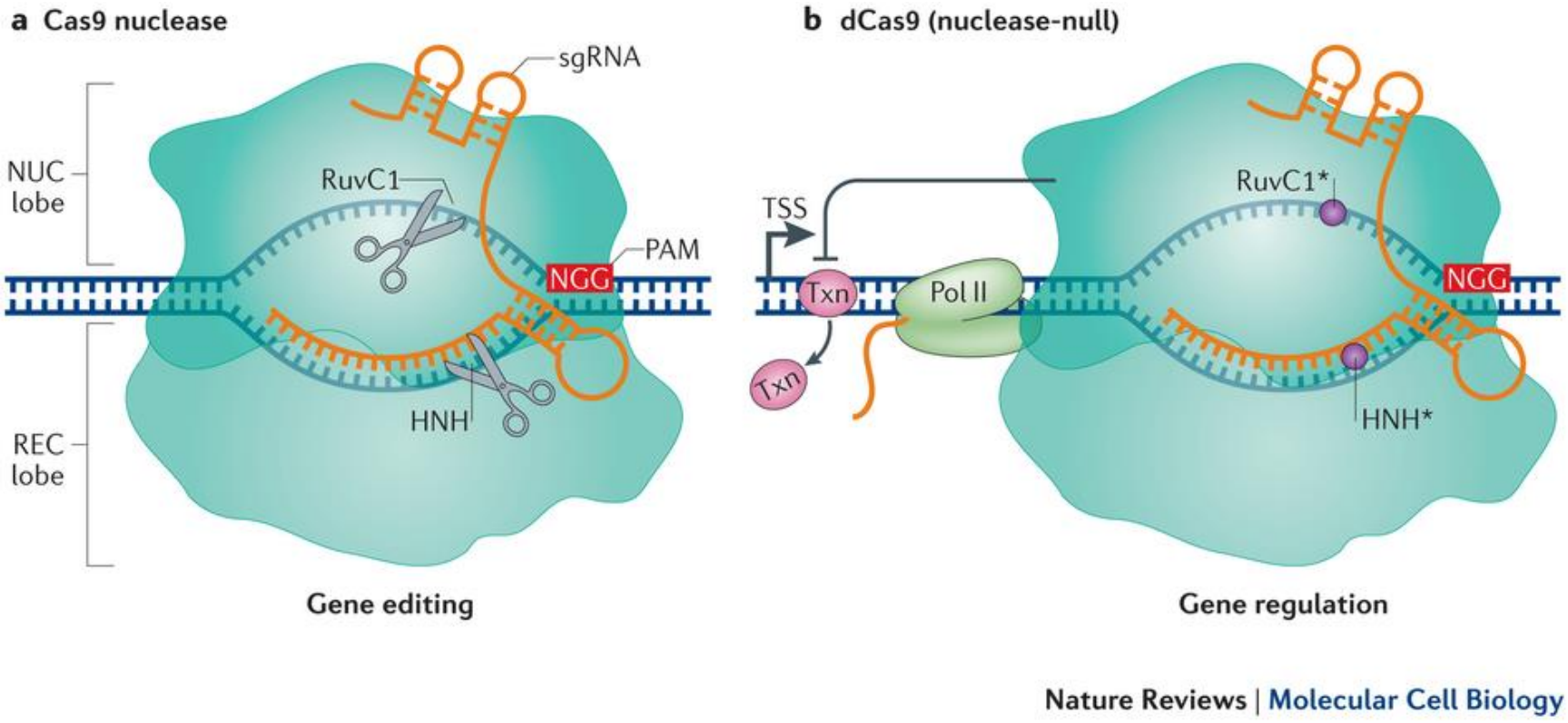
CRISPR/Cas系统的表达







CRIPSR/Cas系统转录调控



Nature Reviews | Molecular Cell Biology

CRIPSR/Cas系统转录调控

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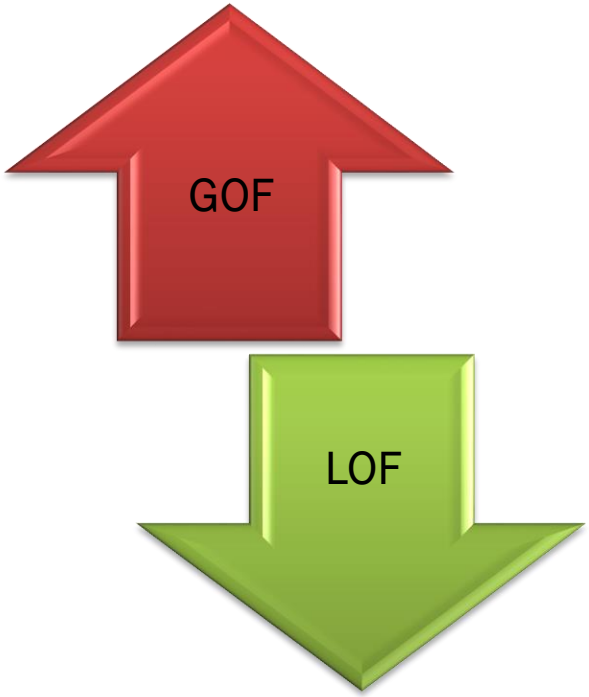
CRISPRi

CRISPRa

CRISPR/Cas与基因功能研究

基因功能研究手段



Gain-of-function(GOF):

OverExpression

CRISPRa

Loss-of-function(LOF):

KnockDown: RNAi, CRISPRi

KnockOut: CRISPR/Cas9, TALEN…

CRISPR/Cas可实现的功能

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- 通过引入Indels, 实现:
  - 编码蛋白基因的敲除(移码突变)
- microRNA的敲除(破坏Seed核心区)
- 大片段删除, 实现:
  - LncRNA的敲除
- 片段插入
  - 基因的敲入
  - LncRNA的转录终止
  - Tagging
- 精确的碱基修改
  - 点突变

肿瘤相关CRISPR/Cas研究案例

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Sci Rep. 2015 Mar 11;5:8997. doi: 10.1038/srep08997.

PIK3R1 negatively regulates the epithelial-mesenchymal transition and stem-like phenotype of renal cancer cells through the AKT/GSK3β/CTNNB1 signaling pathway.

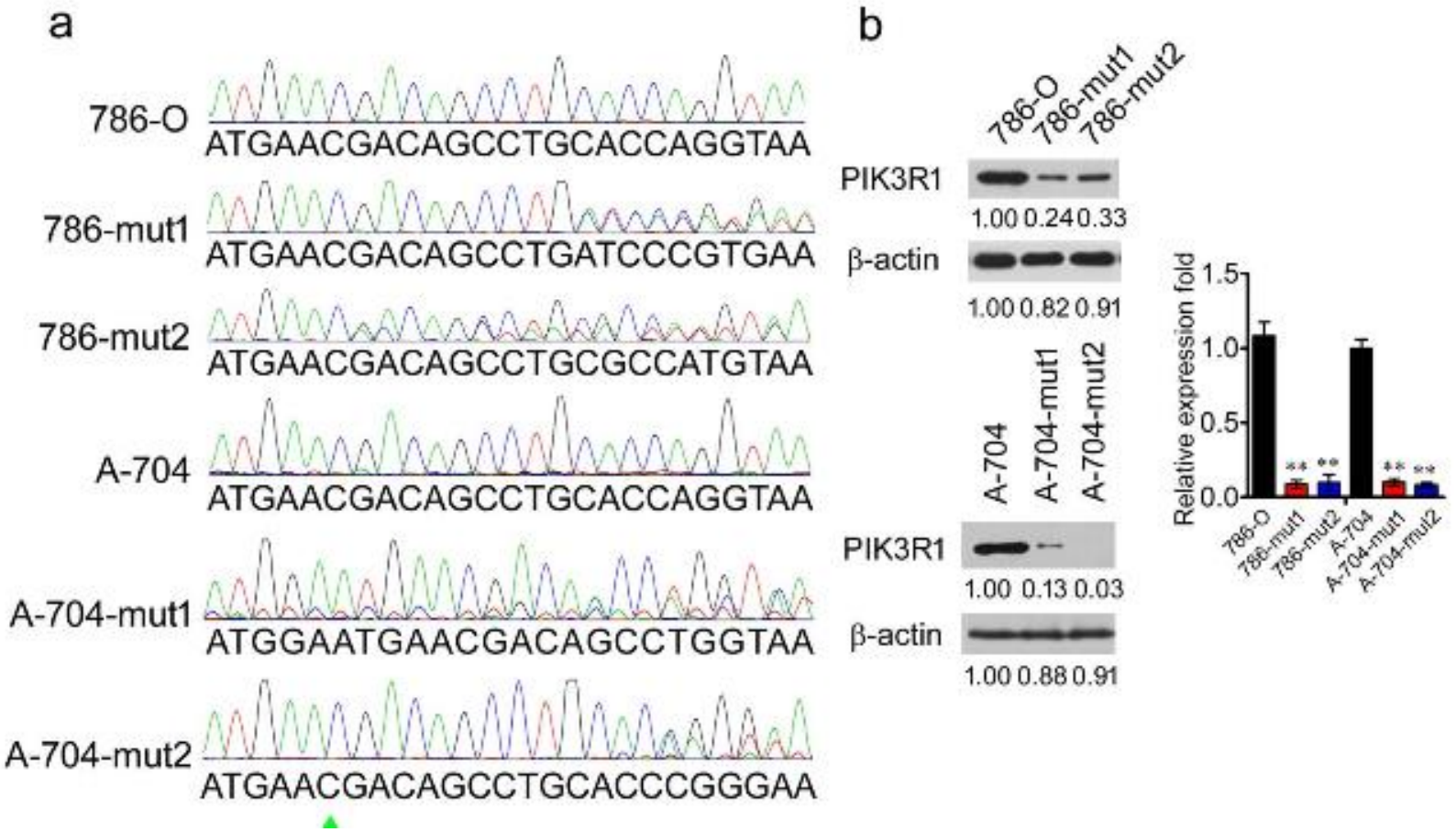
Lin Y[1], Yang Z[2], Xu A[1], Dong P[3], Huang Y[4], Liu H[5], Li F[5], Wang H[6], Xu Q[7], Wang Y[4], Sun D[5], Zou Y[1], Zou X[4], Wang Y[8], Zhang D[5], Liu H[5], Wu X[1], Zhang M[4], Fu Y[4], Cai Z[4], Liu C[1], Wu S[9].

⊕ Author information

Abstract

The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway has been identified as an important pathway in renal cell carcinoma (RCC). We have reported a nonsense mutation in PIK3R1, which encodes the regulatory subunit of PI3K, in a metastatic RCC (mRCC), while the mutation was absent in the corresponding primary RCC (pRCC). To identify the function of PIK3R1 in RCC, we examined its expression in normal kidney, pRCC and mRCC by immunohistochemistry and real-time polymerase chain reaction. The expression of PIK3R1 significantly decreased in pRCC and was further reduced in mRCC compared with normal tissue. Besides, its expression levels were negatively correlated with T-category of tumor stage. Additionally, 786-O and A-704 cells with PIK3R1 depletion introduced by CRISPR/Cas9 system displayed enhanced proliferation, migration and epithelial-mesenchymal transition (EMT), and acquired a stem-like phenotype. Moreover, the PIK3R1 depletion promoted the phosphorylation of AKT in the cells. The knockdown of AKT by shRNA reduced p-GSK3β and CTNNB1 expression in the cells, while the depletion of CTNNB1 impaired stem-like phenotype of the cells. Overall, PIK3R1 down-regulation in RCC promotes propagation, migration, EMT and stem-like phenotype in renal cancer cells through the AKT/GSK3β/CTNNB1 pathway, and may contribute to progression and metastasis of RCC.

在直肠癌细胞系敲除*PIK3R1*基因，证明*PIK3R1*基因具有调节直肠癌细胞的侵袭转移及肿瘤干细胞特性的功


a
786-O
ATGAACGACAGCCTGCACCAGGTAA
786-mut1
ATGAACGACAGCCTGATCCCGTGAA
786-mut2
ATGAACGACAGCCTGCGCCATGTAA
A-704
ATGAACGACAGCCTGCACCAGGTAA
A-704-mut1
ATGGAATGAACGACAGCCTGGTAA
A-704-mut2
ATGAACGACAGCCTGCACCCGGGAA
b
786-O
786-mut1
786-mut2
PIK3R1
1.00 0.24 0.33
β-actin
1.00 0.82 0.91
A-704
A-704-mut1
A-704-mut2
PIK3R1
1.00 0.13 0.03
β-actin
1.00 0.88 0.91
Relative expression fold
1.5
1.0
0.5
0.0
**
786-O
786-mut1
786-mut2
A-704
A-704-mut1
A-704-mut2

编码蛋白基因KO方式

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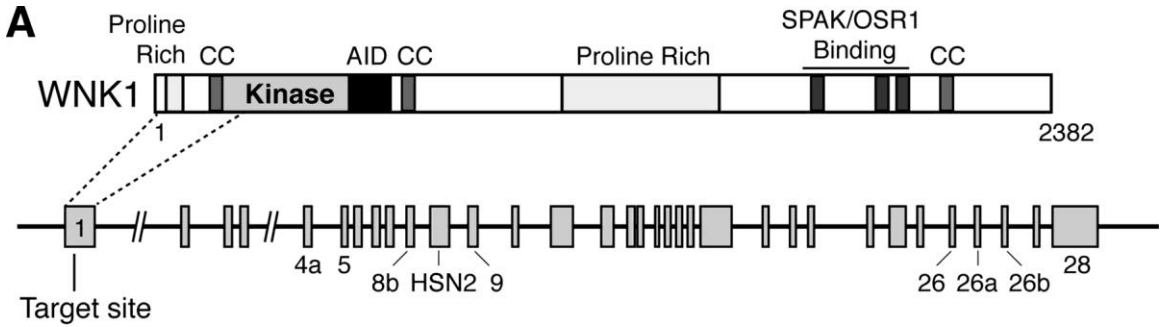
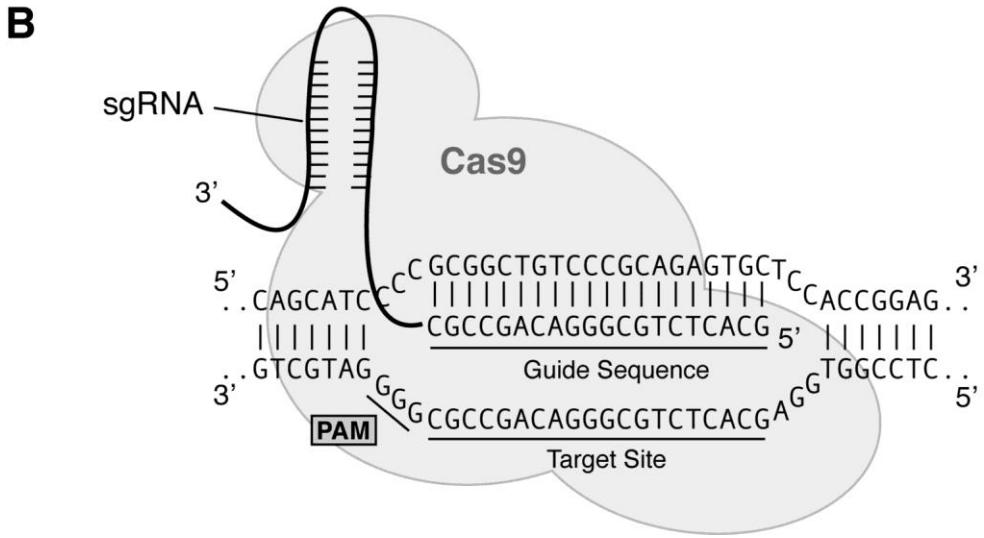
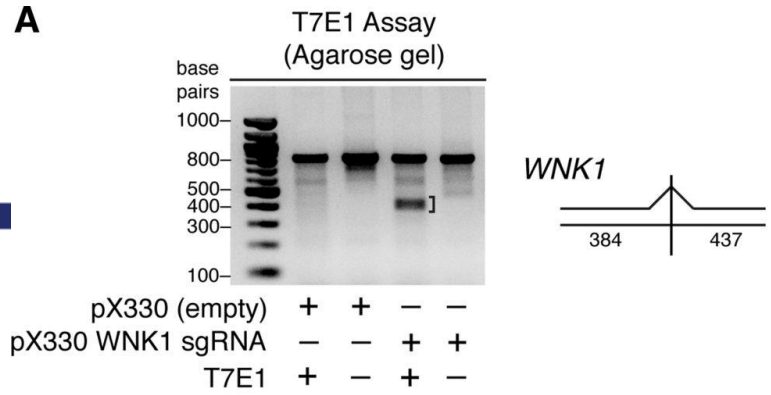
- 移码突变(1 gRNA)
- 靶向重要的蛋白结构域(1 gRNA)
- 切掉外显子(2 gRNAs)
- 大片段删除(2 gRNAs)

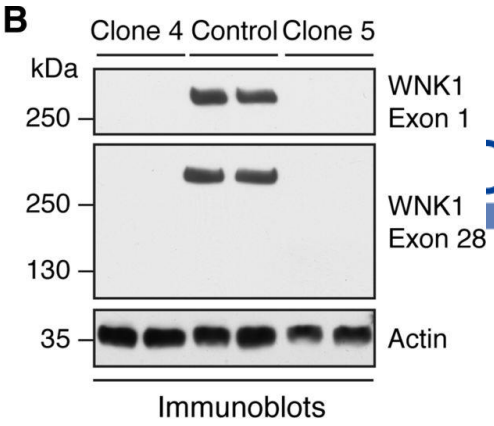
并不一定排斥

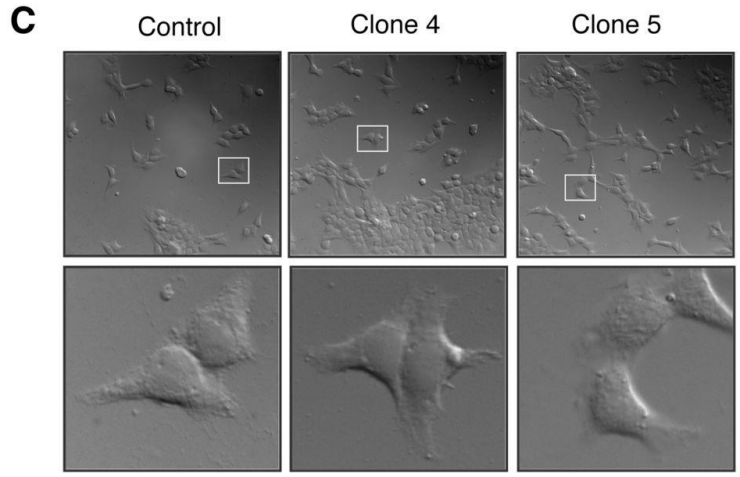
移码突变(1 gRNAs)

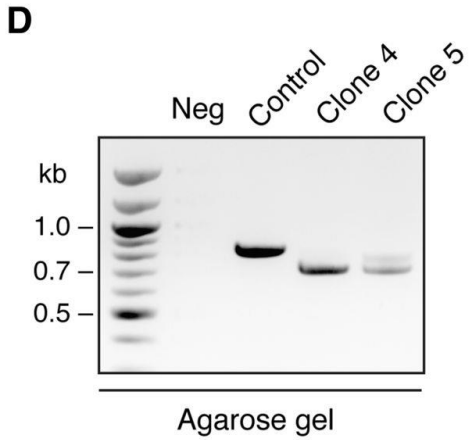

A
Proline Rich
CC
AID
CC
Proline Rich
SPAK/OSR1 Binding
CC
WNK1
Kinase
1
2382
1
4a
5
8b
HSN2
9
26
26a
26b
28
Target site


B
sgRNA
3'
Cas9
5'
3'
CAGCATCC
GCGGCTGTCCCGCAGAGTGCT
CACCGGAG
CGCCGACAGGGCGTCTCACG
5'
GTCGTAG
TGGCCTC
3'
5'
Guide Sequence
PAM
CGCCGACAGGGCGTCTCACG
Target Site









E

```
WT (NG_007984.2)  5736 CACCGCTTCTTCCGCCGGAGCGTCATCTGTGACTCCAATGCCACTGCACT 5785
Control HEK293T        CACCGCTTCTTCCGCCGGAGCGTCATCTGTGACTCCAATGCCACTGCACT
Mutant Allele 1        CACCGCTTCTT-----------------------------------------
Mutant Allele 2        CACCGCTTCTTCCGCCGGAGCGTCATCTGTGACTCCAATGCCACTGCACT

WT (NG_007984.2)  5786 GGAGCTTCCCGGCCTTCCTCTTTCCCTGCCCCAGCCCAGCATCCCCGCGG 5835
Control HEK293T        GGAGCTTCCCGGCCTTCCTCTTTCCCTGCCCCAGCCCAGCATCCCCGCGG
Mutant Allele 1        ------------------------------------------------
Mutant Allele 2        GGAGCTTCCCGGCCTTCCTCTTTCCCTGCCCCAGCCCAGCATCCCC----

WT (NG_007984.2)  5836 CTGTCCCGCAGAGTGCTCCACCGGAGCCCCACCGGGAAGAGACCGTGACC 5885
Control HEK293T        CTGTCCCGCAGAGTGCTCCACCGGAGCCCCACCGGGAAGAGACCGTGACC
Mutant Allele 1        ----------------CCACCGGAGCCCCACCGGGAAGAGACCGTGACC
Mutant Allele 2        ----------------------------ACCGGGAAGAGACCGTGACC
```

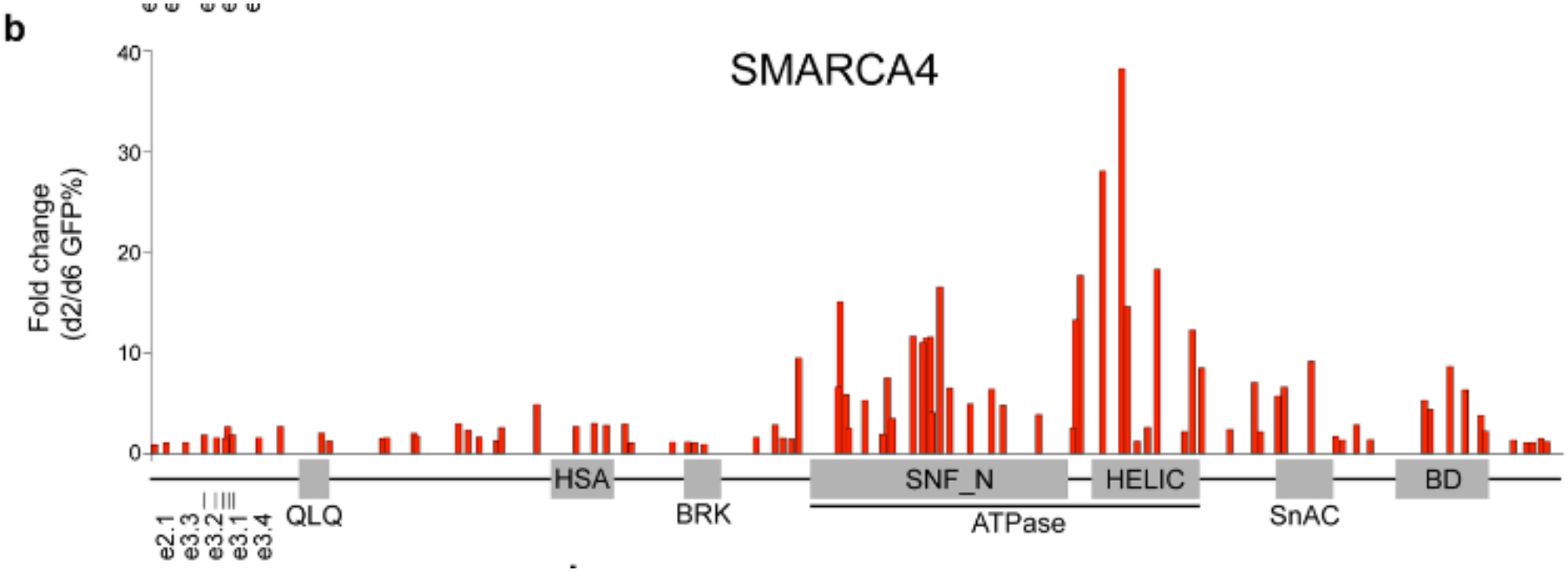
靶向重要的蛋白结构域(1 gRNAs)

Nat Biotechnol. 2015 Jun;33(6):661-7. doi: 10.1038/nbt.3235. Epub 2015 May 11.

Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains.

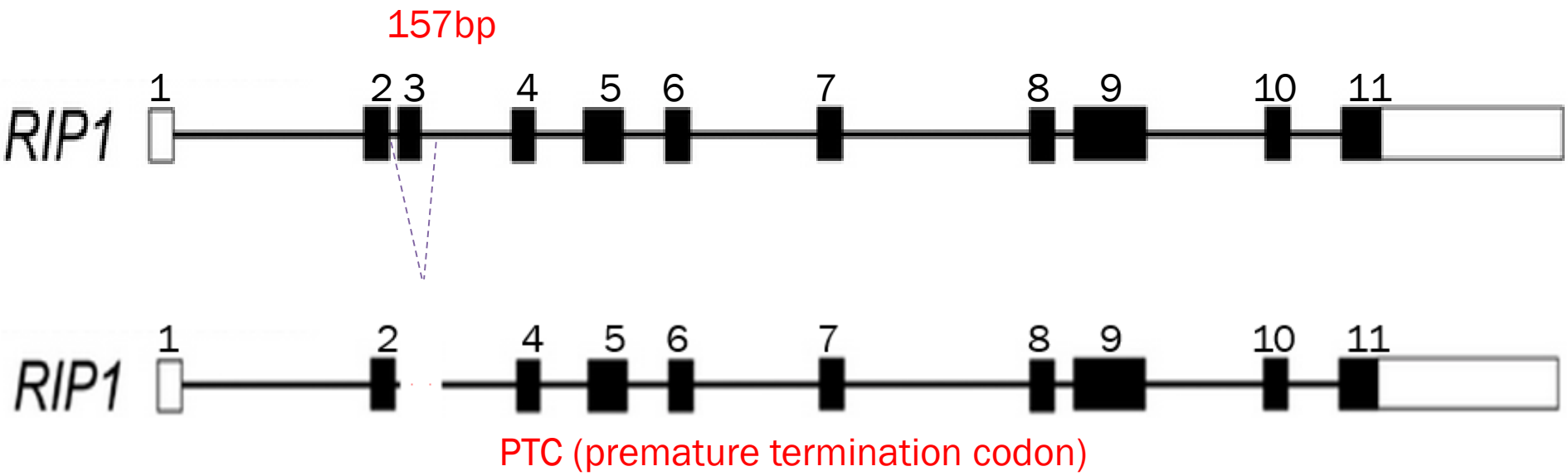
Shi J[1], Wang E[2], Milazzo JP[2], Wang Z[2], Kinney JB[2], Vakoc CR[2].

Author information

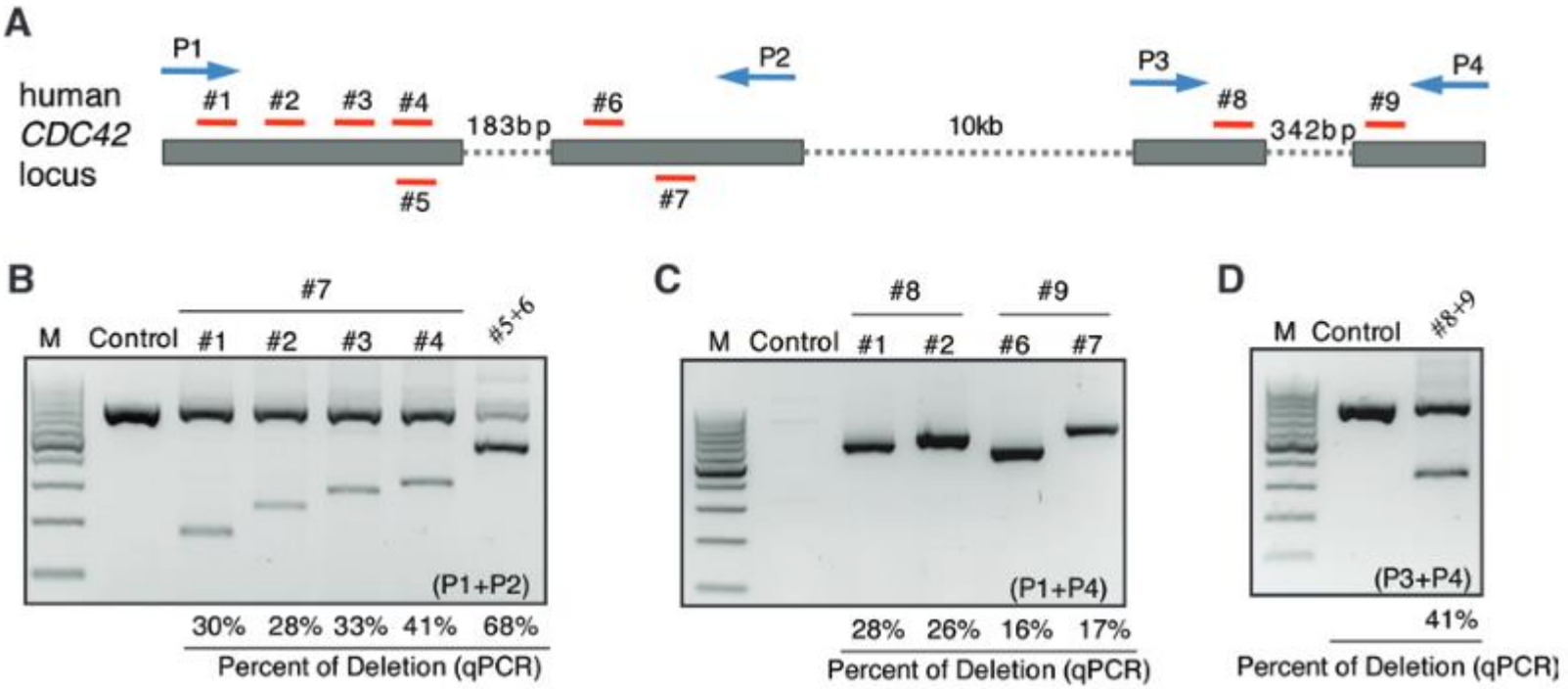


CRISPR mutagenesis of functional protein domains leads to a higher proportion of null mutations and an enhanced severity of negative selection

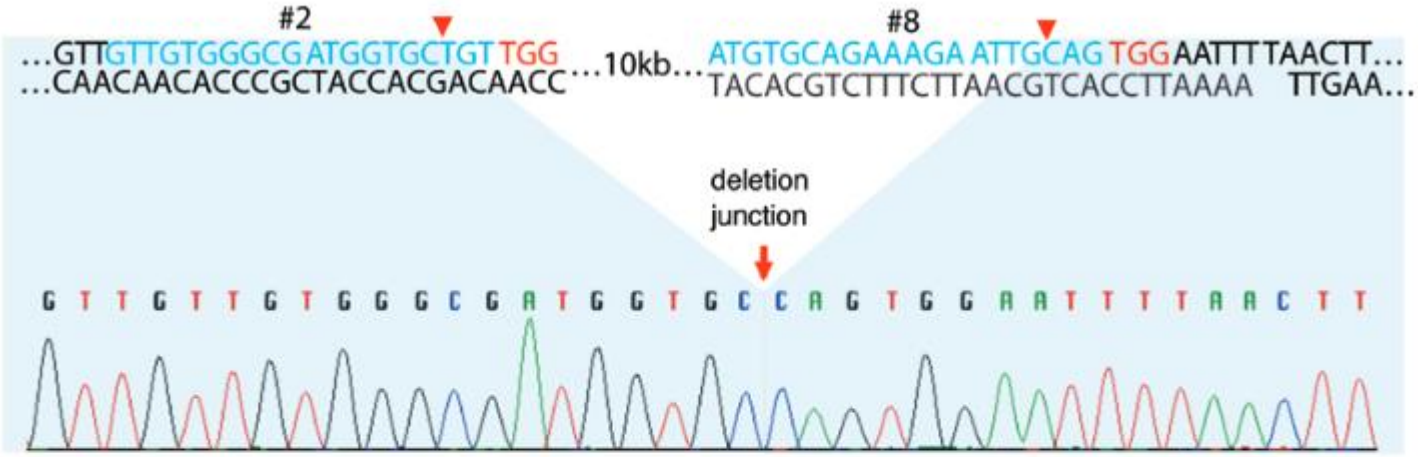
切掉外显子(2 gRNAs)



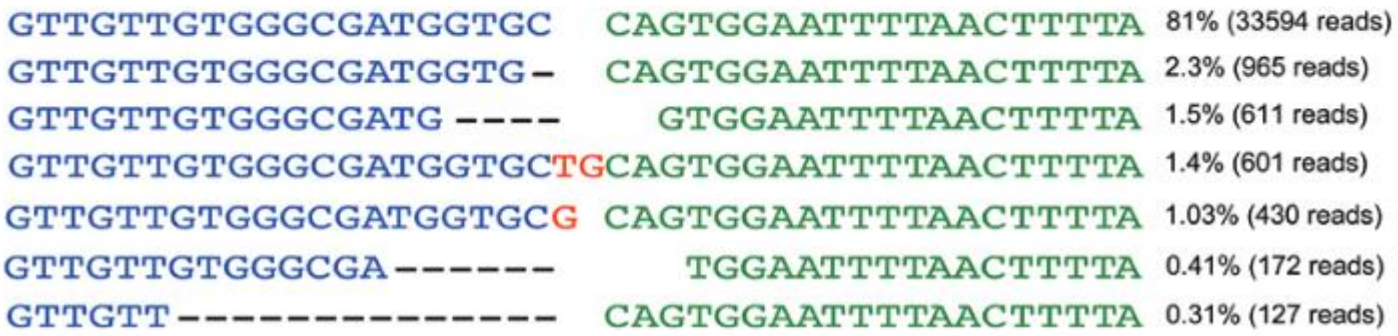
大片段删除(2 gRNAs)



E


#2
#8
…GTTGTTGTGGGCGATGGTGCTGTTGG…10kb…ATGTGCAGAAAGAATTGCAGTGGAATTTTAACTT…
…CAACAACACCCGCTACCACGACAACC TACACGTCTTTCTTAACGTCACCTTAAAA TTGAA…
deletion
junction
G T T G T T G T G G G C G A T G G T G C C A G T G G A A T T T T A A C T T

F

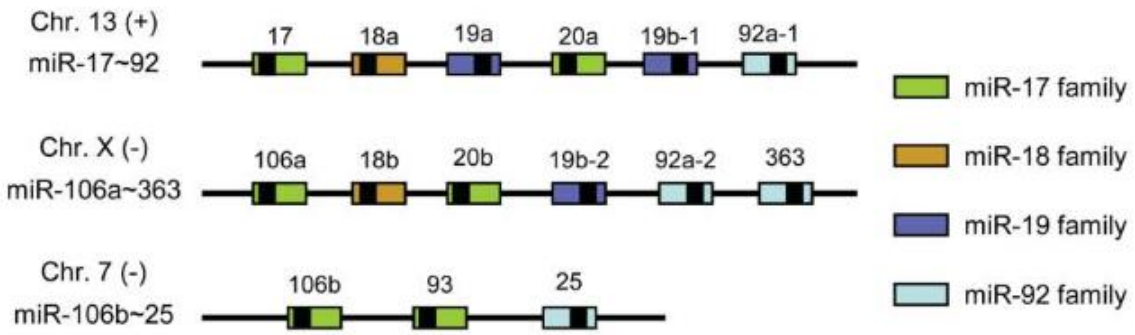

GTTGTTGTGGGCGATGGTGC CAGTGGAATTTTAACTTTTA 81% (33594 reads)
GTTGTTGTGGGCGATGGTG– CAGTGGAATTTTAACTTTTA 2.3% (965 reads)
GTTGTTGTGGGCGATG ---- GTGGAATTTTAACTTTTA 1.5% (611 reads)
GTTGTTGTGGGCGATGGTGCTGCAGTGGAATTTTAACTTTTA 1.4% (601 reads)
GTTGTTGTGGGCGATGGTGCG CAGTGGAATTTTAACTTTTA 1.03% (430 reads)
GTTGTTGTGGGCGA------ TGGAATTTTAACTTTTA 0.41% (172 reads)
GTTGTT---------- CAGTGGAATTTTAACTTTTA 0.31% (127 reads)

miRNA敲除主要方案

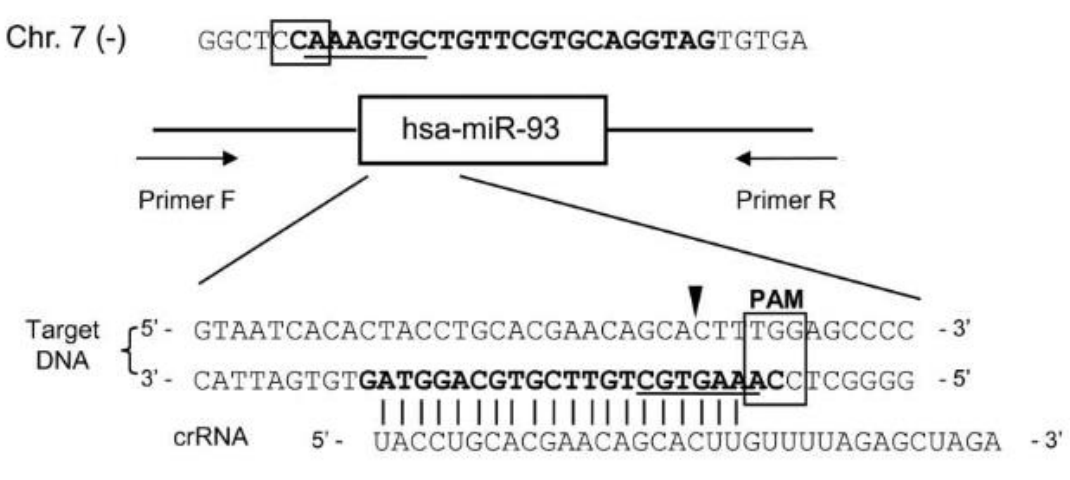
OBiO® 和元上海

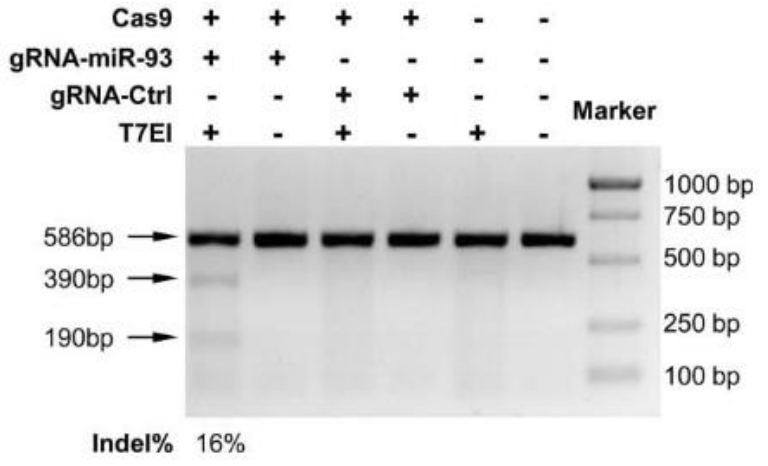
- 针对Seed区
- 前体(pre-miRNA) 区域删除
- 靶向miRNA加工位点

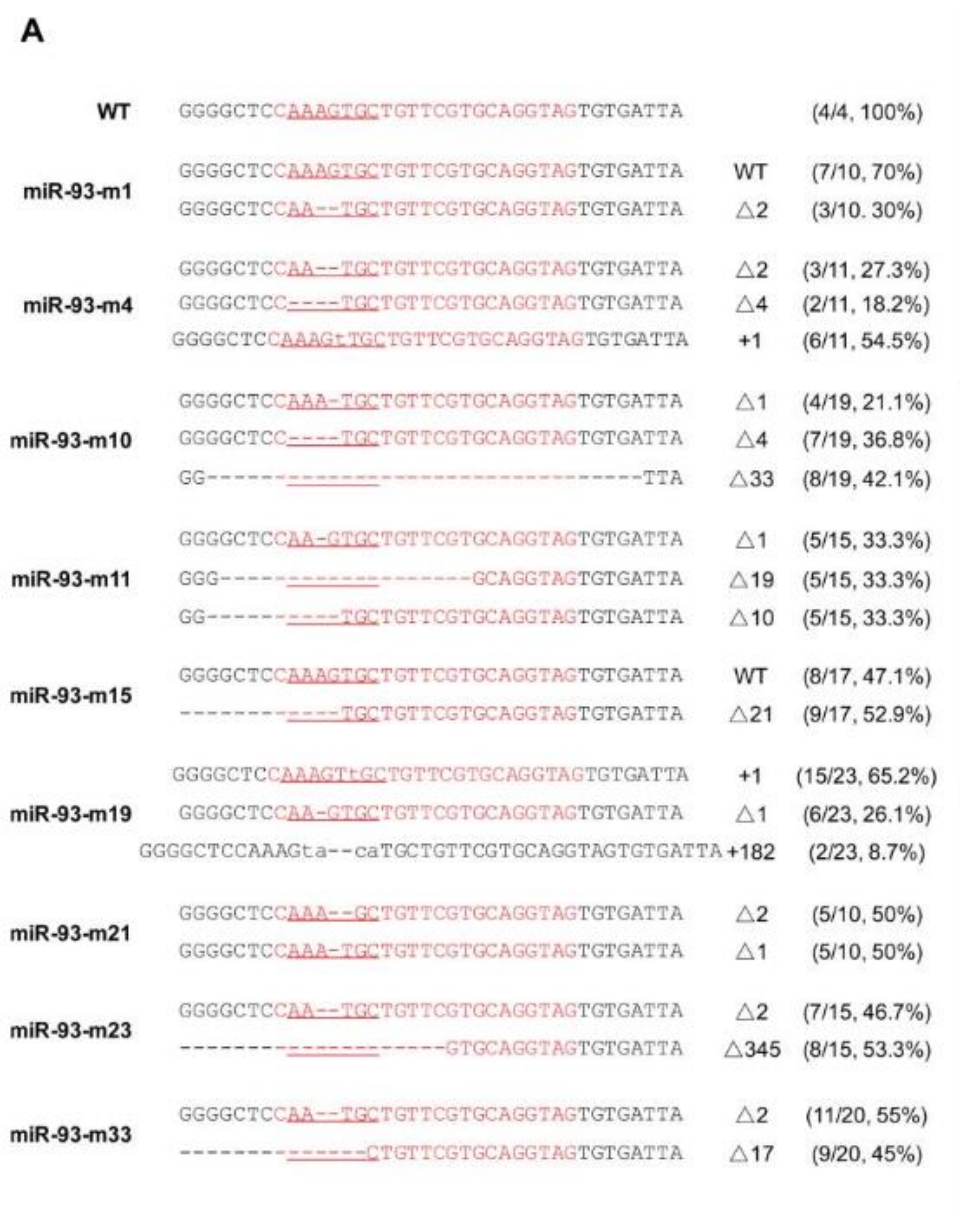
针对Seed区



miR-17	CAAAGUGCUUACAGUGCAGGUAG
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG
miR-20b	CAAAGUGCUCAUAGUGCAGGUAG
miR-106a	AAAAGUGCUUACAGUGCAGGUAG
miR-106b	UAAAGUGCUGACAGUGCAGAU
miR-93	CAAAGUGCUGUUCGUGCAGGUAG



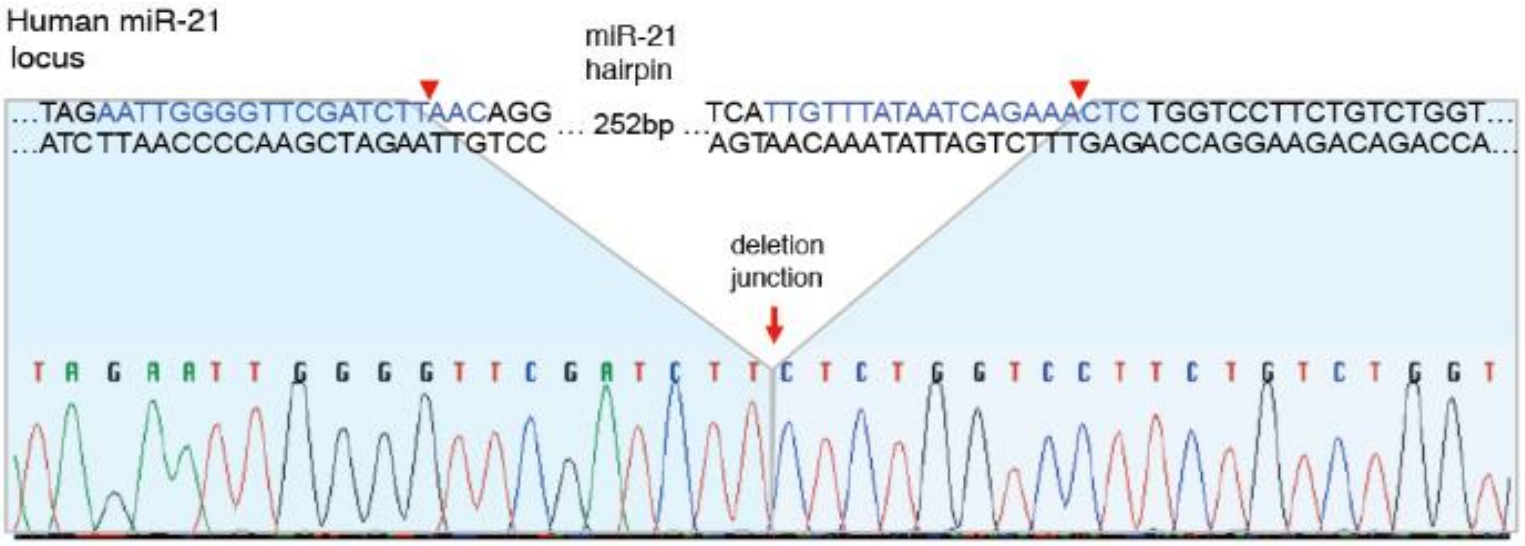


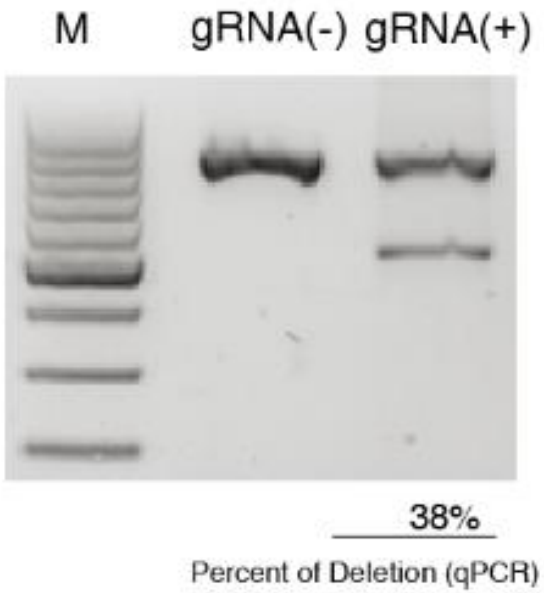


前体(pre-miRNA) 区域删除

miR-21 locus

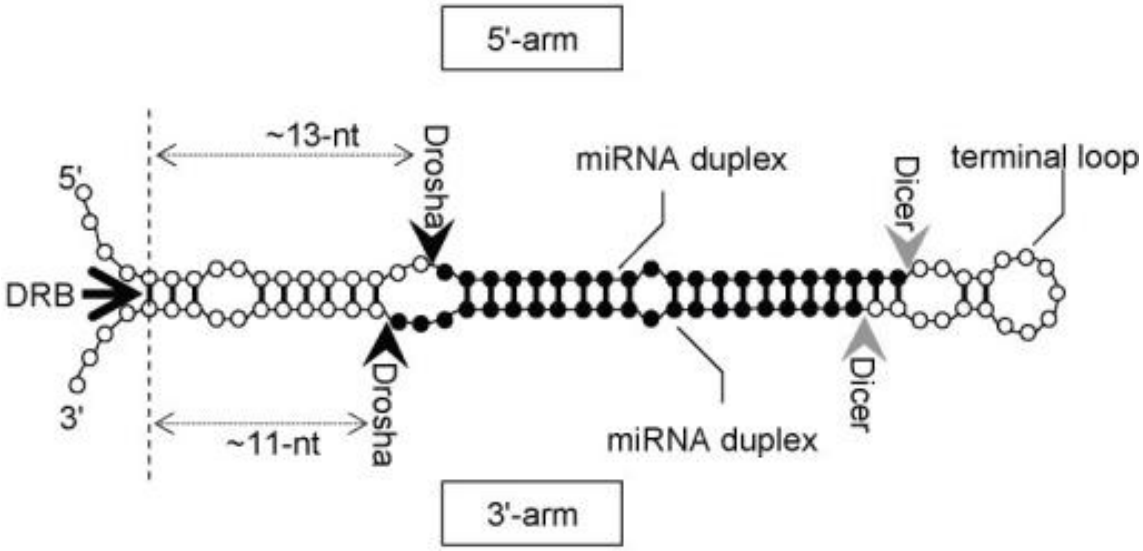
#1 AATTGGGGTTCGATCTTAAC #2 TTGTTTATAATCAGAAACTC

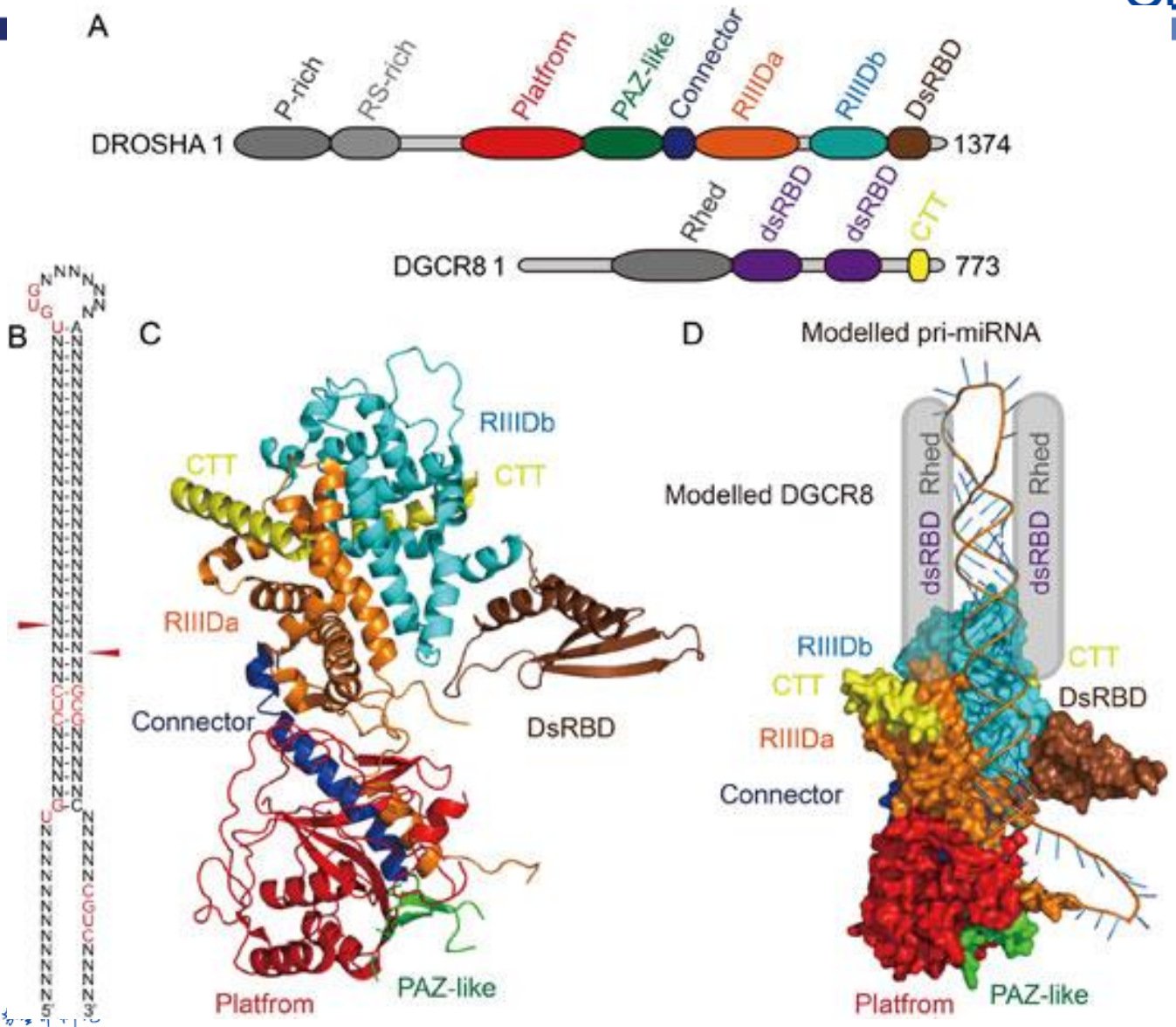




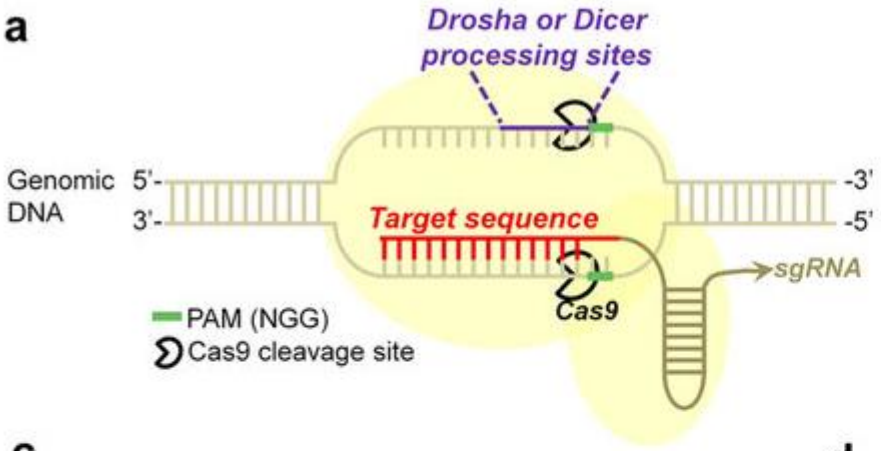
TAGAATTGGGGTTCGATCTT CTCTGGTCCTTCTGTCTGGT	73% (4100 reads)
TAGAATTGGGGTTCGATCT---- GGTCCTTCTGTCTGGT	3.8% (216 reads)
TAGAATTGGGGTTCGATCT-CTCTGGTCCTTCTGTCTGGT	2.8% (160 reads)
TAGAATTGGGGTTCGATCTTACTCTGGTCCTTCTGTCTGGT	1.8% (100 reads)
TAGAATTGG---------- GGTCCTTCTGTCTGGT	1.3% (76 reads)
TAGAATTGGGGTTCGAT -- CTCTGGTCCTTCTGTCTGGT	1.2% (65 reads)
TAGAATTGGGGTTCGAT --------- CTTCTGTCTGGT	0.08% (46 reads)

靶向miRNA加工位点





a



b

miR-17 stem loop

```
          GA        --CA          A  G      G  --  AU
5'- GUCA  AUAAUGU    AAGUGCUU  CA  UGCAG  UAG  UG   A
    ||||  |||||||    ||||||||  ||  ||||||  |||  ||   U
3'- CAGU  UAUUACG    UUCACGGA  GU  ACGUC  AUC  AC   G
      GG        AUG          A  G      --  U  GU
```

sgRNA-miR-17-1
5'-TGTCAAAGTGCTTACAGTGC-3' (5'- Dicer processing site)
sgRNA-miR-17-2
5'-TGAAGGCACTTGTAGCATTA-3' (3'- Drosha processing site)

c

miR-200c stem loop

```
      CU      --        A          U   U  GGU
5'- CC   CGUC  UUACCC  GCAGUGUU  GGG  GC    U
    ||   ||||  ||||||  ||||||||  |||  ||
3'- GG   GUAG  AAUGGG  CGUCAUAA  CUC  UG    G
      AG      U       C          U  --  AGG
```

sgRNA-miR-200c-1
5'-ATACTGCCGGGTAATGATGG-3' (3'- Drosha processing site)

```
3'- GG   GUAG  AAUGGG  CGUCAUAA  CUC  UG    G
      AG      U       C          U  --  AGG
```

sgRNA-miR-200c-2
5'-CTAATACTGCCGGGTAATGA-3' (3'- Drosha processing site)

d

miR-141 stem loop

```
      C      G      U     --U        ----      U        --   UGG   UA
5'-  GGCC  GCCC  GGG   CCAUCUU   CCAG  ACAGUGUU  GGA    UC    A
     ||||  ||||  |||   |||||||   ||||  ||||||||  |||    ||
3'-  UUGG  UGGG  CCC   GGUAGAA   GGUC  UGUCACAA  CCU    AG    U
      C      G     --   UC         AU      --        U   CGA   UG
```

sgRNA-miR-141-1
5'-TCCATCTTCCAGTACAGTGT-3' (5'- Dicer processing site)
sgRNA-miR-141-2
5'-CTAACACTGTCTGGTAAAGA-3' (3'- Drosha processing site)

lncRNA敲除主要方案

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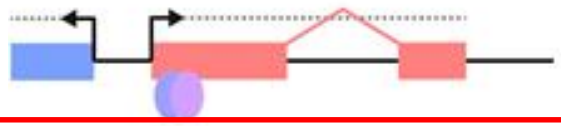
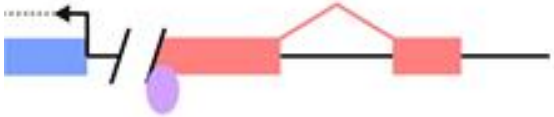
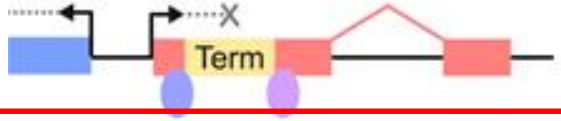
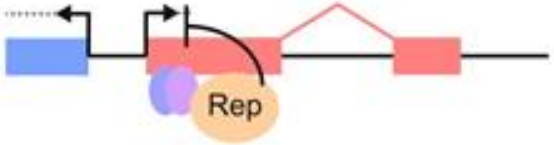
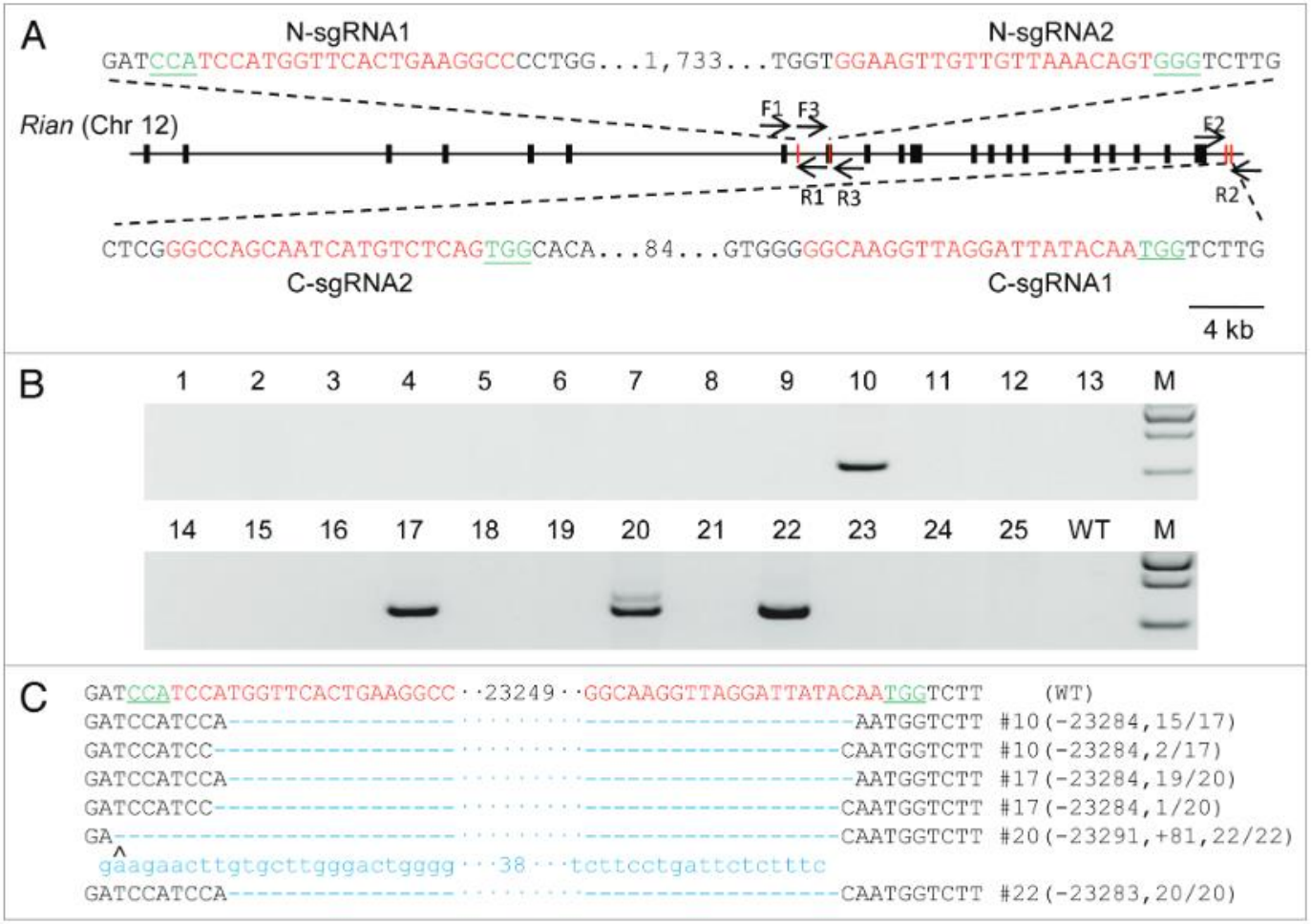
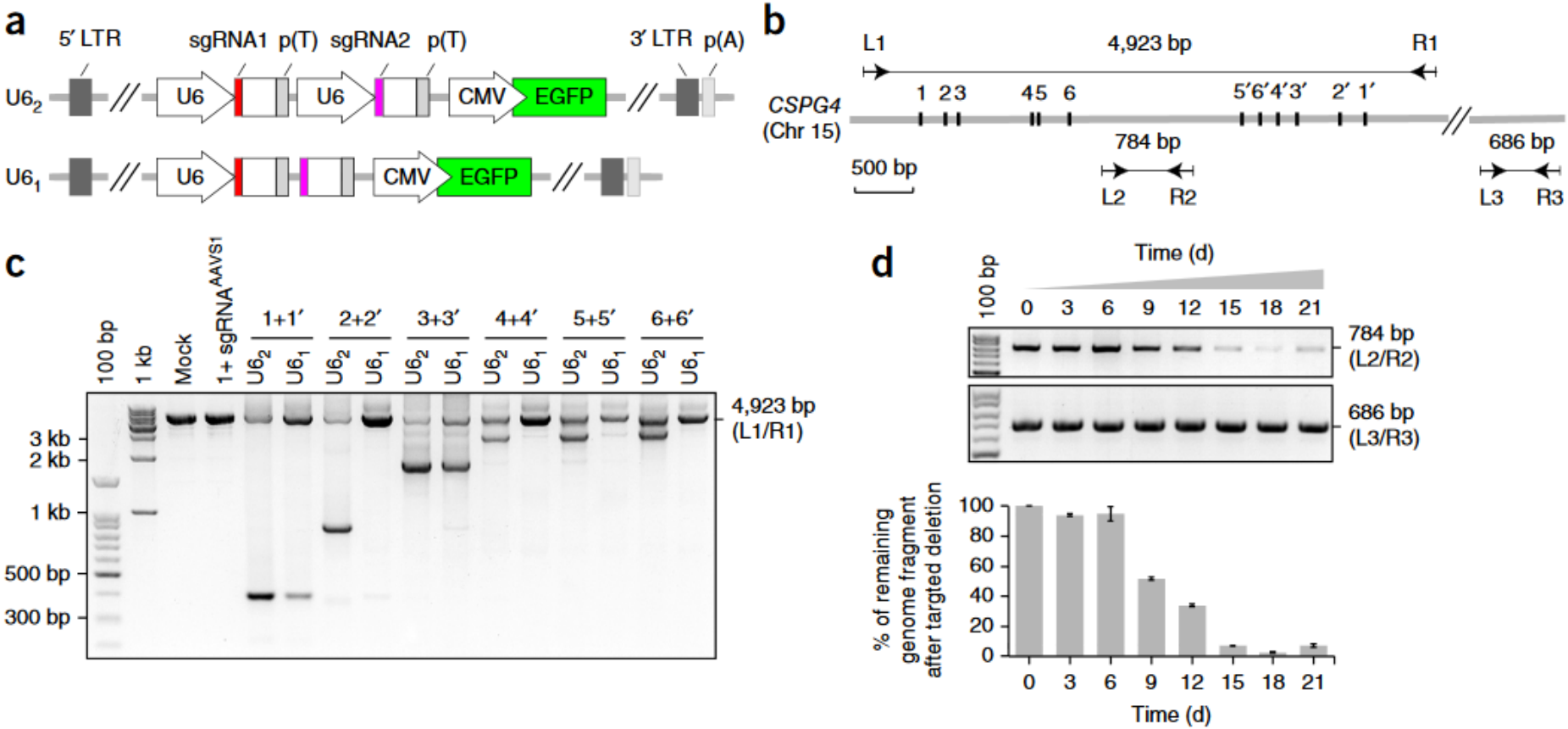
- 大片段删除
- 启动子删除
- Knock In 终止序列(Knock Down)

	Prevents transcription	Disrupts underlying DNA elements	Stable transgenics
			
 Locus deletion	Yes	Removes completely	Yes
 Promoter deletion	Yes	Removes some	Yes
 Transcription terminator insertion	Reduces	Alters spacing	Yes
 CRISPR-on/off TALE-TF/repressor	Reduces	No	Yes

Transcription factors lncRNA exons Neighbouring protein coding gene

大片段删除


A
N-sgRNA1
N-sgRNA2
GATCCATCCATGGTTCACTGAAGGCCCCTGG...1,733...TGGTGGAAGTTGTTGTTAAACAGTGGGTCTTG
Rian (Chr 12)
F1 F3
F2
R1 R3
R2
CTCGGGCCAGCAATCATGTCTCAGTGGCACA...84...GTGGGGGCAAGGTTAGGATTATACAATGGTCTTG
C-sgRNA2
C-sgRNA1
4 kb
B
1 2 3 4 5 6 7 8 9 10 11 12 13 M
14 15 16 17 18 19 20 21 22 23 24 25 WT M
C
GATCCATCCATGGTTCACTGAAGGCC··23249··GGCAAGGTTAGGATTATACAATGCTCTT (WT)
GATCCATCCA----------··------AATGGTCTT #10(-23284,15/17)
GATCCATCC----------··------CAATGGTCTT #10(-23284,2/17)
GATCCATCCA----------··------AATGGTCTT #17(-23284,19/20)
GATCCATCC----------··------CAATGGTCTT #17(-23284,1/20)
GA----------··------CAATGGTCTT #20(-23291,+81,22/22)
gaagaacttgtgcttgggactgggg···38···tcttcctgattctctttc
GATCCATCCA----------··------CAATGGTCTT #22(-23283,20/20)



e

GTCAGTGCAGAGTCTAGTGAGACGGAGGCGTGG.....3,488 bp.....TGCTGGGAGGAGGTTTGAGAGGGGCTCACCCCT	WT, 3/25)
GTCAGTGCAGAGTCTAGTGAGACGGAG----------//----------AGAGGGGCTCACCCCT	(Δ3,476 bp, 19/25)
GTCAGTGCAGAGTCTAGTGAGACGGA----------//----------AGGGGCTCACCCCT	(Δ3,479 bp, 1/25)
GTCAGTGCAGAGTCTAGTGAGACGGAG----------//----------GAGAGGGGCTCACCCCT	(Δ3,475 bp, 1/25)
GTCAGTGCAGAGTCTAGTGAGAC----------//----------GGGCTCACCCCT	(Δ3,484 bp, 1/25)

启动子删除

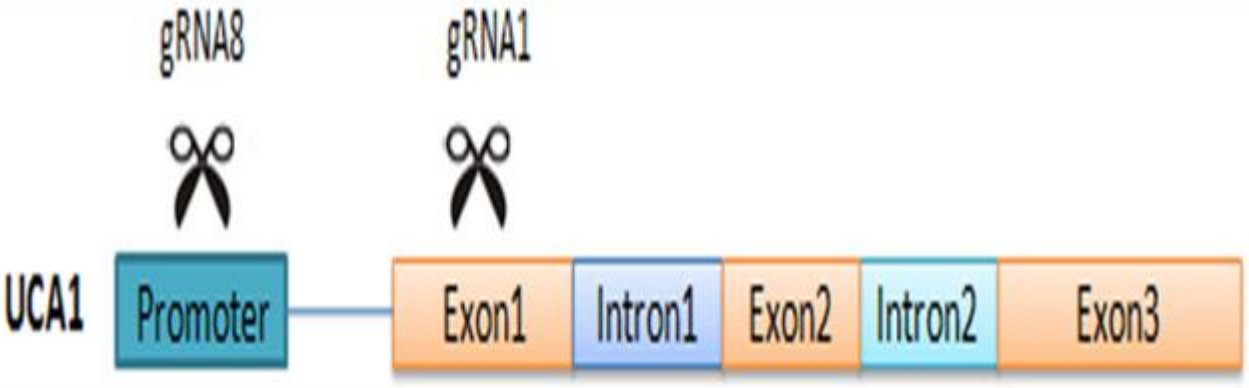
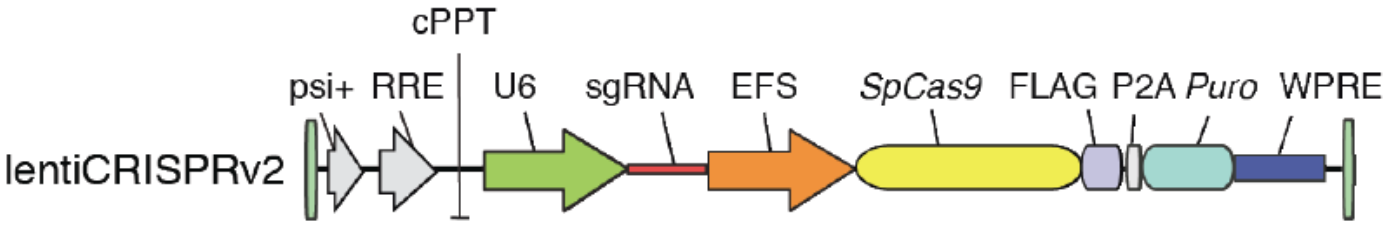


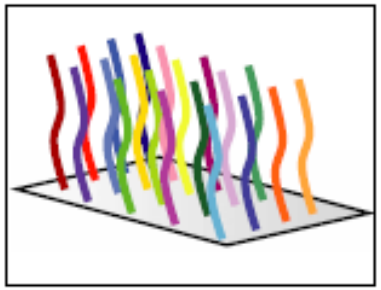
Figure 7: Strategy for targeting UCA1 with co-transfection of CRISPR/Cas9-UCA1-1/8 plasmids.

基于CRISPR/Cas的高通量功能筛选

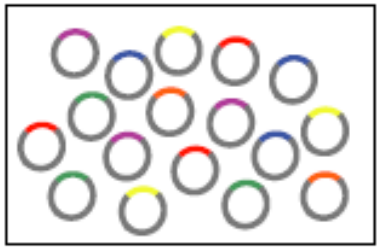


GeCKO v2.0 library specifications

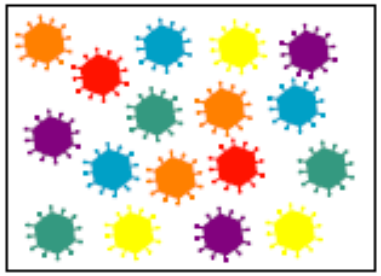
	GeCKO v2 human library	GeCKO v2 mouse library
Species	human	mouse
Number of genes targeted	19,050	20,611
Targeting constructs per gene	6 per gene (3 in Library A, 3 in Library B)	6 per gene (3 in Library A, 3 in Library B)
Number of miRNA targeted	1,864	1,175
Targeting constructs per miRNA	4 per miRNA	4 per miRNA
Control (non-targeting) sgRNAs	1,000	1,000
Total sgRNA constructs	122,411 (65,383 in Library A, 58,028 in Library B)	130,209 (67,405 in Library A, 62,804 in Library B)
Viral plasmid vector	Single and dual vector: lentiCRISPR v2 and lentiGuide-Puro	Single and dual vector: lentiCRISPR v2 and lentiGuide-Puro



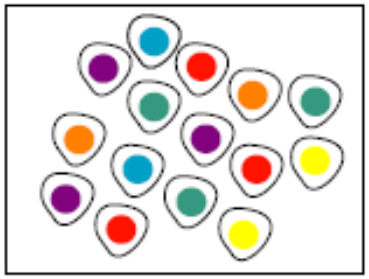
1. Oligo synthesis



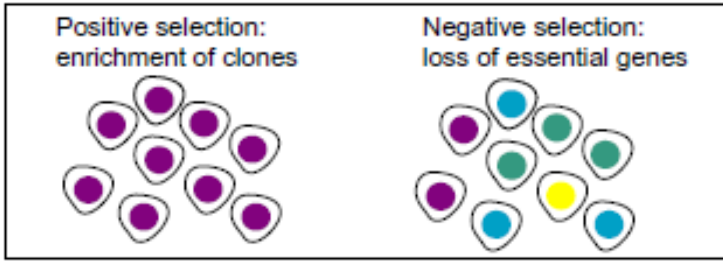
2. Plasmid pool



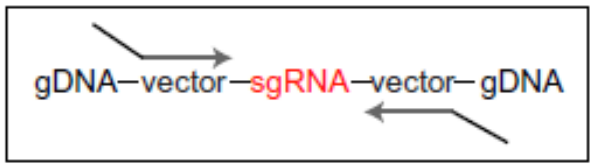
3. Virus production



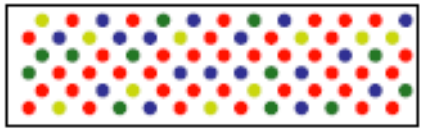
4. Introduce perturbations to cells



5. Conduct a screen with positive or negative selection



6. PCR genomic DNA



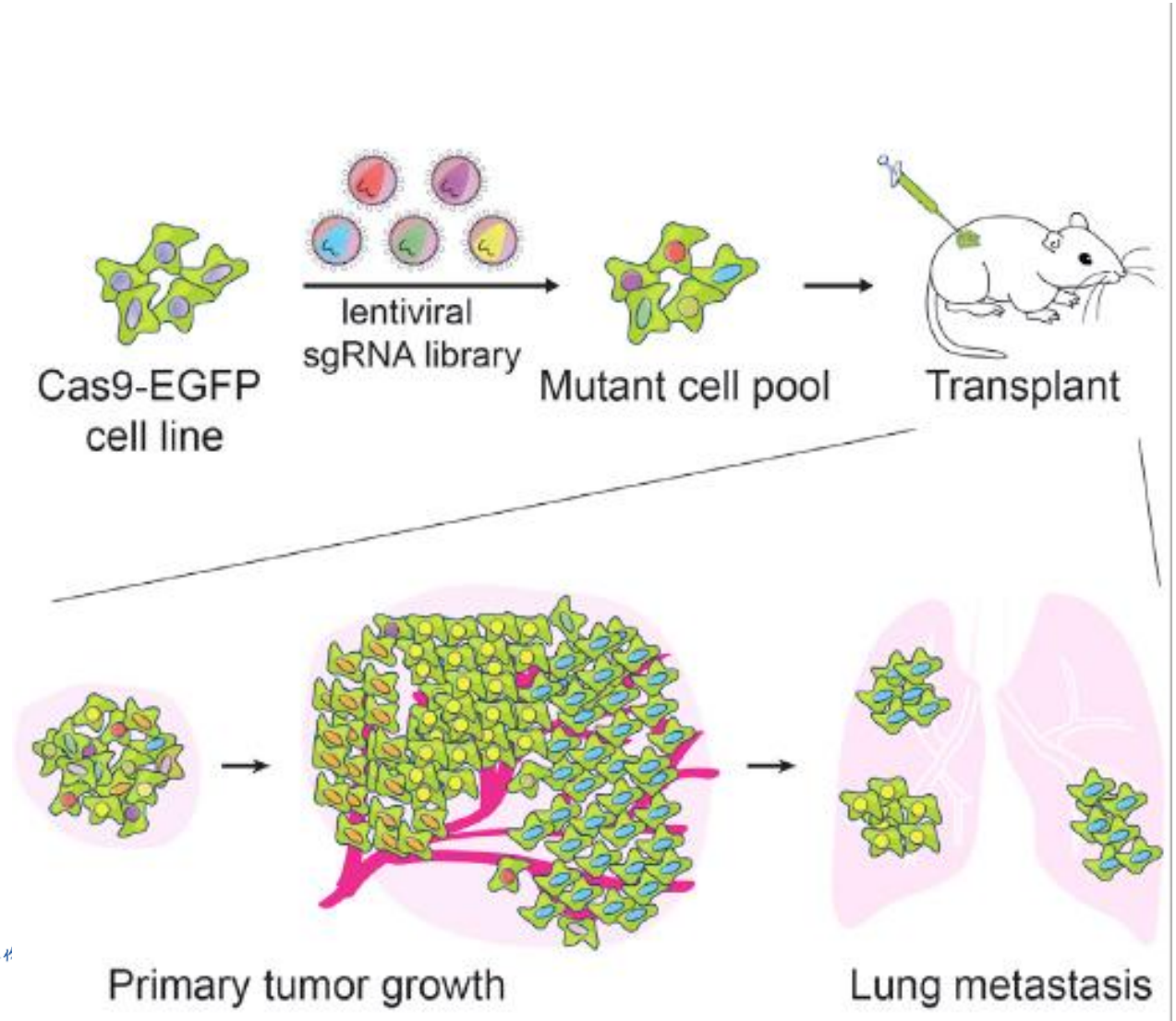
7. Next generation sequencing

sgRNA	Init.	Pos.	Neg.
	16	0	0
	19	100	19
	17	0	18
	18	0	26
	14	0	5
	14	0	0

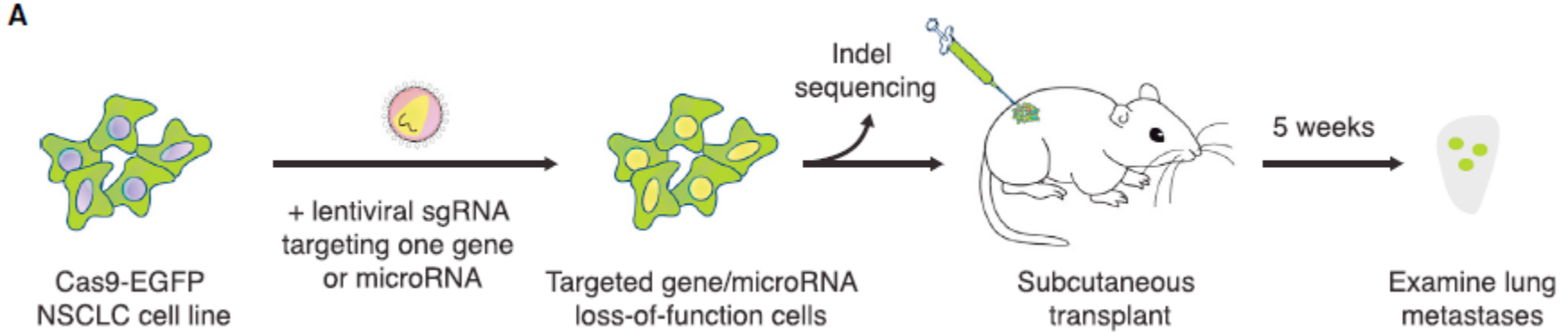
8. Matrix of sgRNA abundances

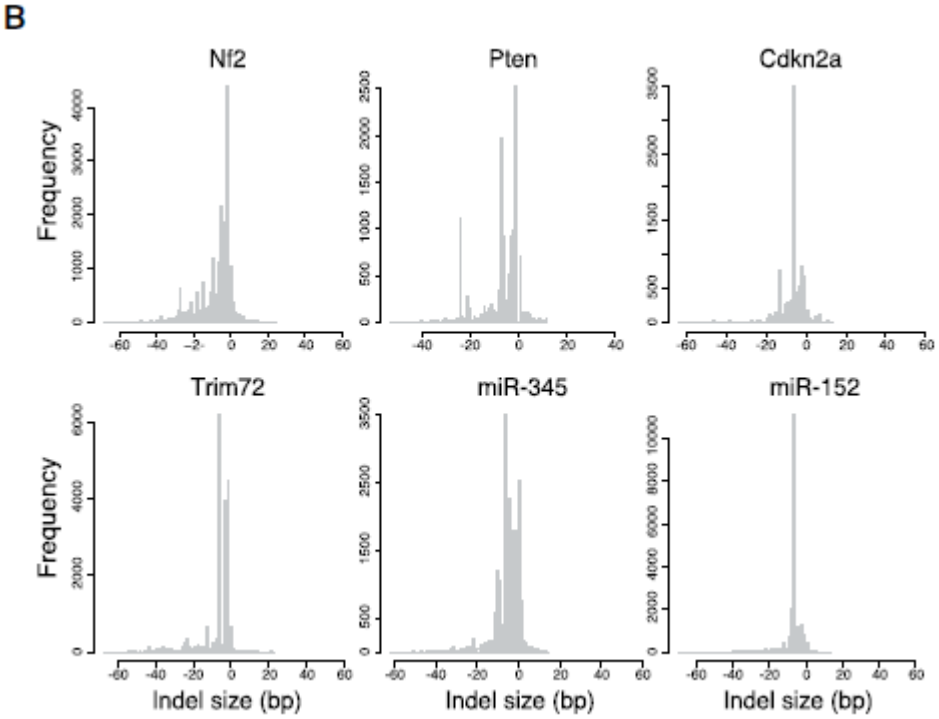
Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis

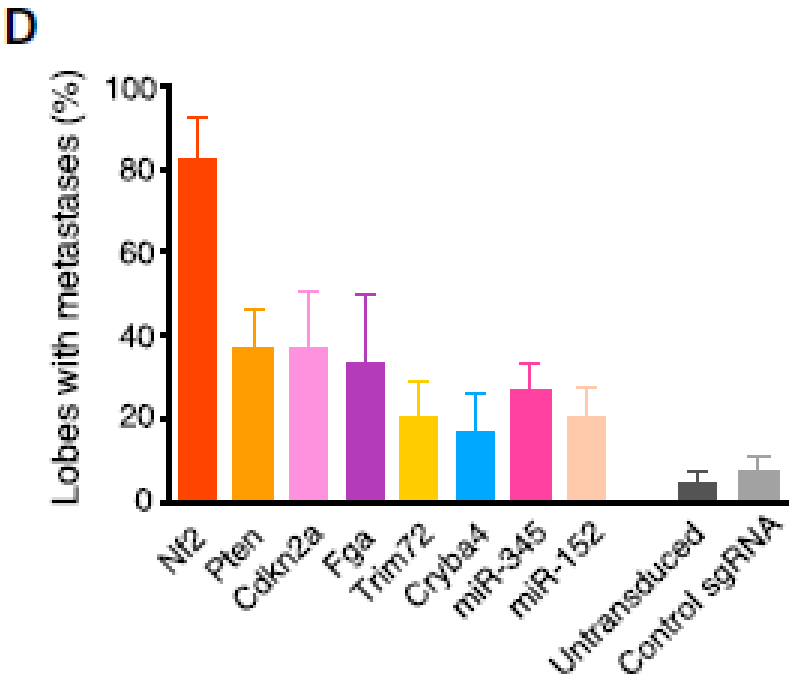
Sidi Chen,[1,2,3,10] Neville E. Sanjana,[3,4,5,6,7,10] Kaijie Zheng,[3,4] Ophir Shalem,[3,4] Kyungheon Lee,[8] Xi Shi,[3,4] David A. Scott,[3,4] Jun Song,[8] Jen Q. Pan,[3,4] Ralph Weissleder,[8,9] Hakho Lee,[8] Feng Zhang,[3,4,5,6,7,*] and Phillip A. Sharp[1,2,*]

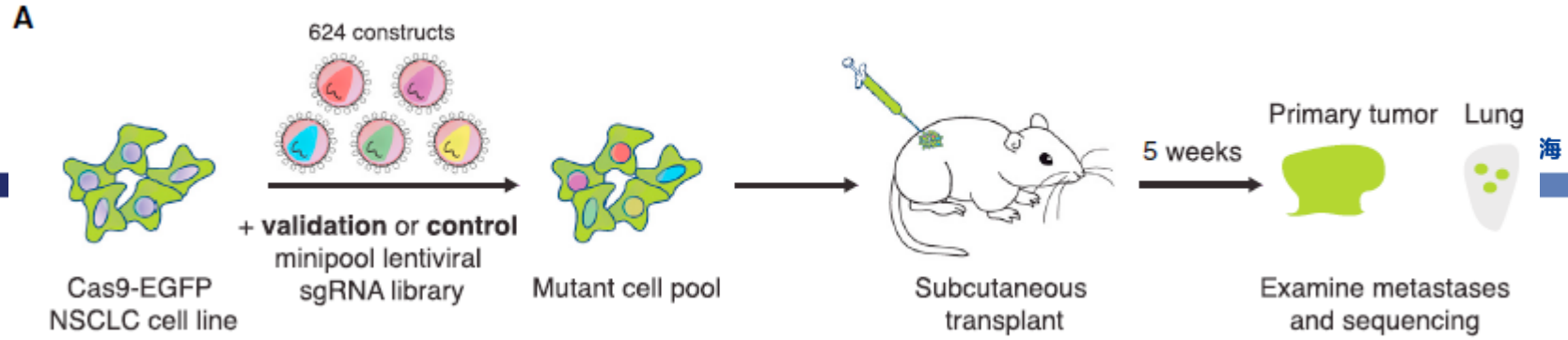


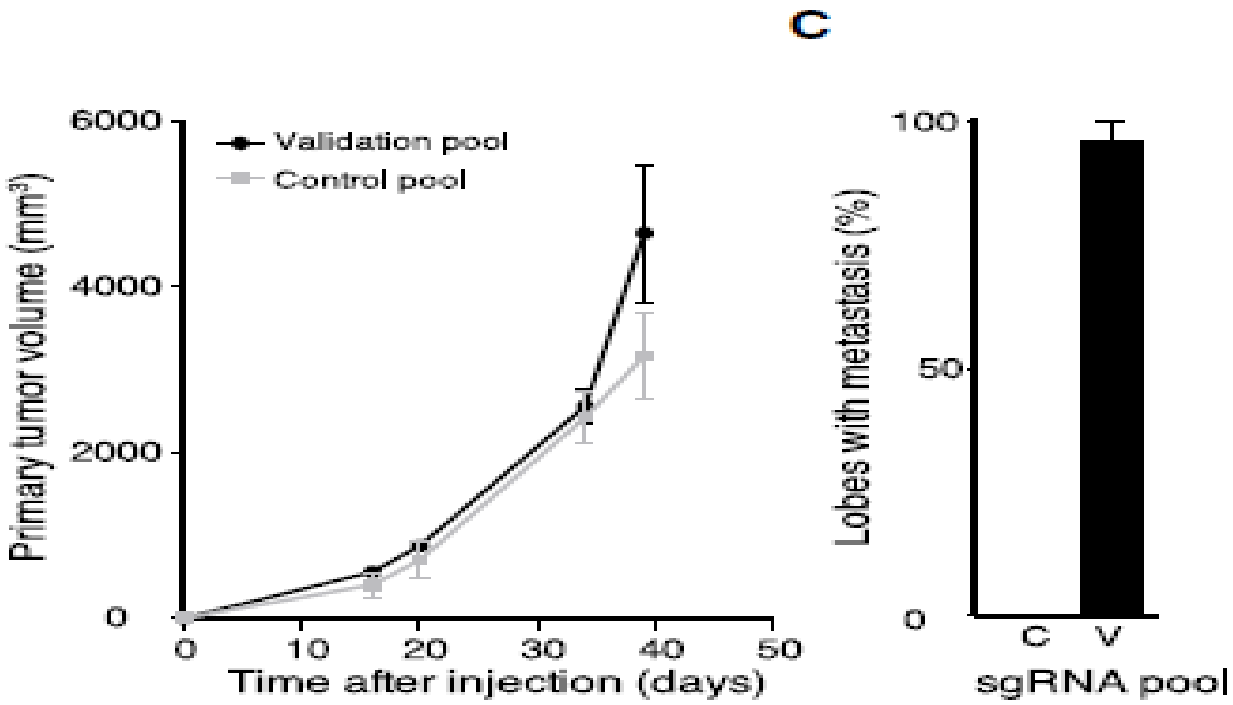
67,405
sgRNAs











CRISPR-Cas9 gRNA类型	物种	类型	覆盖范围
敲除文库	Human	慢病毒单病毒	19,050 Genes + 1,864 miRNAs
敲除文库	Mouse	慢病毒单病毒	20,611 Genes +1,175 miRNAs
敲除文库	Human	慢病毒双病毒	1,880 Unknown function Genes
激活文库	Human	慢病毒双病毒	23,430 gRNAs
激活文库	Mouse	慢病毒三病毒	23,439 gRNAs
定制文库	定制	定制	定制

可选择的筛选表型:

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- **肿瘤转移相关基因筛选(体内筛选)**
- **细胞迁移/侵袭相关基因筛选 (体外筛选)**
- **细胞克隆形成相关基因筛选 (体外筛选)**
- **细胞增殖相关基因筛选 (体外筛选)**
- **肿瘤耐药相关基因筛选(体外筛选)**
- **细胞信号通路相关基因筛选 (体外筛选)**

.

- **其它功能基因筛选**

其它指定的生物学过程, 只要可以通过表型分选细胞, 或者通过报告基因分选的, 都可以进行相关的功能筛选, 比如: 自噬, EMT(上皮间质转化), 细胞分化等等生物学过程都可以先建立报告基因的方式, 再通过CRISPR-Cas9 文库进行高通量筛选.

CRISPR/Cas与动物肿瘤模型

- 多基因突变原发性肿瘤模型

  肺癌 p53、Lkb1、Kras

  结直肠癌 APC、SMAD4 、 p53 、 KRAS和PIK3CA

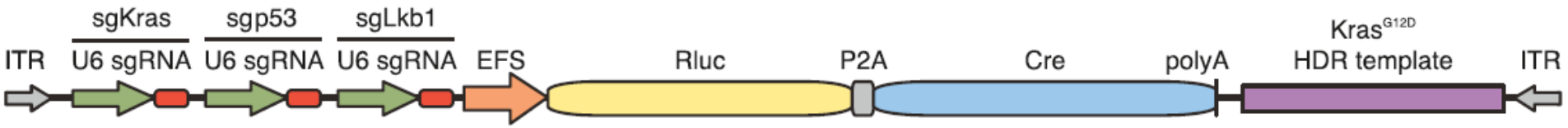
  恶性髓系血液病 Tet2,Runx1,Dnmt3a 和Nf1

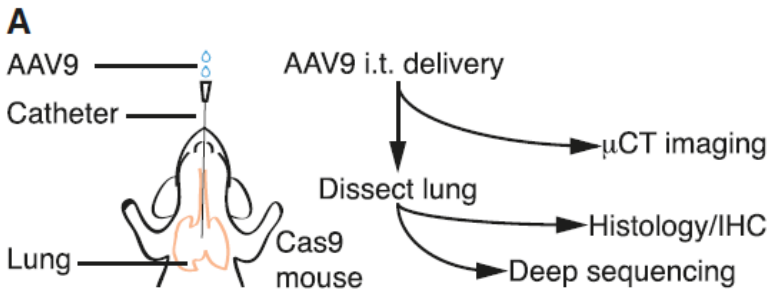
  肝癌 Pten, p53

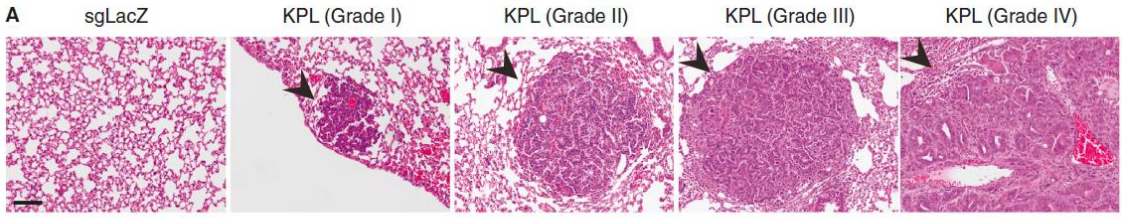
- 染色体异构相关的肿瘤模型

In Vivo Modeling of Multigenic Cancer Mutations 体内肿瘤造模

Lung adenocarcinoma in human (46% for p53, 33% for KRAS and 17% for LKB1)



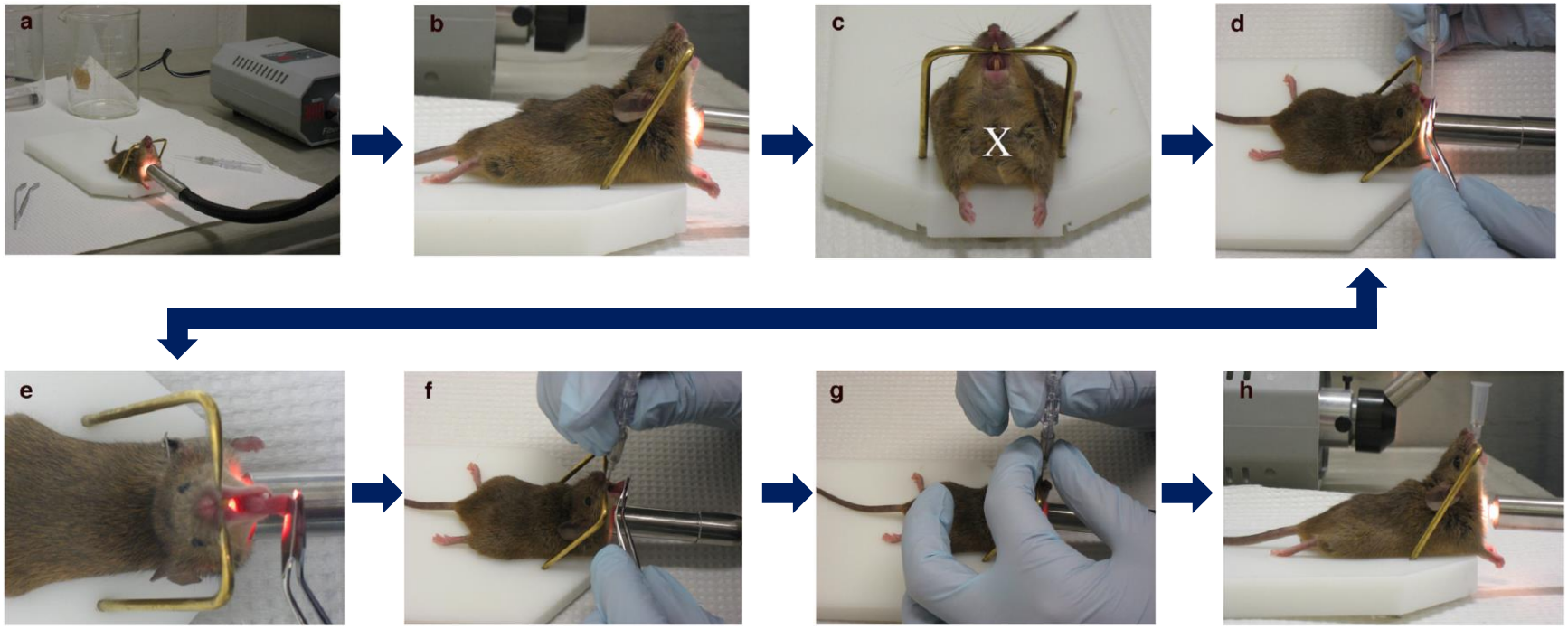




B

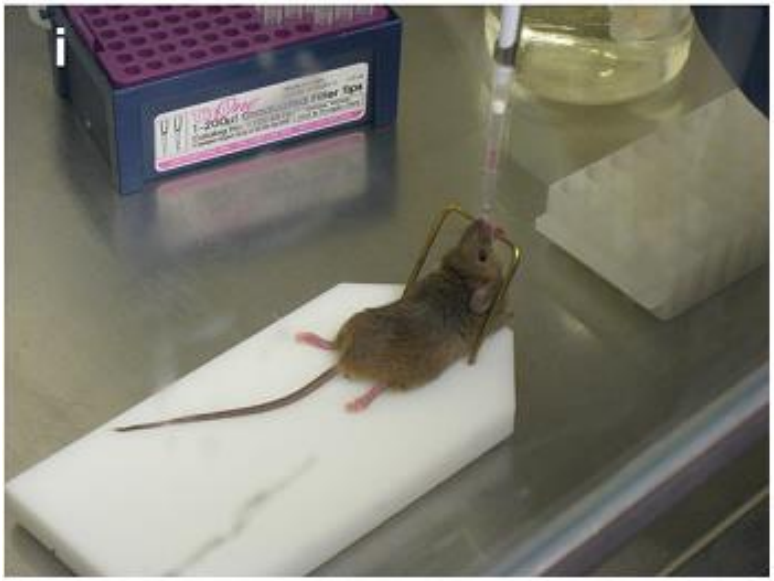
Time	AAV	Tumor grade - Mean number of tumors ± SEM				
		Grade I	Grade II	Grade III	Grade IV	Subtotal
1 month	KPL	4.5 ± 0.05	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.5 ± 0.5
1 month	sgLacZ	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2 months	KPL	6.5 ± 2.7	11 ± 5.5	8.5 ± 5.0	1.0 ± 0.8	27 ± 12
2 months	sgLacZ	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

气管内感染技术操作流程示例



Nat Protoc.
2009

气管内感染



实验相关操作参数

病毒类型：腺相关病毒AAV2/9；
病毒编号： H4048X-3
病毒滴度：5.7E+12
病毒总量：1E+11
感染体积：50-75ul

动物品系：CRISPR/Cas9小鼠
性别：雄性
周龄：8-12周

麻醉剂：戊巴比妥钠

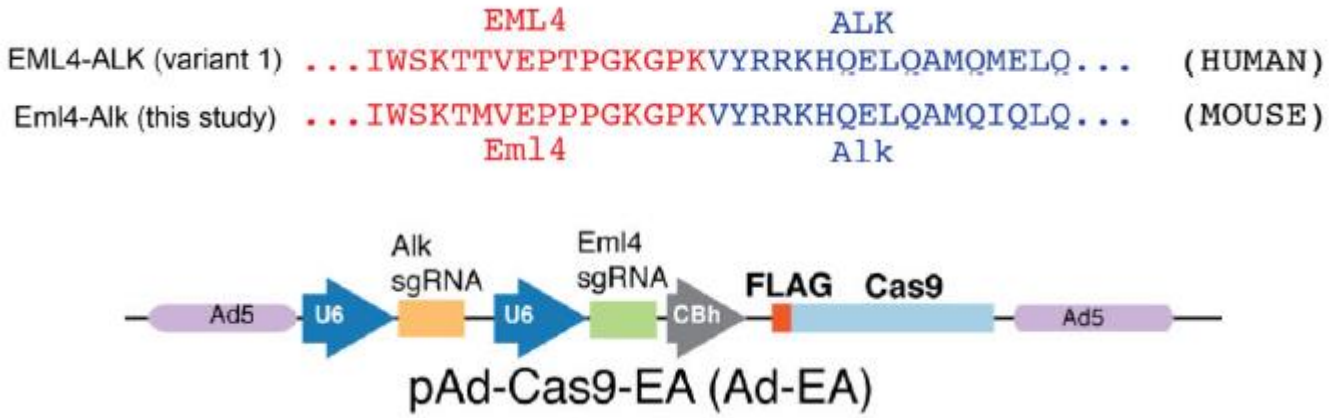
Nat Protoc. 2009　Cell 2014

染色体重排是人类癌症细胞的一个特征，在多种癌症的发病机制及癌症的治疗方案的相关研究中具有重要的地位，如造血系统肿瘤如白血病和淋巴瘤

Nature. 2014 Dec 18;516(7531):423-7. doi: 10.1038/nature13902. Epub 2014 Oct 22.

In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system.

Maddalo D[1], Manchado E[1], Concepcion CP[2], Bonetti C[1], Vidigal JA[1], Han YC[1], Ogrodowski P[1], Crippa A[3], Rekhtman N[4], de Stanchina E[5], Lowe SW[6], Ventura A[1].



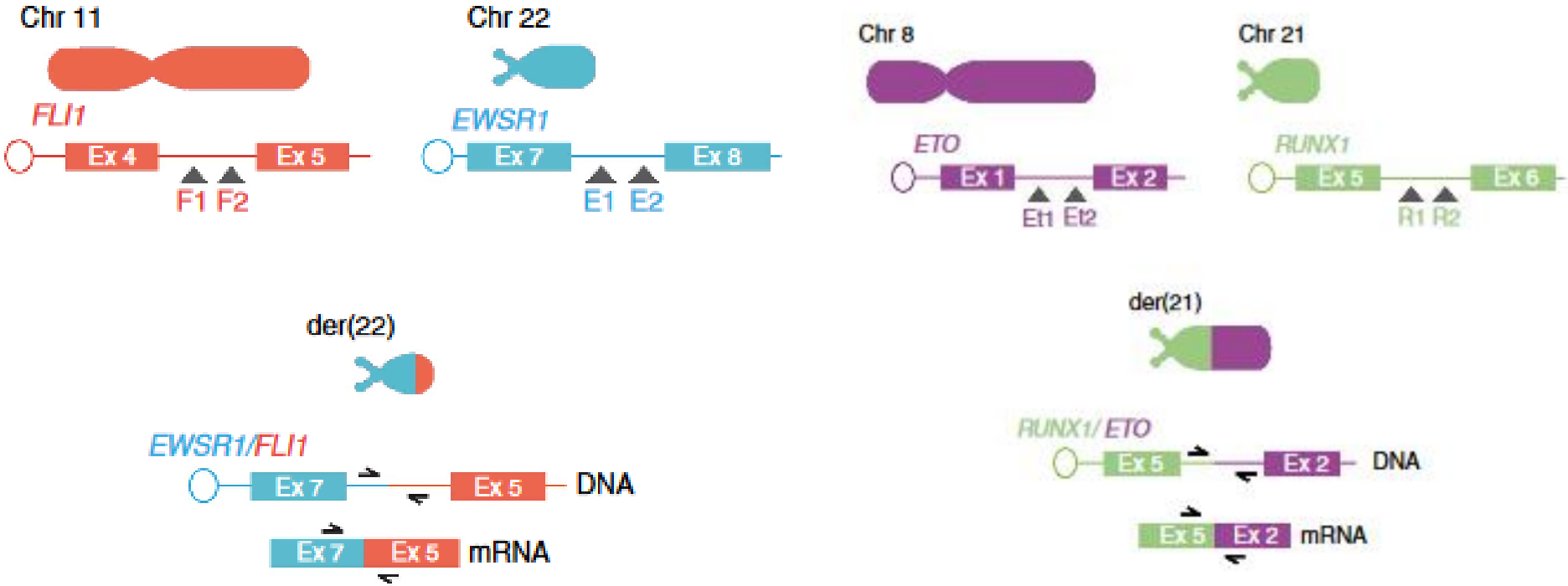
非小细胞肺癌

Nat Commun. 2014 Jun 3;5:3964. doi: 10.1038/ncomms4964.

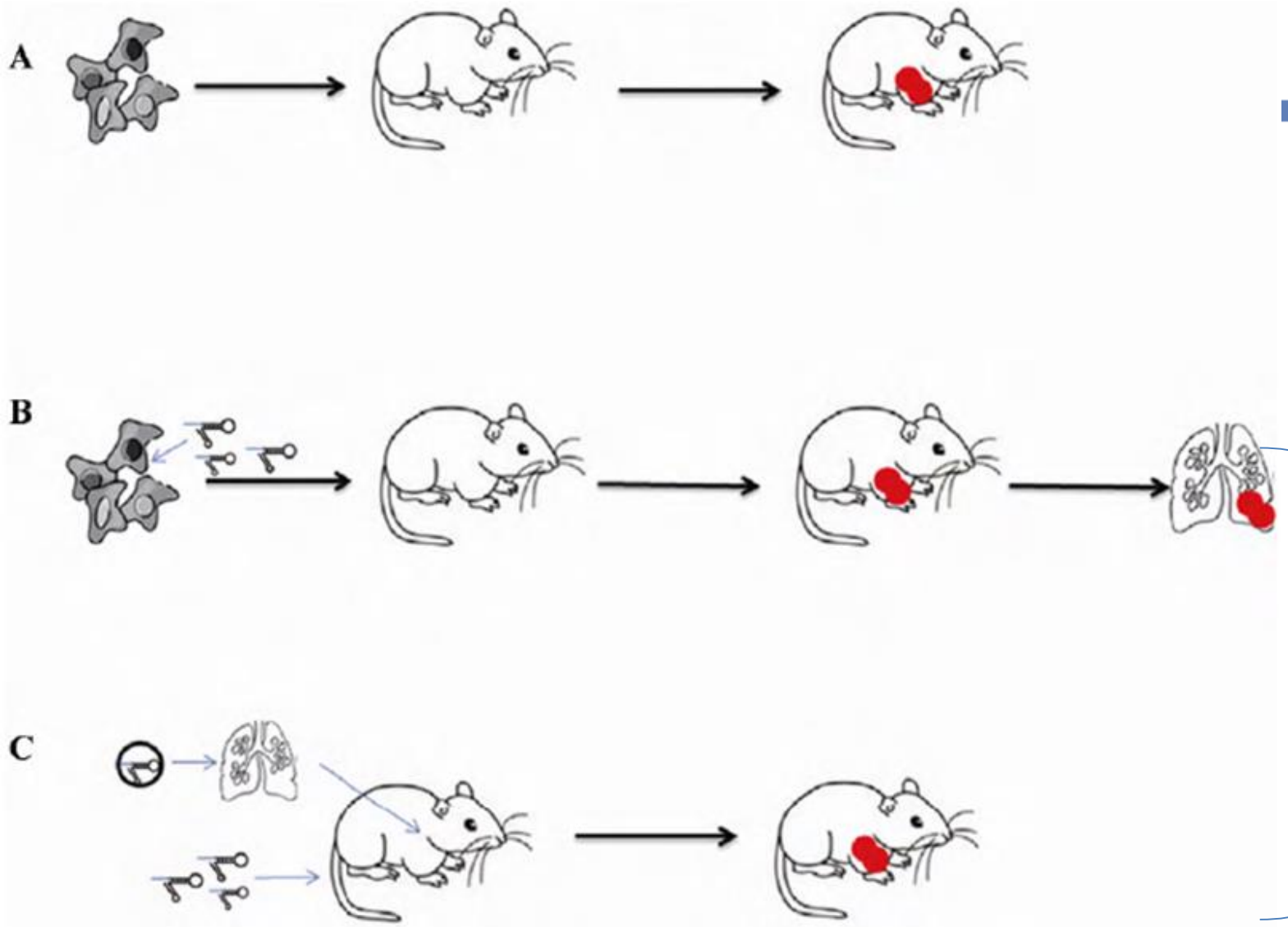
Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system.

Torres R[1], Martin MC[2], Garcia A[1], Cigudosa JC[2], Ramirez JC[1], Rodriguez-Perales S[2].

尤文氏肉瘤EWSR1–FLI1 融合基因



急性粒细胞性白血病RUNX1 与ETO融合基因



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整合你我资源 服务生命科学

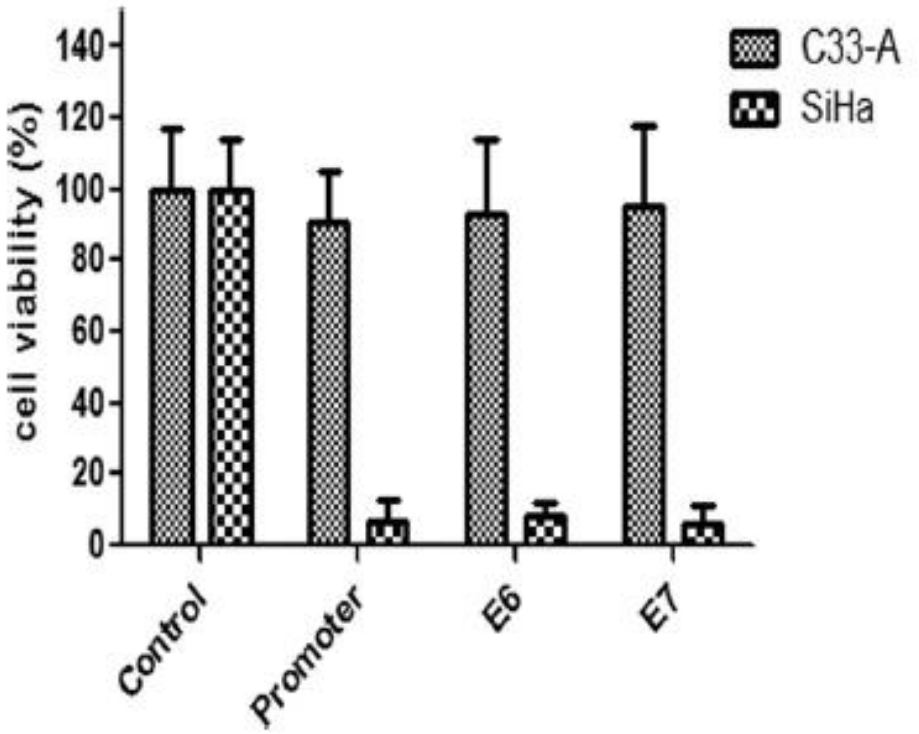
Cas9小鼠肿瘤模型与传统成瘤模型比较

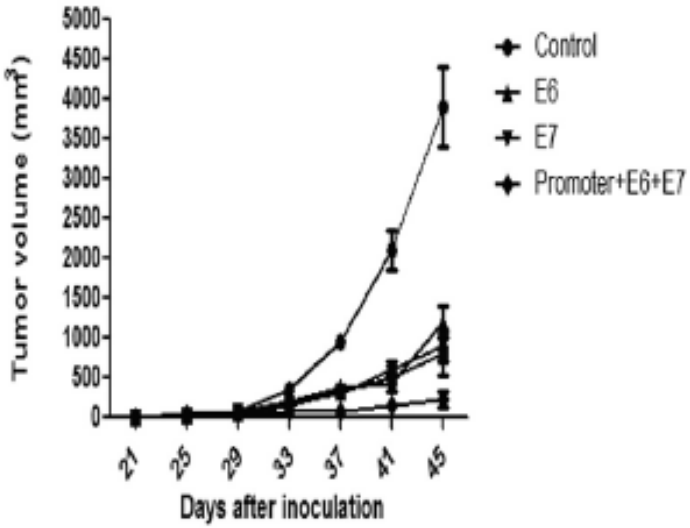
CRISPR/Cas与肿瘤治疗

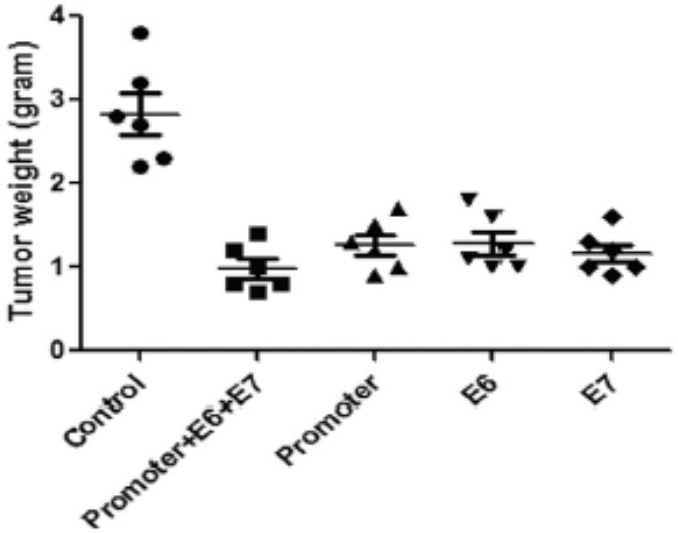
Biochem Biophys Res Commun. 2014 Aug 8;450(4):1422-6. doi: 10.1016/j.bbrc.2014.07.014. Epub 2014 Jul 17.

In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by CRISPR/Cas9.

Zhen S[1], Hua L[2], Takahashi Y[3], Narita S[3], Liu YH[2], Li Y[4].







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Shu Su[1,*], Bian Hu[2,*], Jie Shao[1], Bin Shen[3], Juan Du[1], Yinan Du[2], Jiankui Zhou[2], Lixia Yu[1], Lianru Zhang[1], Fangjun Chen[1], Huizi Sha[1], Lei Cheng[1], Fanyan Meng[1], Zhengyun Zou[1], Xingxu Huang[2,4] & Baorui Liu[1]