DNA-guided genome editing using the *Natronobacterium gregoryi* Argonaute

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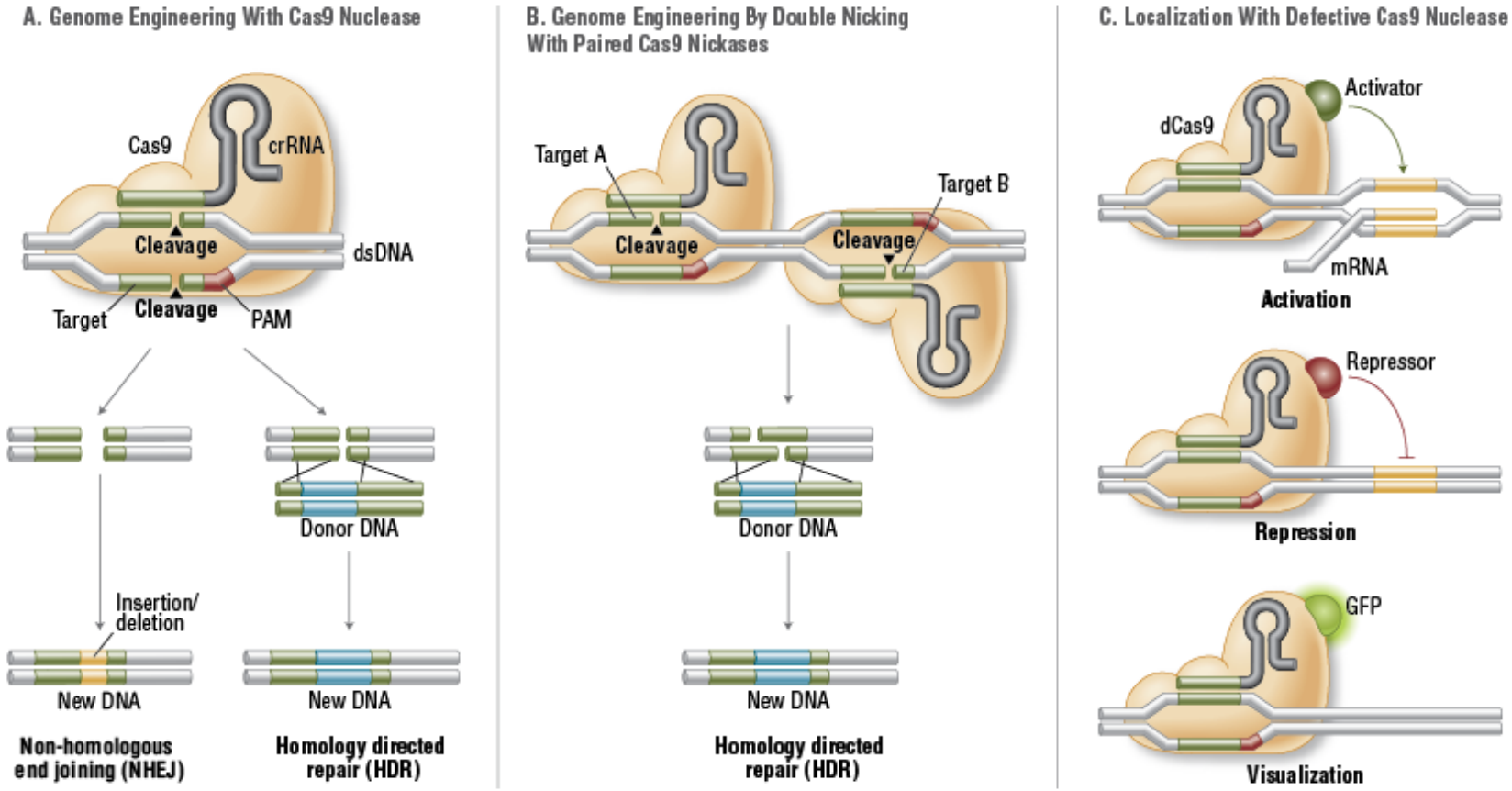
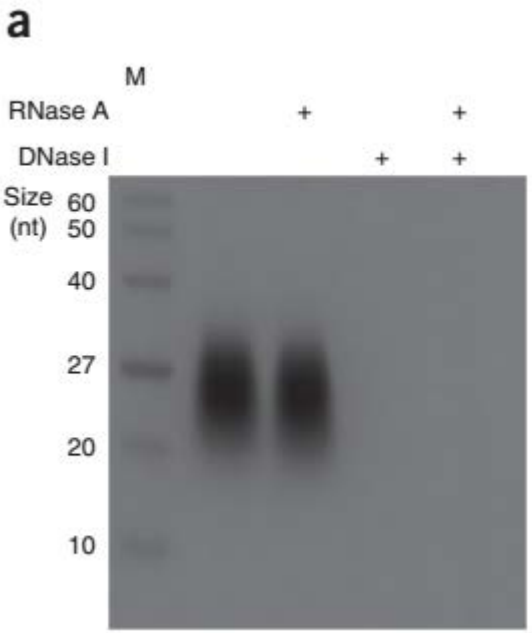
7/25/2016

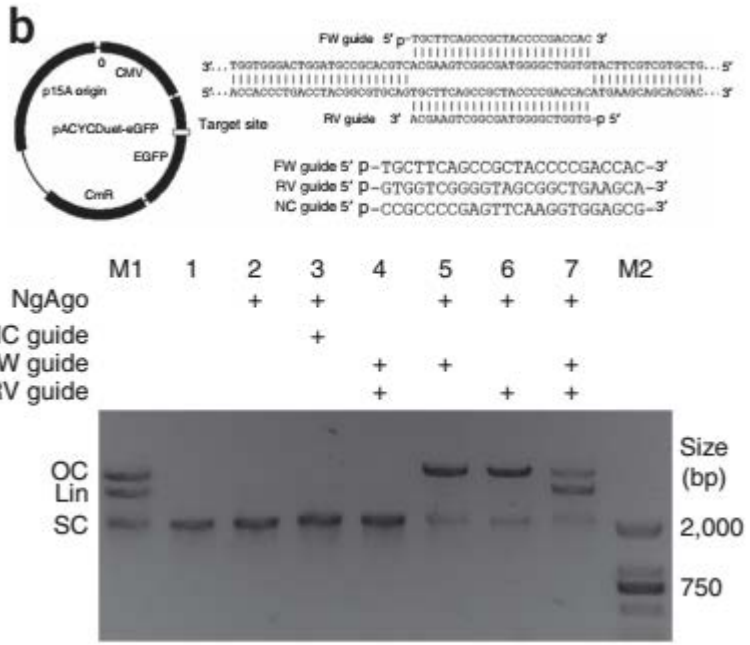
Introduction to CRISPR-Cas9 System

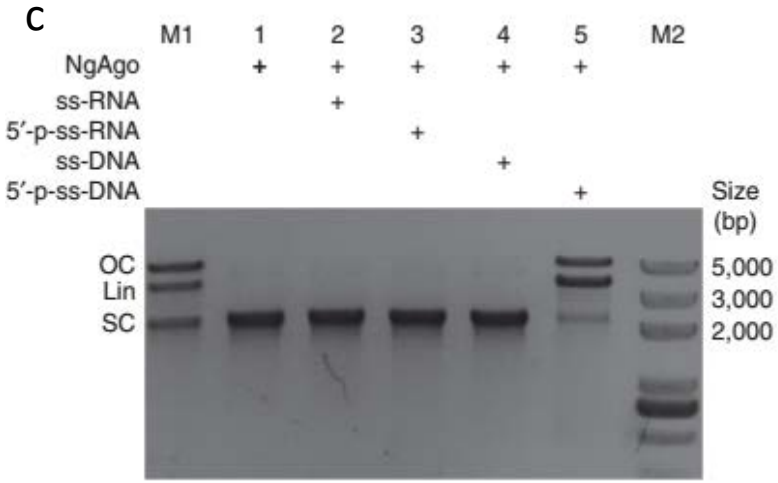


Alex Reis. NEB expressions Issue I, 2014

NgAgo Uses 5′ phosphorylated ssDNA Guides and Cleaves DNA Targets In Vitro





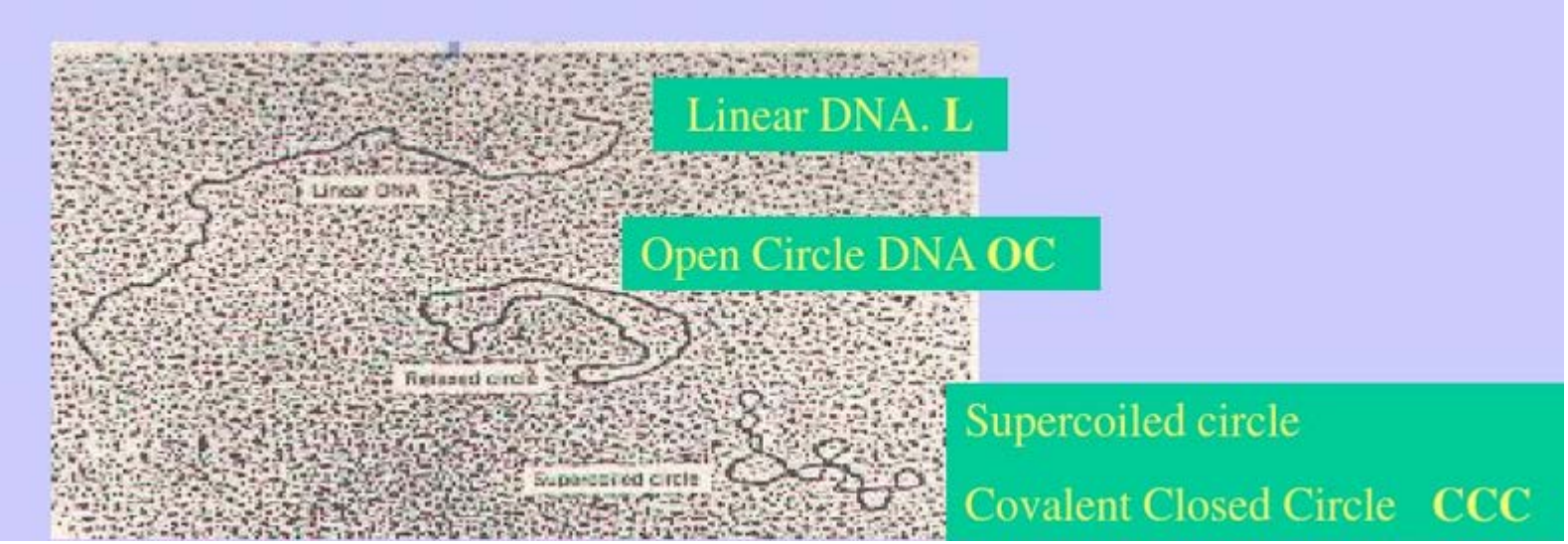


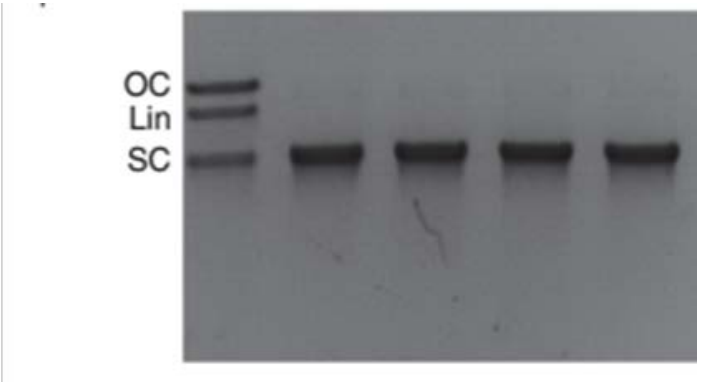
(a) Electrophoresis of GST-NgAgo expressed in E. coli,purified by Sephrose-4B, digested by protein kinase K, and treated with the indicated exonucleases

(b)In vitro plasmid cleavage assay, performed by reloading NgAgo expressed in E. coli with the indicated guide ssDNAs at 55 ° C for 1 h, followed by incubation with the target plasmid at 37 ° C for 12 h, and analysis of the target plasmid by electrophoresis

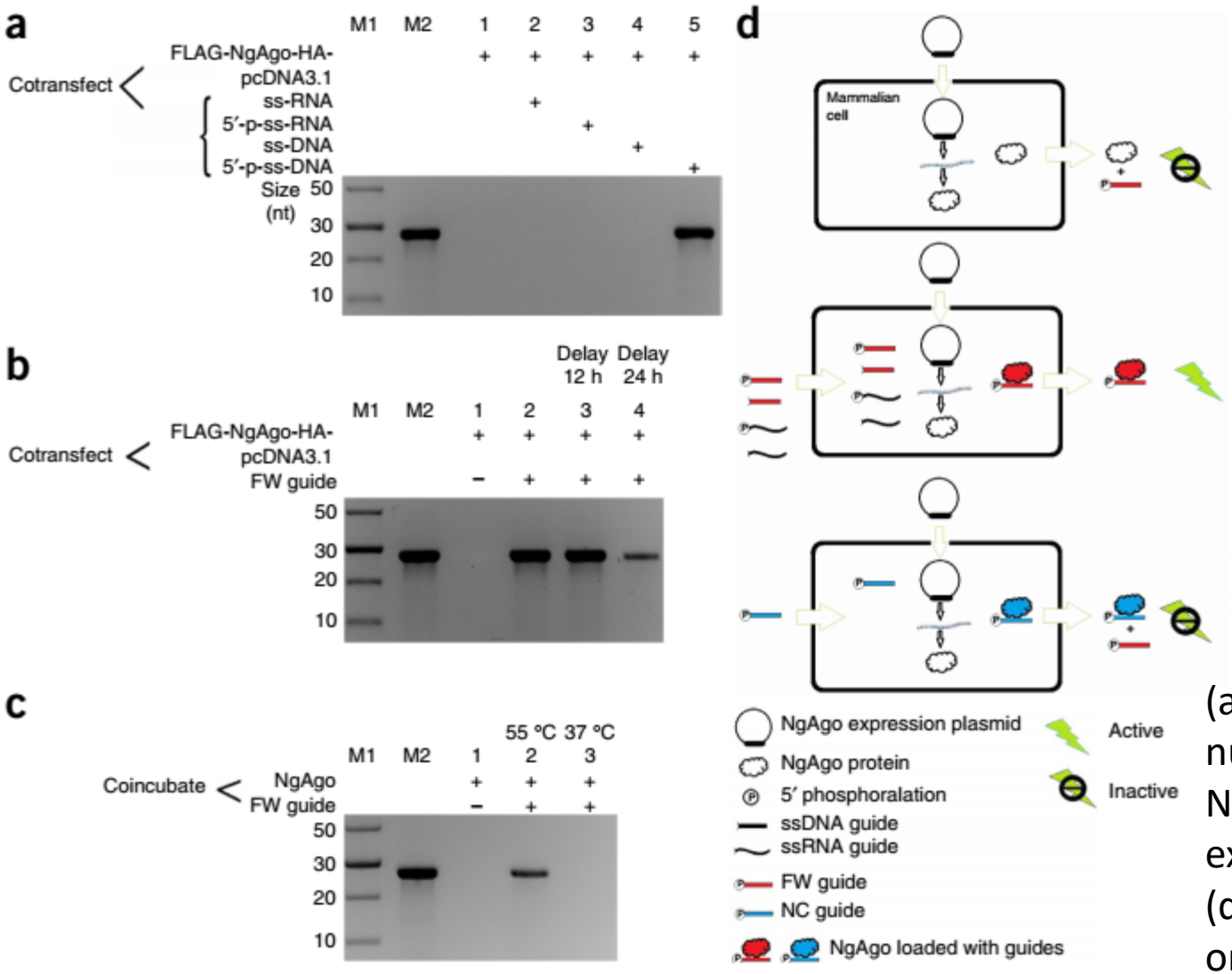
(c) Plasmid cleavage assay performed by using the indicated nucleic acids with or without 5′ phosphorylation

Three Different Conformation of Plasmid



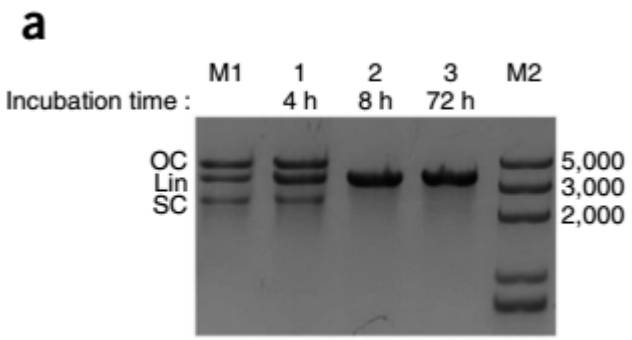


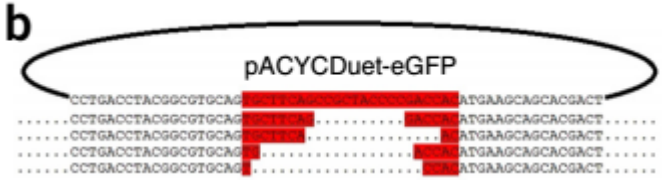
NgAgo Binds ssDNA Guide In A Oneguide-Faithful Manner

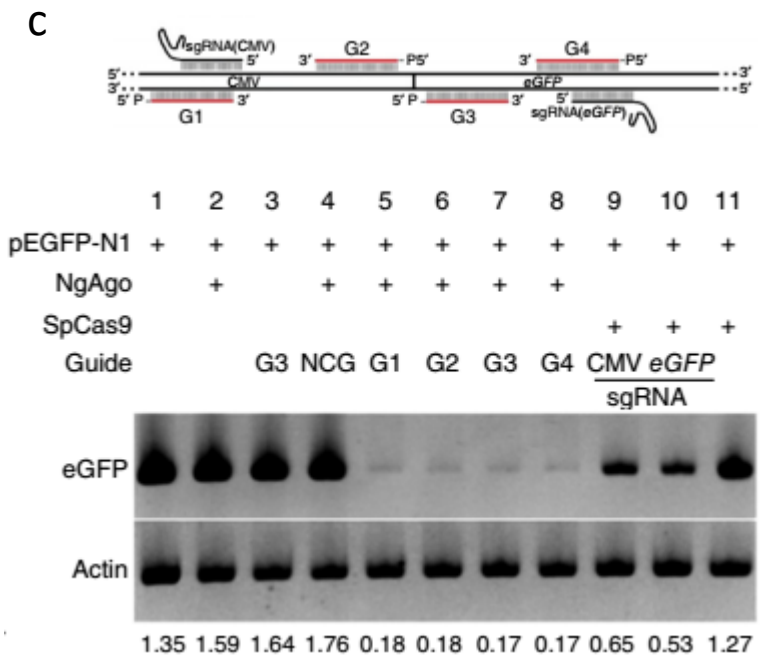


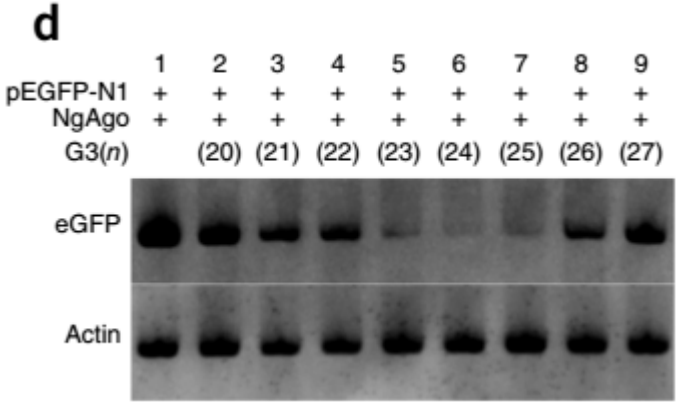
(a,b,c) Electrophoresis of nucleic acids bound to NgAgo in different experimental conditions
(d) Schematic of the one-guide-faithful rule abided by NgAgo

NgAgo Works As An Endonuclease And Can Cleave DNA Targets In Vivo









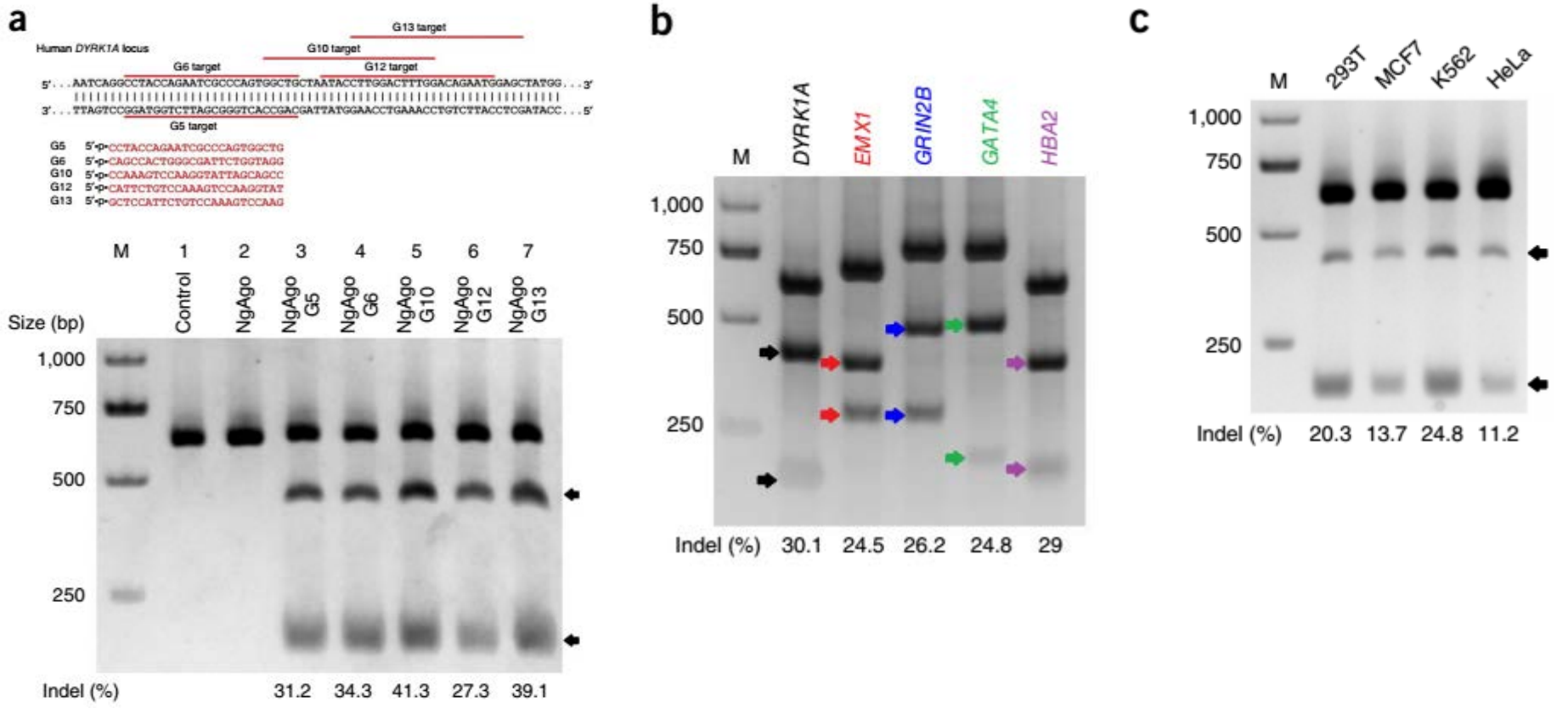
(a)Electrophoresis of target pACYCDuet-eGFP plasmid after it was incubated with purified NgAgo

(b)Examples of sequencing results shows that 1–20 nucleotides within the guide/target region were randomly removed

(3)Comparison between NgAgo and Cas9 system

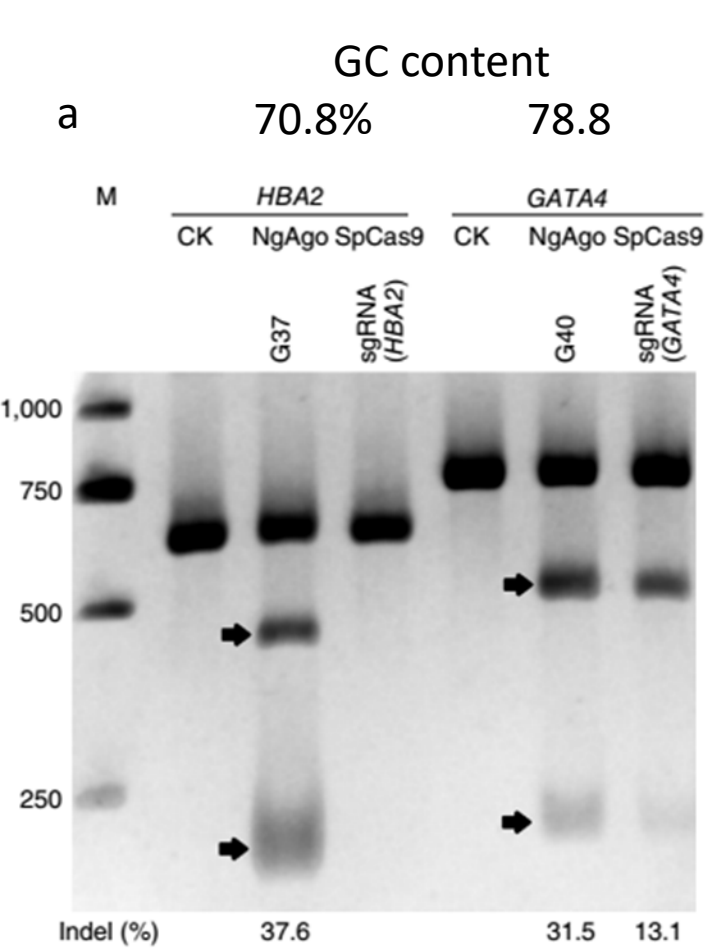
(4) Efficiency of NgAgo with guides of different length

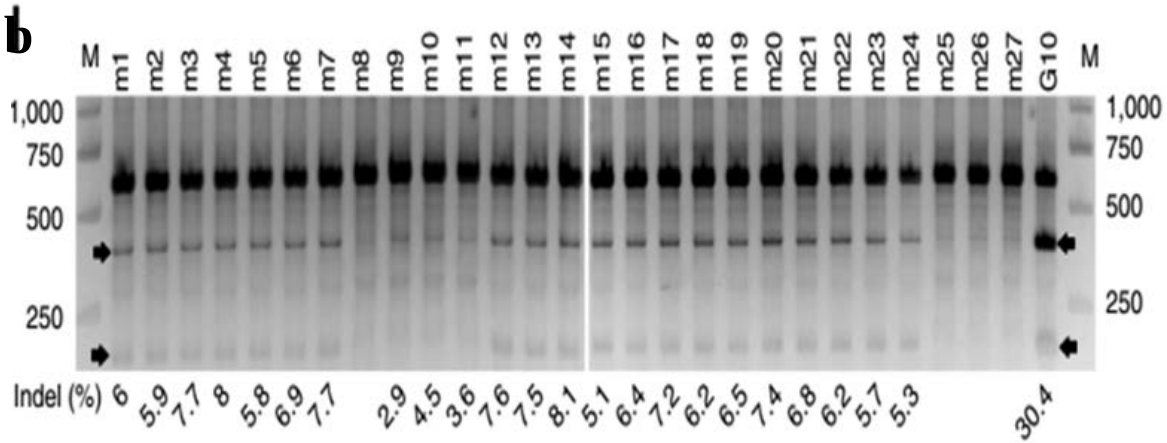
NgAgo Can Make Targeted Double-Strand Breaks In Mammalian Genome



(a)T7E1 assay for NgAgo-mediated insertion-deletions (indels) DYRK1A gene
(b,c) T7E1 assays ofNgAgo-mediated indels at the indicated targets in human genome (b) and at the DYRK1A gene in the indicated cell lines (c)

The Higher Efficiency in (G+C)-Rich Sequences And Fidelity of NgAgo





G10 5′-p-CCAAAGTCCAAGGTATTAGCAGCC

G10 5'P-CCAAAGTCCAAGGTATTAGCAGCC
m1 5'P-TCAAAGTCCAAGGTATTAGCAGCC
m2 5'P-CTAAAGTCCAAGGTATTAGCAGCC
m3 5'P-CCGAAGTCCAAGGTATTAGCAGCC
m4 5'P-CCAGAGTCCAAGGTATTAGCAGCC
m5 5'P-CCAAGGTCCAAGGTATTAGCAGCC
m6 5'P-CCAAAATCCAAGGTATTAGCAGCC
m7 5'P-CCAAAGCCCAAGGTATTAGCAGCC
m8 5'P-CCAAAGTTCAAGGTATTAGCAGCC
m9 5'P-CCAAAGTCTAAGGTATTAGCAGCC
m10 5'P-CCAAAGTCCGAGGTATTAGCAGCC
m11 5'P-CCAAAGTCCAGGGTATTAGCAGCC
m12 5'P-CCAAAGTCCAAAGTATTAGCAGCC
m13 5'P-CCAAAGTCCAAGATATTAGCAGCC
m14 5'P-CCAAAGTCCAAGGCATTAGCAGCC
m15 5'P-CCAAAGTCCAAGGTGTTAGCAGCC
m16 5'P-CCAAAGTCCAAGGTACTAGCAGCC
m17 5'P-CCAAAGTCCAAGGTATCAGCAGCC
m18 5'P-CCAAAGTCCAAGGTATTGGCAGCC
m19 5'P-CCAAAGTCCAAGGTATTAACAGCC
m20 5'P-CCAAAGTCCAAGGTATTAGTAGCC
m21 5'P-CCAAAGTCCAAGGTATTAGCGGCC
m22 5'P-CCAAAGTCCAAGGTATTAGCAACC
m23 5'P-CCAAAGTCCAAGGTATTAGCAGTC
m24 5'P-CCAAAGTCCAAGGTATTAGCAGCT
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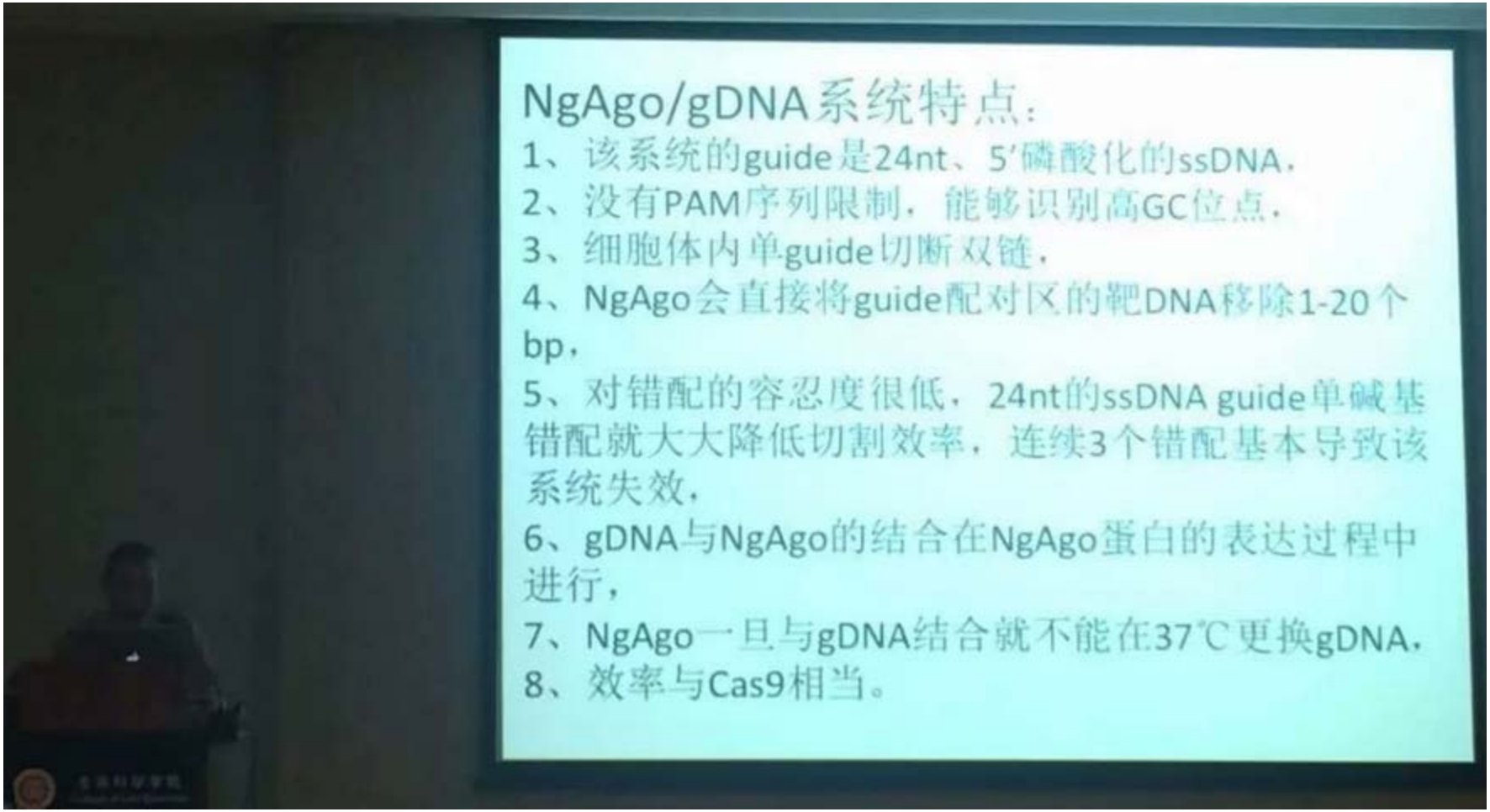
m26 5'P-CCAAAGTCCAAAACATTAGCAGCC
m27 5'P-CCAAAGTCCAAGGTATTAGCAATT

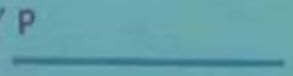

NgAgo/gDNA系统特点：
1、该系统的guide是24nt、5'磷酸化的ssDNA，
2、没有PAM序列限制，能够识别高GC位点，
3、细胞体内单guide切断双链，
4、NgAgo会直接将guide配对区的靶DNA移除1-20个bp，
5、对错配的容忍度很低，24nt的ssDNA guide单碱基错配就大大降低切割效率，连续3个错配基本导致该系统失效，
6、gDNA与NgAgo的结合在NgAgo蛋白的表达过程中进行，
7、NgAgo一旦与gDNA结合就不能在37℃更换gDNA，
8、效率与Cas9相当。

1. gDNA设计极其简便，输送方式直接:

Cas9
gRNA

做转染细胞用的表达gRNA的载体，费时费工！

NgAgo
gDNA



像合成PCR引物一样由公司合成，直接转染细胞

但是无法使用质粒或慢病毒载体输送gDNA。

2. 可切割基因组任何位置

	Cas9	NgAgo
（1）	受PAM区限制 没有靶向序列限制	
（2）	受富含GC序列限制 受GC丰度限制	不

但是gDNA不能再改造，而gRNA可以连接一段RNA不影响功能。

3. 特异性高，低脱靶

Cas9	NgAgo
（1） gRNA 20 nt长	gDNA 24 nt长
（2） 细胞内有大量非特异RNA片段	细胞内鲜有5'P ssDNA片段
（3） 能耐受gRNA与靶序列5个核苷酸错配	对一个错配已经很敏感 三个错配则完全失活

但是还没有高通量测序，NgAgo脱靶还需深入研究。