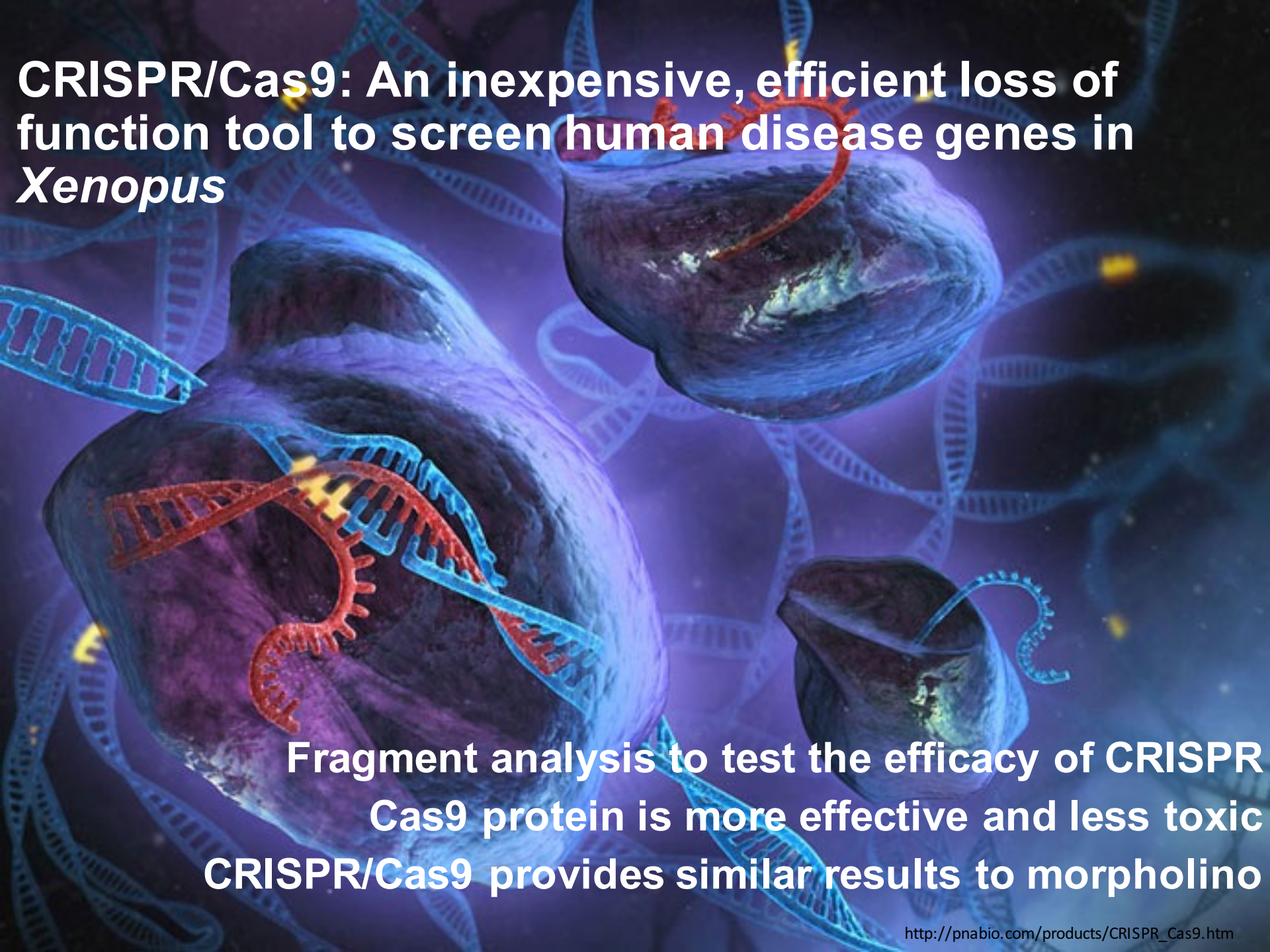
CRISPR/Cas9: An inexpensive, efficient loss of function tool to screen human disease genes in *Xenopus*

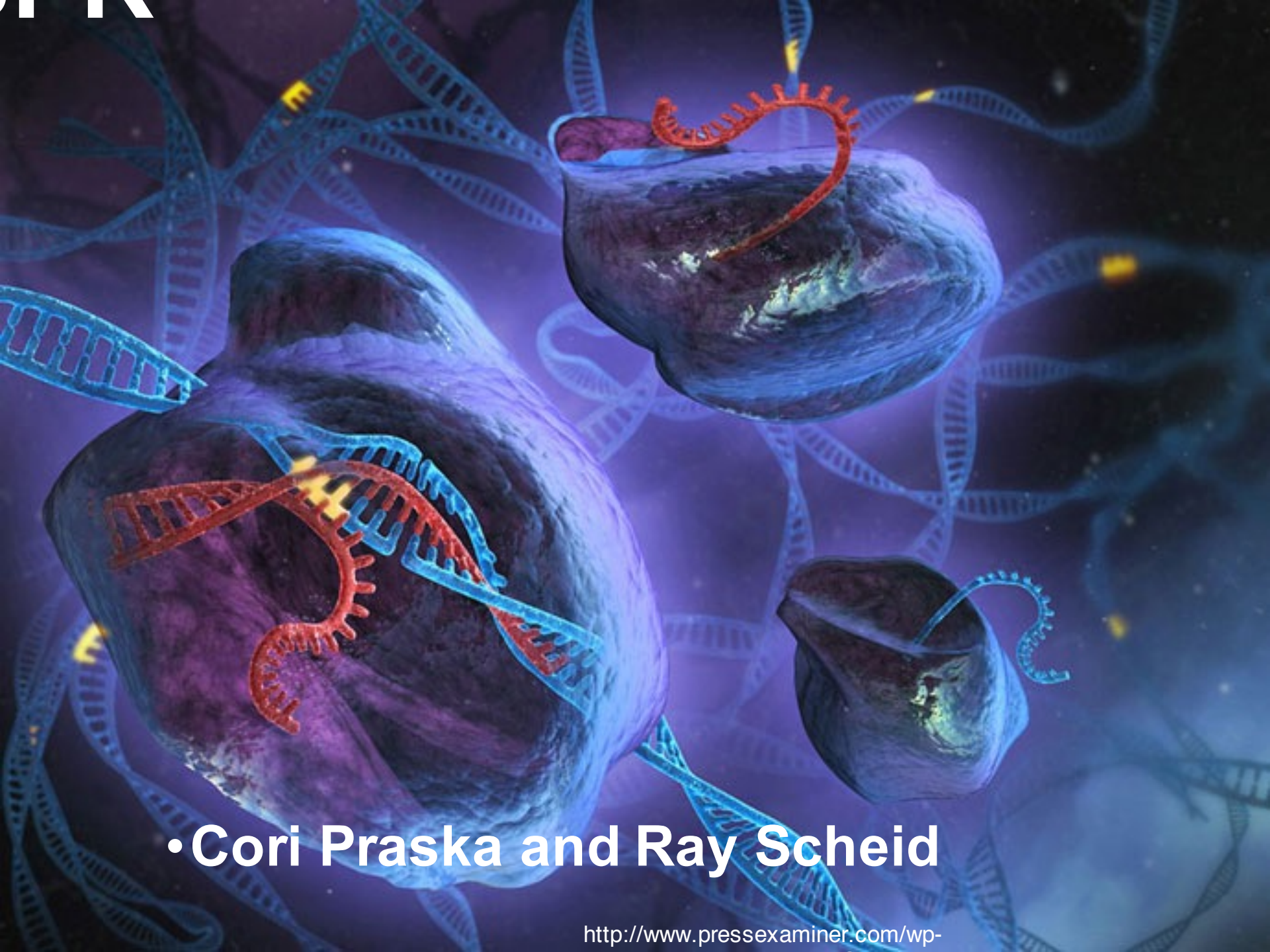


Fragment analysis to test the efficacy of CRISPR

Cas9 protein is more effective and less toxic

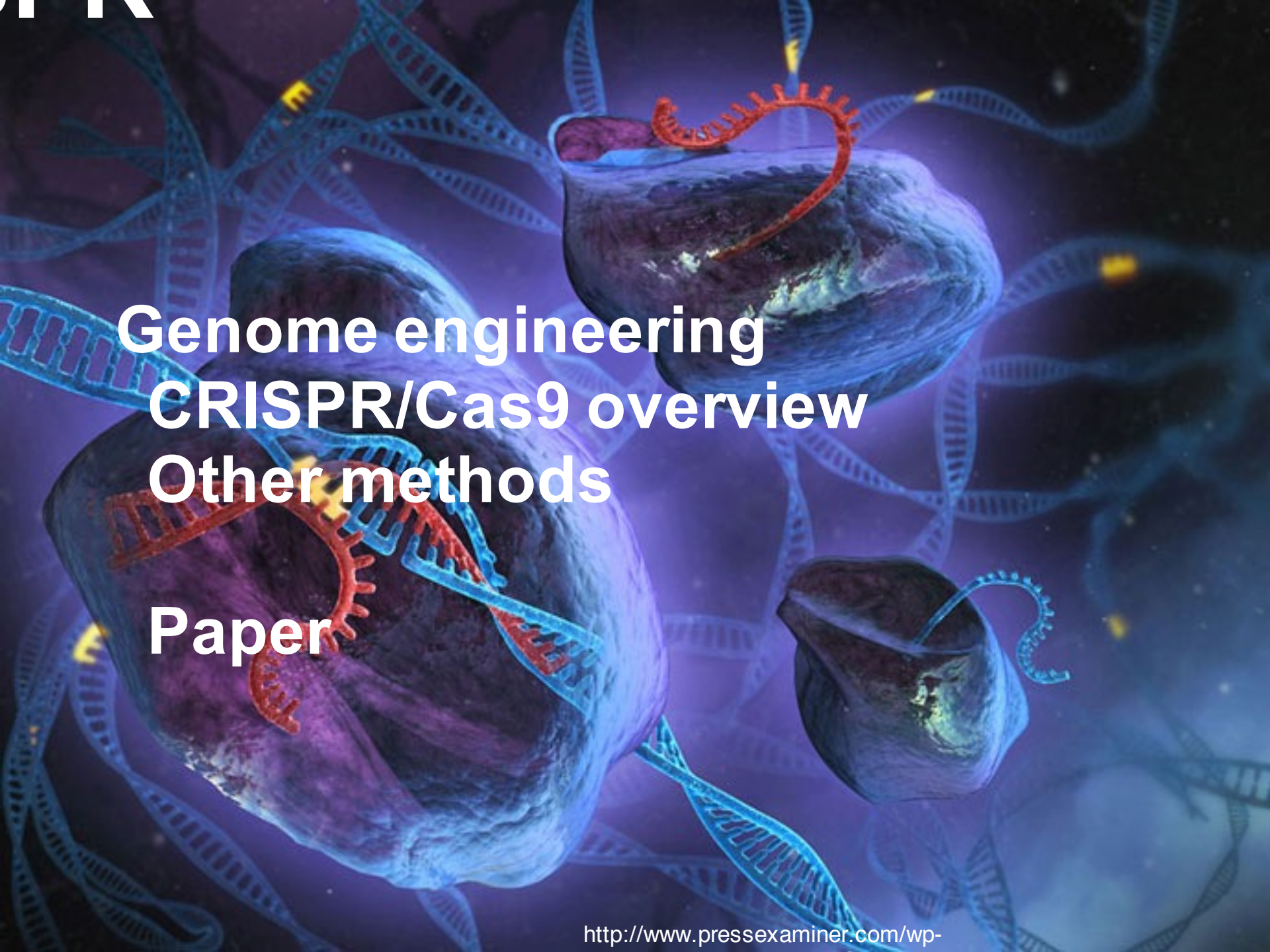
CRISPR/Cas9 provides similar results to morpholino

http://pnabio.com/products/CRISPR_Cas9.htm



- Cori Praska and Ray Scheid

http://www.pressexaminer.com/wp-

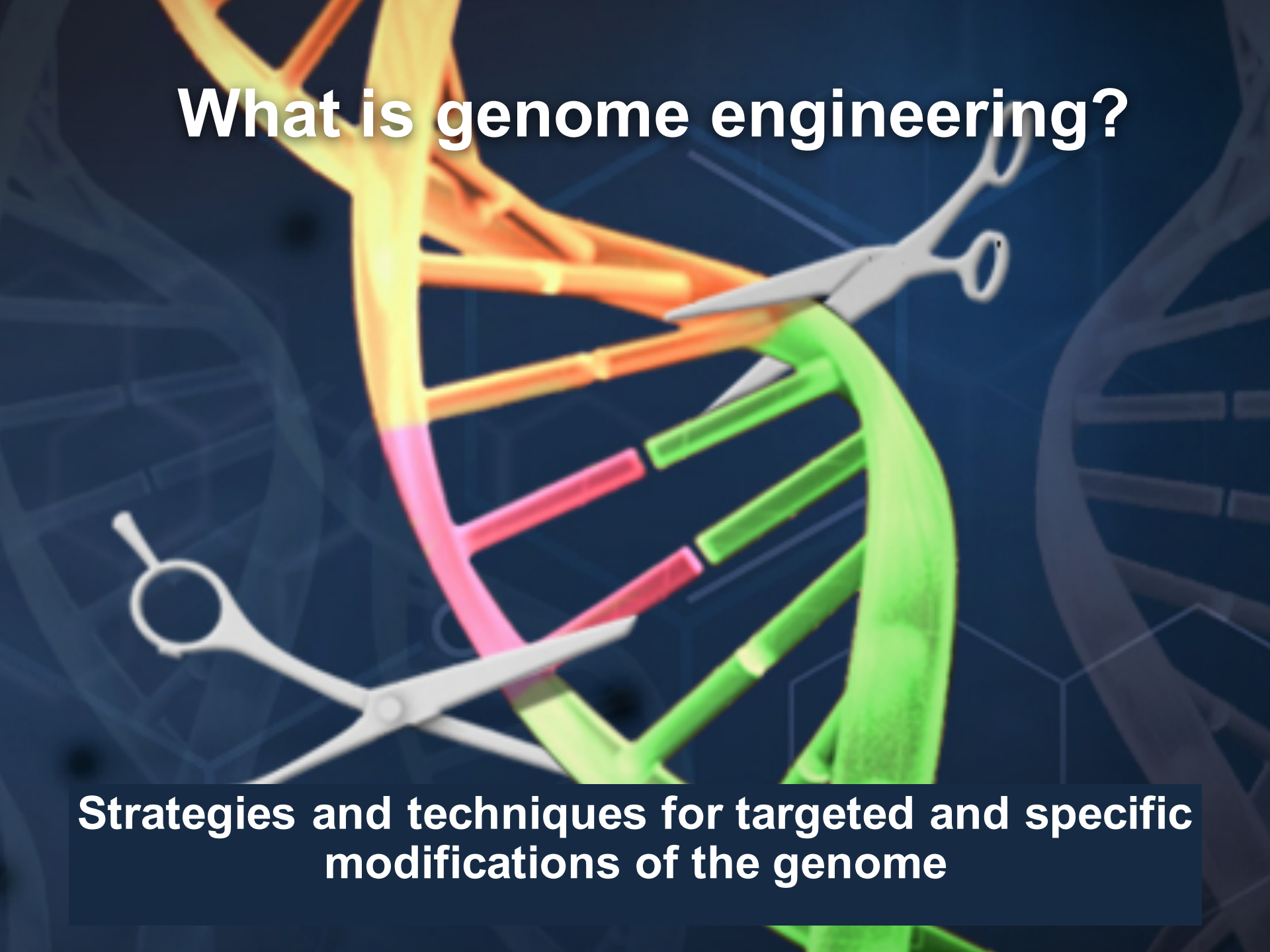


Genome engineering
CRISPR/Cas9 overview
Other methods

Paper

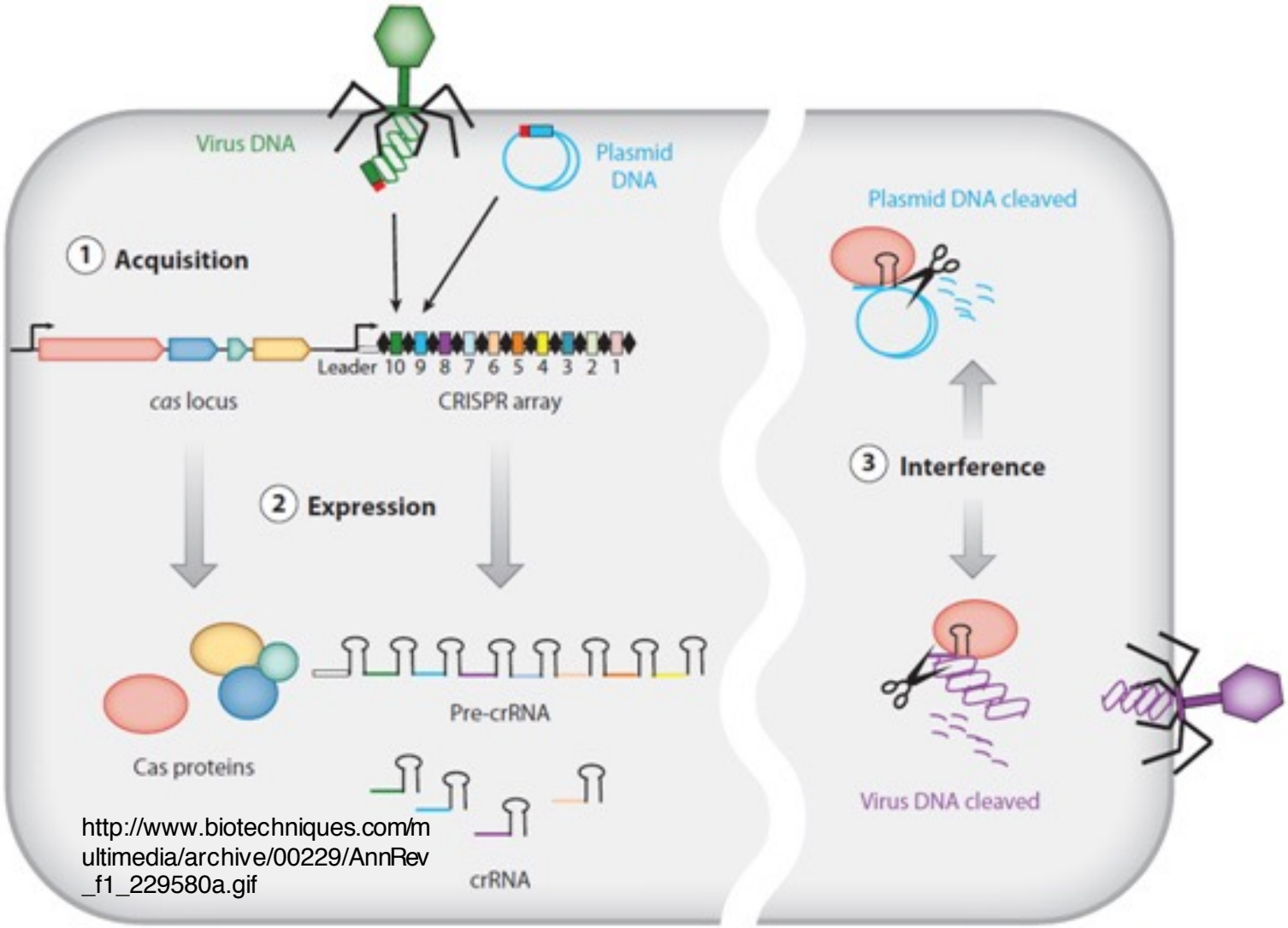
http://www.pressexaminer.com/wp-

What is genome engineering?



Strategies and techniques for targeted and specific modifications of the genome

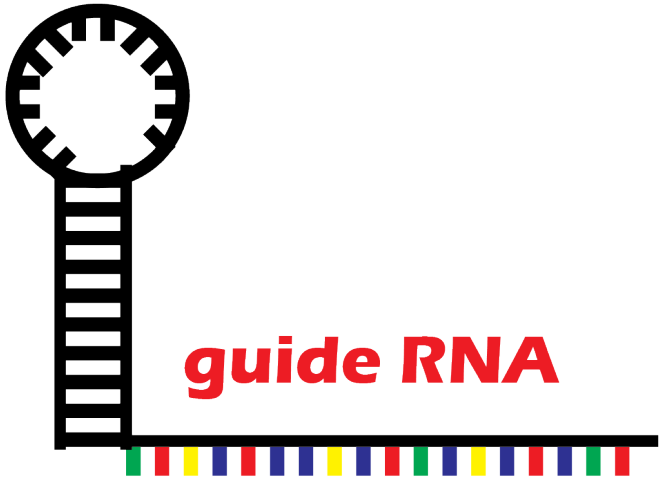
What is CRISPR/Cas9?



http://www.biotechniques.com/multimedia/archive/00229/AnnRev_f1_229580a.gif

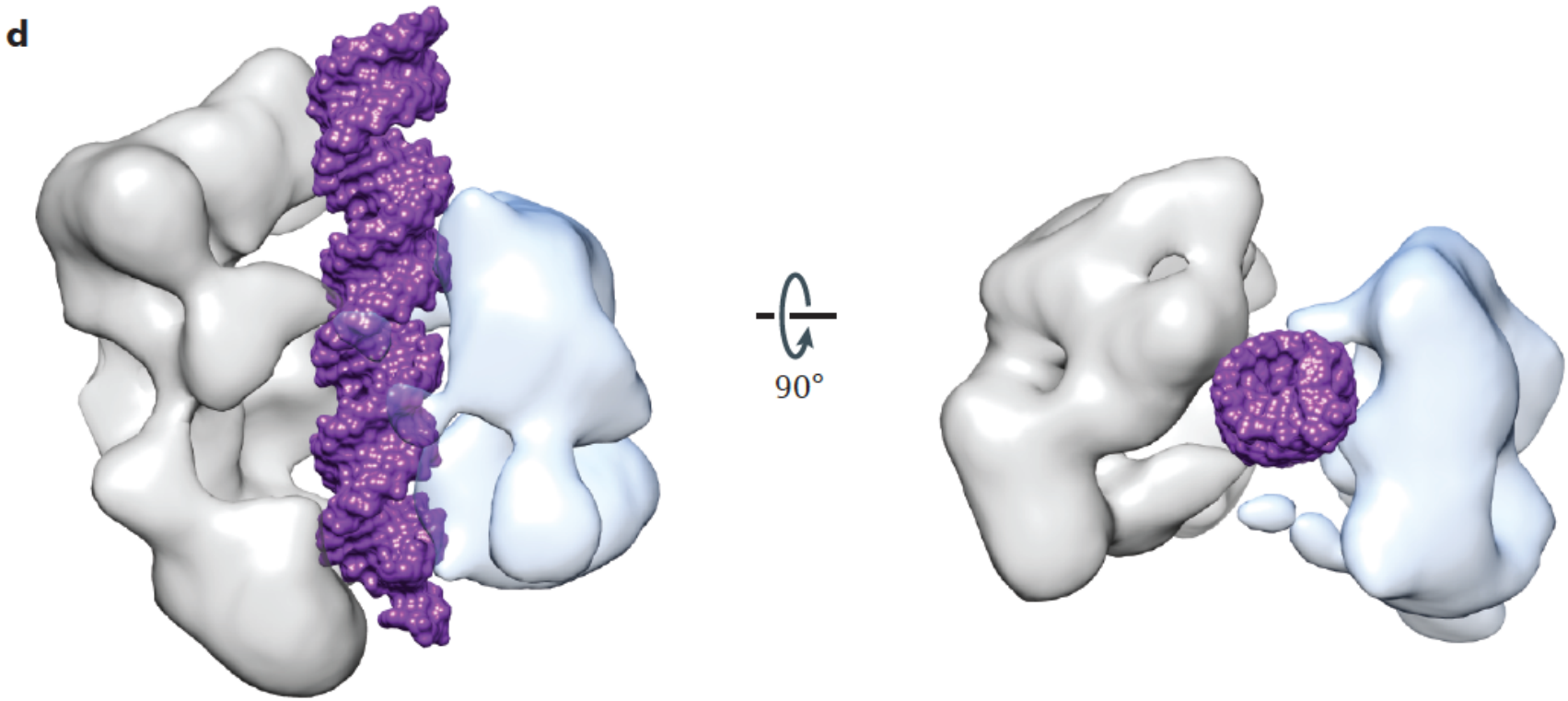
Clustered regularly interspaced short palindromic repeats (CRISPR)

CRISPR-CAS9 TARGETING THE THREE COMPONENTS:



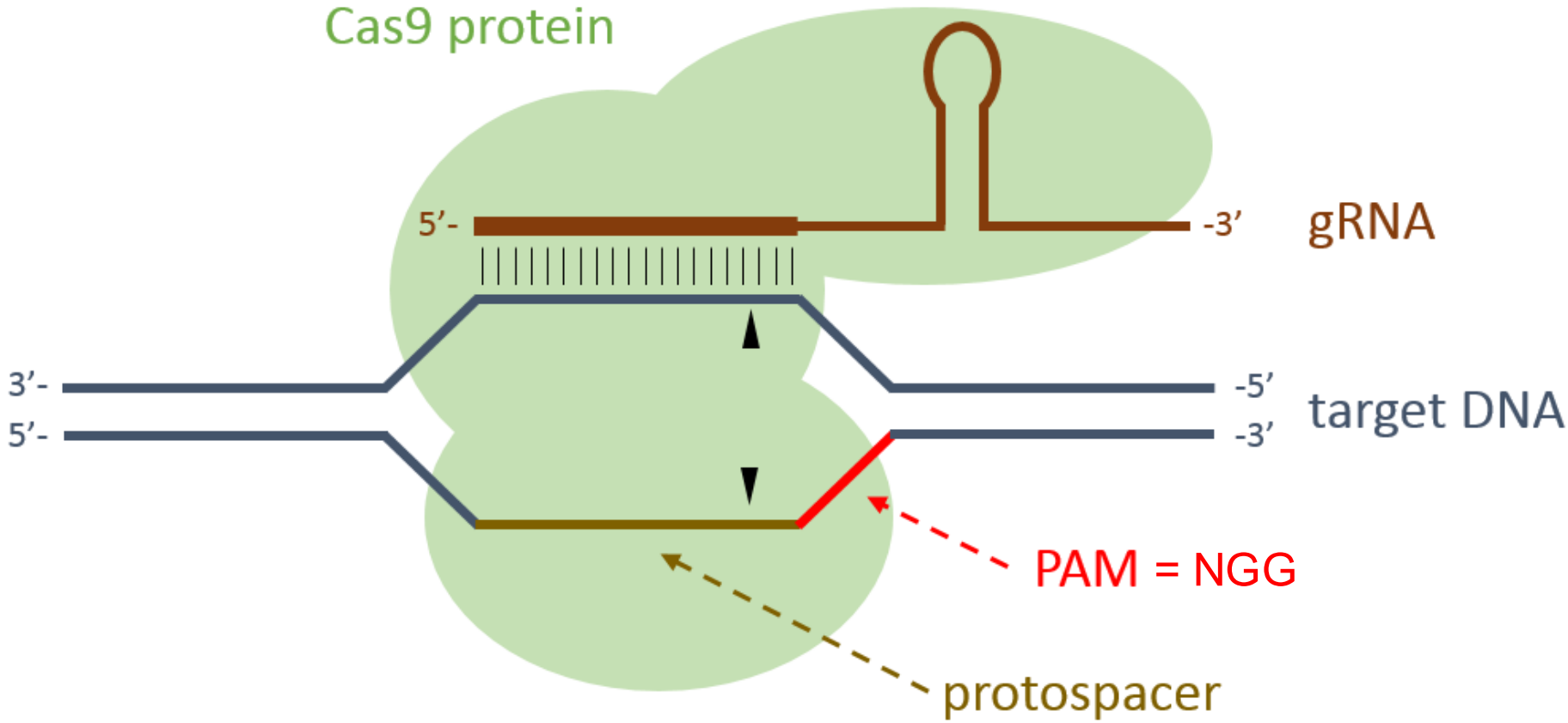
http://2013.igem.org/wiki/images/archive/6/6a/20130926052718!Cas9-VP16schematic

What does the nuclease Cas9 look like?



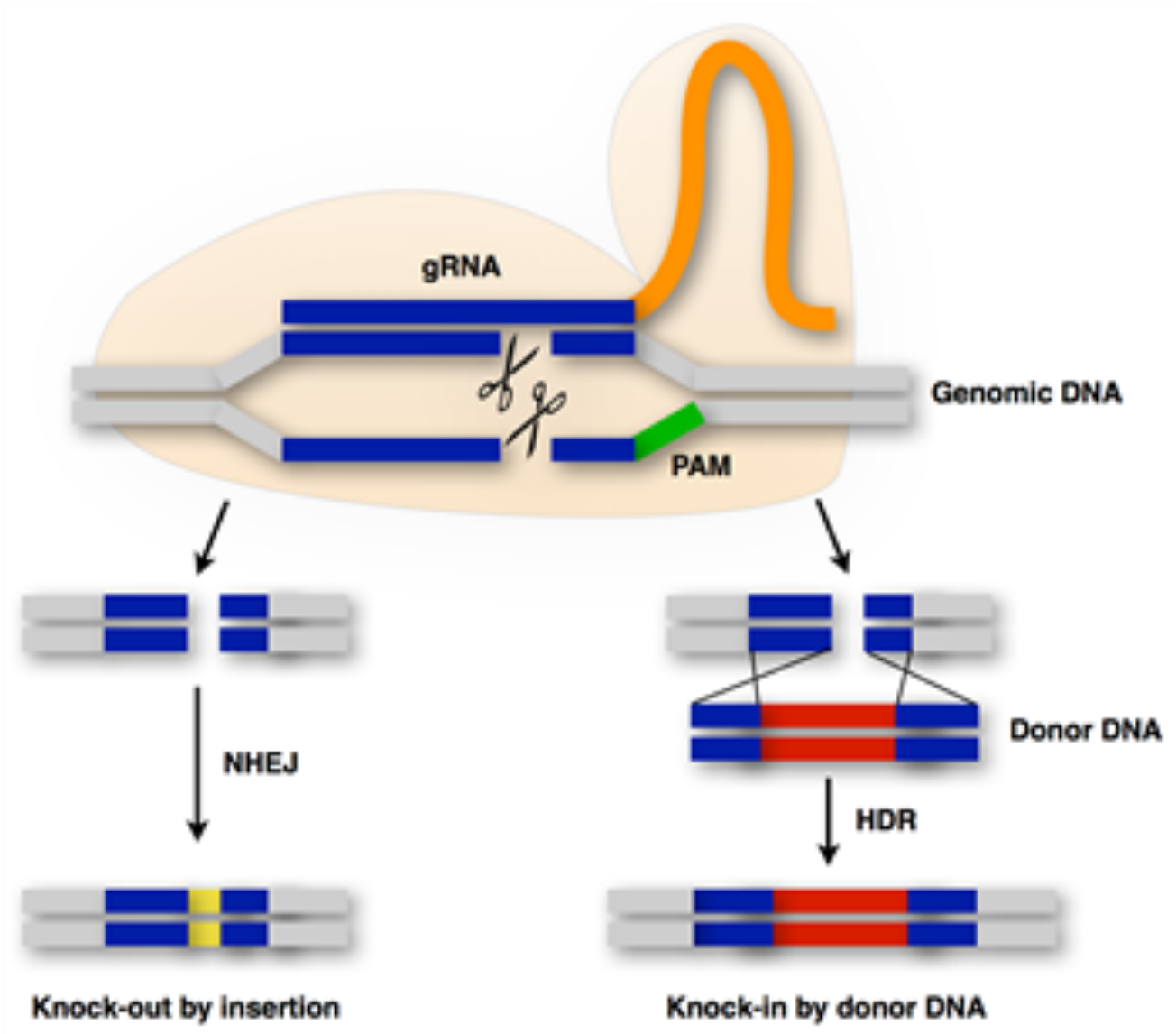
Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

How does the nuclease Cas9 work?



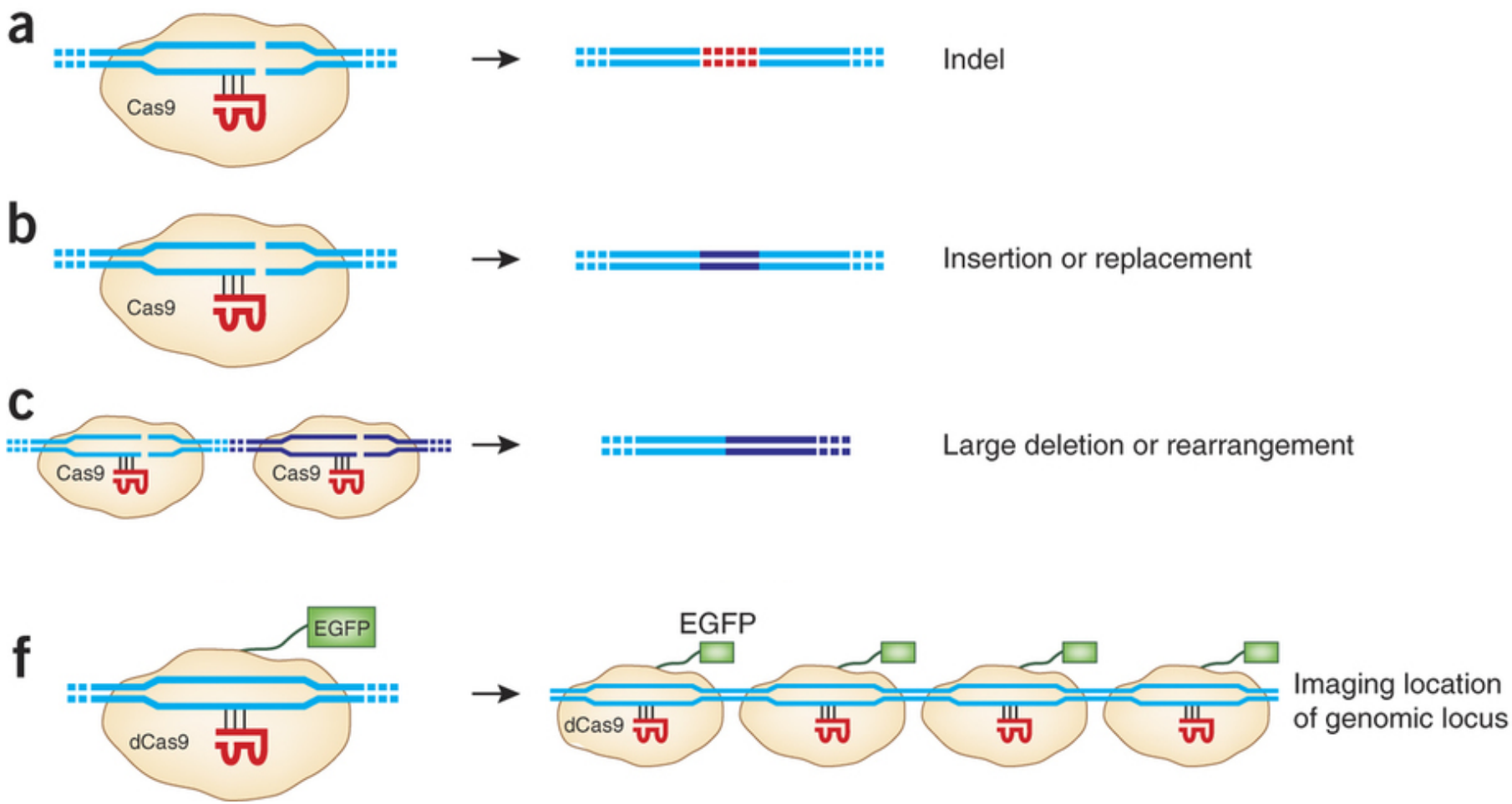
http://eendb.zfgenetics.org/casot/pic/cas9.png

How does the cell repair double strand breaks?



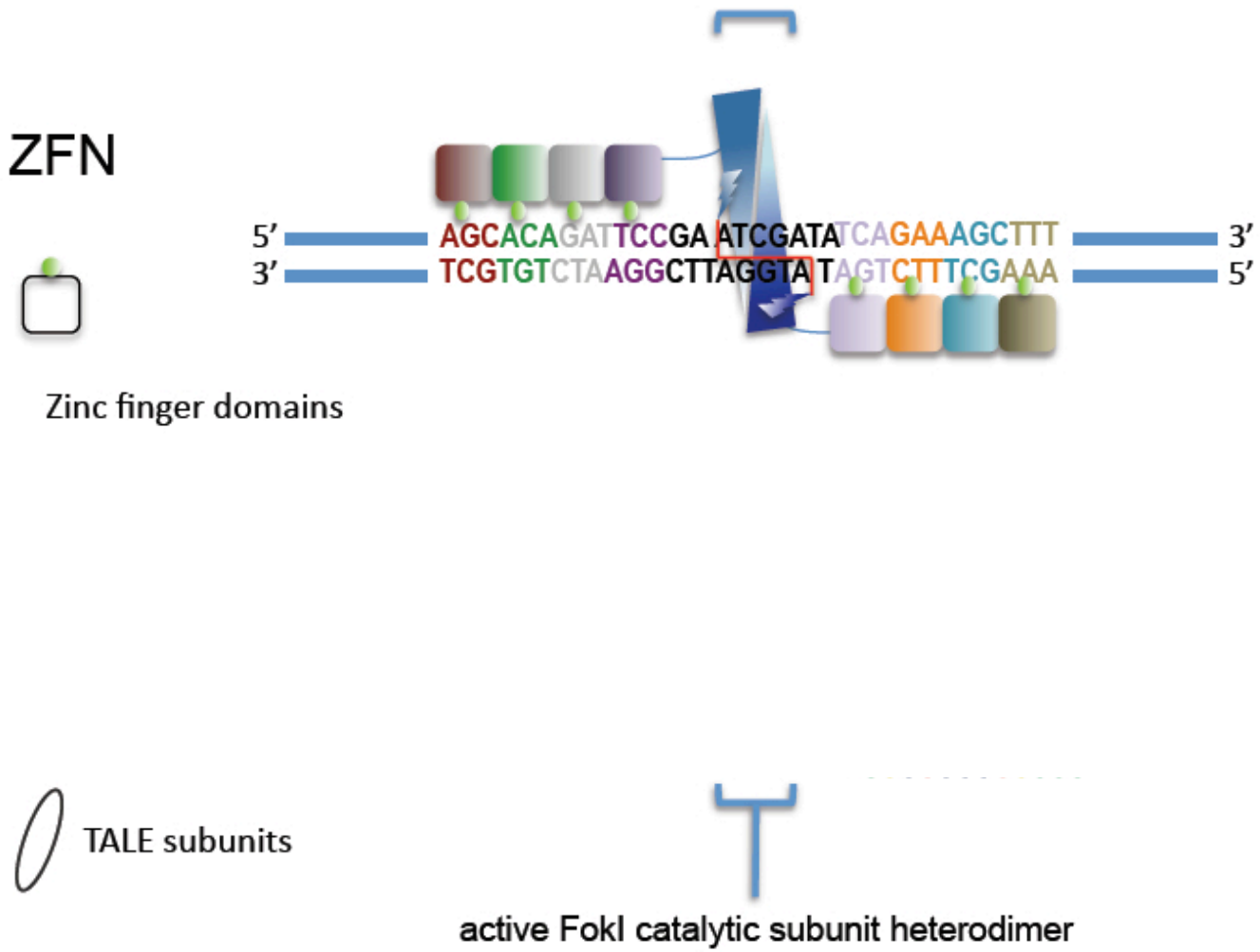
http://www.eurofinsgenomics.eu/media/1034726/crispr_tab3_en_350x308.jpg

What kinds of CRISPR changes can you make?



http://www.nature.com/nbt/journal/v32/n4/images_article/nbt.2842-

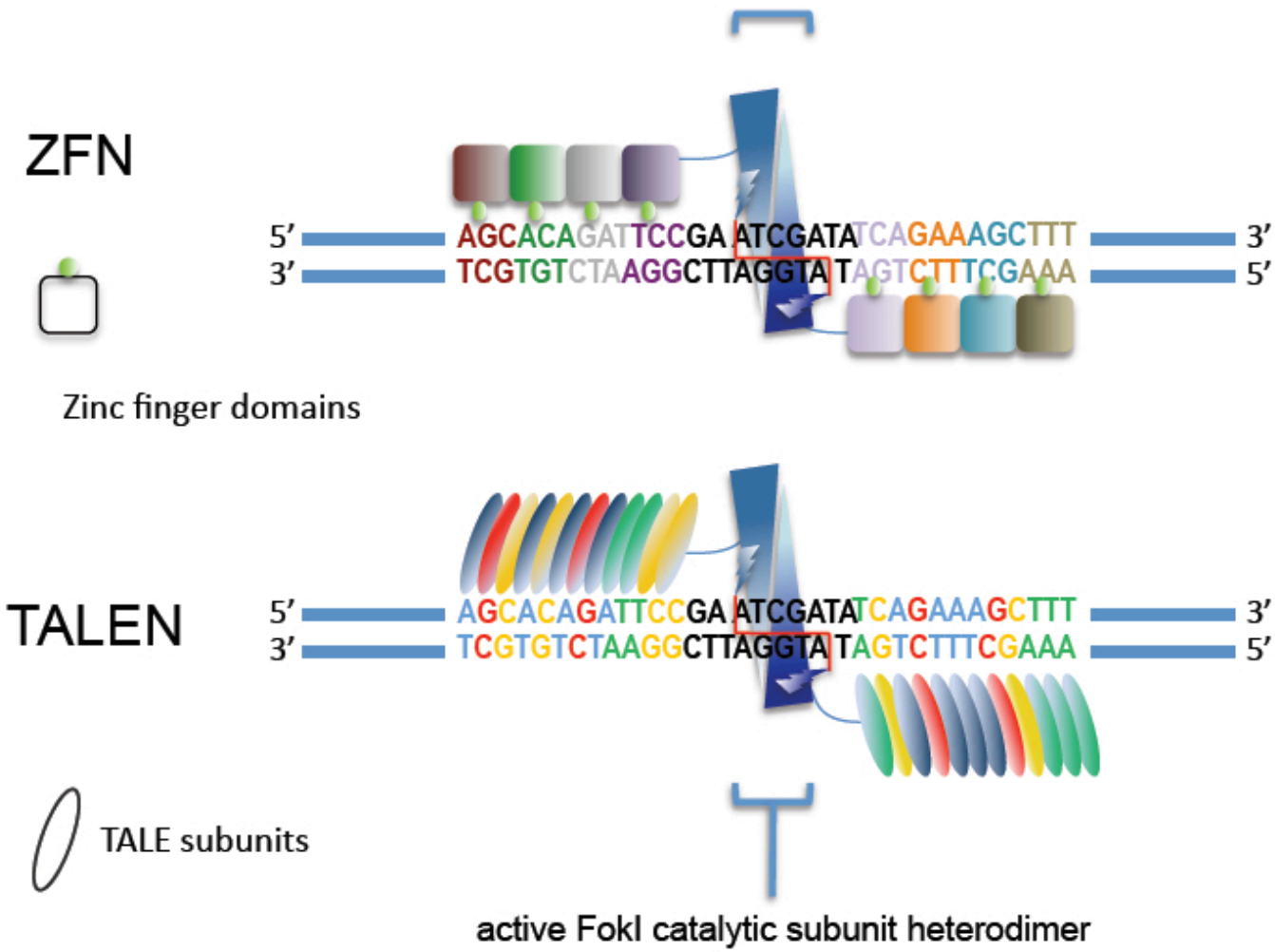
What were previous gene editing methods?



TALENS & ZFNs

https://upload.wikimedia.org/wikipedia/en/c/cd/Engineered_Nucleases.jpg

What were previous gene editing methods?



TALENS & ZFNs

https://upload.wikimedia.org/wikipedia/en/c/cd/Engineered_Nucleases.jpg

How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

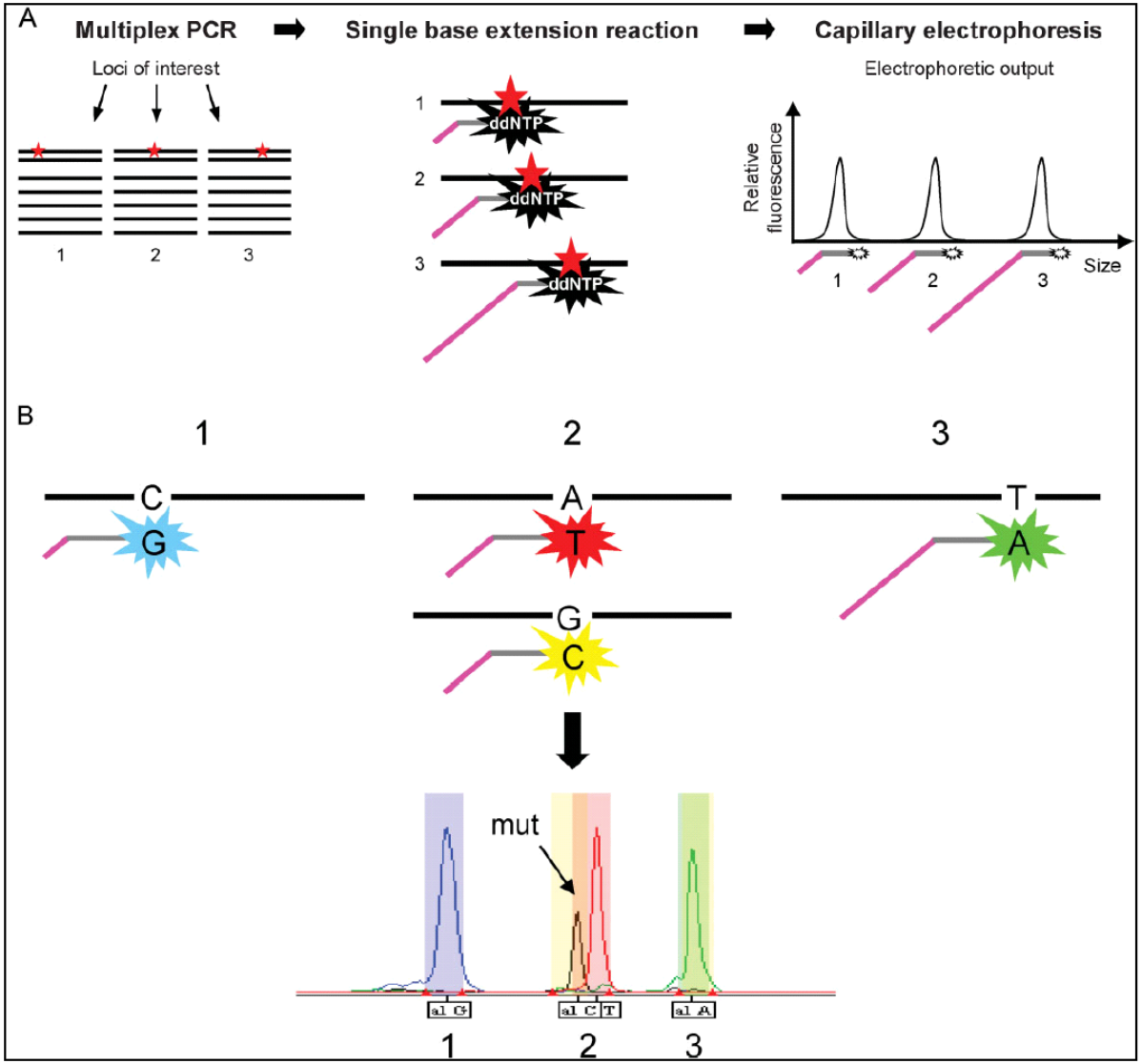
How does CRISPR/Cas9 compare with TALEN and ZFN?

Table 2 | **Comparison of three classes of programmable nucleases***

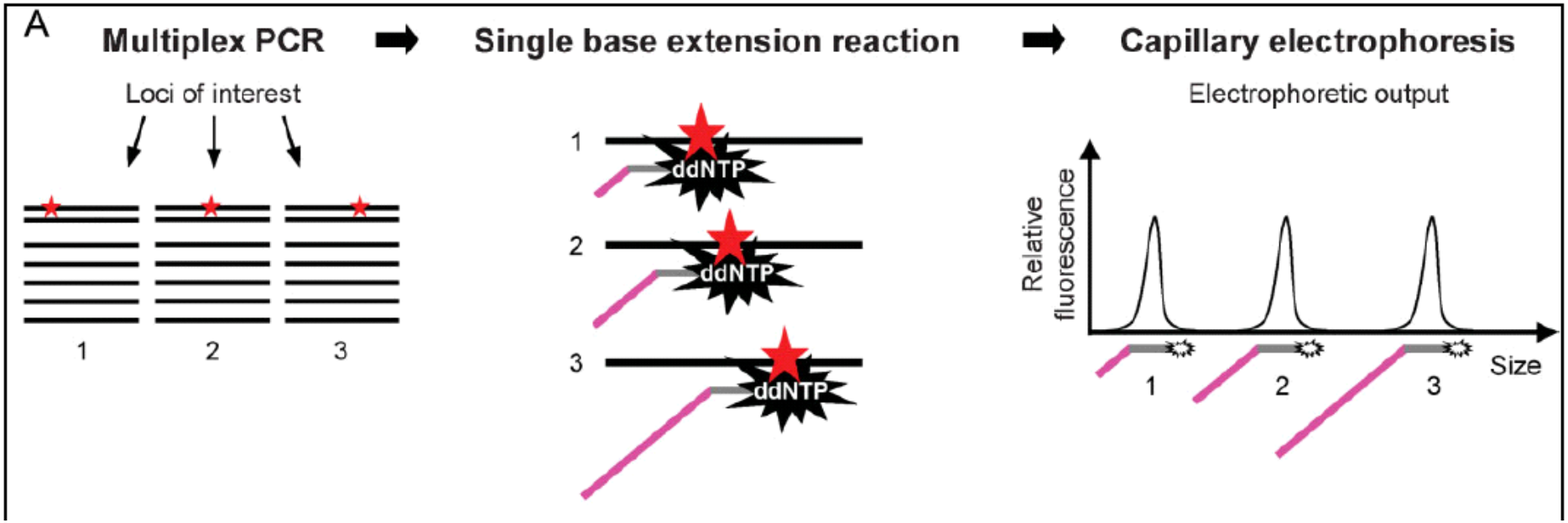
	ZFNs	TALENs	RGENs
DNA targeting specificity determinant	Zinc-finger proteins	Transcription activator-like effectors	crRNA or sgRNA
Nuclease	*Fok*I	*Fok*I	Cas9
Success rate‡	Low (~24%)	High (>99%)	High (~90%)
Average mutation rate§	Low or variable (~10%)	High (~20%)	High (~20%)
Specificity-determining length of target site	18–36 bp	30–40 bp	22 bp (total length 23 bp)
Restriction in target site	G-rich	Start with T and end with A (owing to the heterodimer structure)	End with an NGG or NAG (lower activity) sequence (that is, PAM)
Design density	One per ~100 bp	At least one per base pair	One per 8 bp (NGG PAM) or 4 bp (NGG and NAG PAM)
Off-target effects	High	Low	Variable
Cytotoxicity	Variable to high	Low	Low
Size	~1 kb × 2	~3 kb × 2	4.2 kb (Cas9 from *Streptococcus pyogenes*) + 0.1 kb (sgRNA)

Hyongbum K, Jin-Soo K (2014) *Nature Rev Genet* 15: 321-334

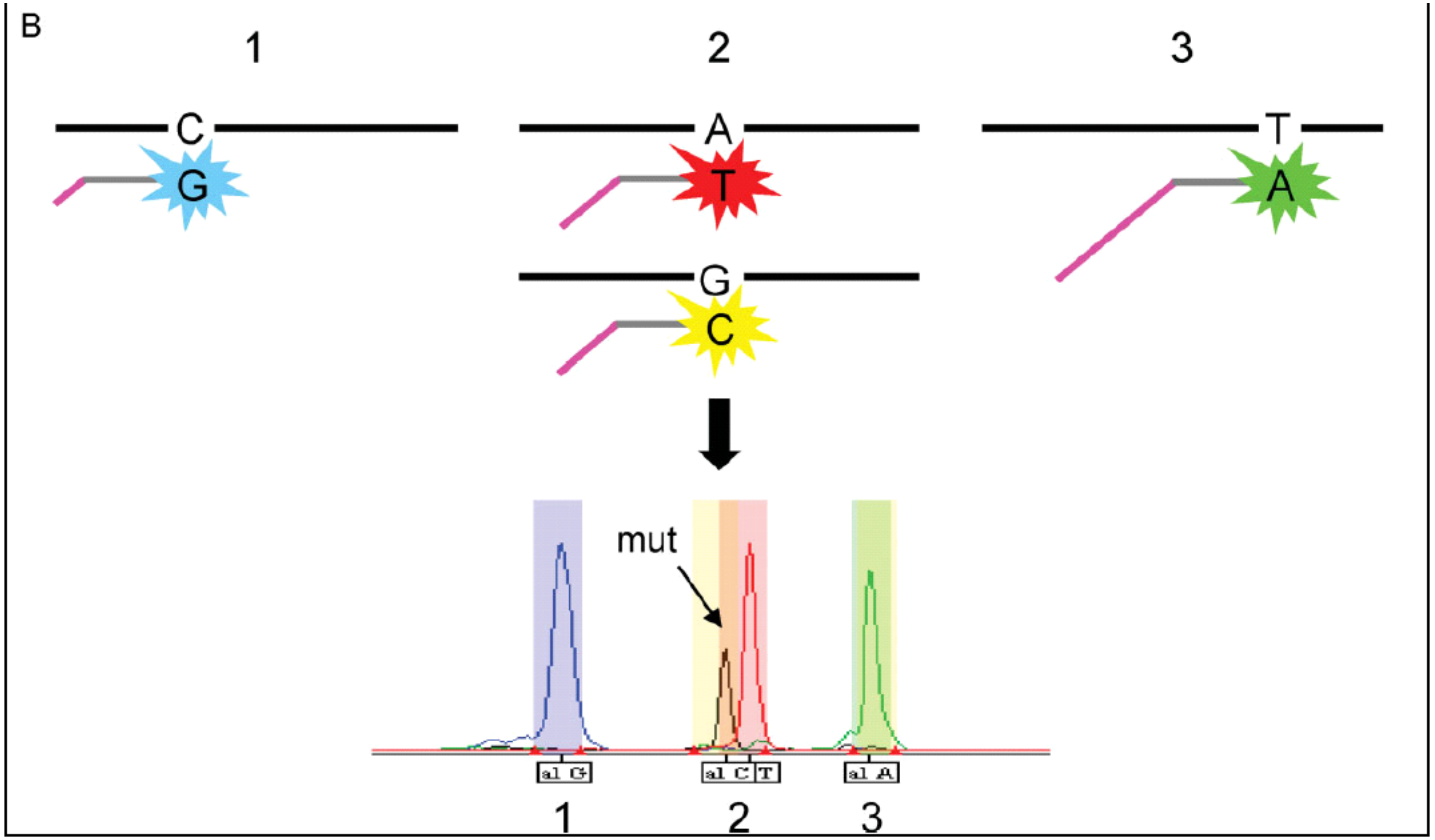
What is Fragment analysis (FA)?



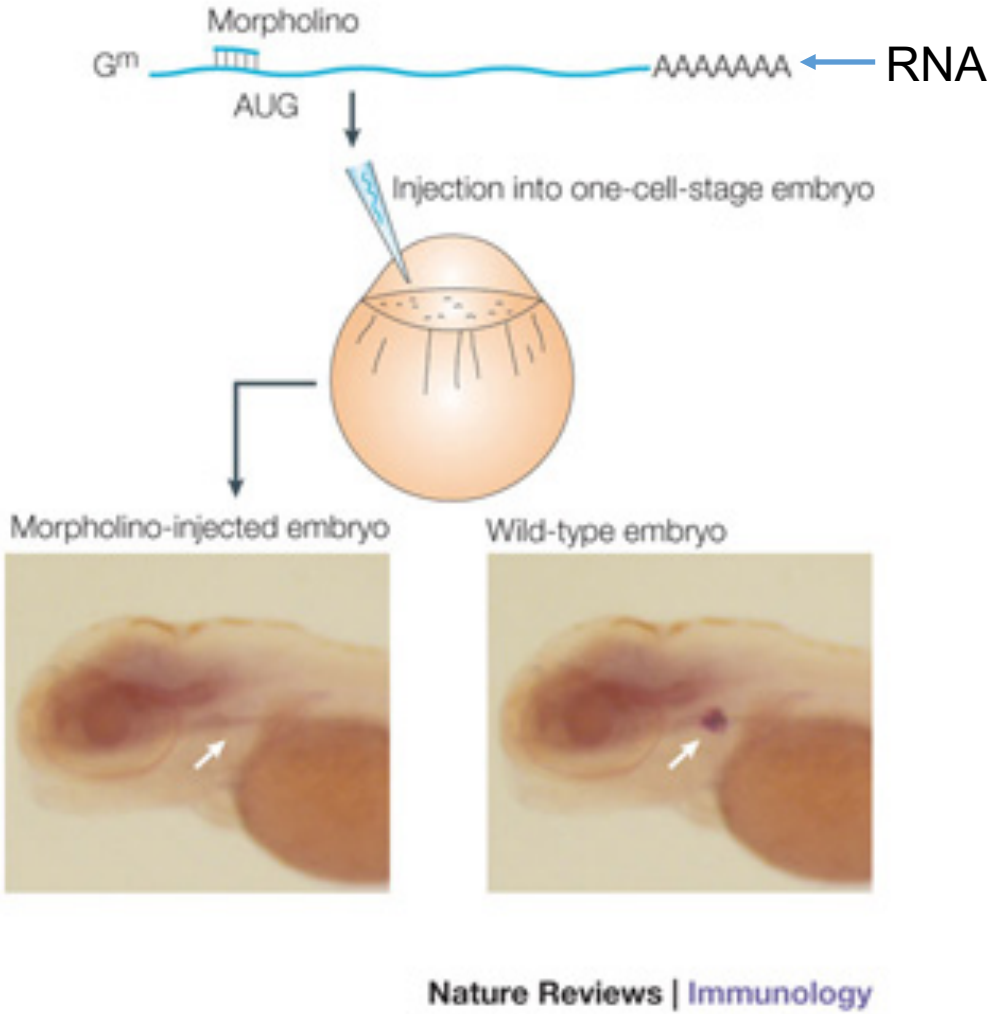
What is Fragment analysis (FA)?



What is Fragment analysis (FA)?



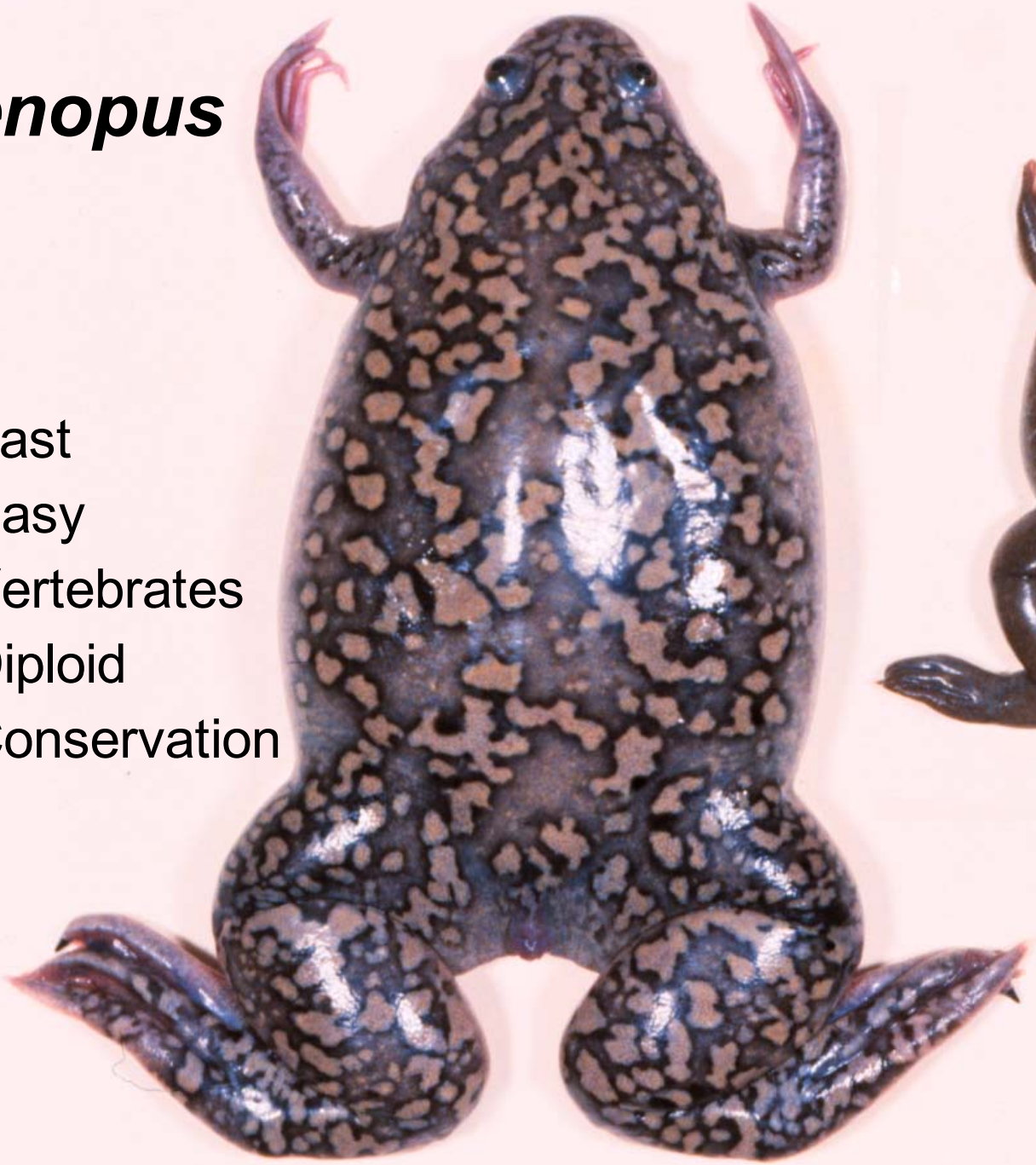
How did they knock down genes in Xenopus and Zebrafish before Cas9?



Morpholino oligos (MOs)

Xenopus

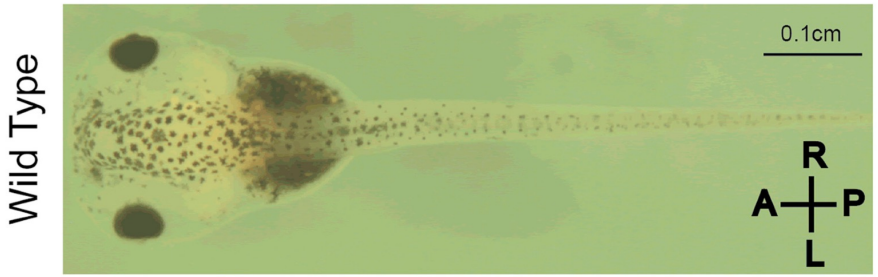
- Fast
- Easy
- Vertebrates
- Diploid
- Conservation

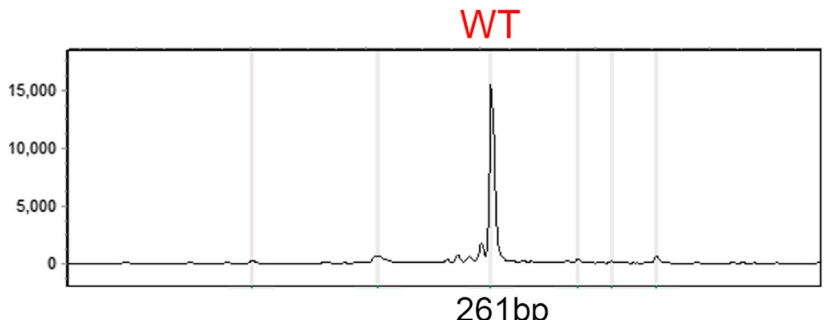


http://genomics.crick.ac.uk/online/xt-fl-db.html

How is fragment analysis used to analyze CRISPR?

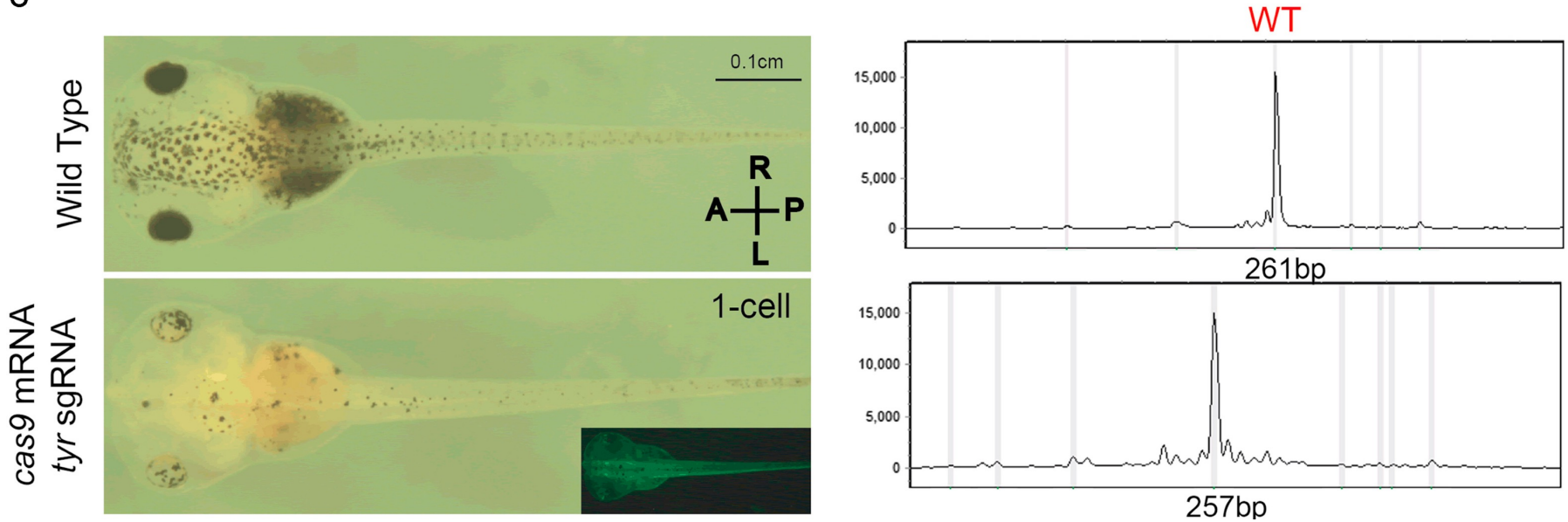
c





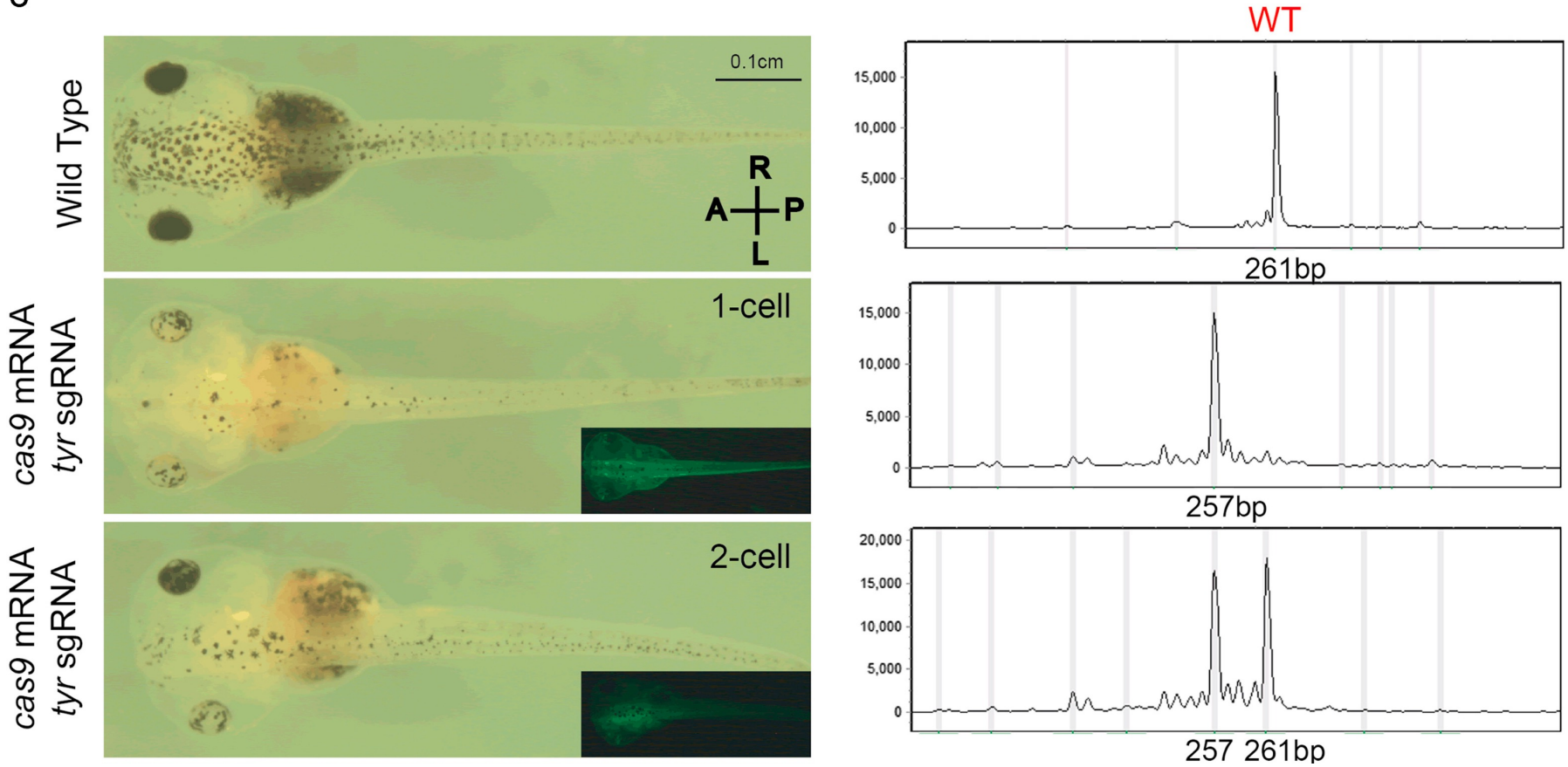
How is fragment analysis used to analyze CRISPR?

c



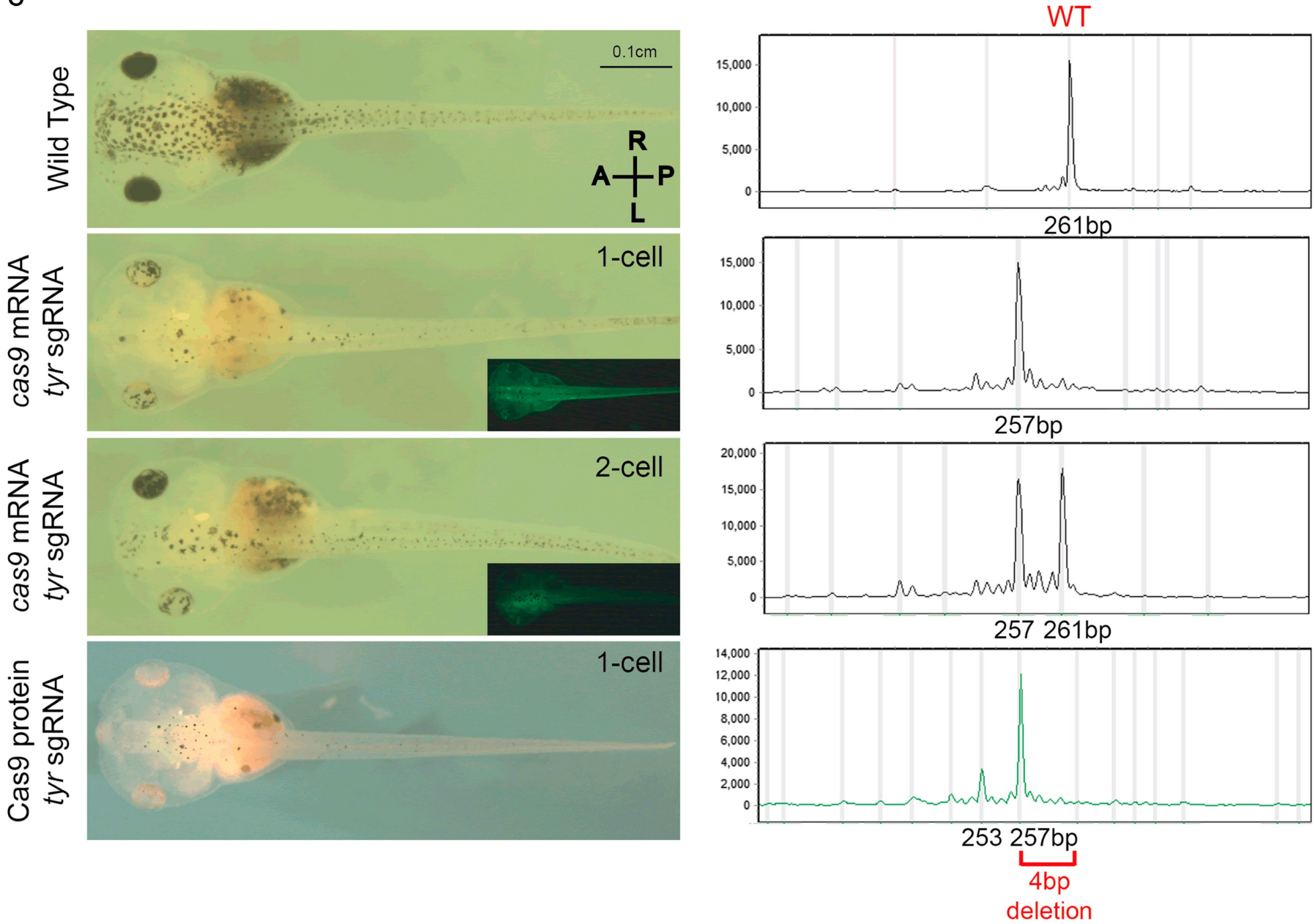
How is fragment analysis used to analyze CRISPR?

c

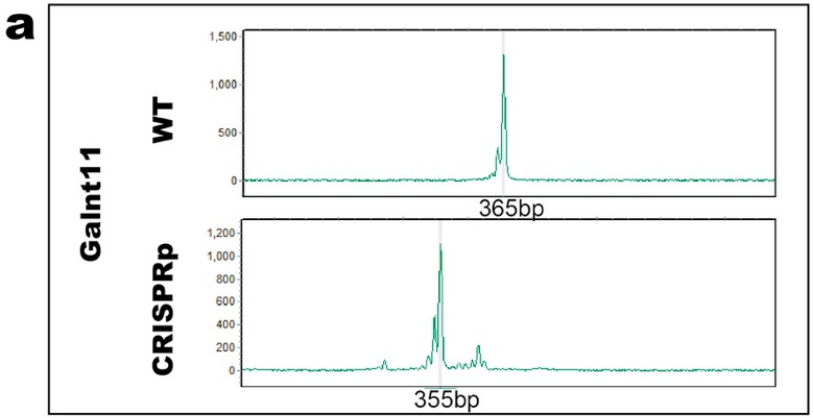


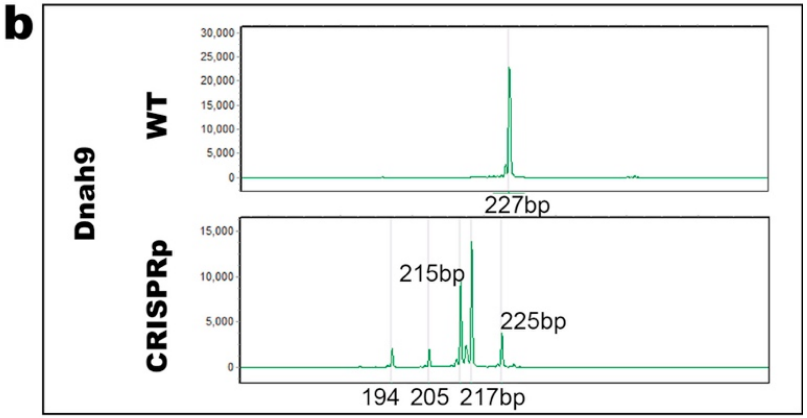
How is fragment analysis used to analyze CRISPR?

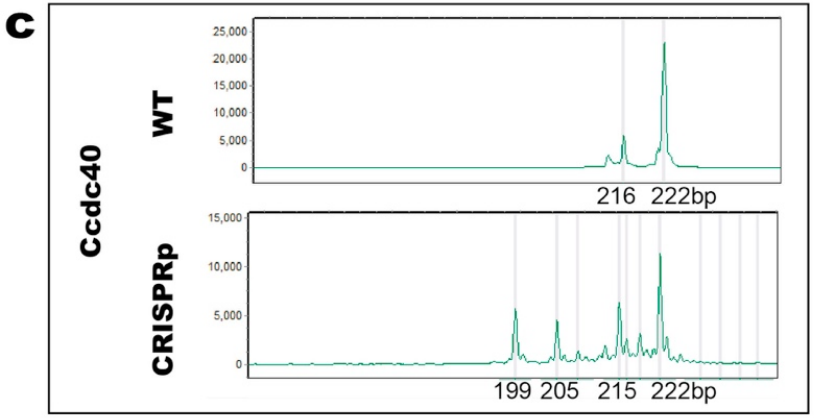
c

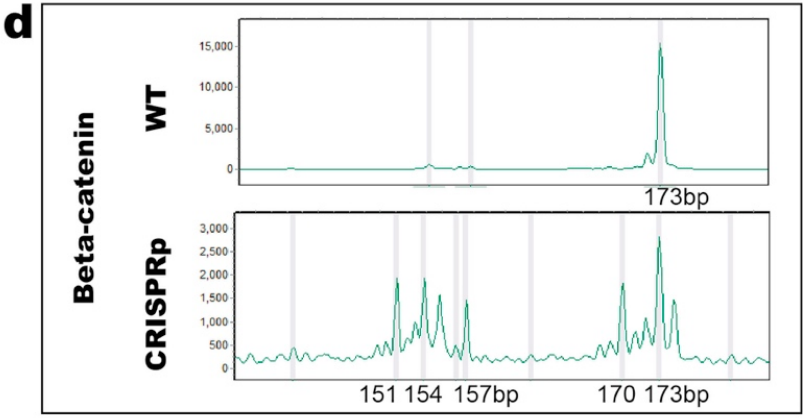


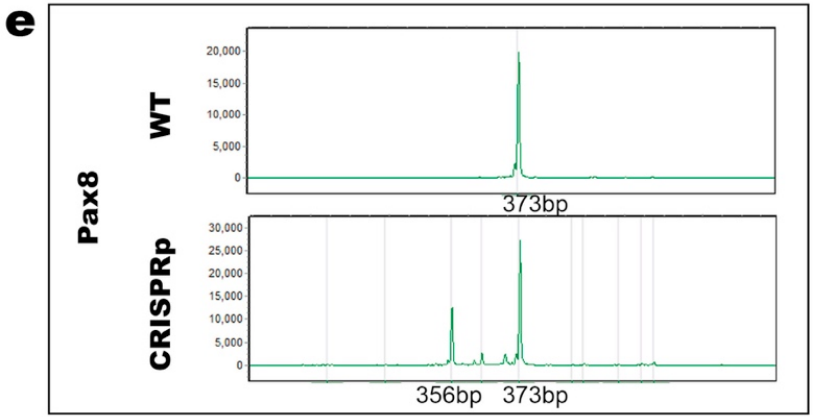
How do different sgRNA's affect results?



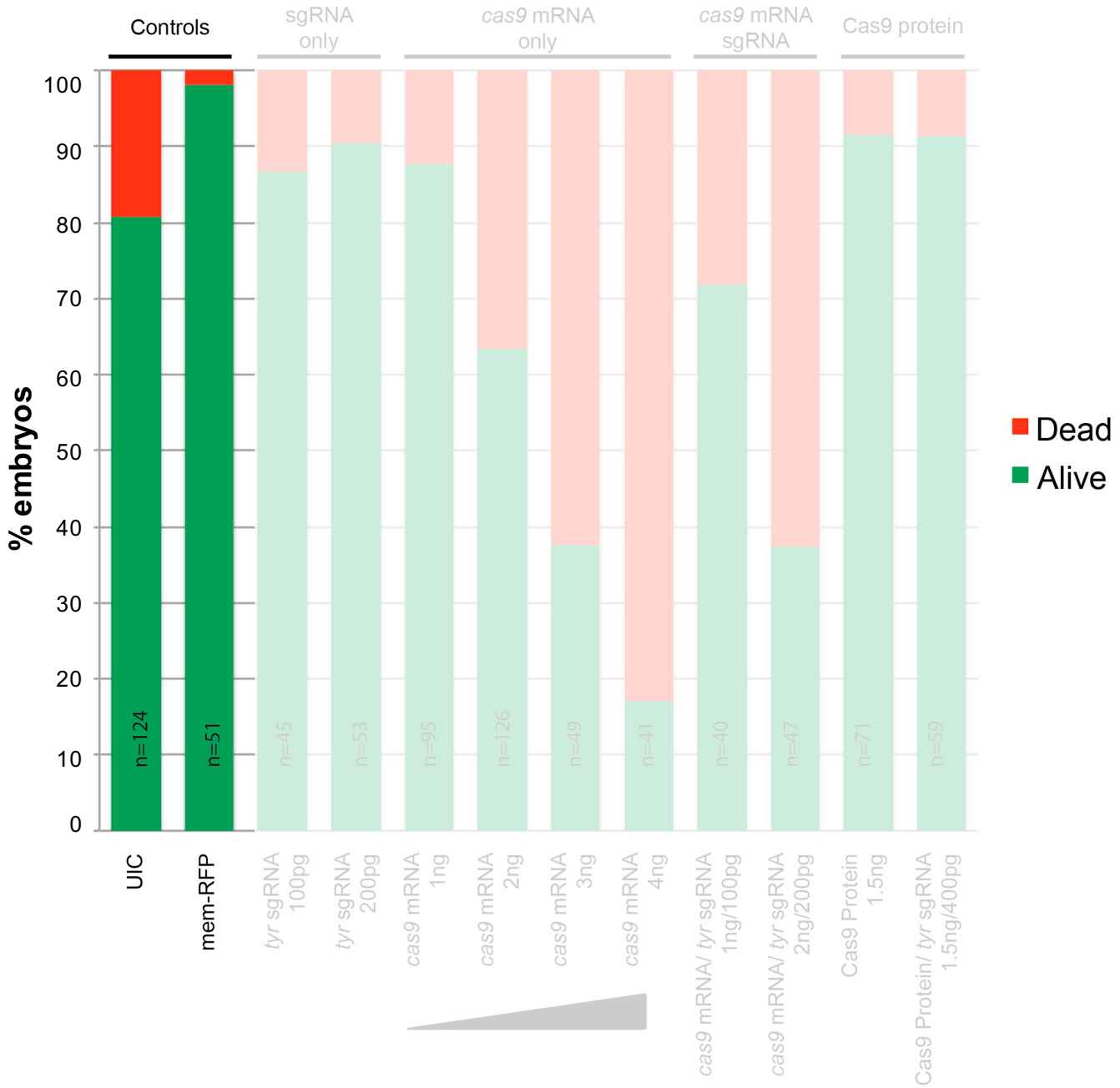




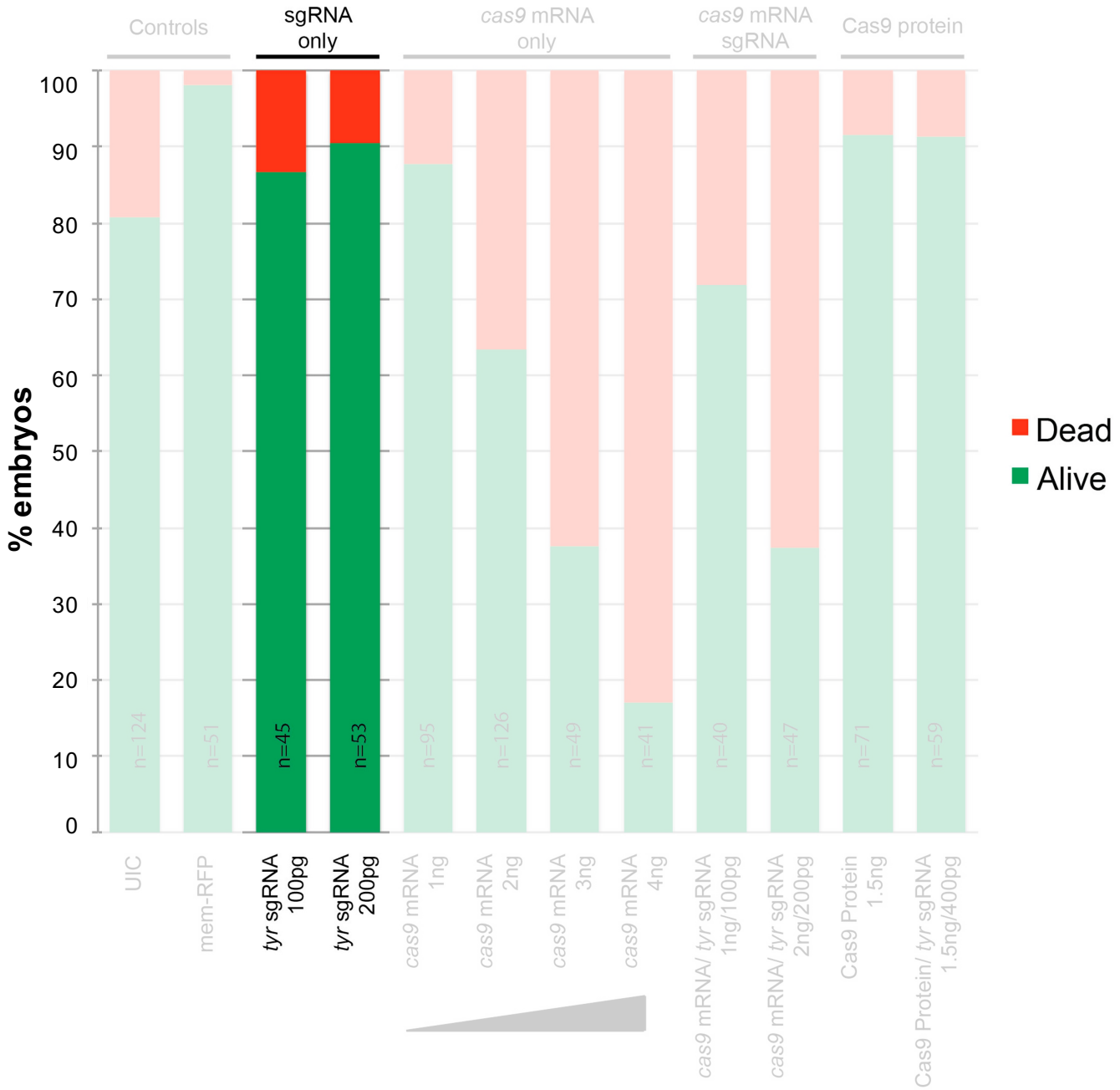




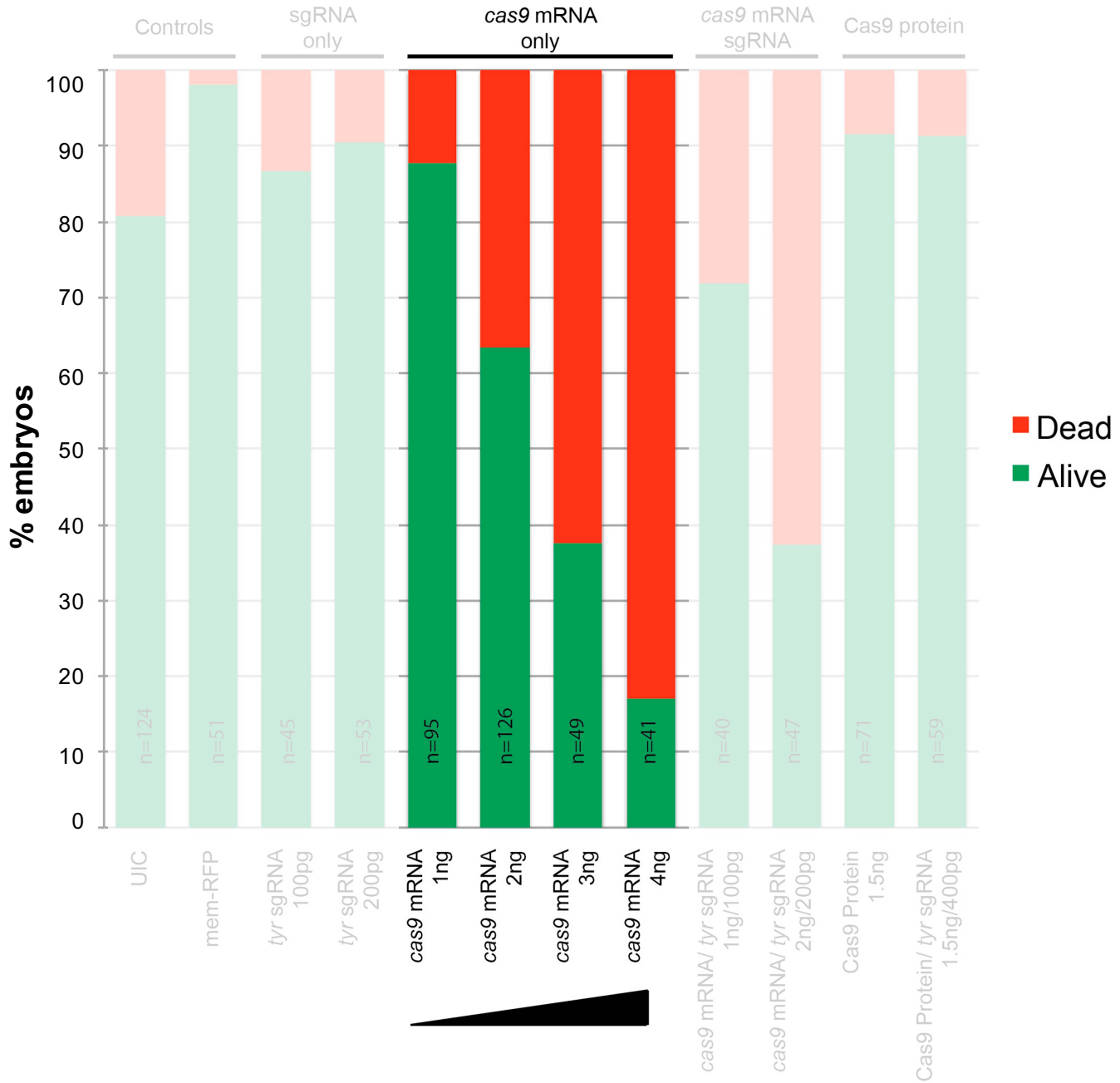
How does Cas9 protein toxicity compare to *cas9* mRNA?



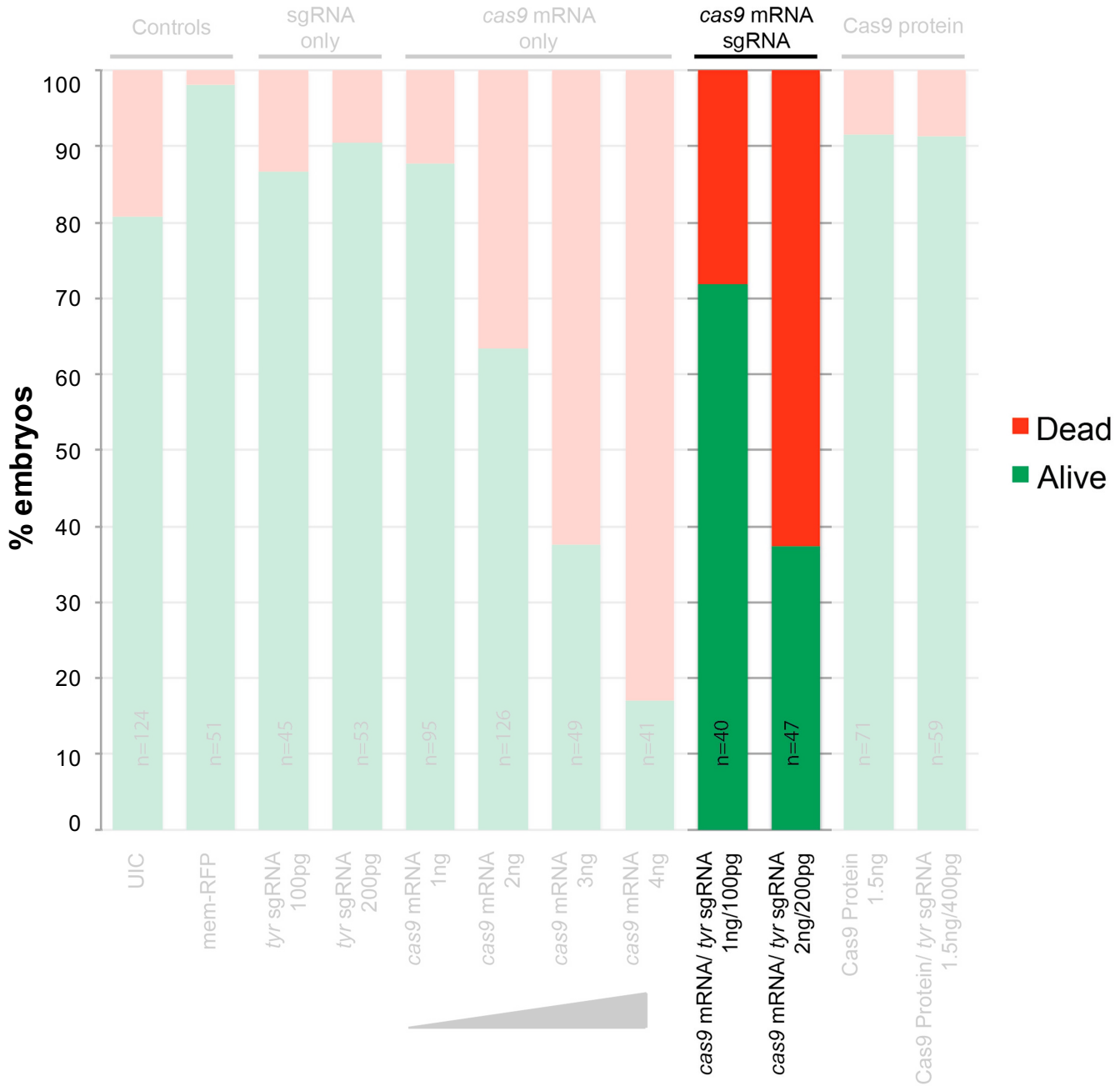
How does Cas9 protein toxicity compare to *cas9* mRNA?



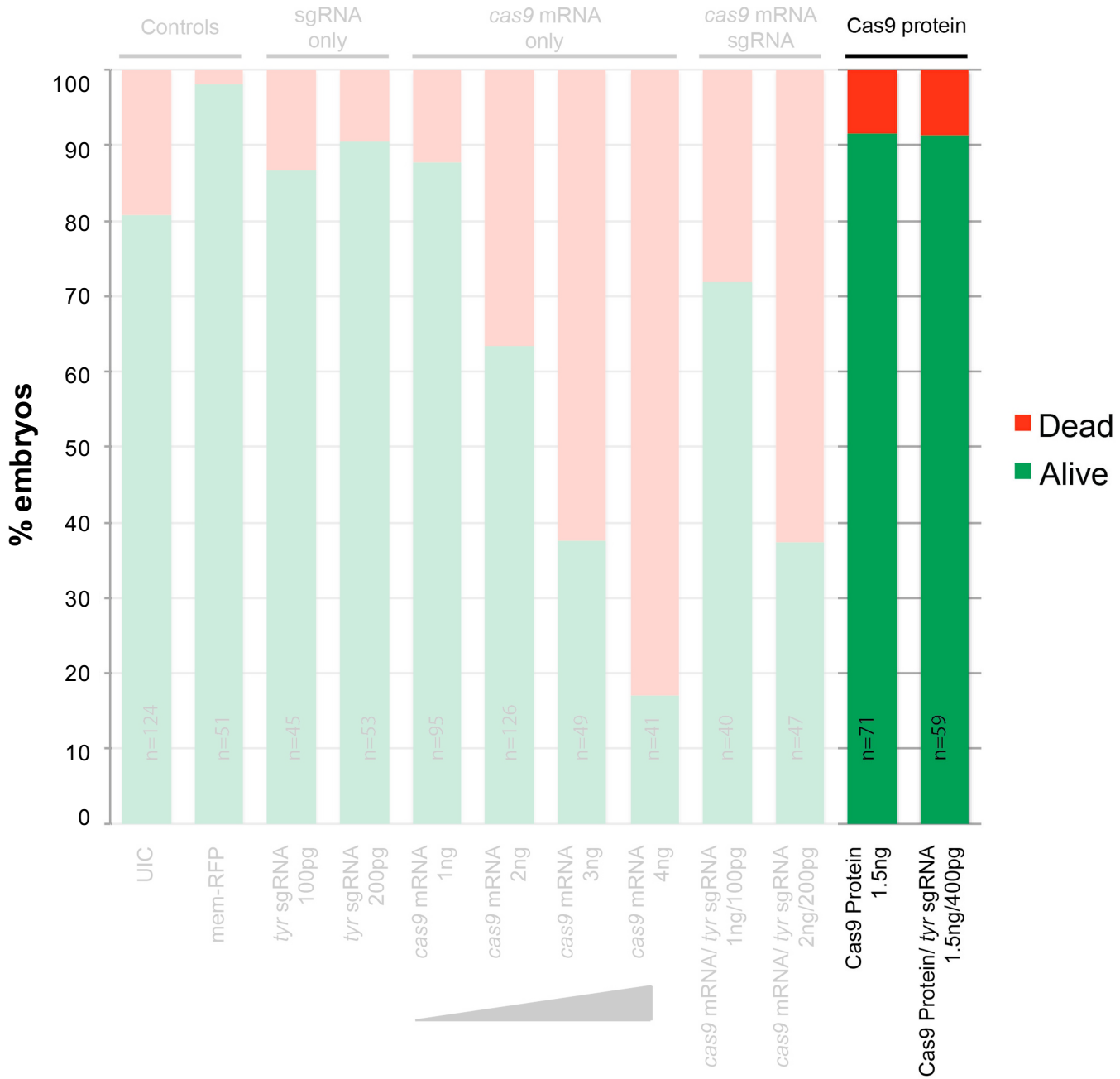
How does Cas9 protein toxicity compare to *cas9* mRNA?



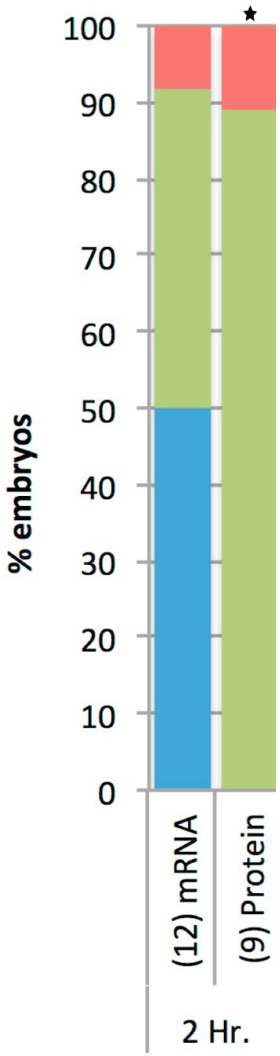
How does Cas9 protein toxicity compare to *cas9* mRNA?

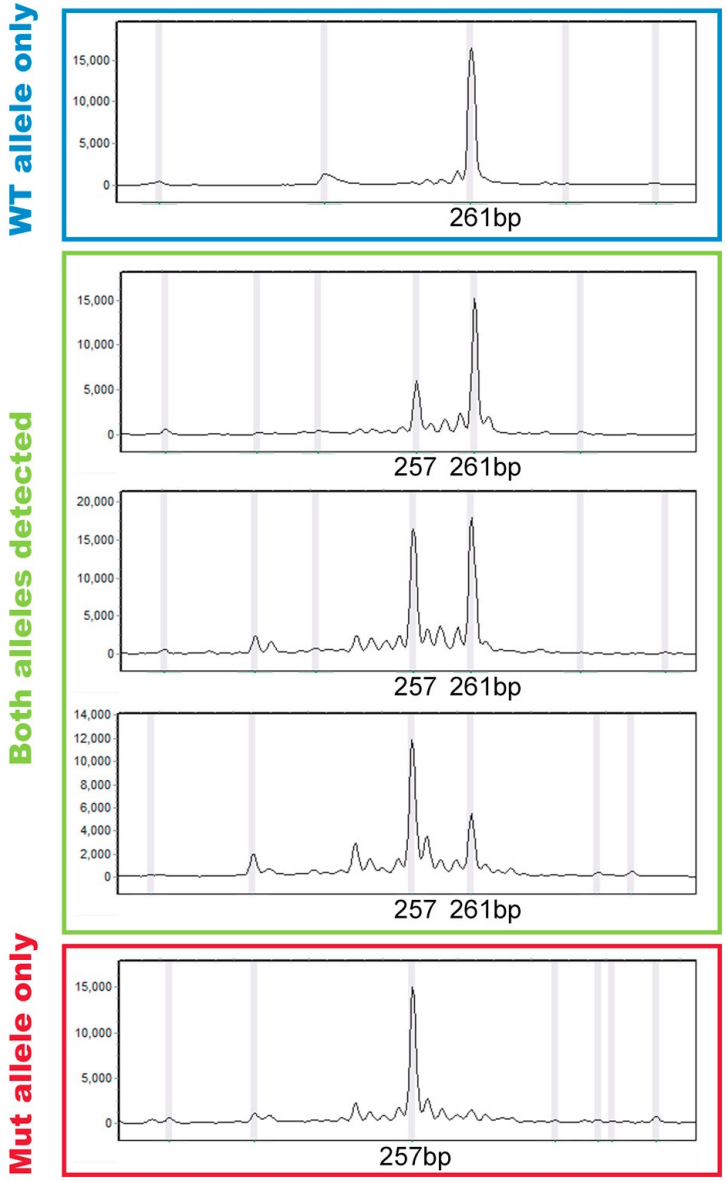


How does Cas9 protein toxicity compare to *cas9* mRNA?

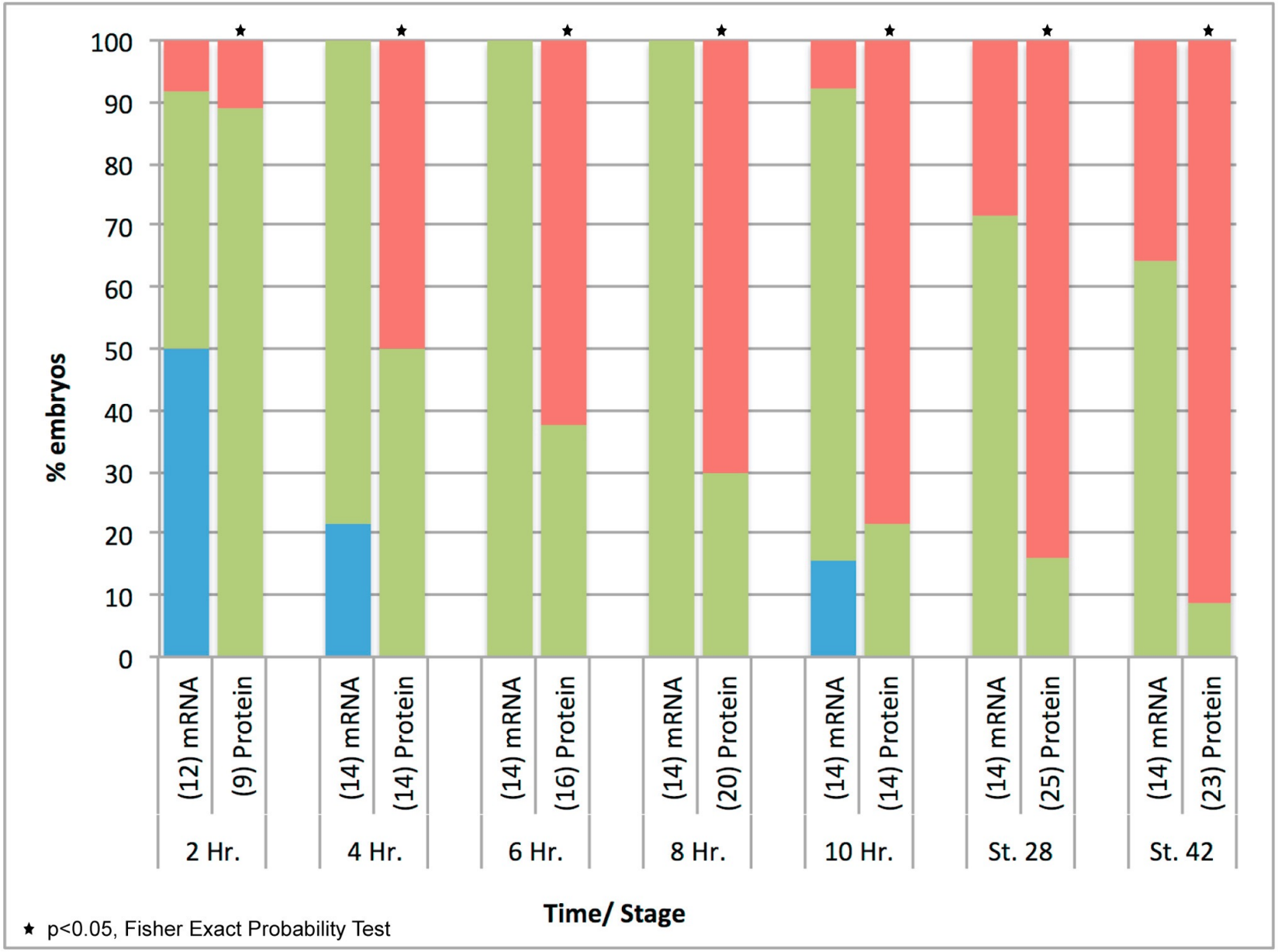


How does Cas9 protein perform compared to *cas9* mRNA?

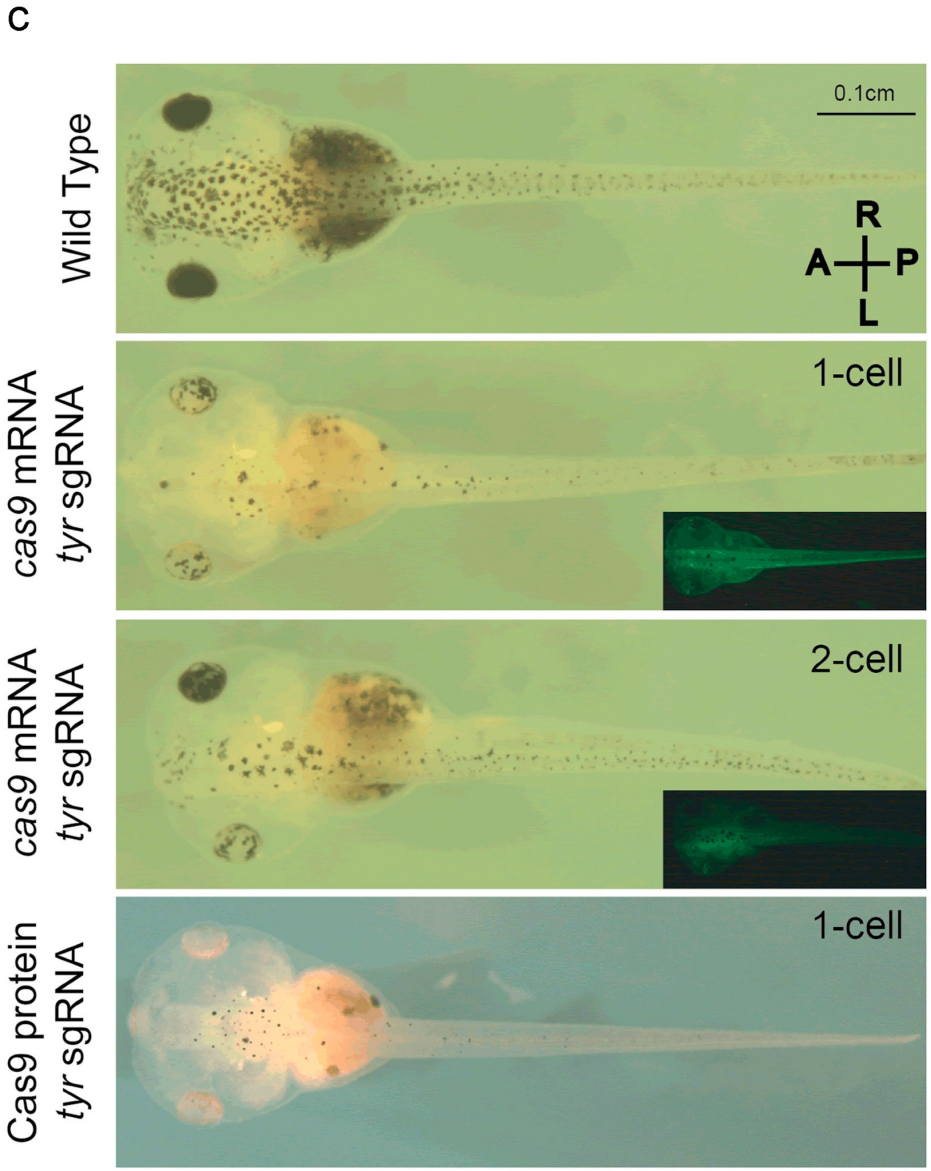




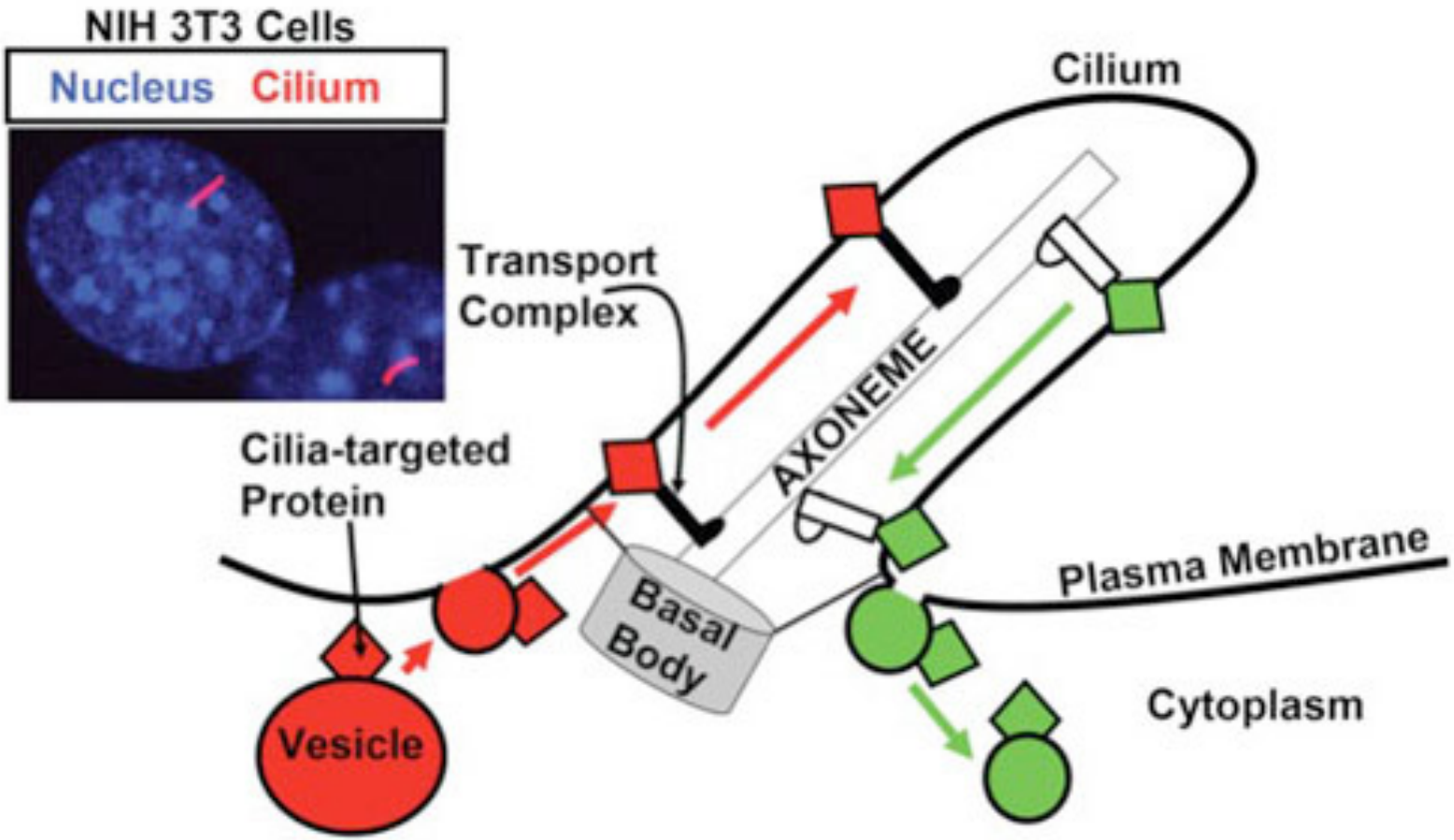
How does Cas9 protein perform compared to *cas9* mRNA?



How does Cas9 protein perform compared to *cas9* mRNA?



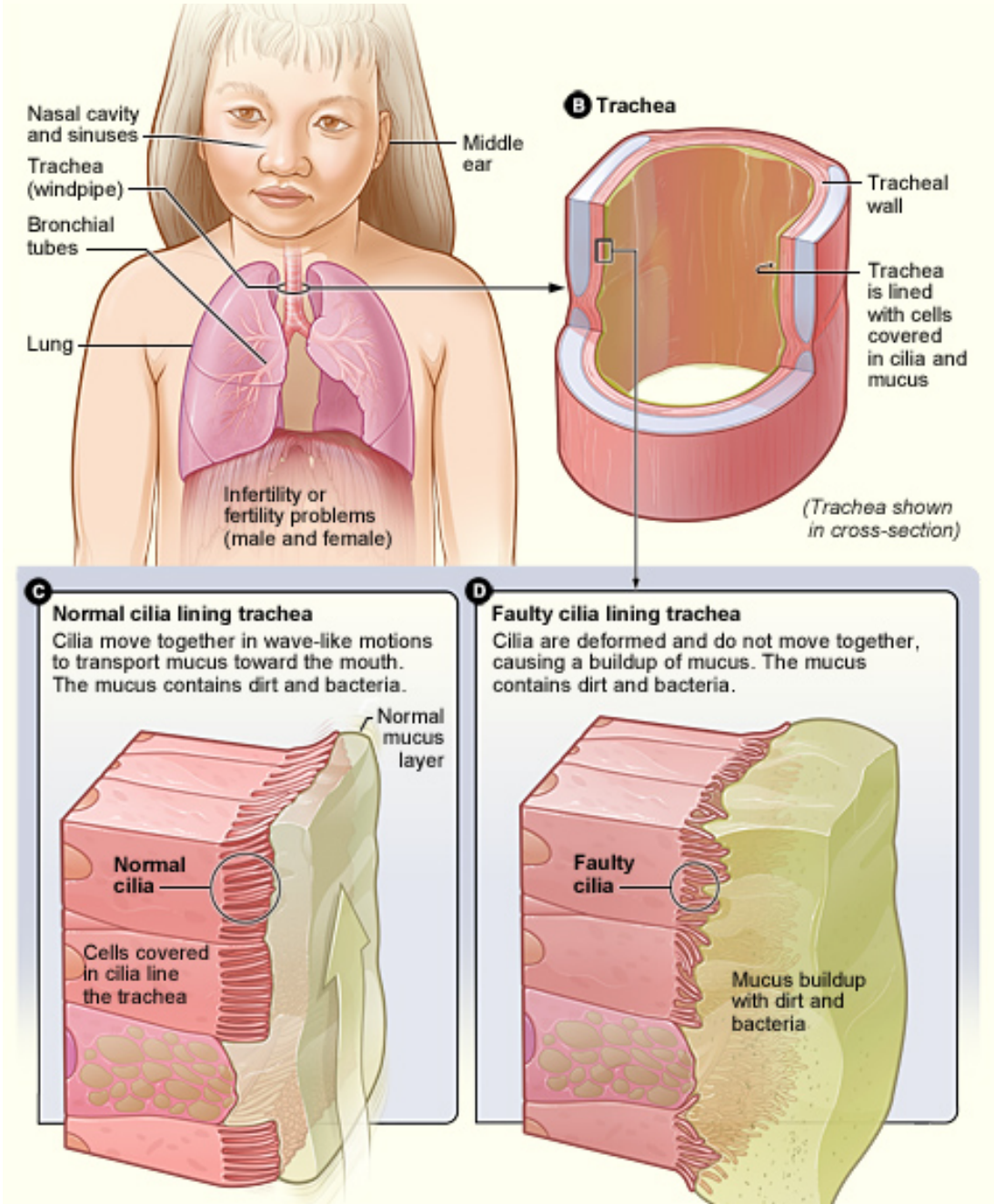
What are primary cilia?



Chemical sensation ***Signal transduction***

Control of cell growth

What are motile cilia?

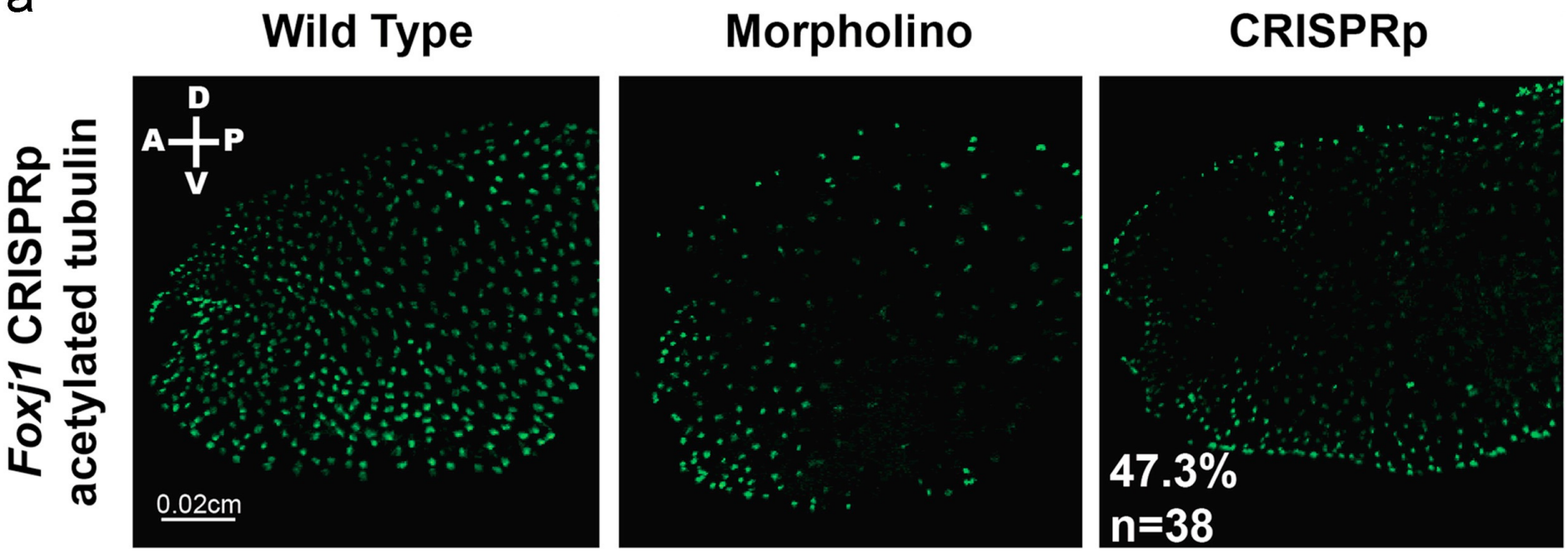


How did CRISPR phenotypes compare to morpholino's?

Gene/ mutation	OMIM disease phenotype	Morpholino/mutant line phenotype	CRISPR phenotyope (% of embryos)
Foxj1	Allergic rhinitis, Autoimmune disease	90% decreased epidermal cilia	68% decreased epidermal cilia
Ccdc40	Primary ciliary dyskinesia	Decreased and abnormal epidermal cilia	48% decreased and abnormal epidermal cilia
Dnah9	Primary ciliary dyskinesia	30% laterality defect	28% A + L heart loops
Galnt11	Heterotaxy	25% A + L heart loops; increased epidermal cilia	23% A + L heart loops; 50% increased epidermal cilia
Pax8	Kidney agenesis, Hypothyroidism	59% malformed pronephros	40% malformed pronephros
Tyrosinase	Oculocutaneous albinism	Albinism	~100% albinism
Beta-catenin	Cancer, intellectual disability	Ventralization of embryo	No venralization

How does CRISPRp in foxj1 affect surface cilia development?

a

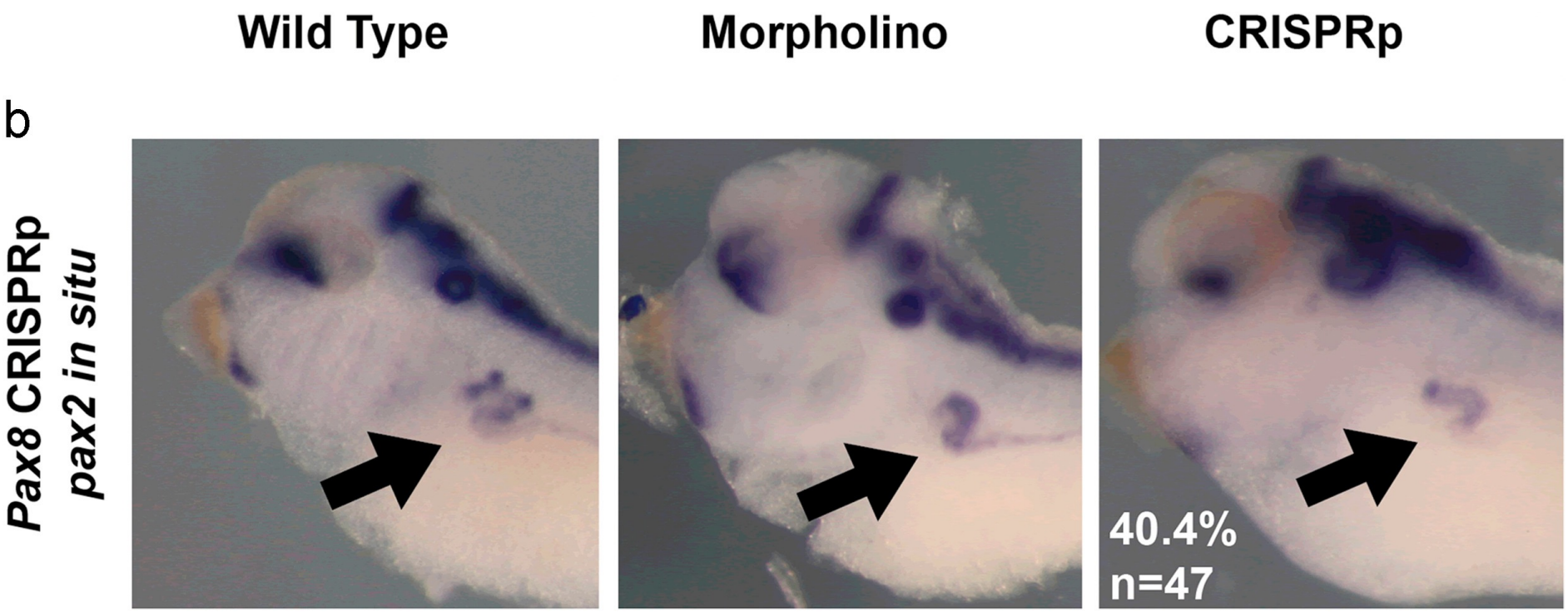


(*dnah9*). *Foxj1* MO knockdown leads to decreased epidermal cilia (Stubbs et al., 2008) and 68% of our *foxj1* CRISPRp injected embryos ($n=38$) had grossly reduced epidermal ciliated cells (Fig. 3,

How did CRISPR phenotypes compare to morpholino's?

Gene/ mutation	OMIM disease phenotype	Morpholino/mutant line phenotype	CRISPR phenotyope (% of embryos)
Foxj1	Allergic rhinitis, Autoimmune disease	90% decreased epidermal cilia	68% decreased epidermal cilia
Ccdc40	Primary ciliary dyskinesia	Decreased and abnormal epidermal cilia	48% decreased and abnormal epidermal cilia
Dnah9	Primary ciliary dyskinesia	30% laterality defect	28% A + L heart loops
Galnt11	Heterotaxy	25% A + L heart loops; increased epidermal cilia	23% A + L heart loops; 50% increased epidermal cilia
Pax8	Kidney agenesis, Hypothyroidism	59% malformed pronephros	40% malformed pronephros
Tyrosinase	Oculocutaneous albinism	Albinism	~100% albinism
Beta-catenin	Cancer, intellectual disability	Ventralization of embryo	No venralization

How does CRISPRp in *pax8* affect kidney development?



How did CRISPR phenotypes compare to morpholino's? ★

Gene/ mutation	OMIM disease phenotype	Morpholino/mutant line phenotype	CRISPR phenotyope (% of embryos)
Foxj1	Allergic rhinitis, Autoimmune disease	90% decreased epidermal cilia	68% decreased epidermal cilia
Ccdc40	Primary ciliary dyskinesia	Decreased and abnormal epidermal cilia	48% decreased and abnormal epidermal cilia
Dnah9	Primary ciliary dyskinesia	30% laterality defect	28% A + L heart loops
Galnt11	Heterotaxy	25% A + L heart loops; increased epidermal cilia	23% A + L heart loops; 50% increased epidermal cilia
Pax8	Kidney agenesis, Hypothyroidism	59% malformed pronephros	40% malformed pronephros
Tyrosinase	Oculocutaneous albinism	Albinism	~100% albinism
Beta-catenin	Cancer, intellectual disability	Ventralization of embryo	No ventralization

Summary

- Cas9 protein is a more effective option than *Cas9* mRNA
- Fragment analysis provides an effective mechanism to test the efficacy of the CRISPR
- CRISPR/Cas9 can reproduce MO phenotypes with fewer cons
- CRISPR is an efficient screening tool for testing congenital malformation candidate genes in *Xenopus*

http://www.artofthecell.com/animation/crispr-cas9-gene-editin

What next?

NATIONAL GEOGRAPHIC

Photograph by Eiko Jones, My Shot

What next?

sgRNA activity and efficiency
Model organisms
Specificity
Off-target effects


Questions?