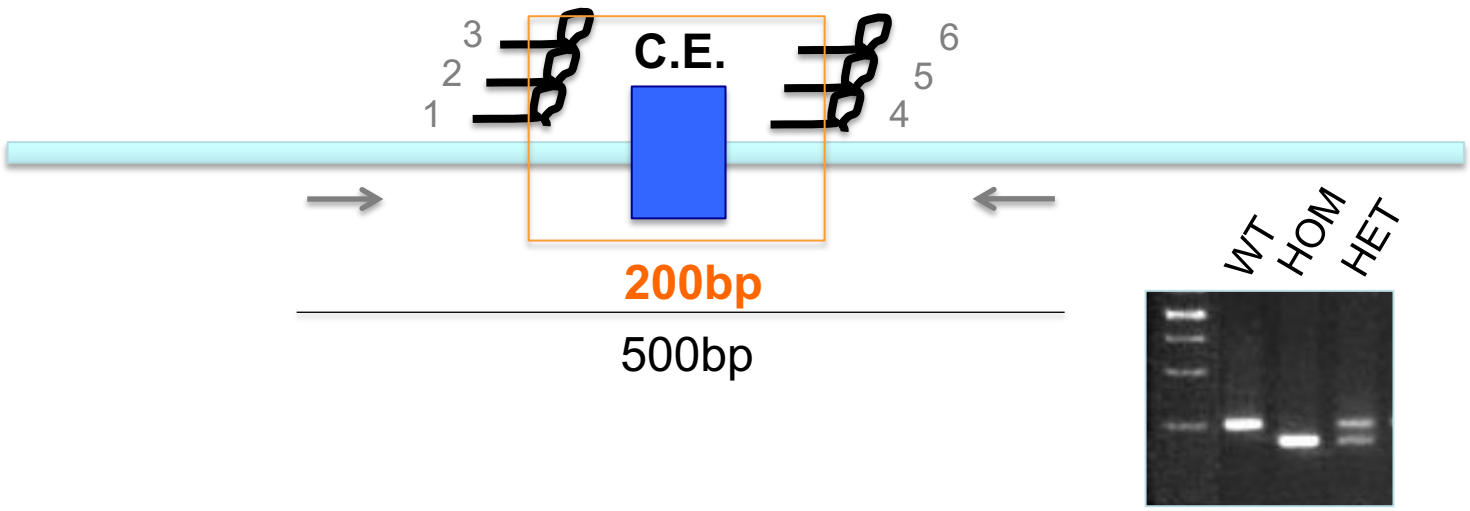
Screening: Deletion-KO by Cas9

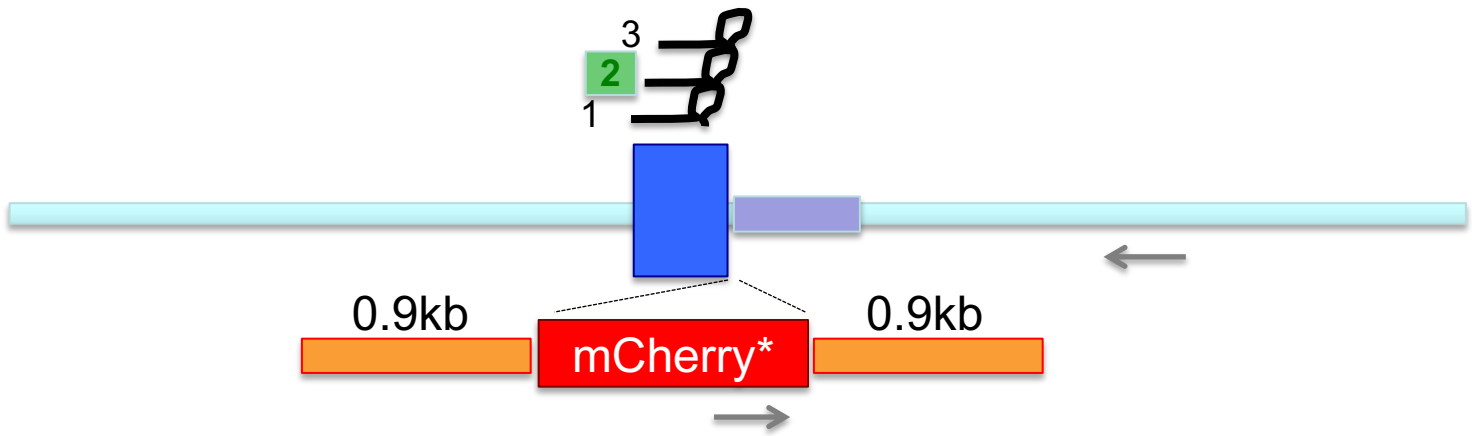


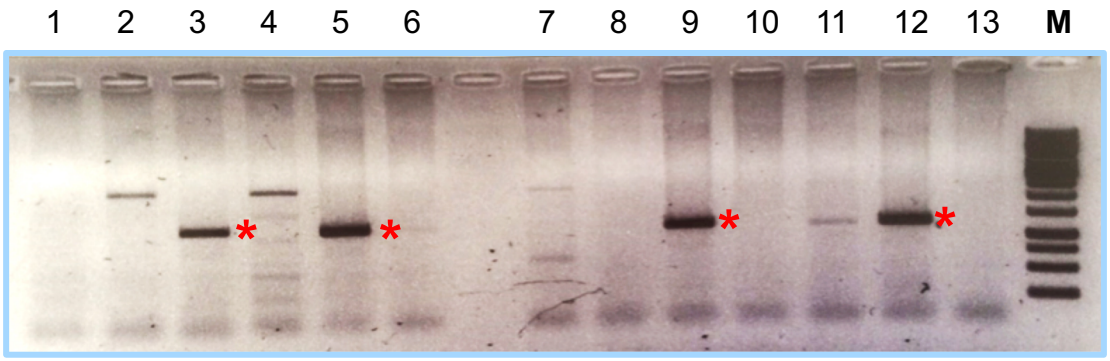
Initial screen by PCR: 500 (WT) vs. 300 (Δ) in **one PCR rxn**.
Even better: it is **irrespective of ploidy**.

→ Culture and sequence only predominant 300bp clones.

Hublitz, unpublished
Lonowski *et al.*, Nature Protocols, 2017

Screening: HDR Events

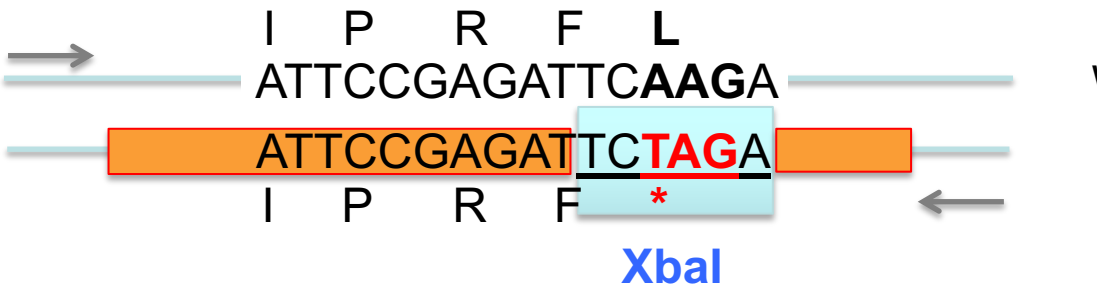


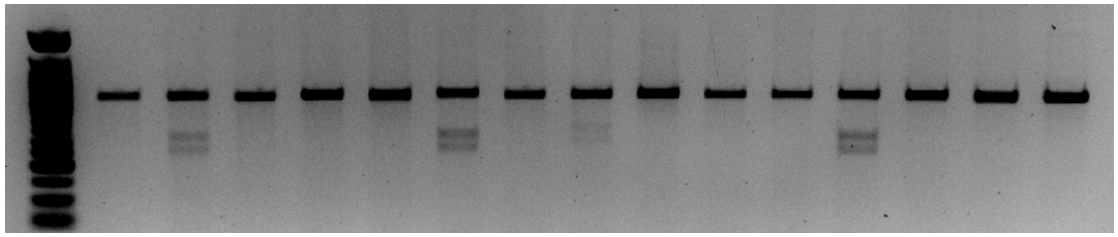


Type	Frequency	
n.d.	69%	9/13
HDR	**31%**	4/13

Chagraoui *et al.*, Nature Communications, 2018

SNP-Screening by PCR and REN





Type	Frequency	
WT	73%	11/15
1n/2n	**27%**	4/15
3n	0%	0/15

Dr. Katherine Garnett

Adjusting the Level of QC

Mouse: Breeding removes SNPs.

Cell lines: Test several independent clones.

Clinical Applications: All that is possible.

Table 1 | Summary of variants detected by whole-genome sequencing

sgRNA	No. of mismatches	No. of NGG genomic sites	No. of NAG genomic sites	No. of off-target sites
Ar-a	0	1	0	N/A
	1	2	2	0
	2	3	6	1[a]
	3	26	38	0
	4	358	446	0
	5	2,432	3,130	0
Ar-b	0	1	0	N/A
	1	0	0	N/A
	2	0	0	N/A
	3	9	3	0
	4	98	82	0
	5	920	884	0
Total	–	**3,850**	**4,591**	**1**

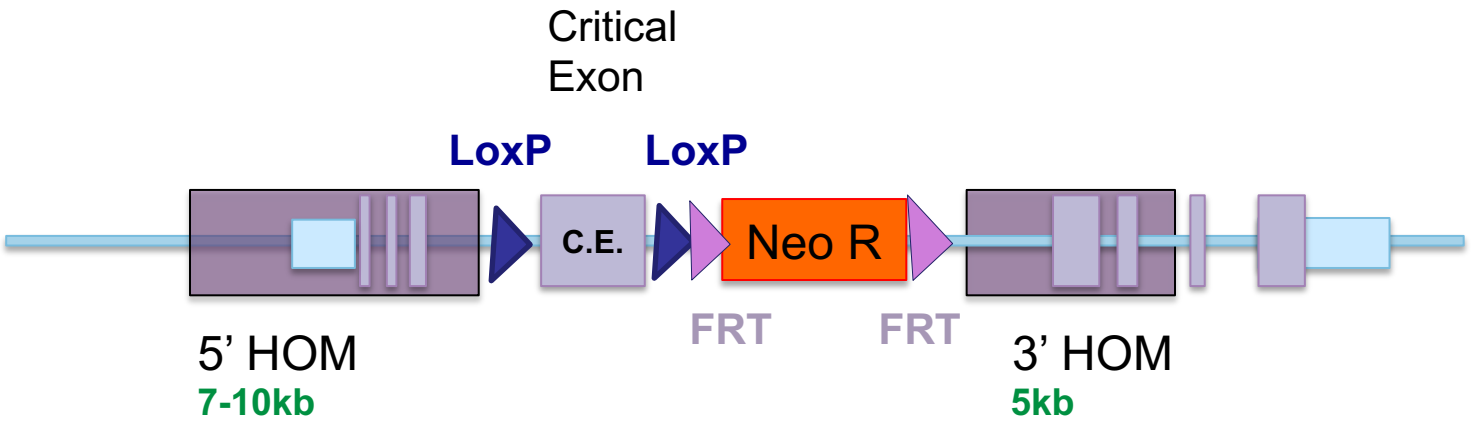
[a]OTS3 (chromosome 8: 121017178), a 2-base-pair deletion experimentally validated in F_1 animal F18-9.

Iyer et al., 2015, Nature Methods

BLISS, BLESS, GUIDE-Seq, Digenome-Seq, Circle-Seq, WGS.

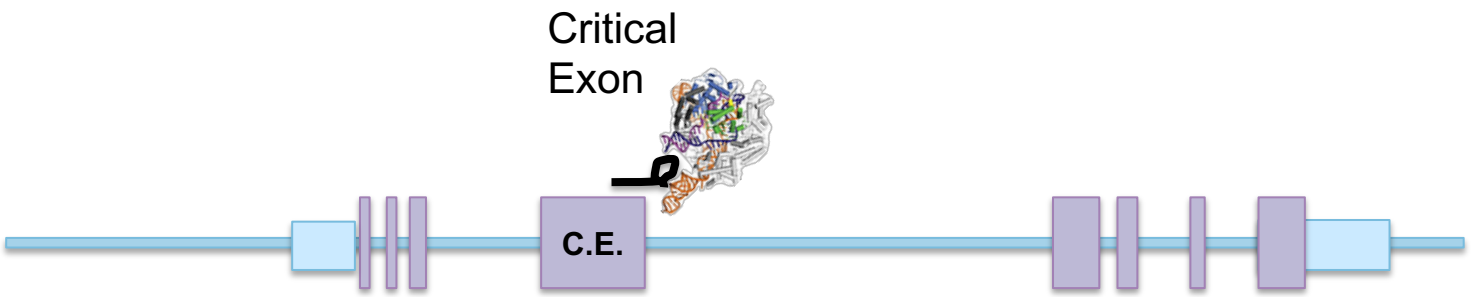
Bolukbasi *et al.*, 2015, Nature Methods
Tsai *et al.*, 2017, Nature Methods

Mouse Models: Targeting Constructs



B.C.
Before **C**RISPR.

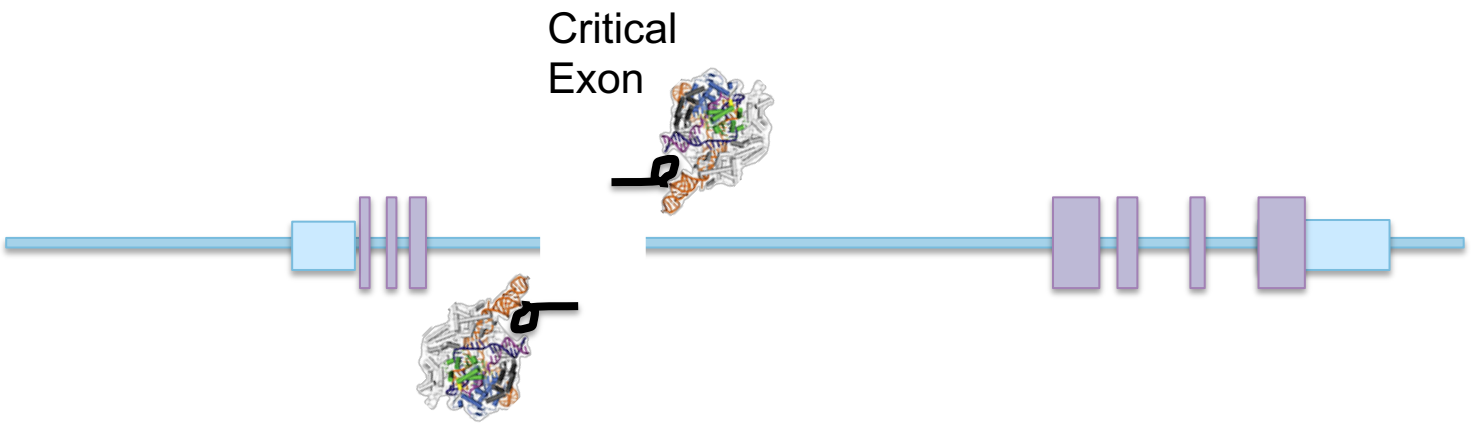
KO by Cas9 induced NHEJ



KO by Cas9-induced NHEJ.
Injection of **RNP** into oocytes.
~30% with frame-shift, **2/3 useful**.
Well-established protocols available.

A.C.
After **C**RISPR.

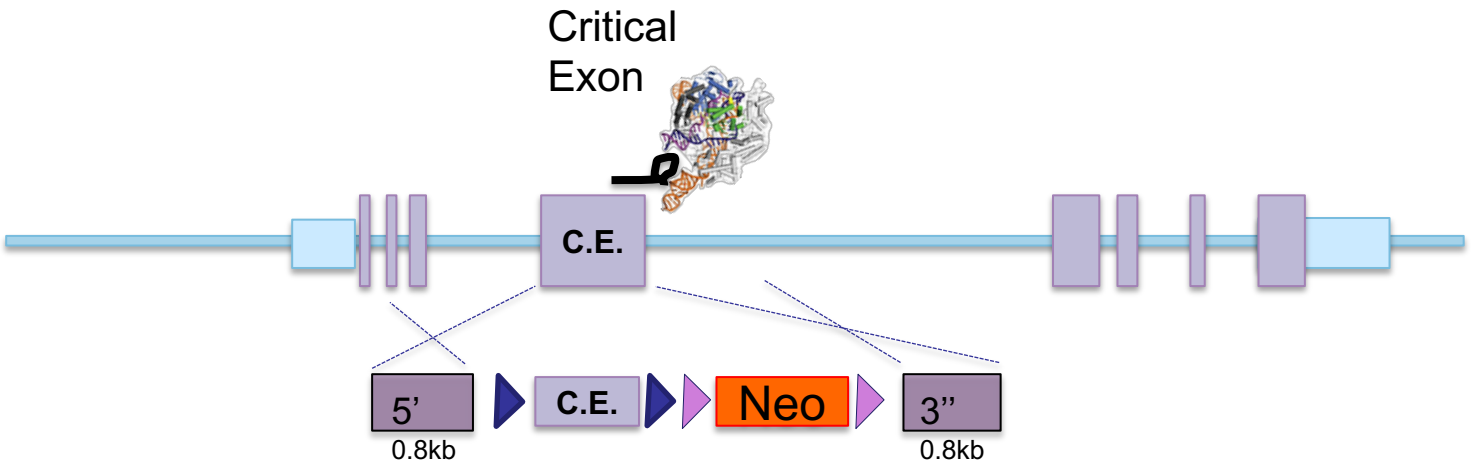
KO by Exon Deletion



KO by Cas9 mediated exon excision.
Injection of **RNPs** into oocytes.
5-10% **with defined** KO.
Well-established protocols available.

A.C.
After **C**RISPR.

KI by Homologous Recombination



KI works well in mESCs (**> 20-50%**).
Very much **shorter** homology arms required.
Oocyte injection: 0 to <10%...?

A.C.
After **C**RISPR.

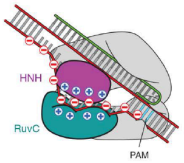
Refining the Technology

1 Use of RNPs instead of Cas9 mRNA.

Empirically determined.

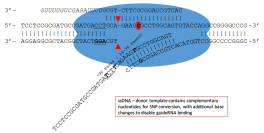
2 HiFi versions of Cas9 with less OFF-targets.

Slaymaker *et al.*, Science, 2015
Kleinstiver *et al.*, Nature, 2016
Chen *et al.*, Nature, 2017



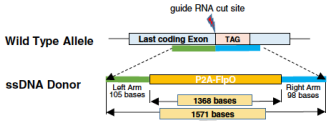
3 Asymmetric ssODN donors.

Richardson *et al.*, NBT, 2016



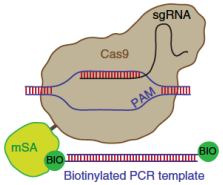
4 Long ssDNA donors.

Quadros *et al.*, Genome Biology, 2017

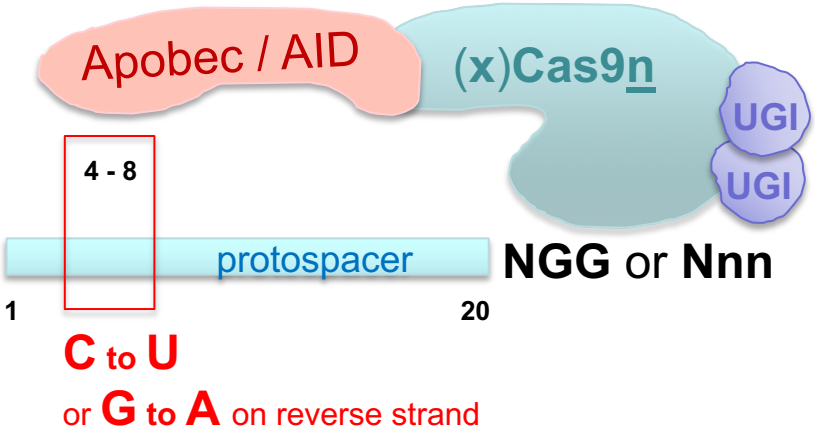


5 2C-HR-CRISPR and Biotin-tagging

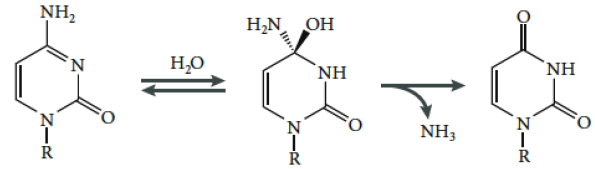
Gu *et al.*, NBT, 2018

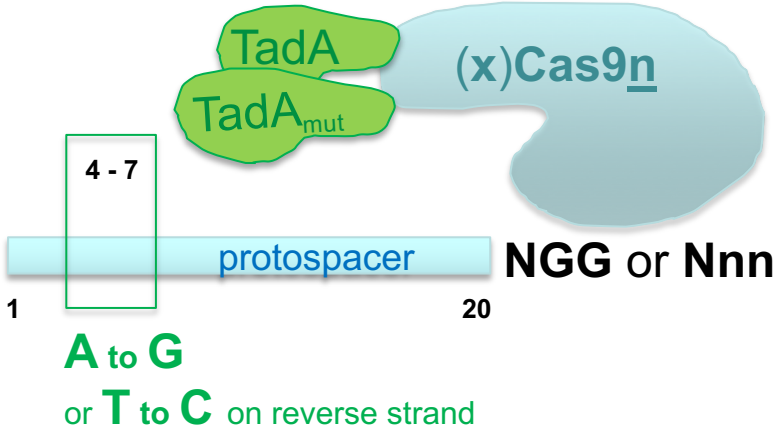


Base Editing

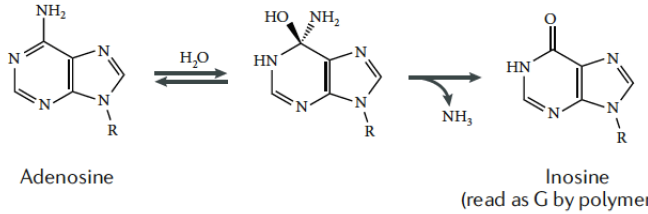


BE4
BE4 max



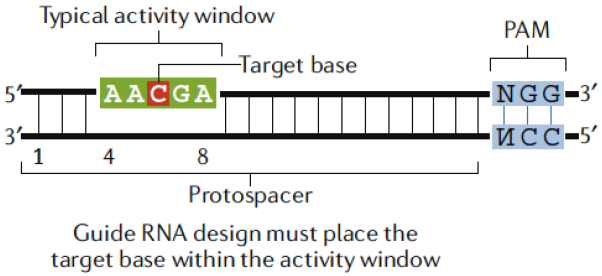


ABE7.10

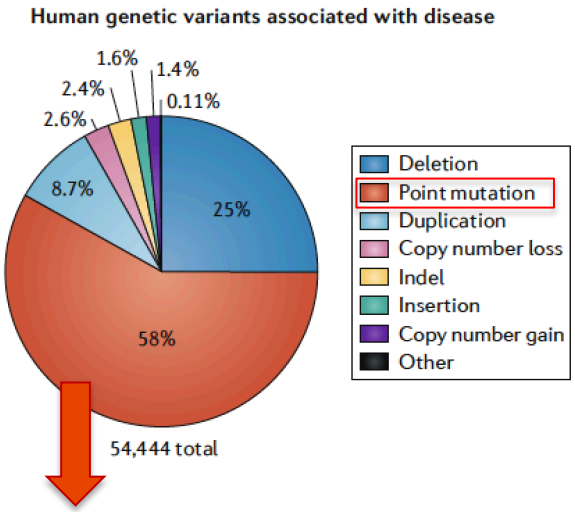


Modified after Rees and Liu, NRG, 2018

Base Editing: Challenges / Applications

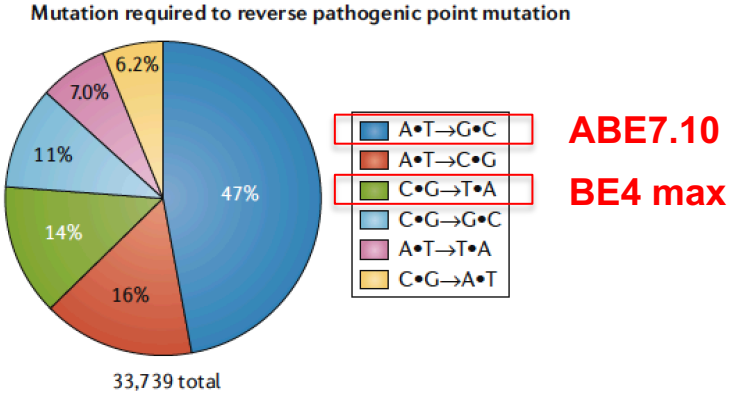


Guide RNA design must place the target base within the activity window



58% approachable.
61% thereof treatable.
→ **40% of total SNPs**.

26% of that available to WT Cas9, but **95%** for xCas9…

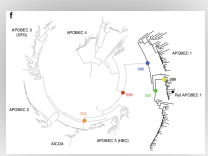


Rees and Liu, NRG, 2018

Refining the Technology II

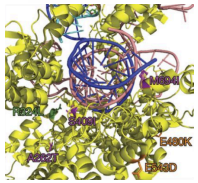
6 Better Base Editors by expression optimization and ancestral reconstitution: BE4 max.

Koblan et al., NBT, 2018



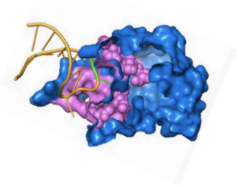
7 xCas9 versions with equal specificity and lower PAM stringency: broader target spectrum.

Hu et al., Nature, 2018



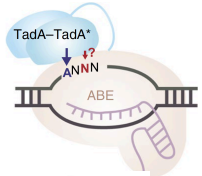
8 Base editor evolution: higher specificity, narrower target window, less OFF-targets also in mRNA.

Gruenewald et al., NBT, 2019
Thuronyi et al all., NBT, 2019
Cheng et al., Nature Communications, 2019



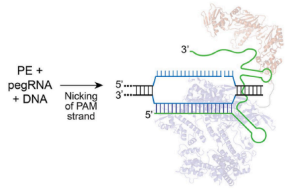
9 Potential A to C base editing…?

Kim *et al.*, NBT, 2019



10 Prime editing with pegRNA

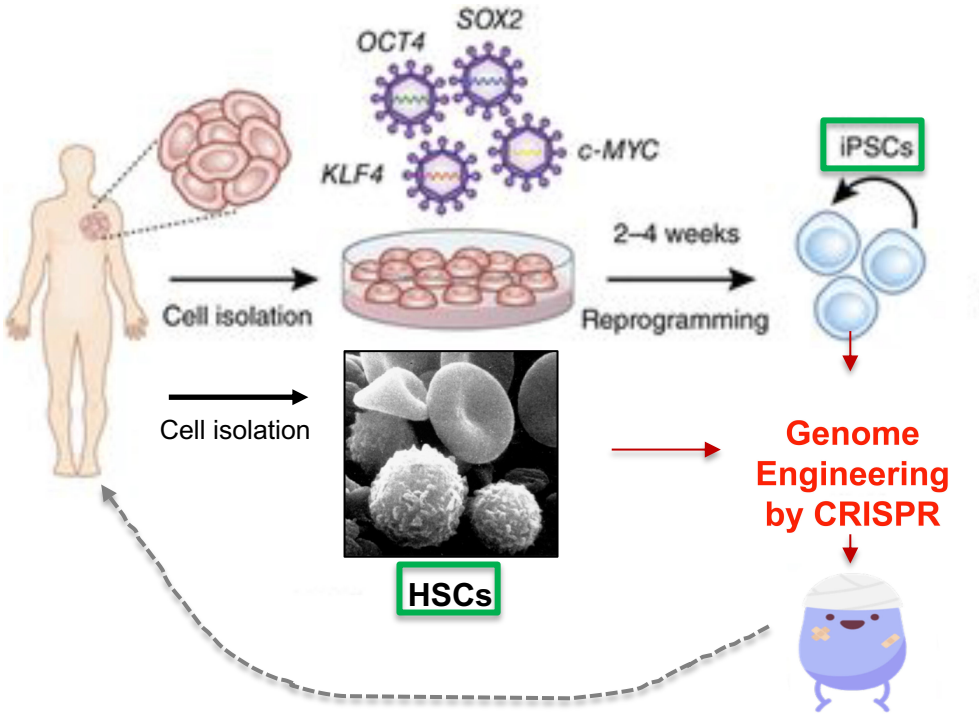
Anzalone *et al.*, Nature, 2019



100% of all SNPs

Clinical Applications

Ex vivo approaches



Several 100's of publications 2013 to 2019

In vivo

First in vivo CRISPR candidate enters the clinic

Editas Medicine and its partner Allergan have advanced AGN-151587 into a phase I/II trial for patients with Leber congenital amaurosis type 10, a rare and inherited form of blindness.

AGN-151587, previously called Edit-101, is the first CRISPR–Cas9 genome-editing medicine that is administered directly to patients. Doctors inject the adeno-associated virus-based candidate subretinally, so that it can cut out a mutation in the *CEP290* gene in photoreceptor cells in the eye. Spark Therapeutics and Novartis's voretigene neparvovec, the first gene therapy to gain approval in the US, corrects a different form of the inherited eye disease, by introducing a normal copy of the *RPE65* gene to patients with Leber congenital amaurosis type 2.

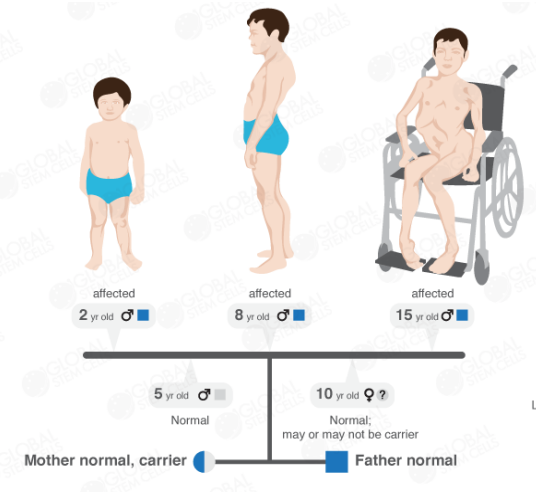
Mullard, Nature Reviews Drug Discovery, September 2019

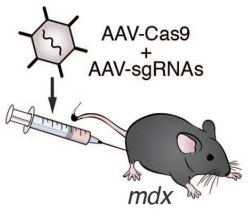
Clinical Example: Muscular Dystrophy

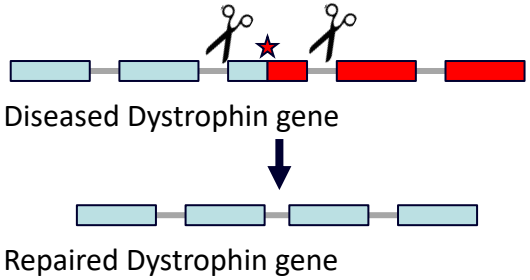
A key muscle protein – **Dystrophin**.

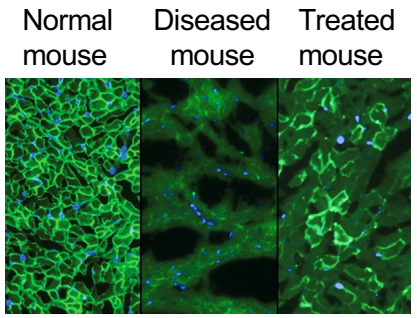
Patients with damaged Dystrophin gene have severe muscle wasting, known as **Muscular Dystrophy**.

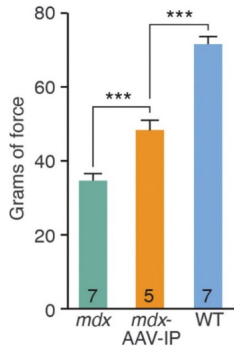
Genome editing by CRISPR-Cas9 is applied to tackle this mutation in a mouse model.





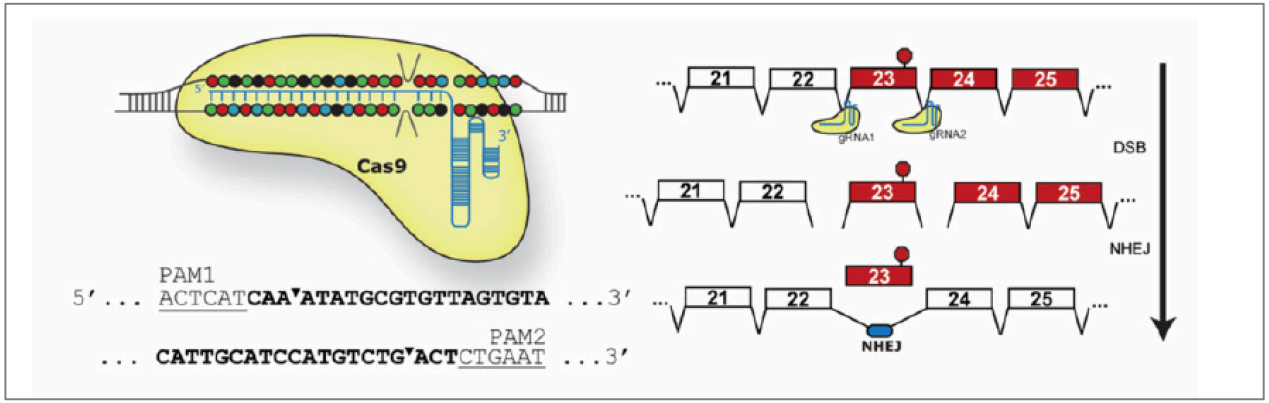






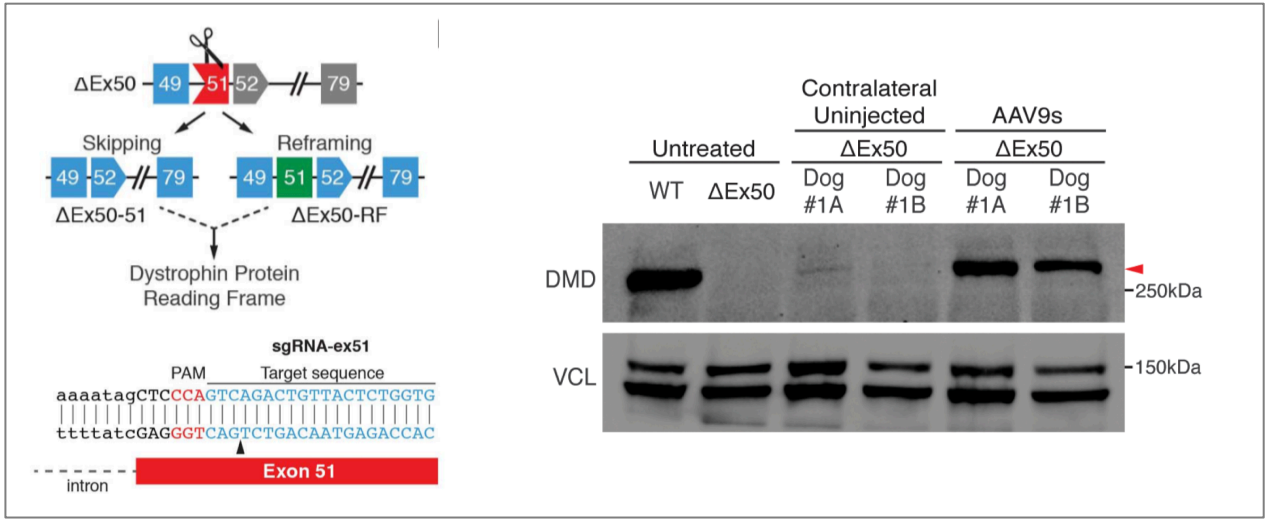
Long *et al.*, Science, 2015

Clinical Example: Muscular Dystrophy



2015.
2 sgRNAs.
In vivo, mouse.

Nelson *et al.*, Science, 2015
Long *et al.*, Science, 2015



2018.
1 sgRNA.
No OFF !!!
In vivo, dog

Amoasii *et al.*, Science, 2018

Clinical Example: HIV

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT September 11th, 2019

CRISPR-Edited Stem Cells in a Patient with HIV and Acute Lymphocytic Leukemia

Lei Xu, M.D., Ph.D., Jun Wang, M.D., Ph.D., Yulin Liu, B.S., Liangfu Xie, B.S.,

CRISPR-edited *CCR5*-ablated donor HSPCs (CCR5-null are largely resistant to HIV1 infection) were transplanted into an HIV+/ALL+ patient. The acute lymphoblastic leukemia was in complete remission with full donor chimerism, and donor cells carrying the ablated *CCR5* persisted for more than 19 months without gene editing-related adverse events. The percentage of *CCR5* disruption in reconstituted lymphocytes was only approximately 5%, **indicating the need for further research into this approach**.

Germline Editing…

NEWS · 18 OCTOBER 2019 · CORRECTION 18 OCTOBER 2019

Russian 'CRISPR-baby' scientist has started editing genes in human eggs with goal of altering deaf gene

Denis Rebrikov also told *Nature* that he does not plan to implant gene-edited embryos until he gets regulatory approval.

The Economist, 2015

Germline Editing

NEWS · 24 SEPTEMBER 2019

CRISPR might be the banana's only hope against a deadly fungus

Researchers are using the gene-editing tool to boost the fruit's defences and prevent the extinction of a major commercial variety.

Workers inspect a banana harvest at a farm in Australia.

Nature, 24.09.2019

Rice infested with bacterial blight, which can cause crop losses as high as 75%. Credit: Nigel Cattlin/Alamy

CRISPR-CAS9 GENOME EDITING · 29 OCTOBER 2019

A crop that feeds billions freed from blight by CRISPR

Bacteria that infect rice are thwarted by changes to rice genes involved in sugar transport.

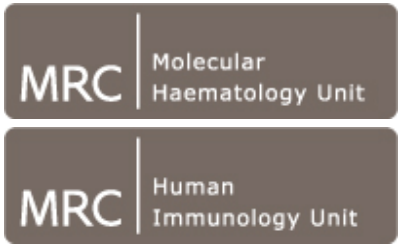
Nature, 08.11.2019

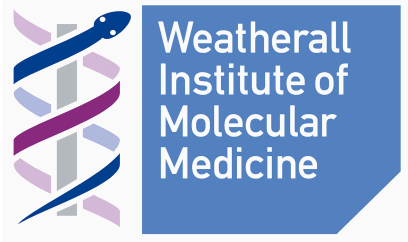
Acknowledgments

Prof. Claus Nerlov
Prof. Douglas Higgs

Dr. Zoe Holloway
Dr. Ryan Beveridge

Dr. Yale Michaels





https://www.imm.ox.ac.uk/research/facilities/**genome-engineering-facility**

philip.hublitz@ndcls.ox.ac.uk