Tools and methodologies Transgenesis in organoids

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INRAE

EuroFAANG

INNOVATIVE CELLULAR MODELS FOR ANIMAL SCIENCE

Summer School – 11-15th September 2023

Tools and methodologies
Transgenesis in organoids

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UMR1313 GABI

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Functional analysis : Timeline

Gurdon
Yamanaka

Charpentier
Doudna

Cell culture
1910…

Transgenic mouse
1980

Embryonic Stem cells isolated
1981

Knock-in mouse Homology recombination
1987

iPScells
2006

CRISPR gene editing
2012

Preclinical studies with organoids
2020

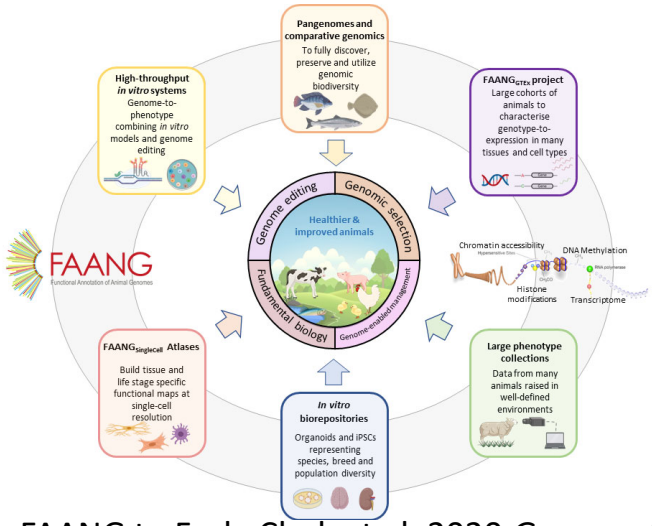
Mid-1970
Restriction enzymes

1981
Meganucleases

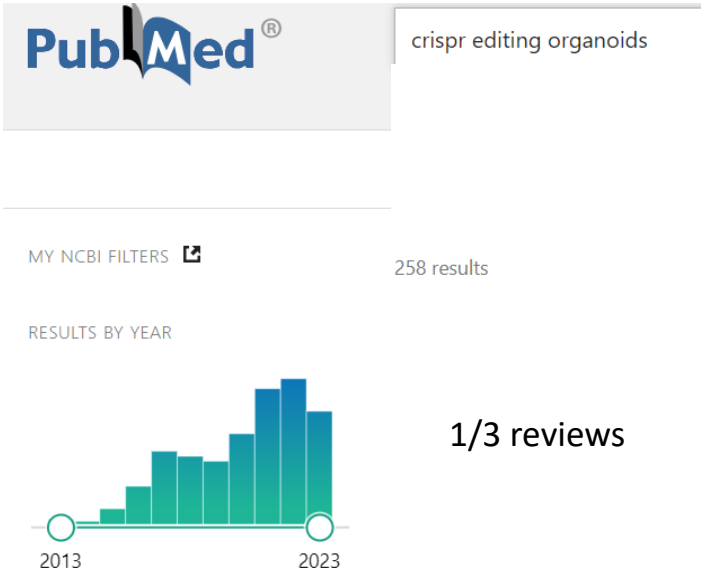
1995
ZFN
I-SceI

2009
3D organoids from ASC

2013
CRISPR editing on organoids



From FAANG to Fork. Clark et al. 2020 *Genome Biology*



1/3 reviews

Transgenesis in organoids

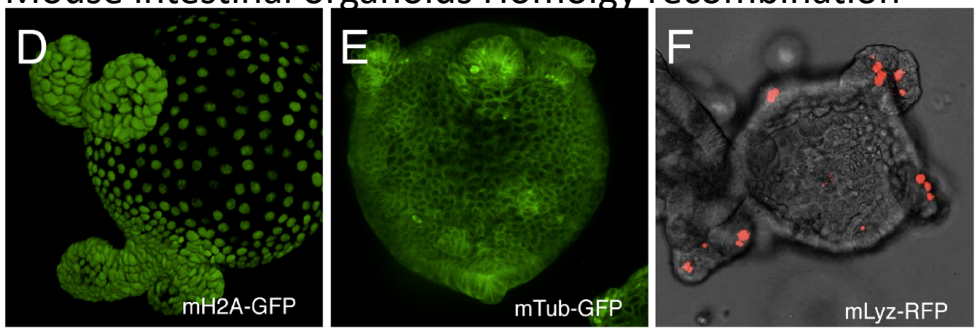
Published: 04 December 2011

Controlled gene expression in primary *Lgr5* organoid cultures

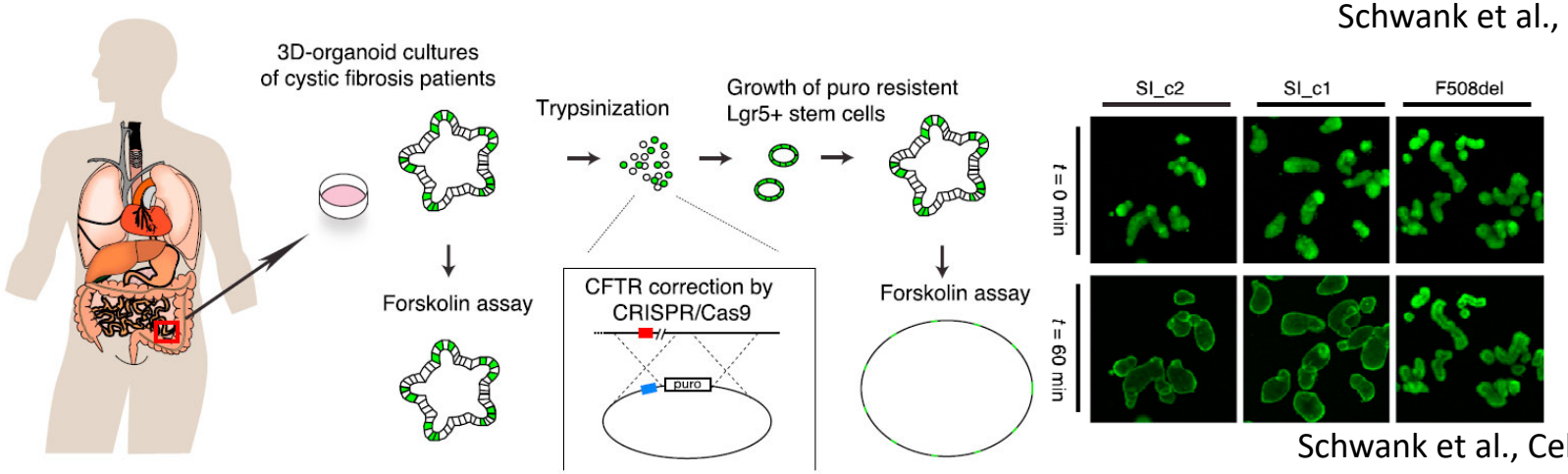
Bon-Kyoung Koo, Daniel E Stange, Toshiro Sato, Wouter Karthaus, Henner F Farin, Meritxell Huch, Johan H van Es & Hans Clevers

Nature Methods **9**, 81–83 (2012) Cre inducible retroviral vector, mouse

Mouse intestinal organoids Homolgy recombination



Schwank et al., PLoS One 2013



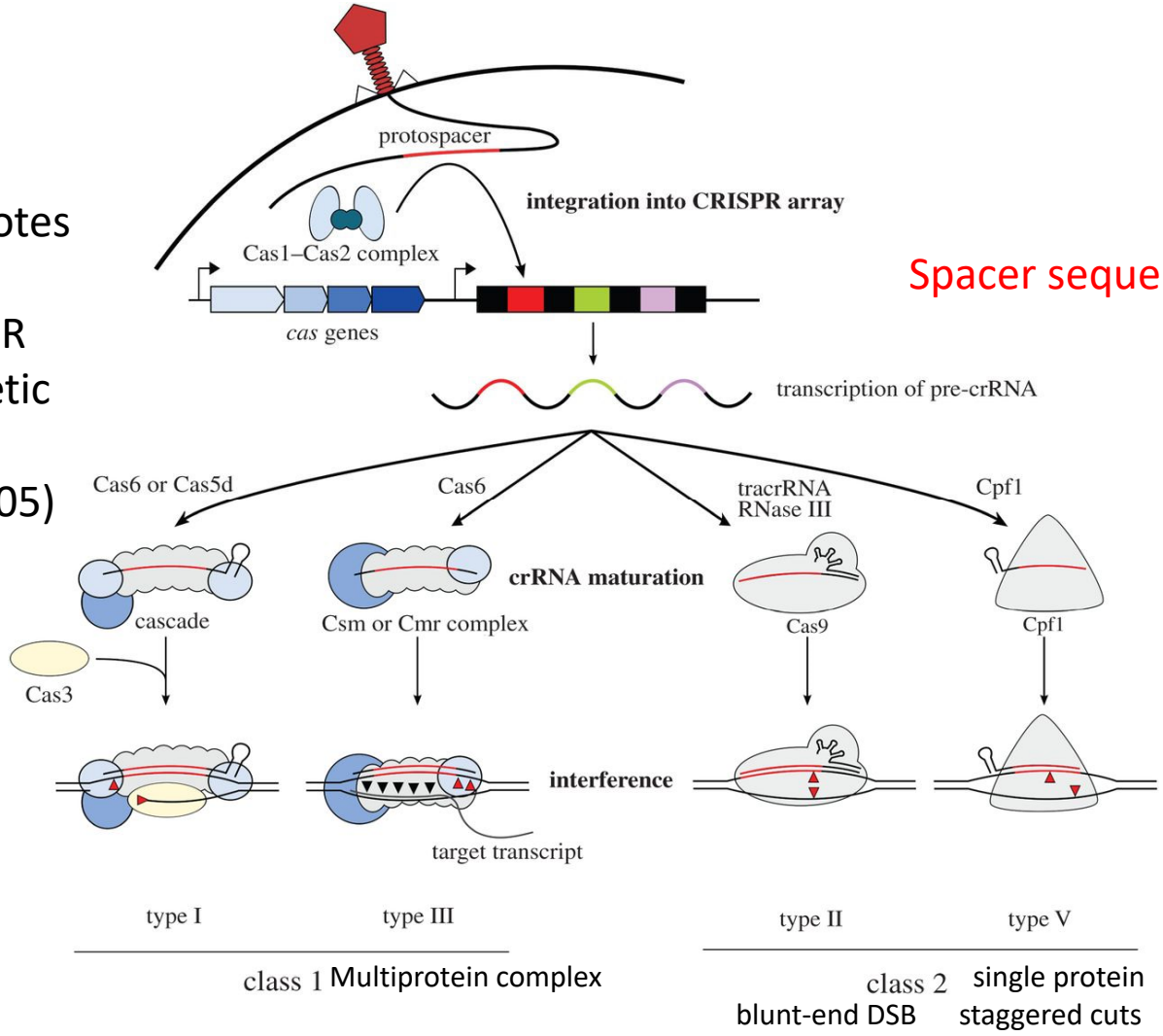
Schwank et al., Cell Stem Cell 2013

Clustered Regularly Interspaced Short Palindromic Repeats

Simplified model of the immunity mechanisms of class 1 and class 2 CRISPR-Cas systems.

CRISPR-Cas is an adaptive immune system in prokaryotes

Unique sequences in CRISPR originate from mobile genetic elements (bacteriophages, transposons, plasmids) (2005)

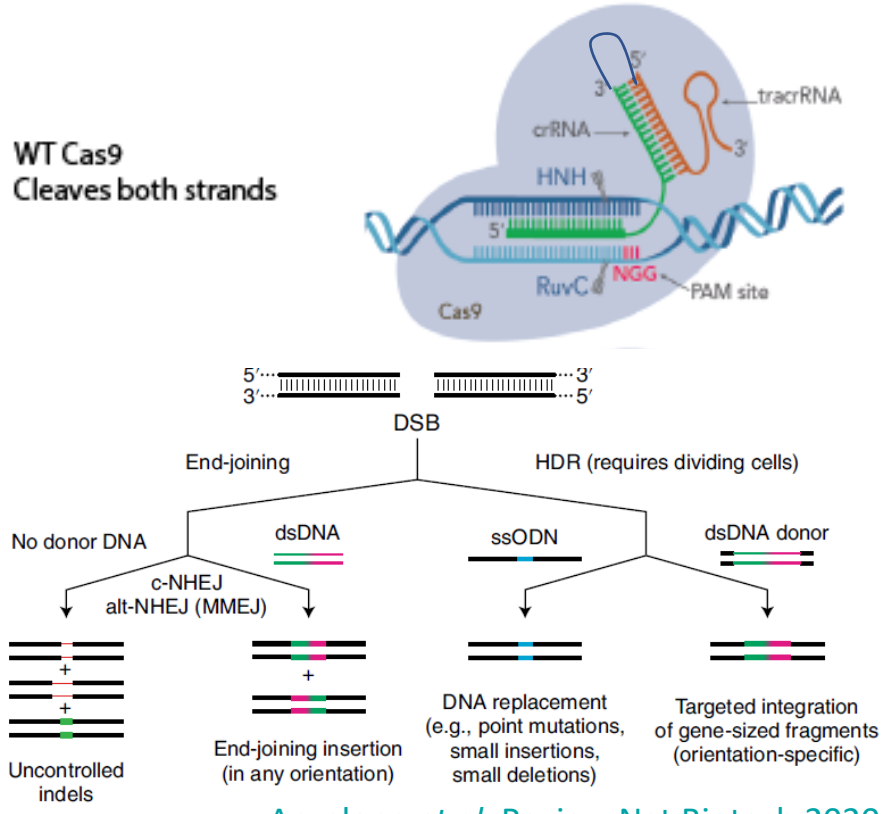


Spacer sequences

Frank Hille, and Emmanuelle Charpentier Phil. Trans. R. Soc. B 2016;371:20150496

CRISPR/Cas9 programmable dual-RNA-guided DNA endonuclease

Cas9 programmed by single chimeric RNA



Anzalone *et al.* Review Nat Biotech 2020

crRNA-tracrRNA chimera

20 nt Protospacer PAM

Protospacer Adjacent Motif: requirement protecting genomic DNA encoding the guides

NGG : restricting target sequences

Non-target DNA strand displaced by the guide RNA spacer

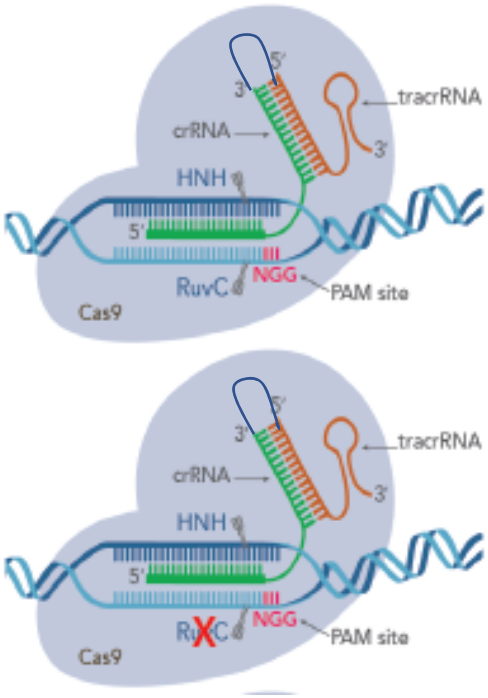
Adapted from Jinek *et al.* Science 2012 & IDT-DNA.com

CRISPR/Cas9 programmable dual-RNA-guided DNA endonuclease

Cas9 programmed by single chimeric RNA

WT Cas9
Cleaves both strands

Cas9 D10A nickase
Inactivated RuvC domain
Cleaves target strand



crRNA-tracrRNA chimera

20 nt Protospacer PAM

Protospacer Adjacent Motif: requirement protecting genomic DNA encoding the guides

NGG : restricting target sequences

Non-target DNA strand displaced by the guide RNA spacer

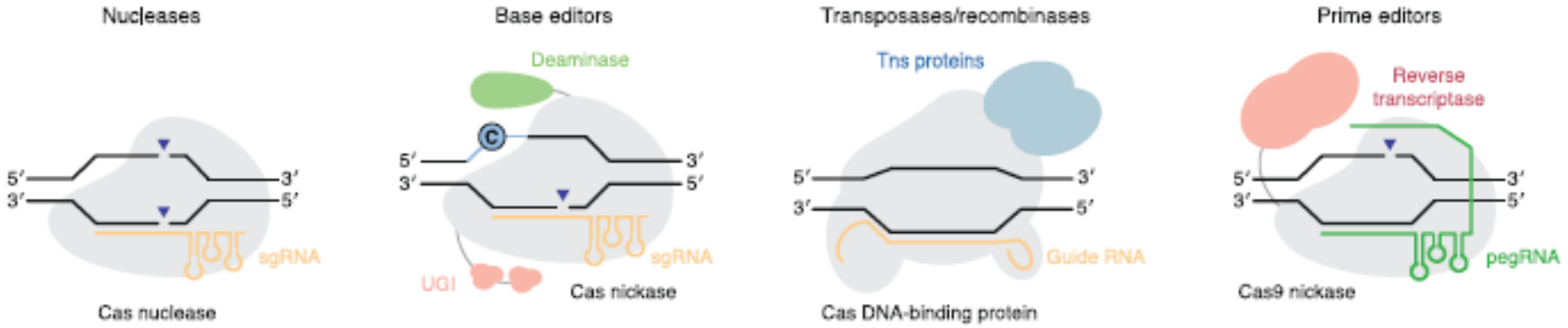
dCas9 : dead Cas9 still bind specific DNA sequences

Adapted from Jinek *et al.* Science 2012 & IDT-DNA.com

CRISPR-Cas genome editing technologies

Aim : to Install any genetic change at any position within the genome of any living cell with minimal unwanted genome modification or cellular perturbation

- Targeted alteration of genomic DNA sequence
- Transcriptional regulation
- Epigenetic modifications
- RNA editing
- Nucleic acid detection



Anzalone *et al.* Review Nat Biotech 2020

Genome editing w/o DSB: Base editing: C→T, G→A substitutions

Catalytically impaired CRISPR/Cas nuclease are fused to ssDNA deaminase
These enzymes do not exist
Extensive directed evolution of existing ones to obtain base editors
Editing window is away from the Cas9 DSB site

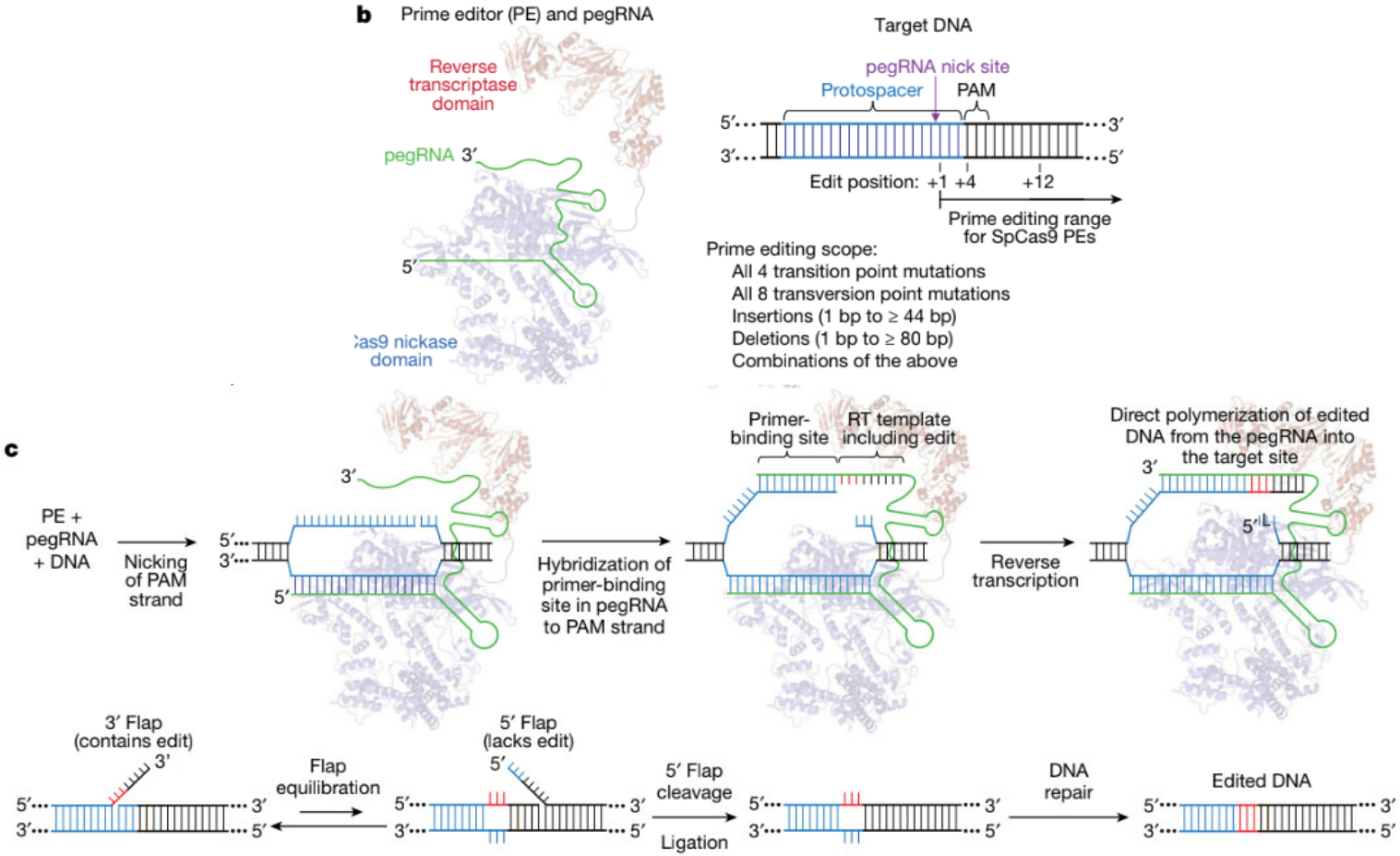
Figure 4 | BE3-mediated correction of two disease-relevant mutations in mammalian cells. The sequence of the protospacer is shown to the

Two nearby Cs are also converted to Ts, but with no change to the predicted sequence of the resulting protein. Identical treatment of these

Komor et al., Nature 2016

Base editors A/T→G/C, Gaudelli et al., Nature 2017

Genome editing w/o DSB or donor DNA: Prime editor



BE performed better C.G to T.A conversion for bases in the center of the window

Efficiency of prime editing exceedes that of base editing (no by stander editing)

Anzalone et al., Nature 2019

Genome editing with relaxed PAM requirement

doi:10.1038/nature26155

Evolved Cas9 variants with broad PAM compatibility and high DNA specificity

Johnny H. Hu[1,2,3], Shannon M. Miller[1,2,3], Maarten H. Geurts[1,2,3], Weixin Tang[1,2,3], Liwei Chen[1,2,3], Ning Sun[1,2,3], Christina M. Zeina[1,2,3], Xue Gao[1,2,3], Holly A. Rees[1,2,3], Zhi Lin[1,2,3] & David R. Liu[1,2,3]

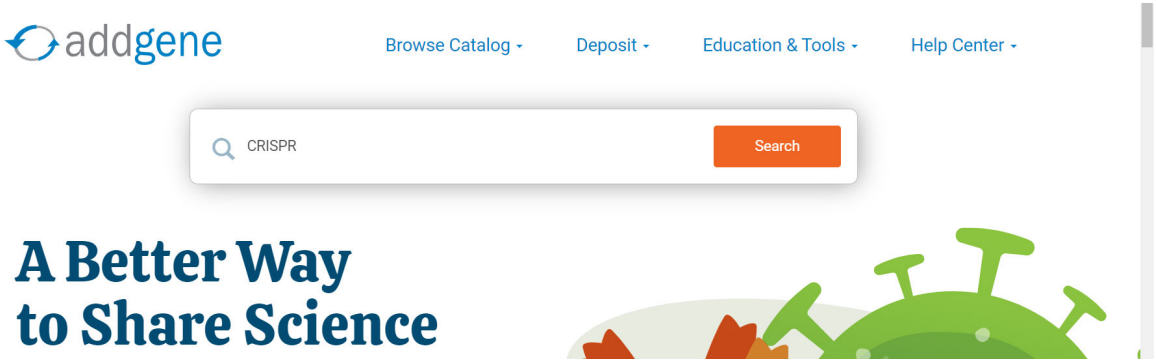
RESEARCH

Nature 2018
Science 2018

BIOTECHNOLOGY

Engineered CRISPR-Cas9 nuclease with expanded targeting space

Hiroshi Nishimasu[1*], Xi Shi[2,3], Soh Ishiguro[4,5,6], Linyi Gao[2,7], Seiichi Hirano[1], Sae Okazaki[1], Taichi Noda[8], Omar O. Abudayyeh[2,3,9], Jonathan S. Gootenberg[2,3,9], Hideto Mori[4,5,6], Seiya Oura[8,10], Benjamin Holmes[2,3], Mamoru Tanaka[4], Motoaki Seki[4], Hisato Hirano[1], Hiroyuki Aburatani[4], Ryuichiro Ishitani[1], Masahito Ikawa[8,10,11], Nozomu Yachie[1,4,5,6], Feng Zhang[2,3,7,9], Osamu Nureki[1*]



CRISPR genome editing efficient in livestock

CRISPR-mediated gene ko in livestock: Agricultural applications

Biomedical applications

CRISPR-mediated knockin in livestock

TABLE 3 | CRISPR-mediated gene knockin in livestock.

Species	Gene	Purpose of manipulation	Type of KI	Approach	SCNT or MI	KI Animals produced	Mosaicism (%)	References
		Agriculture: improvements in						
Sheep	*SOCS2*	Reproductive traits	Point mutation	Crispr/Cas9 BE	MI	3/4 (25%)	3/3 (100%)	Zhou et al. (2019)
	BMPR1B	Reproductive traits	Point mutation	Crispr/Cas9	MI	5/21 (23.8%)	Not stated	Zhou et al. (2018)
Goat	*Tβ4*	CCR5-targeted KI, cashmere yield	Gene insertion	Crispr/Cas9	SCNT	1	N/A	Li X. et al. (2019)
	FGF5	Cashmere yield	Point mutation	Crispr/Cas9 BE	MI	5/5 (100%)	5/5 (100%	Li G. et al. (2019)
	GDF9	Reproductive traits	Point mutation	Crispr/Cas9	MI	4/17 (23.5%)	2/4 (50.0%)	Niu et al. (2018)
	FAT-1	Disease resistance	Gene insertion	Crispr/Cas9	SCNT	1 from 8 pregnancies	N/A	Zhang J. et al. (2018)
Cattle	*Pc*	Generation of a polled genotype	Gene insertion	Crispr/Cas12a	SCNT	1, died on D1 after birth	N/A	Schuster et al. (2020)
	NRAMP1	Tuberculosis resistance	Gene insertion	Crispr/Cas9n	SCNT	9	N/A	Gao et al. (2017)
	IARS	Correction of IARS syndrome	Gene insertion	Crispr/Cas9	SCNT	5 viable fetuses	N/A	Ikeda et al. (2017)
Pig	*PBD-2*	Disease-resistant pigs	Gene insertion	Crispr/Cas9	SCNT	5 pigs	N/A	Huang et al. (2020)
	MSTN	Meat production	Gene insertion	Crispr/Cas9	SCNT	2 pigs	N/A	Zou Y.-L. et al. (2019)
	UCP1	Reproduction traits	Gene insertion	Crispr/Cas9	SCNT	12 piglets	N/A	Zheng et al. (2017)
	MSTN	Meat production	Point mutation	Crispr/Cas9	SCNT	1 stillborn piglet	N/A	Wang K. et al. (2016)
	MSTN	MSTN-KO without selectable marker	Gene insertion	Crispr/Cas9	SCNT	2 piglets	No	Bi et al. (2016)
	RSAD2	Generation of pigs with viral resistance	Gene insertion	Crispr/Cas9	SCNT	1 pig	No	Xie et al. (2020)
		Biomedical applications:						
Sheep	*ALPL*	Model of hypophosphatasia	Point mutation	Crispr/Cas9	MI	6/9 (66.6%)	No	Williams et al. (2018)
	PPT1	Infantile neuronal ceroid lipofuscinoses	Point mutation	Crispr/Cas9	MI	6/24 (25.0%)	Not stated	Eaton et al. (2019)
	tGFP	Rosa26-targeted KI	Gene insertion	Crispr/Cas9	MI	1/8 (12.5%)	Not stated	Wu et al. (2016)
	OTOF	Hearing loss phenotype	Point mutation	Crispr/Cas9	MI	8/73 (11.0%)	2/8 (25.0%)	Menchaca et al. (2020b)
Cattle	*CMAH*	Xenotransplantation	Point mutation	Crispr/Cas12a	SCNT	2	N/A	Perota et al. (2019)
Pig	*hF9*	Gene therapy for hemophilia B pigs	Gene insertion	Crispr/Cas9	SCNT	5 pigs	N/A	Chen et al. (2020)
	BgEgXyAp	Salivary gland as bioreactor	Gene insertion	Crispr/Cas9	SCNT	4 piglets (1/4 alive)	N/A	Li G. et al. (2020)
	hIAPP	Type 2 diabetic miniature pig model	Gene insertion	Crispr/Cas9	SCNT	24	N/A	Zou X. et al. (2019)
	SNCA	Parkinson's disease model	Gene insertion	Crispr/Cas9	SCNT	8 piglets	N/A	Zhu et al. (2018)
	HTT	Huntingtin KI model	Gene insertion	Crispr/Cas9	SCNT	6 piglets	N/A	Yan et al. (2018)
	GGTA1	Xenotransplantation	Gene insertion	FokI-dCas9	SCNT	2 piglets	N/A	Nottle et al. (2017)
	tdTomato	porcine Oct4 reporter system	Gene insertion	Crispr/Cas9	SCNT	2 piglets	N/A	Lai et al. (2016)
	hALB	Tg animals as bioreactors	Gene insertion	Crispr/Cas9	MI	16/16 (100%)	1/16 (6.3%)	Peng et al. (2015)
	GFP	H11-targeted KI	Gene insertion	Crispr/Cas9	SCNT	1 piglet	N/A	Ruan et al. (2015)

somatic cell nuclear transfer; MI, zygote microinjection; BE, base editing; N/A, not applicable.

Perisse et al., Frontiers in Genetics 2021

Guide RNA design other than mouse and human

eu.idtdna.com/site/order/designtool/index/CRISPR_PREDESIGN

PRODUCTS & SERVICES ▾ APPLICATIONS & SOLUTIONS ▾ SUPPORT & EDUCATION ▾ TOOLS ▾ COMPANY ▾

Custom Alt-R™ CRISPR-Cas9

Generate CRISPR-Cas9 guide RNAs (gRNAs, such as crRNA
human, mouse, rat, zebrafish, or *C. elegans* genes are availabl

Search for predesigned gRNA | **Design custom gRNA**

Species: Other species (no off-target analy

Input format: FASTA Sequence

Paste/Type input | Upload file

Enter up to 10 FASTA Sequences.
Please enter sequences in standard FASTA formatting.
No more than 1000 bases accepted.

Non sécurisé | chopchop.cbu.uib.no

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CHOPCHOP

Target: e.g. EXT1
RefSeq/ENSEMBL/gene name or genomic coordinates.

In: Sus scrofa (Sscrofa11.1)

Using: CRISPR/Cas9 nickase

For: knock-out

e adjusted in Options.

- Oryzias latipes (ASM223467v1)
- Oncorhynchus mykiss (Omyk_1.0)
- Oreochromis niloticus (ASM185804v2)
- Ovis aries (Oar_v3.1)
- Peromyscus californicus (GCA_007827085.3)
- Rattus norvegicus (rn6)
- Rattus norvegicus (mRatBN7.2)
- Salmo salar (GCF_000233375.1)
- Salmo salar (Ssal_v3.1)
- Salvelinus namaycush (GCF_016432855.1)

Haeussler *et al. Genome Biology* (2016) 17:148
DOI 10.1186/s13059-016-1012-2

Genome Biology

RESEARCH Open Access

Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR

Maximilian Haeussler[1*], Kai Schönig[2], Hélène Eckert[3], Alexis Eschstruth[4], Joffrey Mianné[5], Jean-Baptiste Renaud[6], Sylvie Schneider-Maunoury[4], Alena Shkumatava[3], Lydia Teboul[5], Jim Kent[1], Jean-Stephane Joly[6] and Jean-Paul Concordet[7*]

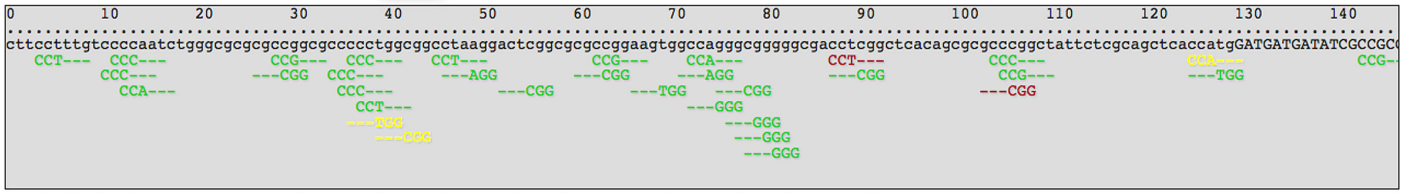
Main output of CRISPOR – sg design tool

At the top, a graphical representation of the input sequence with variants and CRISPR sg

Homo sapiens (hg38), chr7:5529546-5529784, reverse genomic strand

Found 52 possible guide sequences in input (239 bp). Click on a PAM NGG match to show its 20 bp guide sequence.
Shown below are the PAM site and the expected cleavage position located -3bp 5' of the PAM site.
Colors green, yellow and red indicate high, medium and low specificity of the PAM's guide sequence in the genome.

Variant database: dbSNP 148 Common SNPs Update Missing a variant database? We can probably add it.



Download for: SerialCloner (free) - ApE (free) - GenomeCompiler - Benchling - SnapGene - Geneious - Vector NTI - LaserGene - Genbank - FASTA

Predicted guide sequences for PAMs

Ranked by default from highest to lowest specificity score (Hsu et al., Nat Biot 2013). Click on a column title to rank by a score.
If you use this website, please cite our CRISPOR paper in Gen Biol 2016.

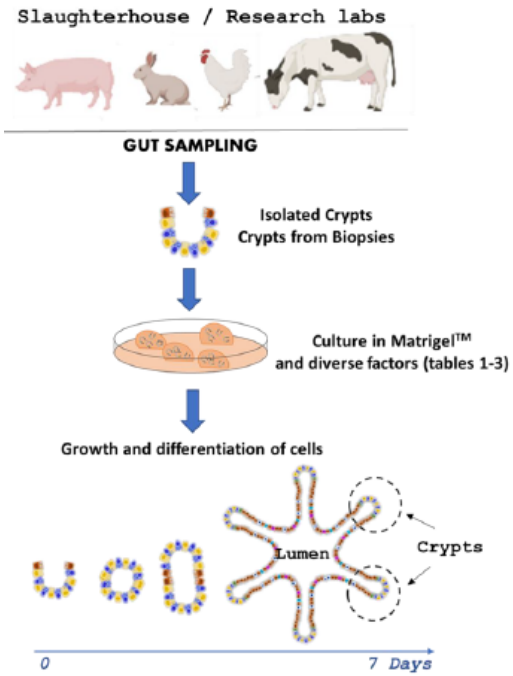
Download as Excel tables: Guides / Off-targets / Saturating mutagenesis assistant

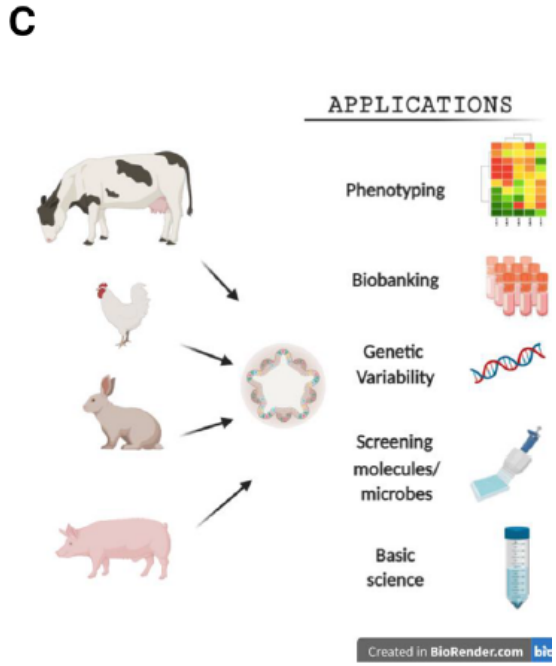
Position/ Strand	Guide Sequence + *PAM* + Restriction Enzymes + Variants Only G- Only GG- Only A-	Specificity Score	Predicted Efficiency Show all scores Doench '16	 Mor.-Mateos	Out-of-Frame score Click on score to show micro-homology	Off-targets for 0-1-2-3-4 mismatches + next to PAM	Genome Browser links to matches sorted by CFD off-target score exons only chr7 only
46 / rev	TTCCGGCGCGCCGAGTCCTT AGG Enzymes: *BshFI, Bse21I, TauI, BstDEI, SsiI, Fsp4HI* **Cloning / PCR primers**	97	49	57	67	0-0-0-0-15 0-0-0-0-1 15 off-targets	4:intergenic:SYNJ2BP-COX16/SYNJ2BP-RP11-486O13.4 4:intergenic:AC013275.2-SCTR 4:intron:CR1L show all...
12 / rev	CGCCGGCGCGCGCCCAGATT GGG ⚠ High GC content Enzymes: *BslFI, BsII* **Cloning / PCR primers**	96	21	58	71	0-0-0-3-28 0-0-0-0-0 31 off-targets	4:exon:LMF1 4:intergenic:MIR4291-RP11-53B5.1 4:intron:DAZAP1 show all...

Allows evaluation of **off-target effects**: Indels/mutations away from target, present with nuclease use

Nucleic Acids Res, Volume 46, Issue W1, 2 July 2018, Pages W242–W245, https://doi.org/10.1093/nar/gky354

Organoids from livestock





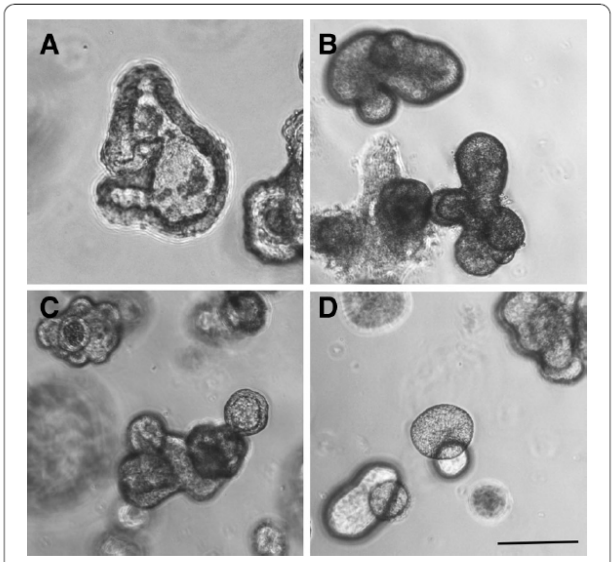


Figure 3 Morphological features of porcine gut organoids obtained from frozen tissues. Organoids are derived from: **A** duodenum (7 days, passage 3), **B** jejunum (7 days, passage 1), **C** ileum (7 days, passage 3) and **D** colon (8 days, passage 1). Observation by phase contrast microscopy. Bars: 200 µm. Images are representative of organoids obtained from 4 pigs for each digestive segment.

Expansion
Differentiation
Screening

Beaumont *et al.* Vet Res 2021

Virtually all organs: brain, skin, airways, gut, liver, kidney, pancreas, testis, retina
But they are not immortalized cell lines.

Editing strategies depend on phenotype selection

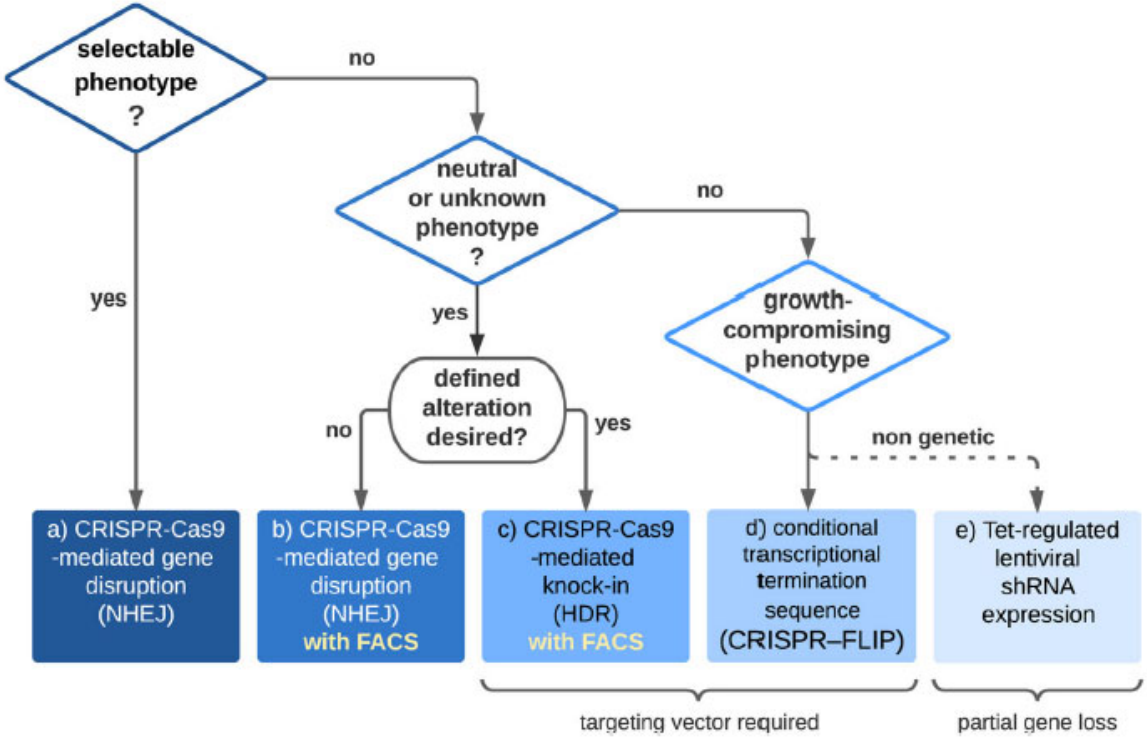


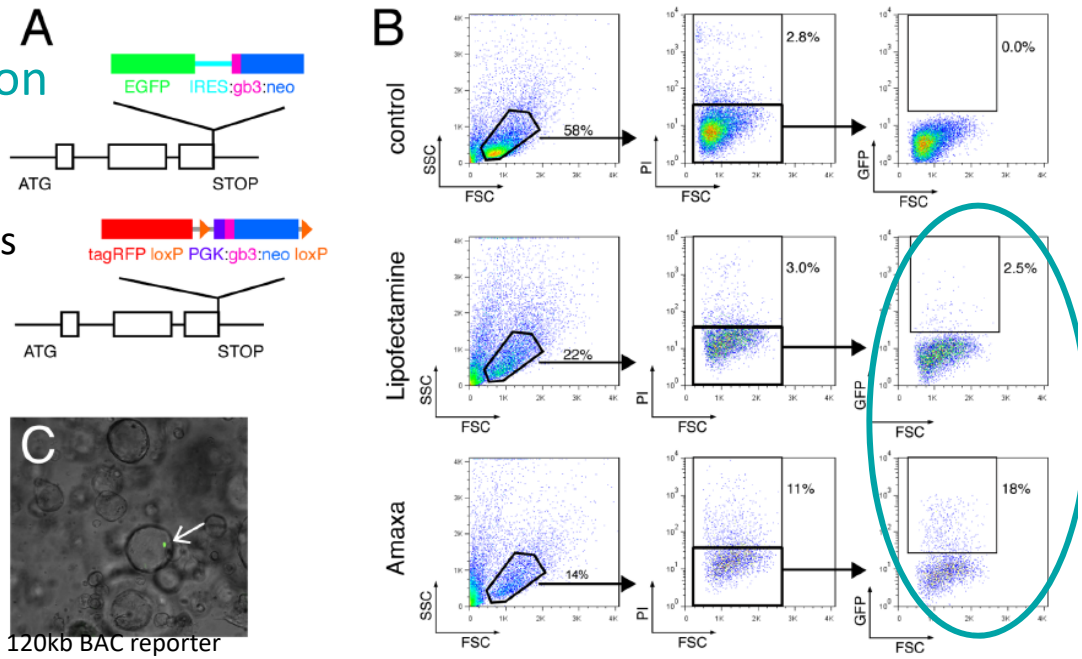
Fig. 4 Considerations for successful modification of gene function in organoids. Flowchart depicting key questions that determine which strategies are most effective to obtain a desired transgenic organoid line. A factor is the anticipated phenotype of the gene of interest. Positively selectable traits such as growth advantages or discernable morphologic changes can be used to identify clonal organoids after NHEJ-mediated gene loss (a). For modifications that result in neutral or unknown phenotypes, a pre-enrichment strategy (e.g., by FACS) should be considered (b). If a defined alteration rather than gene loss is desired, classical HDR-mediated knock-in is recommended (c). To address phenotypes that result in compromised growth, inducible strategies, e.g., insertion of a conditional transcriptional termination sequence (d) or by knockdown using Tet-regulated lentiviral shRNA (e), are required. For additional information on the different approaches (a–e), please refer to Table 1.

Menche and Farin, Exp Mol Med 2021

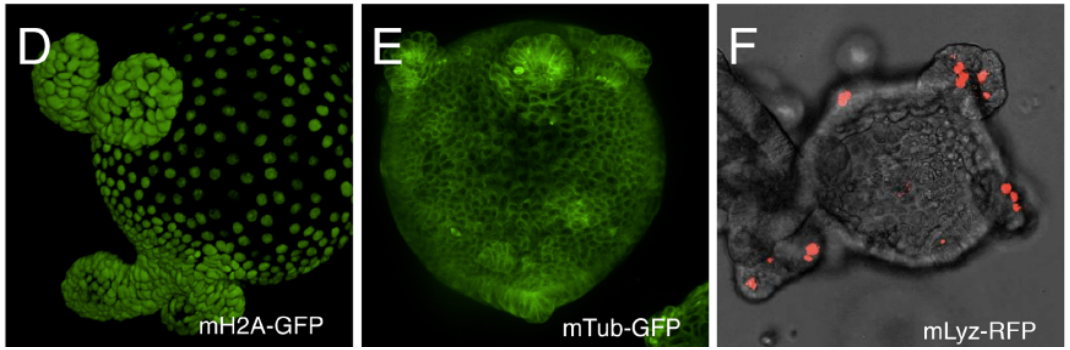
Delivery methods and Efficiency of transgenesis in organoids

Electroporation outperforms lipofection

- Trypsinized organoids
- Transfection by electroporation or liposomes
- Resuspension in cold matrigel
- After 2 days: selection of stable organoids
- After selection: budding organoids medium



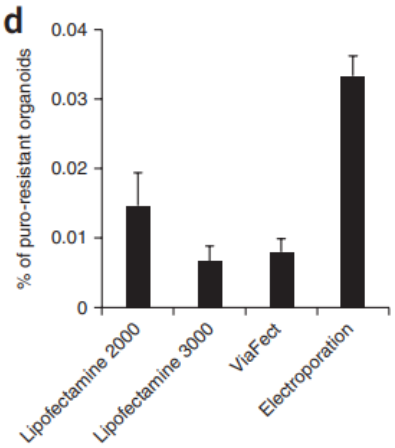
Efficiency of delivery in organoids (mouse/human) is LOW!

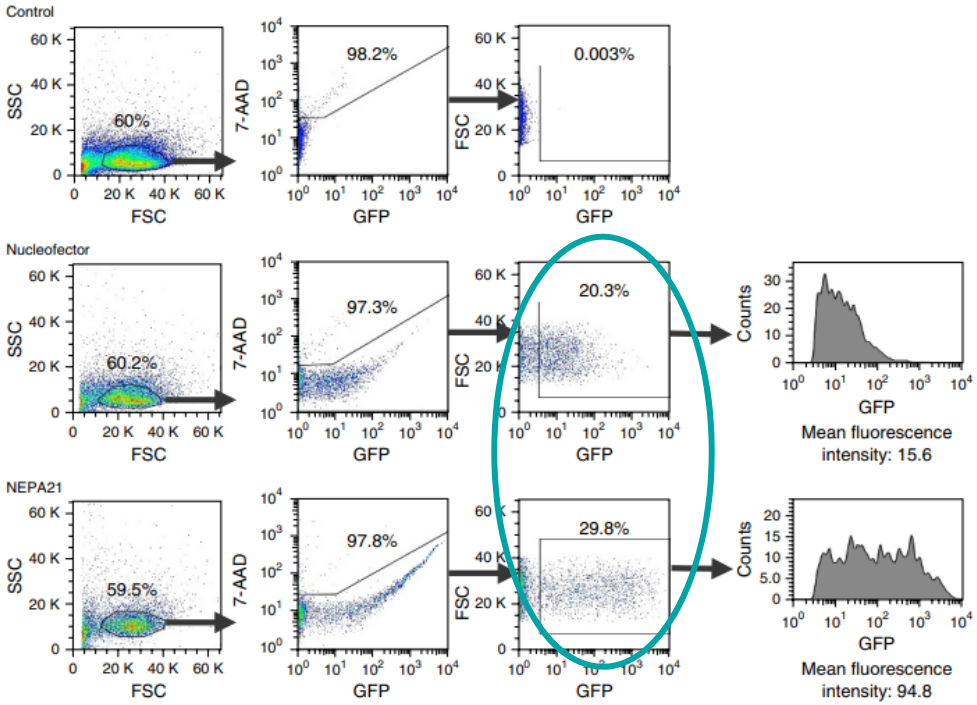


Schwank et al., PLoS One 2013

Delivery methods and Efficiency of transgenesis in organoids

Electroporation gene delivery protocol for organoids





- Trypsinized organoids
- Transfection in NEPA21 electroporator
- Recover cells 30min RT
- Resuspension in cold matrigel + inh Wnt&ROCK
- After 7 days: selection of stable organoids

piggyBAC transposase system or CRISPR/Cas9 with refined protocol: BTXpress, DMSO

Limitations of the method:

6 wells /24WP 24 wells /24WP

Fujii et al Nat Protocols 2015

Delivery methods and Efficiency of transgenesis in organoids

CRISPR-Cas9 editing system to Ko lentiviral transduction and single cell cloning

- Lentiviral transduction
- genetic knockout HIE line within 2-3 months
- HIEs as an *ex vivo* model to assess host restriction factors for viral replication by ko host attachment factors or innate immunity genes

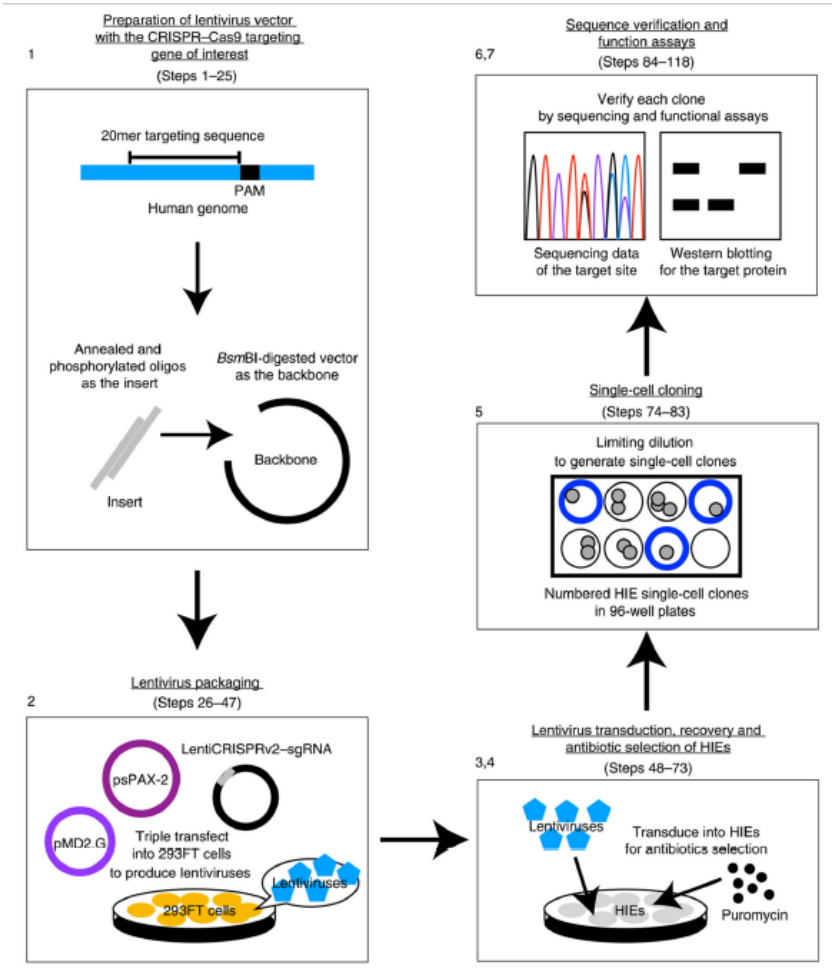


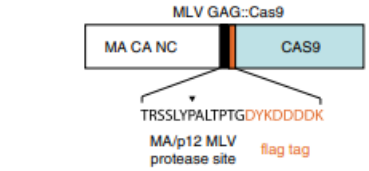
Fig. 1 | The steps of lentivirus packaging and transduction. The protocol has seven parts: (1) preparation of lentivirus vector with the CRISPR–Cas9 targeting gene of interest; (2) lentivirus packaging; (3) lentivirus transduction of HIEs; (4) HIE recovery and antibiotic selection; (5) single-cell cloning; (6) sequencing verification of single-cell clones; (7) using KO lines to evaluate host gene function in viral replication or other biological processes.

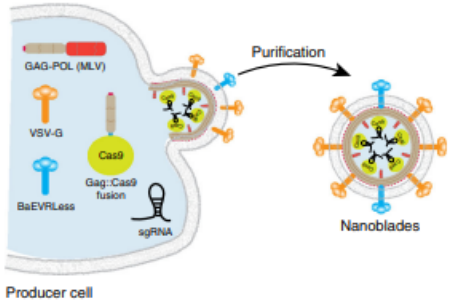
Lin *et al.*, Nature Protocols 2022

Delivery methods and Efficiency of transgenesis in organoids

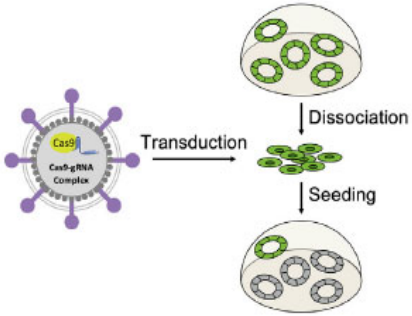
Nanoblades achieve high % gene editing in organoids with low toxicity

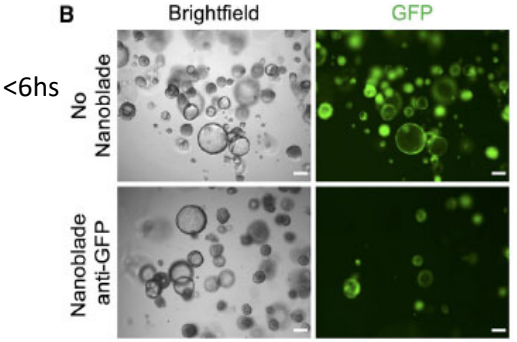
Nanoblades: protein-delivery vector (MLV) tranfering Cas9-sgRNA RNP in vitro & in vivo

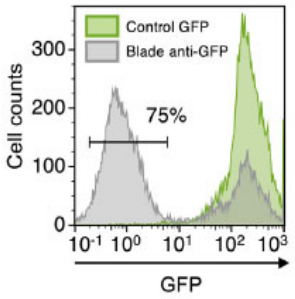


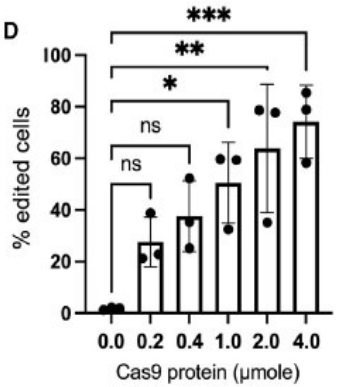


Mangeot et al., Nat Comm 2019



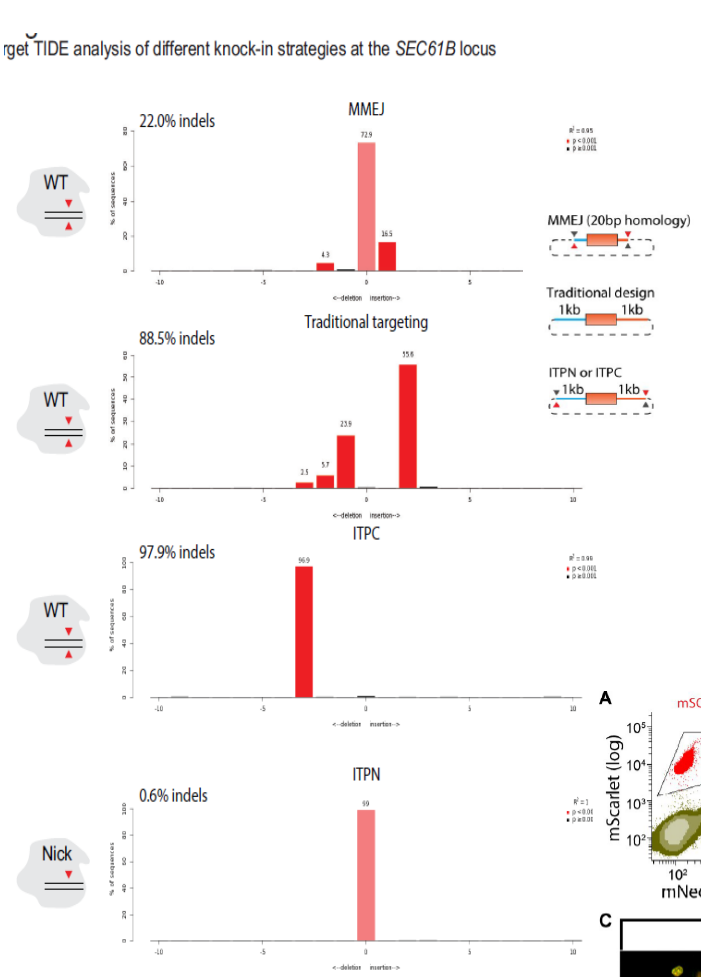


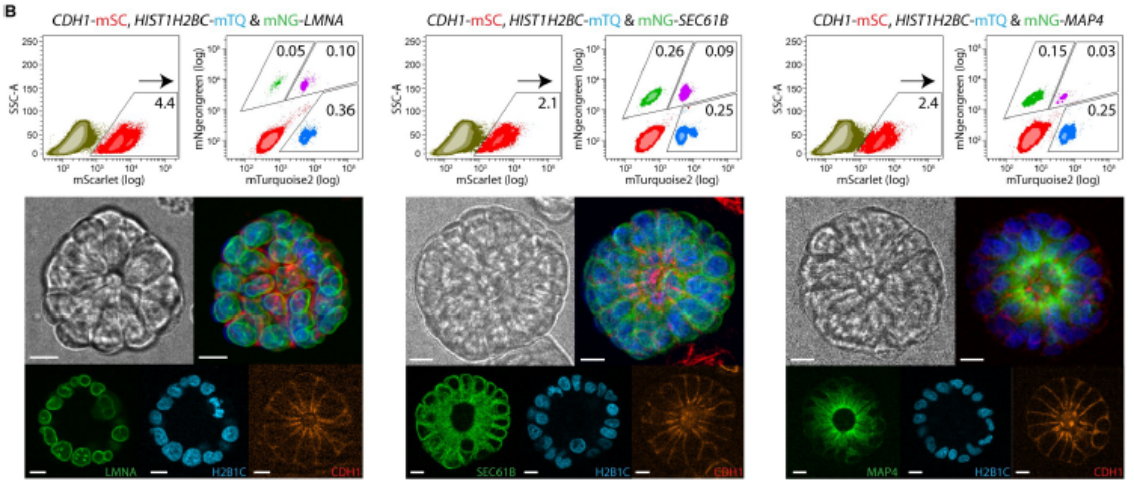




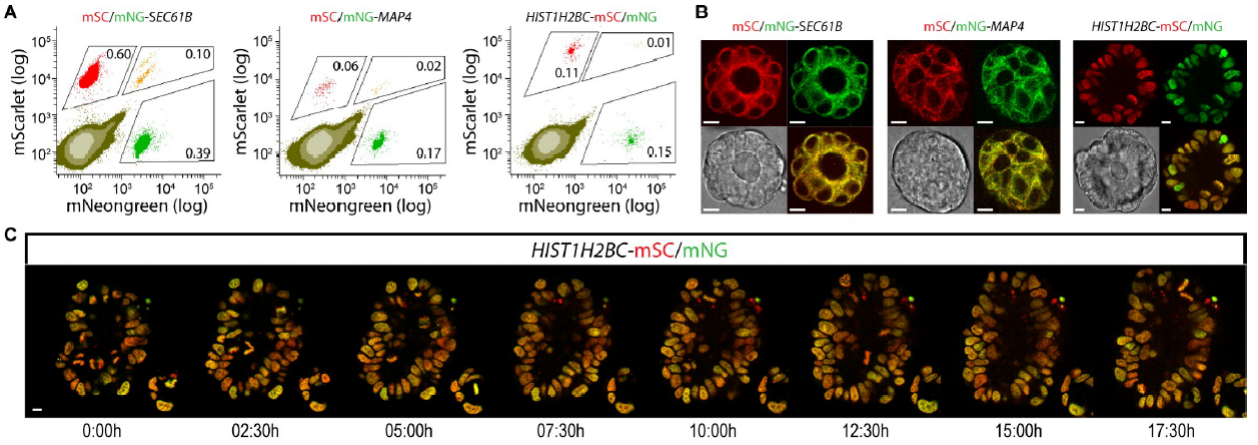
Tiroille et al., Mol Therapy 2023

In trans paired-nicking w/o DSB allows allelic analysis



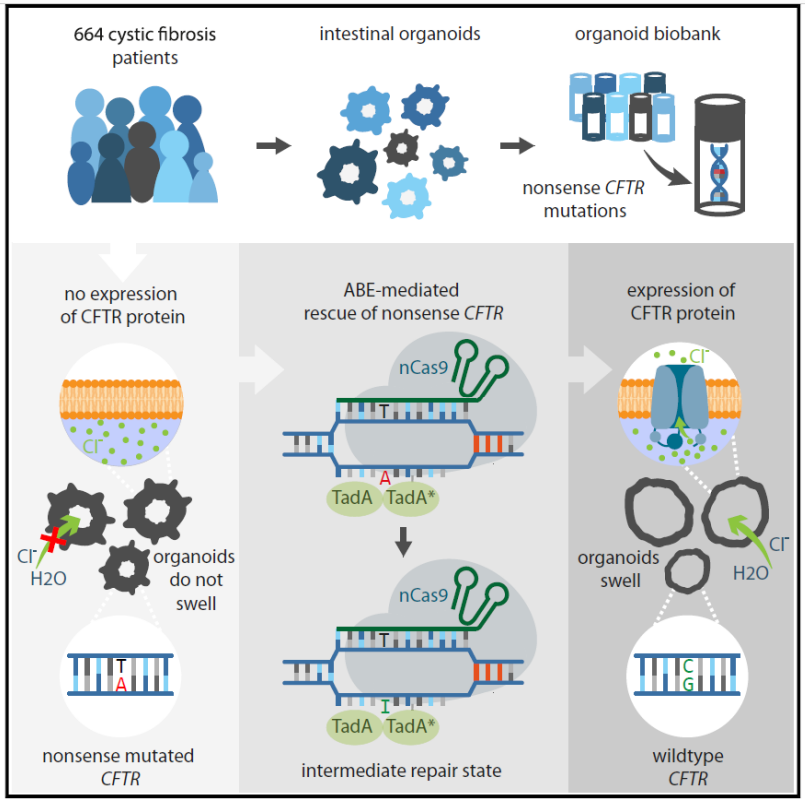


E-cadherin, histone1, laminA, ER translocation complex, microtubule-associated prot



Bollen et al, PLoS Biol 2022

Base editors to correct a genetic defect: CFTR



Maarten H. Geurts, Eyleen de Poel, Gimano D. Amatngalim, ..., Cornelis K. van der Ent, Jeffrey M. Beekman, Hans Clevers

Correspondence

j.beekman@umcutrecht.nl (J.M.B.), h.clevers@hubrecht.eu (H.C.)

In Brief

Here, we show the generation of an extensive cystic fibrosis patient-derived intestinal organoid biobank. We use this biobank to study gene correction by adenine base editors and show genetic repair of four selected nonsense mutations in *CFTR* without any genome-wide off-target effects on canonical and non-canonical PAMs.

Plasmids electroporation (Fijii method) with BE Cas9-ABE or xCas9-ABE
Editing efficiencies vary, depending on Cas9 and sgRNA usage, with a max of 9,3%
10% of residual CFTR function is associated with mild disease
No genome-wide off-targets (multiple consecutive ABE treatment possible)

Cell Stem Cell 2020

CRISPaint : modular base-specific gene tagging through NHEJ

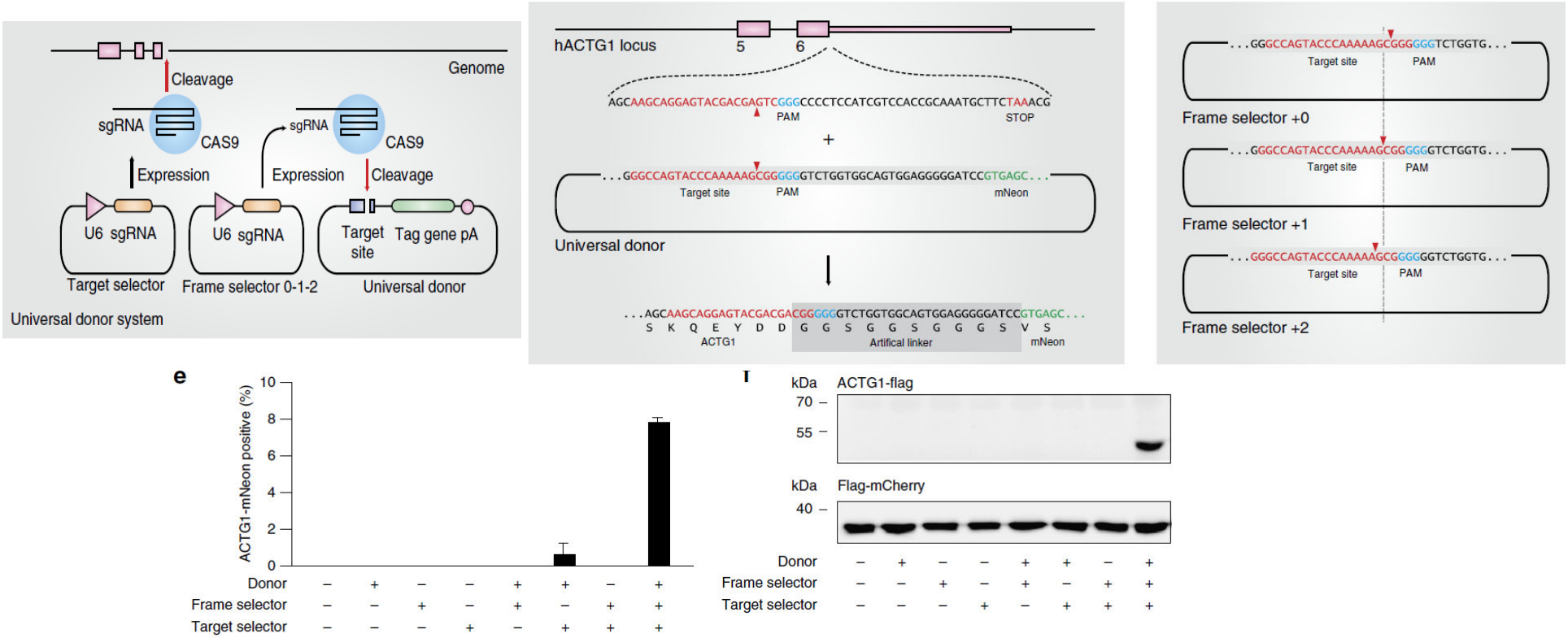
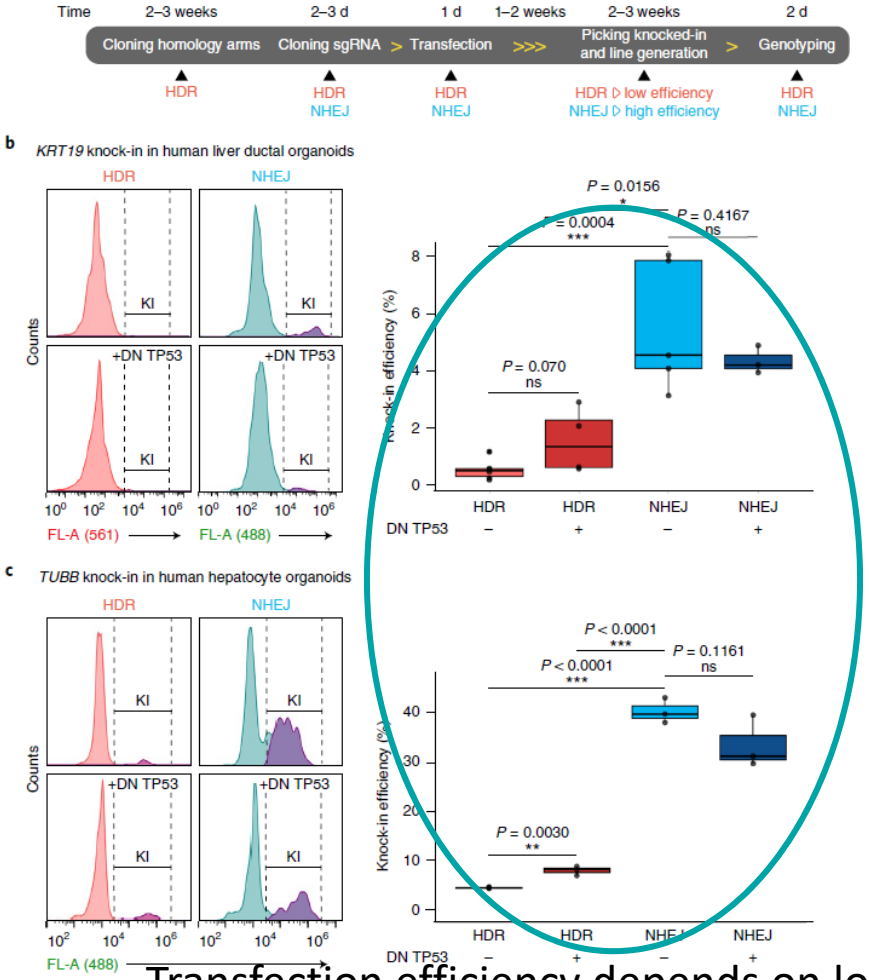


Figure 2 | Modular three-plasmid gene tagging system. (**a**) Three-plasmid tagging system. A target selector plasmid expresses an sgRNA targeting a gene of interest. A frame selector plasmid expresses an sgRNA targeting the donor plasmid. A universal donor plasmid contains the tag gene. (**b**) Sequence details of the universal donor plasmid when integrating into the human *ACTG1* gene. (**c**) Due to a poly-G stretch within the target site of the universal donor plasmid it can be cleaved at three adjacent nucleotide positions, which allows specifying the frame of integration at the time of transfection. (**d**) Fluorescence imaging and deep sequencing analysis of *ACTG1*-mNeon gene tagging using a three-plasmid system and different plasmid combinations. ND, not determined. (**e**) Image quantification of *ACTG1*-mNeon-positive cells. Shown are mean values + s.e.m. from three independent biological replicates. (**f**) Immunoblotting of *ACTG1*-Flag gene tagging using a three-plasmid system and different plasmid combinations.

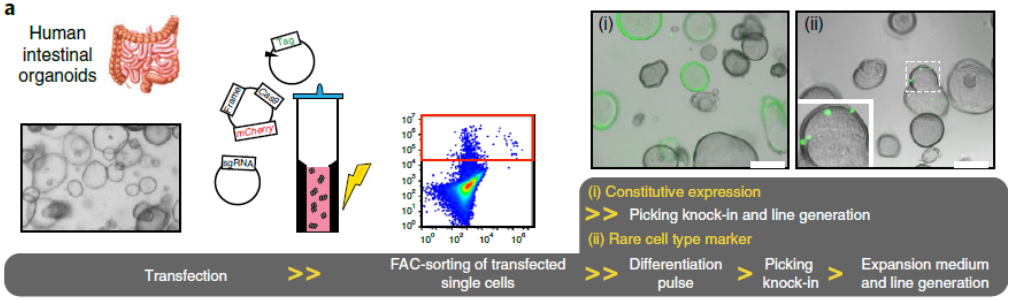
Schmidt-Burgk et al Nature Comm 2016

CRISPR-HOT minimizes the need of molecular cloning

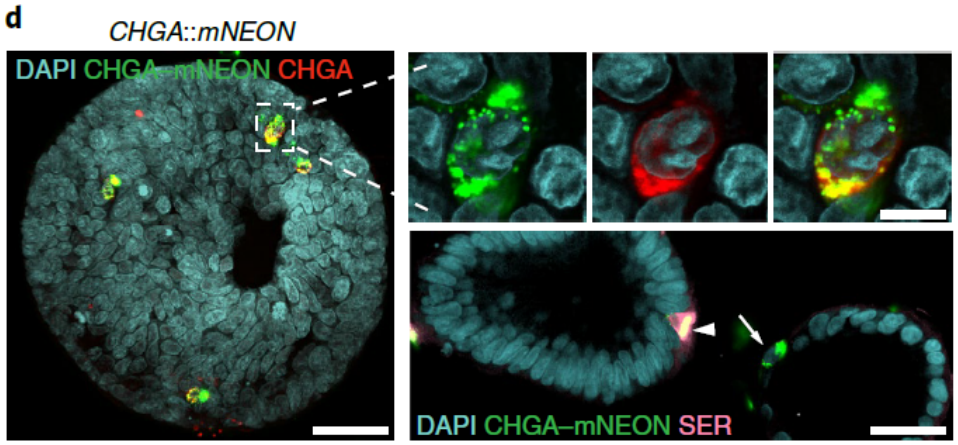
Using CRISPaint:



Tagging rare differentiated cells in organoids, with differentiation pulse to detect fluo organoids



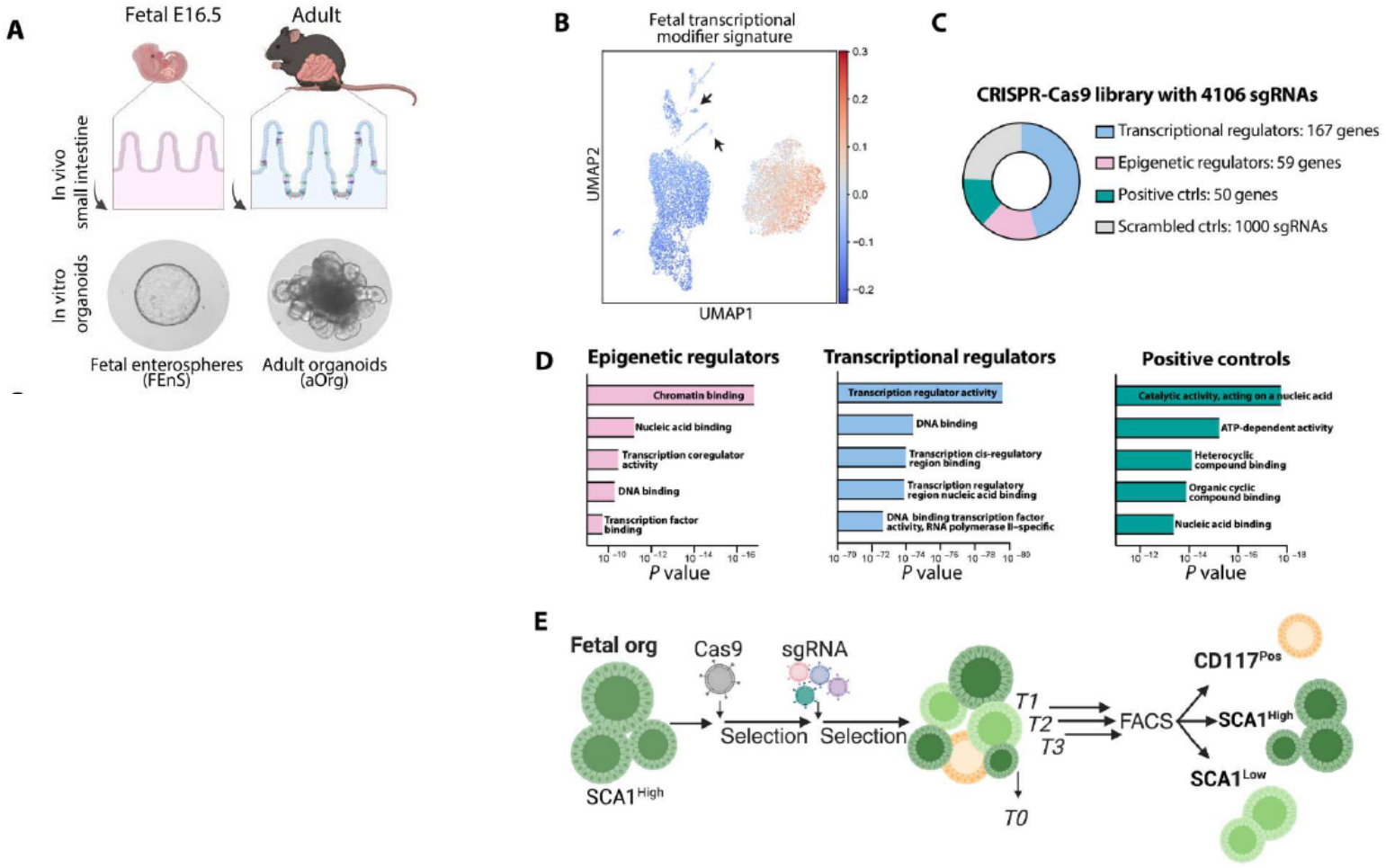
Electroporation as Fujii 2015, sorting day7



Transfection efficiency depends on locus

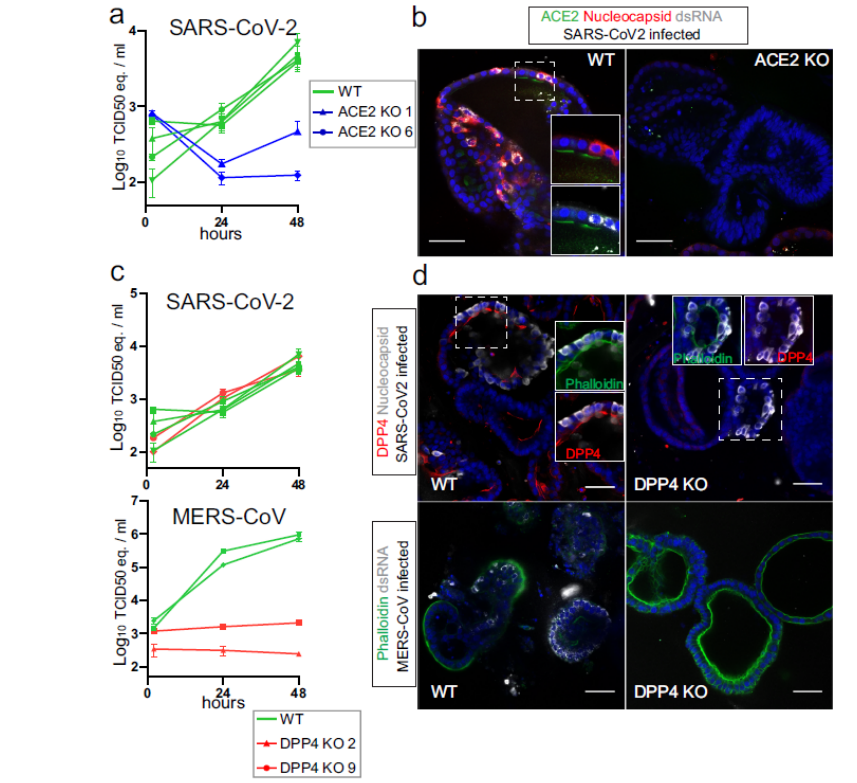
Artegiani et al Nature Cell Biol 2020

Regulators of intestinal epithelial maturation and cell fate

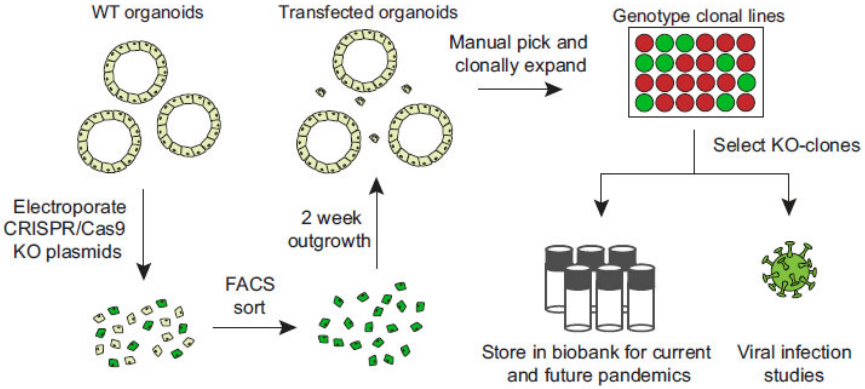


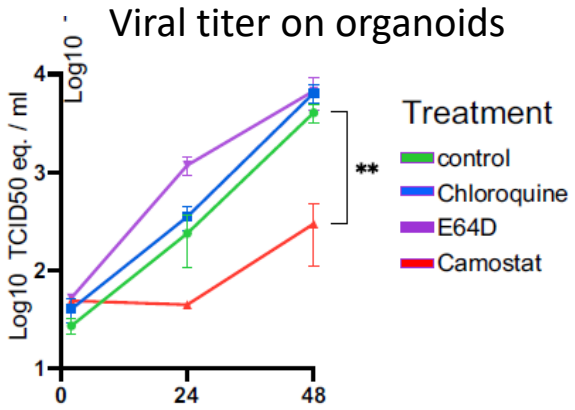
Hansen *et al* Sciences Advances 2023

Organoid biobanks to identify therapeutic targets



ACE2 and DPP4 are the obligate entry receptors for SARS-CoV-2 and MERS-CoV, respectively. a qPCR analysis targeting the



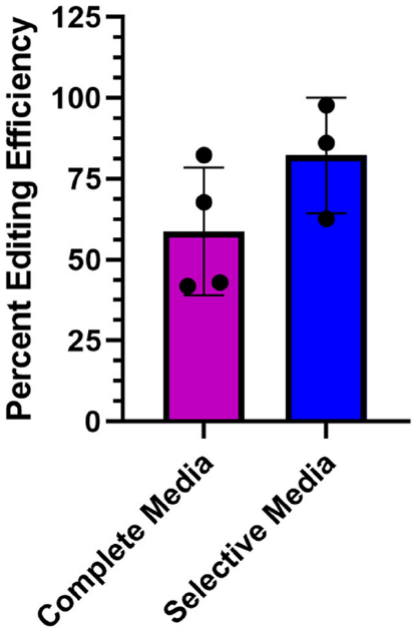


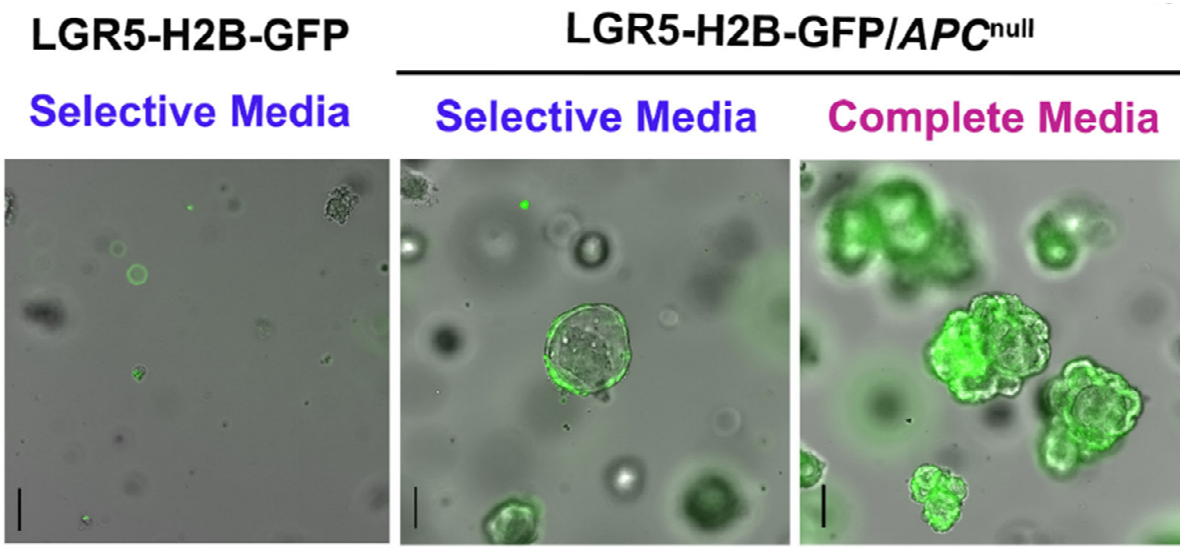
While chloroquine effectively inhibited viral replication in VeroE6 cells

Beumer *et al* Nat Comm 2021

LGR5-H2B-GFP Pigs organoids *APC*$^{-/-}$ developed cystic growth

- LGR5-H2B-GFP colonoids dissociated to single cells (TrypLE + Y-27632)
- 250 000 cells + 5µg sgRNA + 5µg Cas9/nucleofection
- Washed cells plated 5000 cells/50µl Matrigel
- Selection: growth factors reduced media (w/o Wnt-3A, R-spondin, CHIR)





Schaaf *et al.*, FASEB J, 2023

Editing Organoids

Pros

- Time saver & 3R complient
- Non transformed cell cultures, relevant *in vitro* models for normal physiology
- Biobanking : reduce geographic proximity to research lab, platforms to study developmental diseases
- Virtually from any ASC type from animal realm (less for iPS)
- Establishing genotype-phenotype correlations
- High throughput
- Imaging
- Potentially complexifiable with autologous vascularization, muscle or glial cells, fibroblasts
- Physiologic, immunologic, nutritional differences between mouse, human, livestock impede immediate translation of findings
-

Cons

- Standards Operation Protocols still to be optimized
- Some off-targets & difficulties creating complex modifications
- Targeting of SC necessary for maintenance of genetic modifications
- Expansion of SC before genetic manipulation to increase efficacy of colony outgrowth
- Loss of variability of stem cells -neutral drift- during organoid passaging,
- Gentle digestion of organoids necessary to preserve stem cells
- Addition of niche factors or activators of stem cells

Potential uses

- Relaunch species specific fundamental biology
- Understanding different levels of interactions regulating tissue homeostasis
- Confirm genetic association of a mutation with a disease phenotype/ trait
- Bring back functioning alleles

nature biotechnology

Article

https://doi.org/10.1038/s41587-023-01857-x

Evolutionary mining and functional characterization of TnpB nucleases identify efficient miniature genome editors

INRAE

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Thank you for your attention

INNOVATIVE CELLULAR MODELS FOR ANIMAL SCIENCE
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