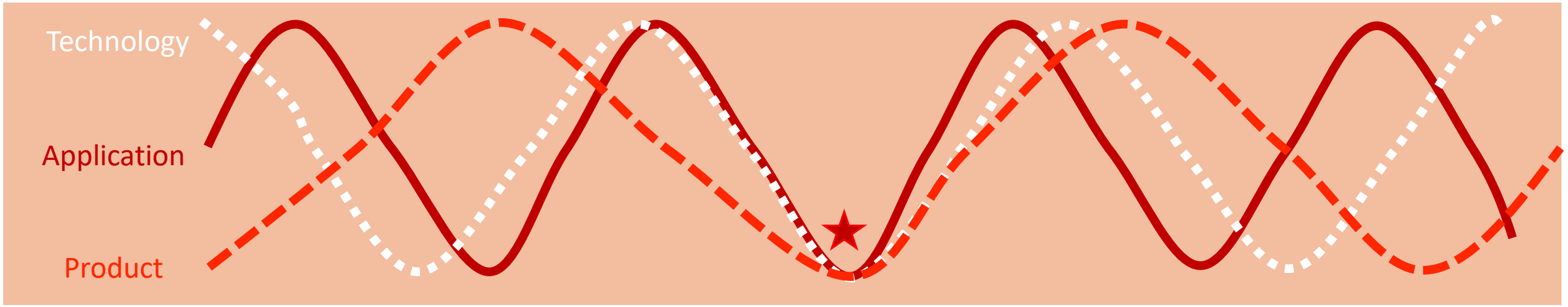
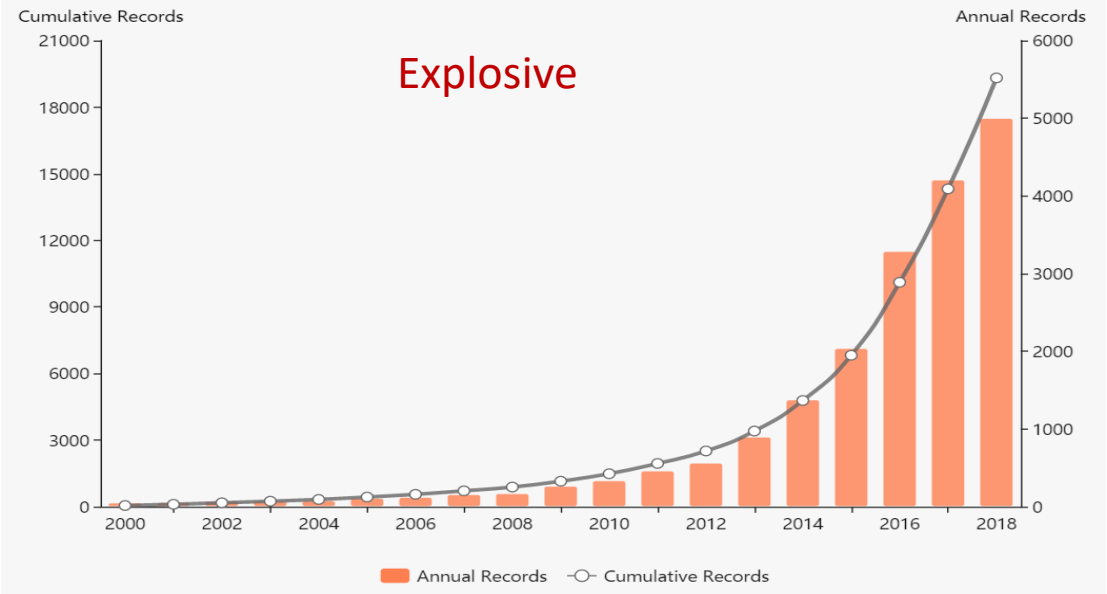
Applications of CRISPR-Cas systems in LAB

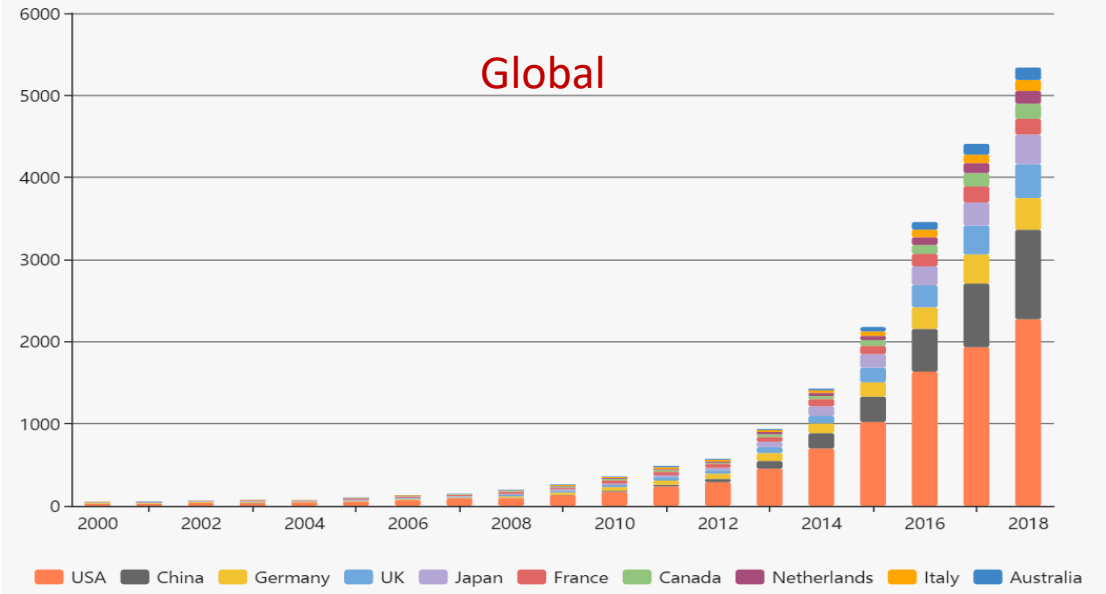
Rodolphe Barrangou | NC State | @CRISPRlab



Science → Technology → Applications → Products → Acceptance

The Innovator's dilemma


Cumulative Records
Annual Records
Explosive
21000
18000
15000
12000
9000
6000
3000
0
6000
5000
4000
3000
2000
1000
0
2000
2002
2004
2006
2008
2010
2012
2014
2016
2018
Annual Records
Cumulative Records


Global
6000
5000
4000
3000
2000
1000
0
2000
2002
2004
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2008
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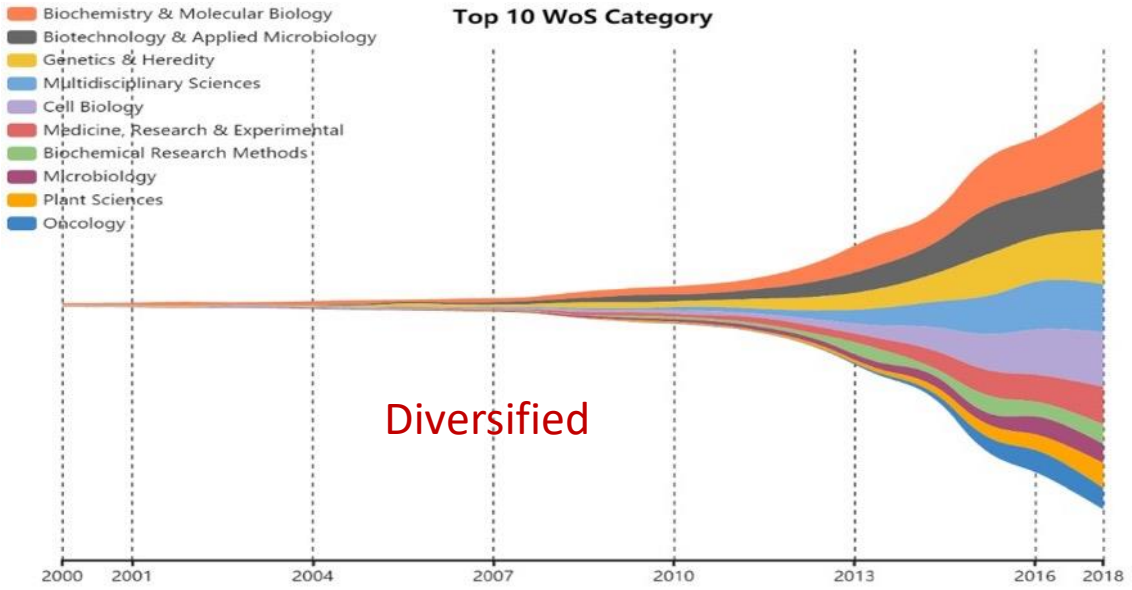
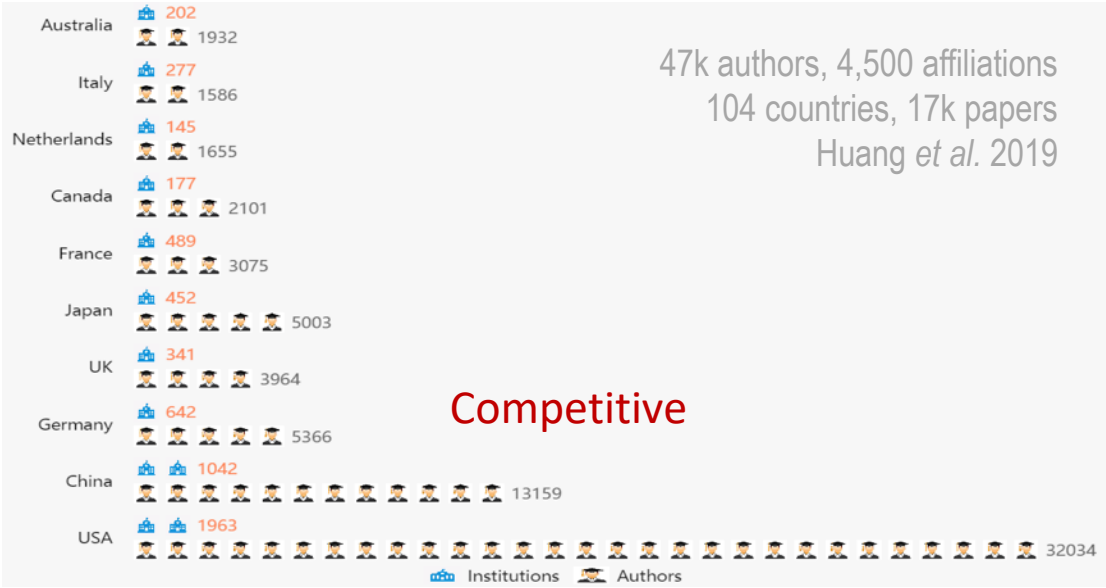
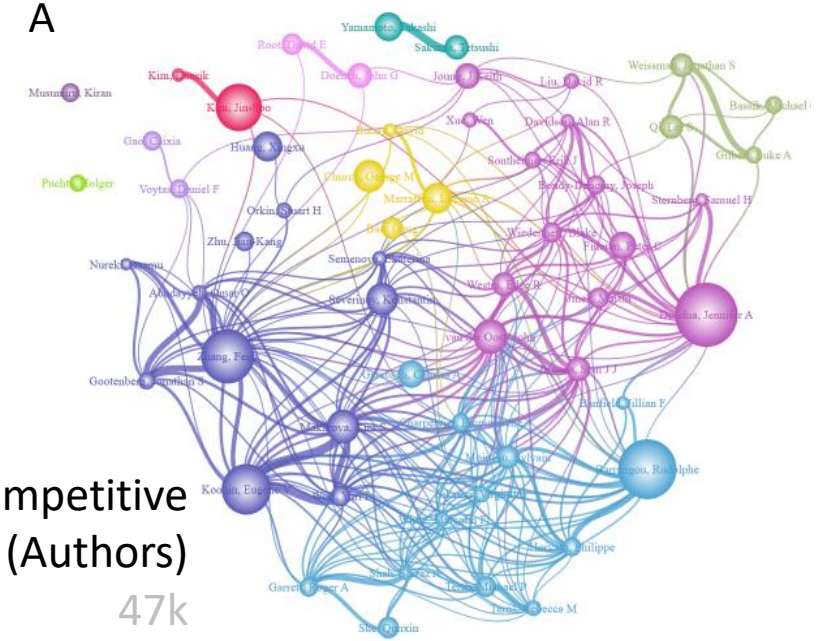
2012
2014
2016
2018
USA
China
Germany
UK
Japan
France
Canada
Netherlands
Italy
Australia


Top 10 WoS Category
Biochemistry & Molecular Biology
Biotechnology & Applied Microbiology
Genetics & Heredity
Multidisciplinary Sciences
Cell Biology
Medicine, Research & Experimental
Biochemical Research Methods
Microbiology
Plant Sciences
Oncology
Diversified
2000
2001
2004
2007
2010
2013
2016
2018

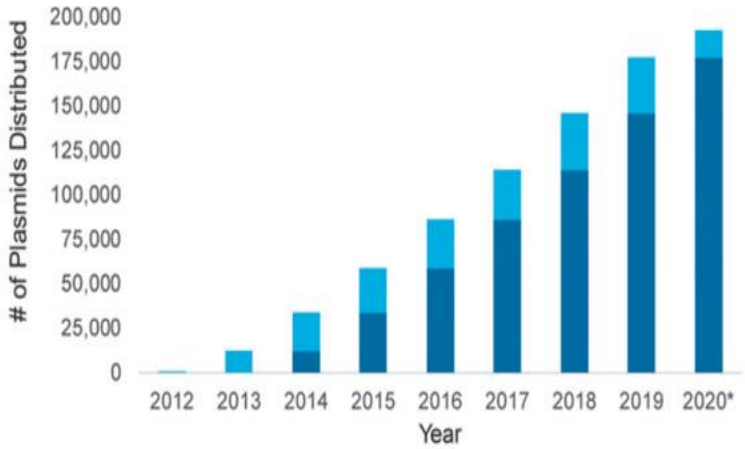

Australia 202 1932
Italy 277 1586
Netherlands 145 1655
Canada 177 2101
France 489 3075
Japan 452 5003
UK 341 3964
Germany 642 5366
China 1042 13159
USA 1963 32034
47k authors, 4,500 affiliations
104 countries, 17k papers
Huang et al. 2019
Competitive
Institutions
Authors

A



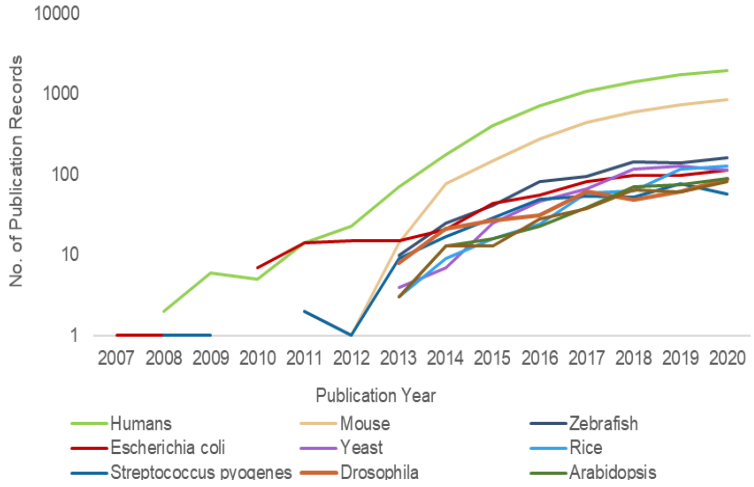
Competitive (Authors)

47k



Explosive (Constructs)

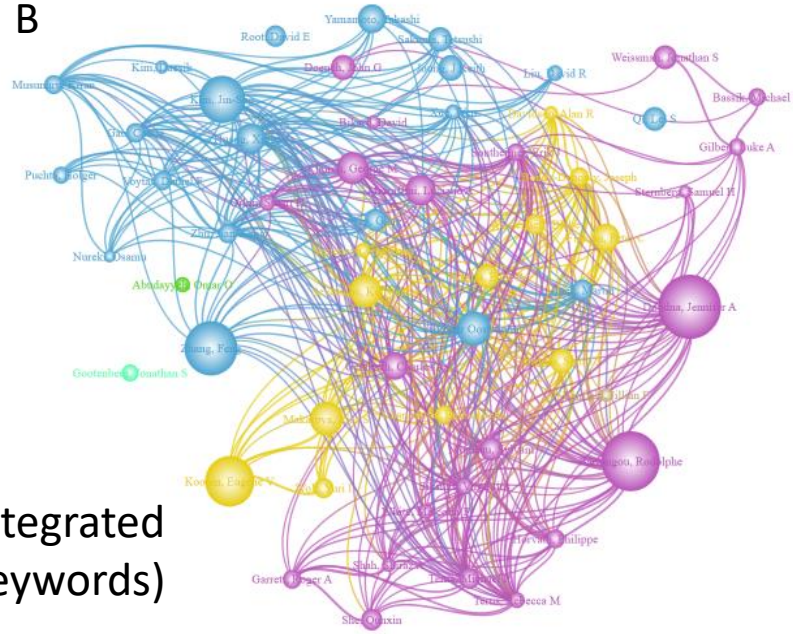
200k



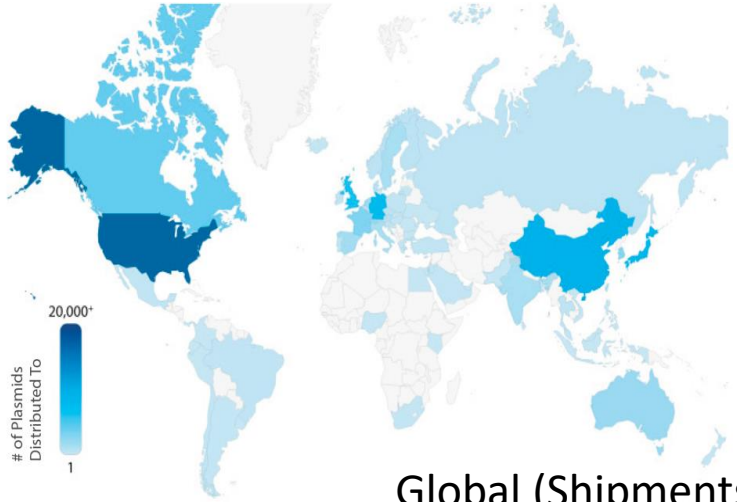
Biased (Organisms)

20k papers

B

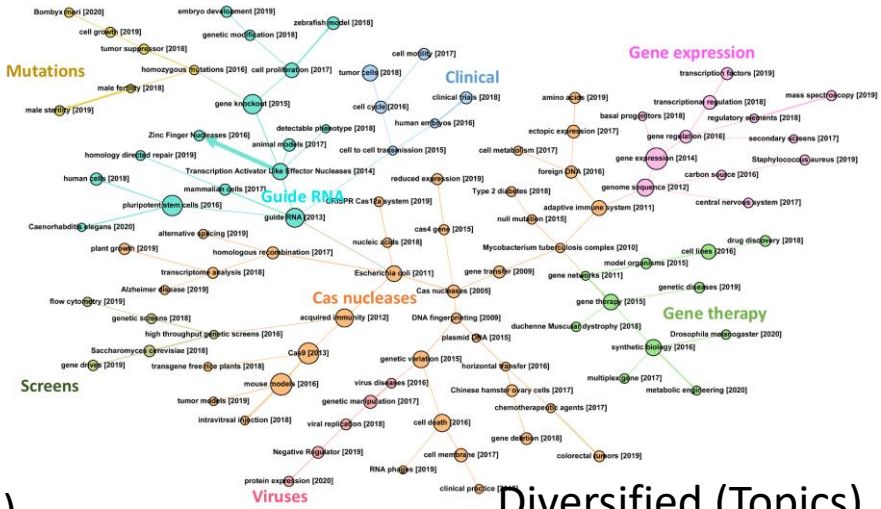


Integrated (Keywords)



Global (Shipments)

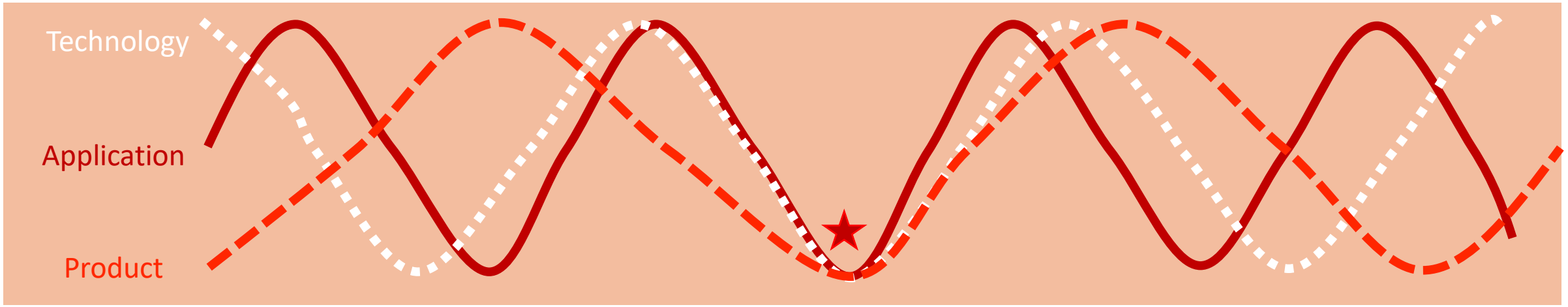
4.5k affiliations



Diversified (Topics)

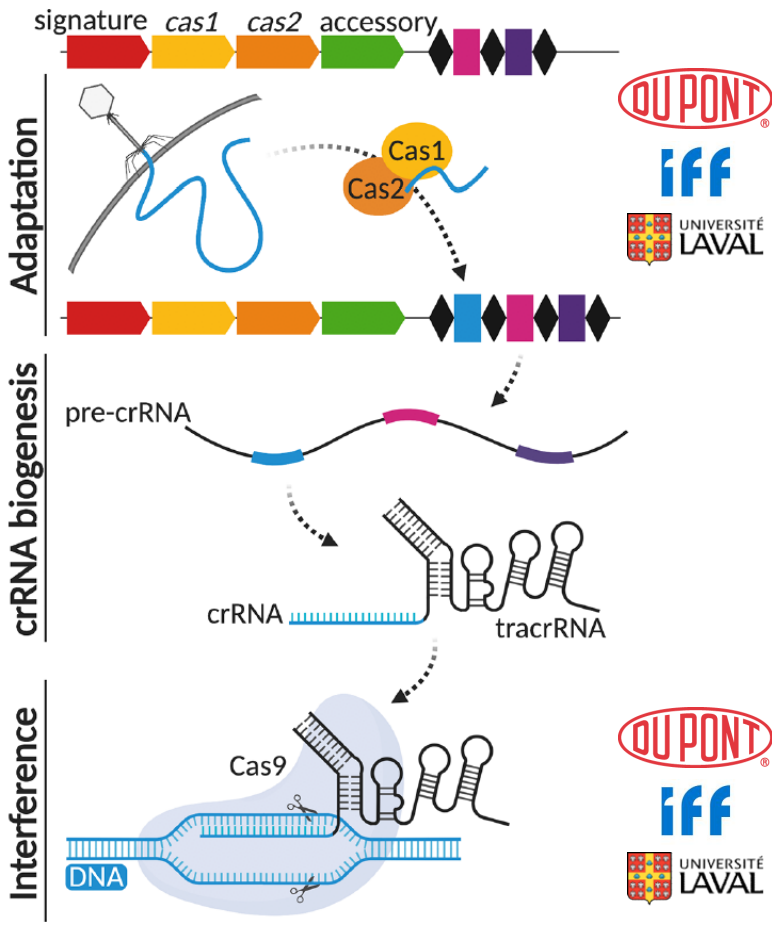
Huang *et al. in press*

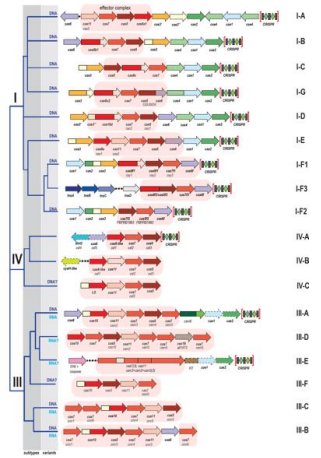
LaManna *et al.* 2020



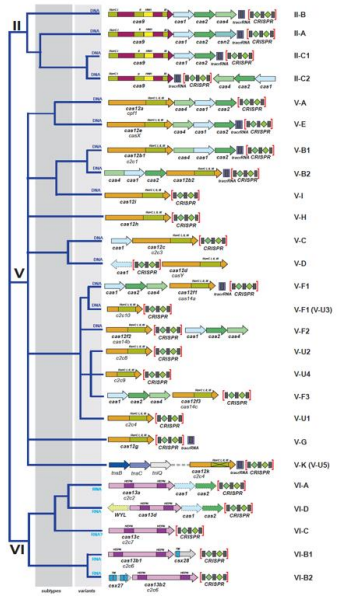
Science → Technology → Applications → Products → Acceptance

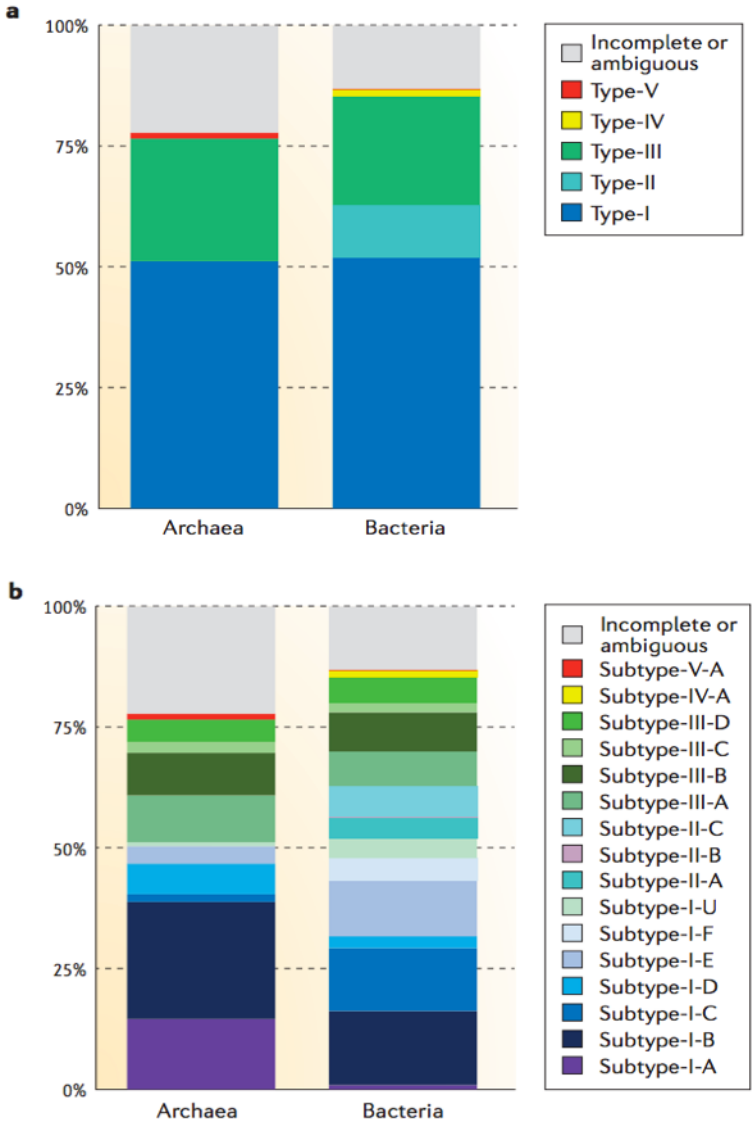
The innovator's dilemma





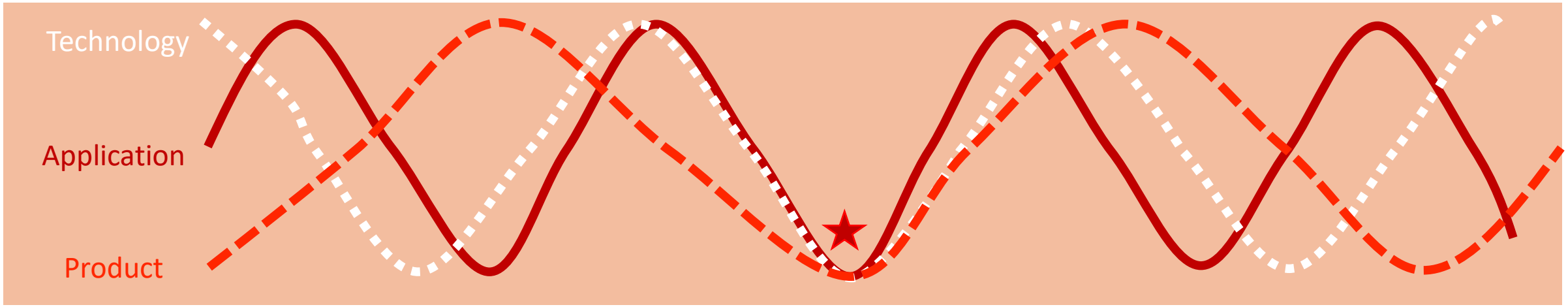
2 classes
6 types
44 subtypes



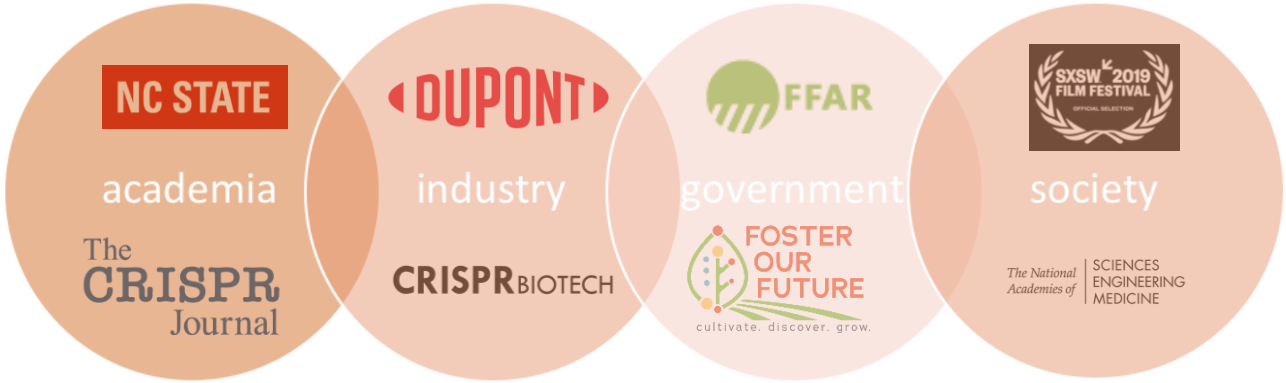


CRISPR[101]

Makarova *et al.* 2011
updated in Makarova *et al.* 2015
updated in Makarova *et al.* 2019



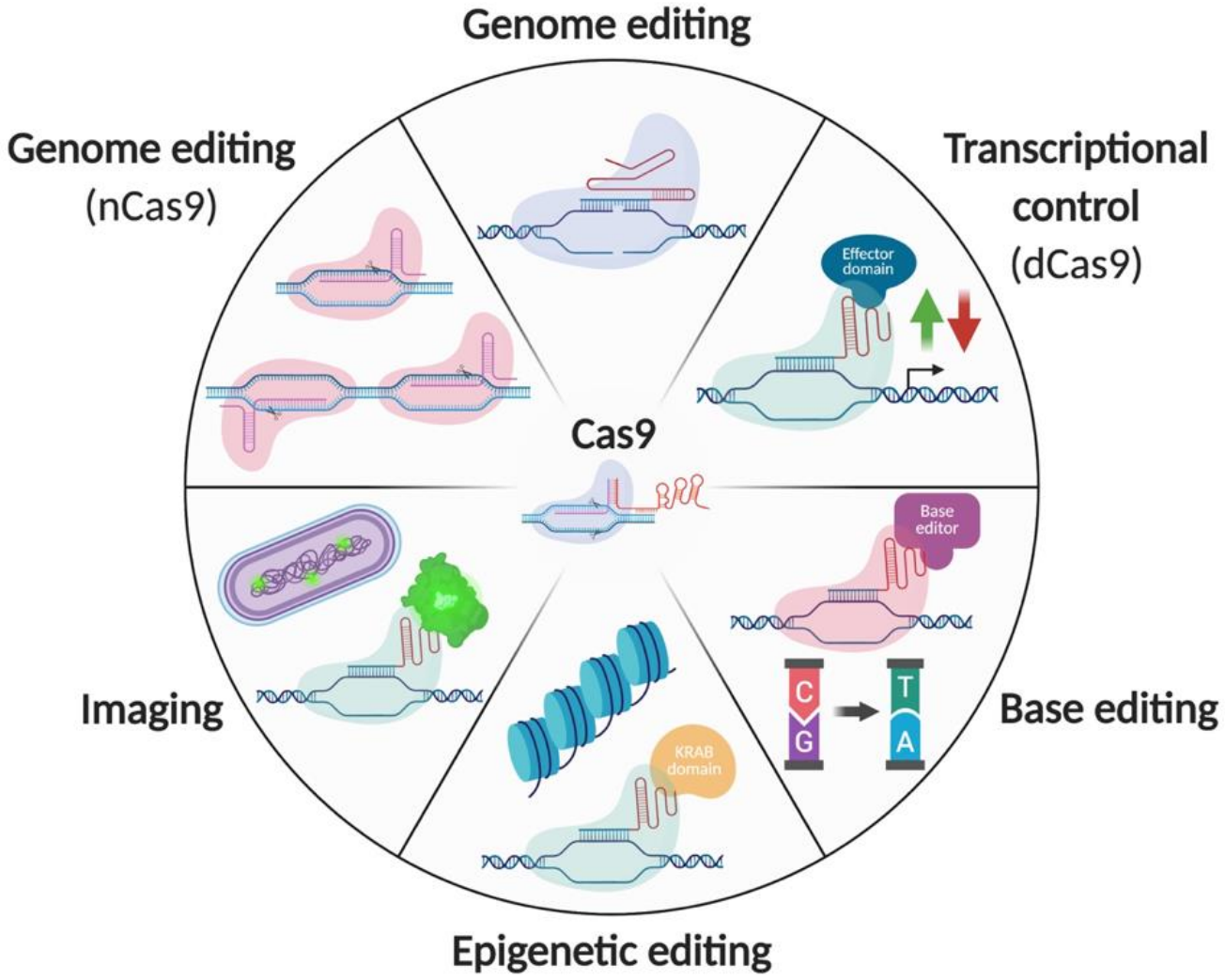
Science | Technology | Applications | Products | Acceptance



The innovator's dilemma

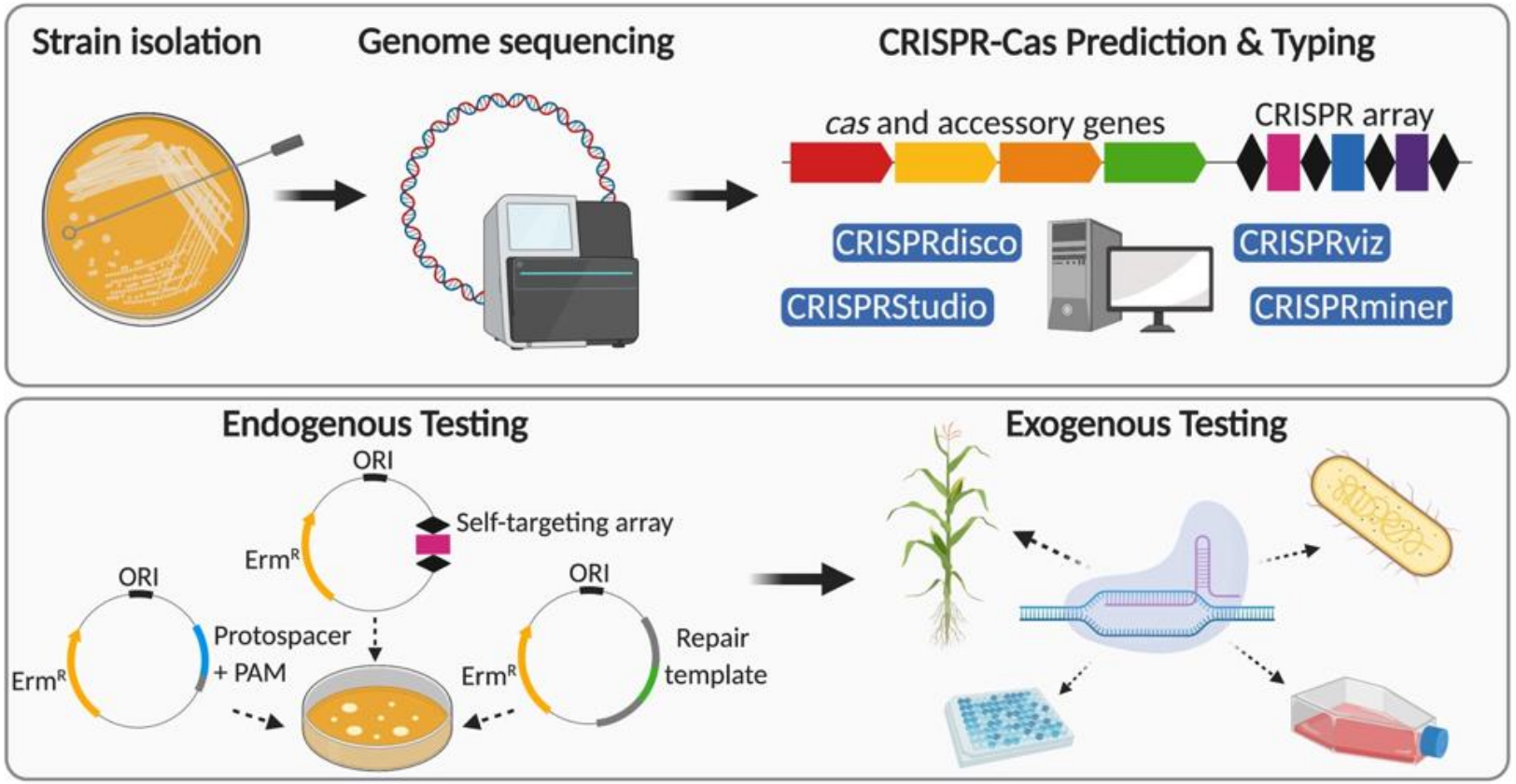
Genome editing technology@Nobel2020

Barrangou & Doudna 2016



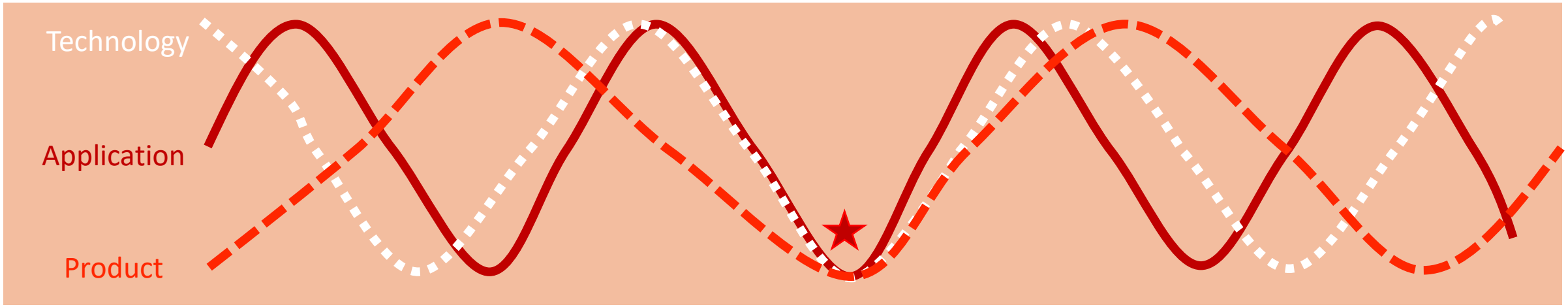
Genome Editing $^{2.0}$ $_{(d/n)}$Cas^{9} | Cas$^{3\ 12\ Tn\ BE}$

Roberts & Barrangou 2020

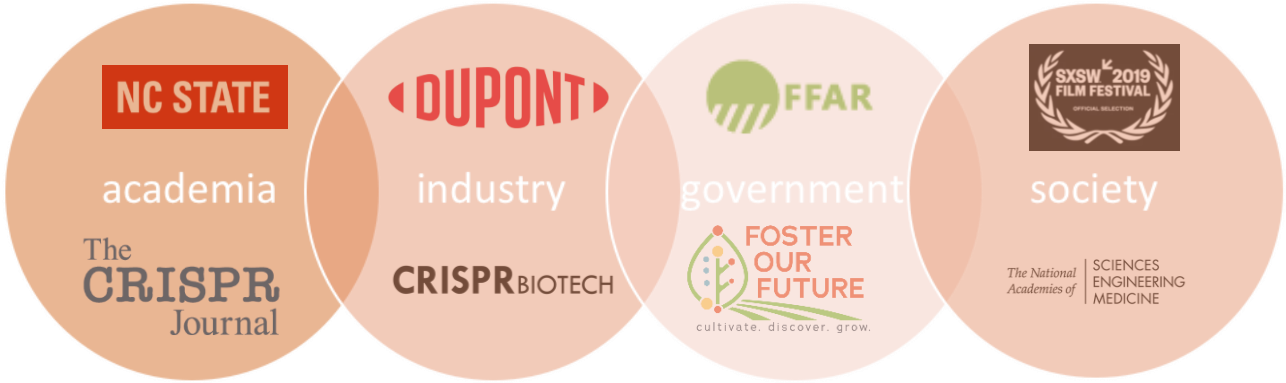


Characterization & validation of CRISPR: bottleneck

Roberts & Barrangou 2020



Science → Technology → Applications → Products → Acceptance



The innovator's dilemma

Fields of use

RESEARCH

Tools
Guides
Enzymes
Software
Plasmids
Delivery
Kits
Primers
Cell lines

BIOTECHNOLOGY

Bacteria
Yeast
Algae

Food
Biomanufacturing
Household care
BioEnergy

AGRICULTURE

Plants
Animals
Microbes
Forestry
Flowers & ornamentals
Aquaculture

THERAPEUTICS

Gene therapies
Antivirals & inf. disease
Microbiomes
Antimicrobials
Cell / immuno therapies
Xeno transplants
Invasive species/Drives
Diagnostics
Petcare

CARIBOU BIOSCIENCES a

Intellia THERAPEUTICS *

CRISPR THERAPEUTICS

ers GENOMICS

editas MEDICINE

Beam THERAPEUTICS

MammothBiosciences

SHERLOCK BIOSCIENCES

egenesis ENGINEERING LIFE

CASZYME

arbor biotechnologies

LifeEDIT RTP

CASTAG RTP BioSciences

acrigen BIOSCIENCES

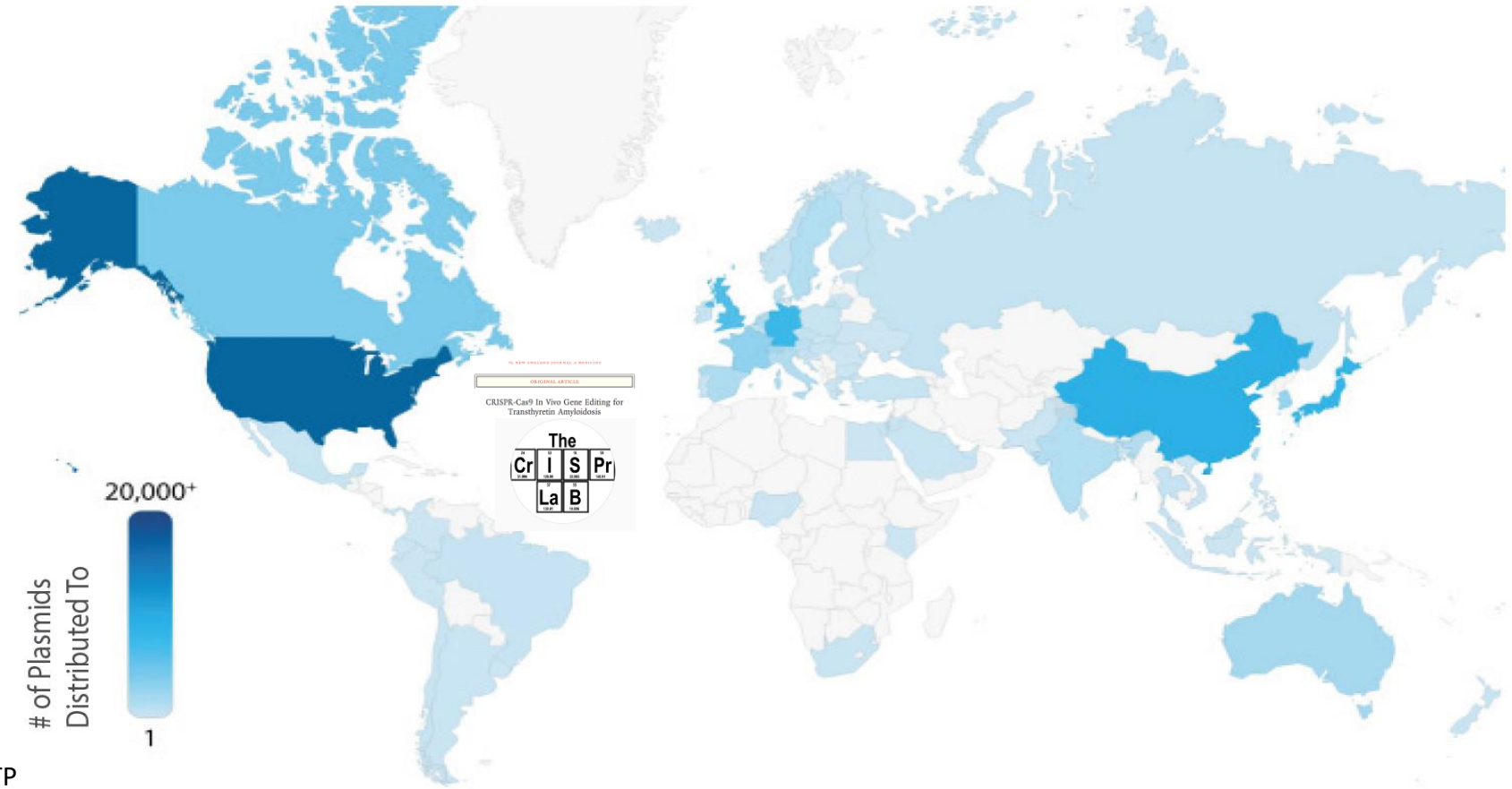
TWELVE.BIO

CRISPR BIOTECH ENGINEERING

METAGENOMI

CRISPR BIOTECH * RTP

CRISPR QC



CASPR BIOTECH

EXCISION

TESSERA

GRAPHITE BIO

scribe THERAPEUTICS

Navega Therapeutics

life edit RTP

EDIGENE

prime medicine

INARI a

pairwise RTP

TREECO * RTP

ELIGO BIOSCIENCE * RTP

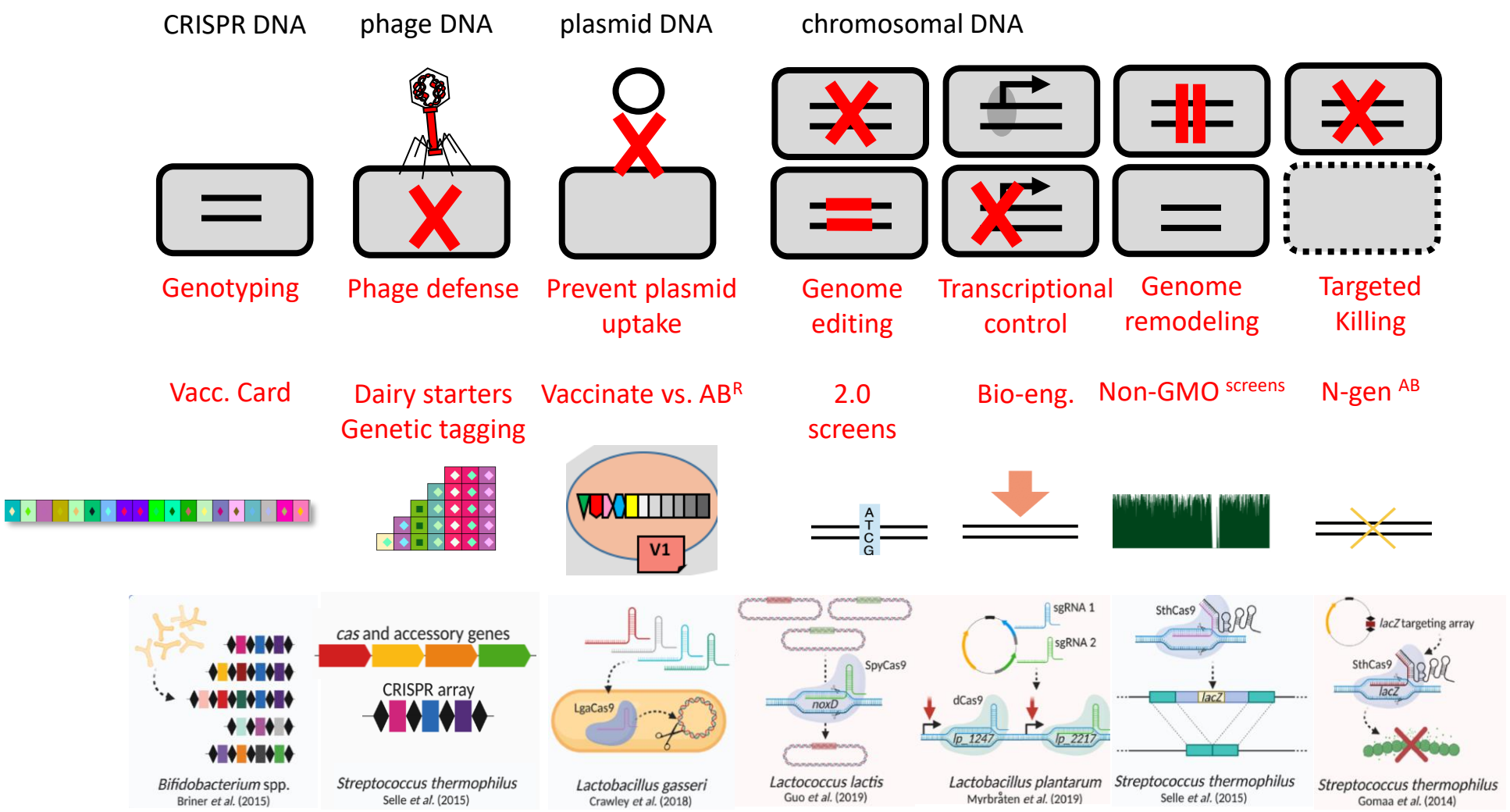
LOCUS BIOSCIENCES

SNIPRBIOME A CRISPR COMPANY

Ancilia BIOSCIENCES RTP *

NEXTBIOTICS

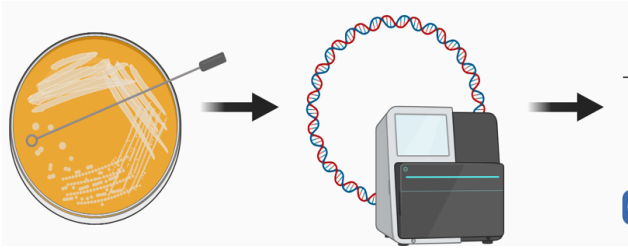
NASDAQ $33B



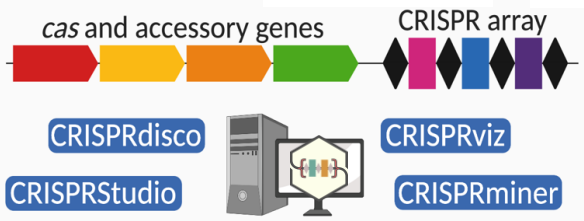
CRISPR microbial applications

Roberts & Barrangou 2020
Barrangou & Horvath 2017
Barrangou & Doudna 2016

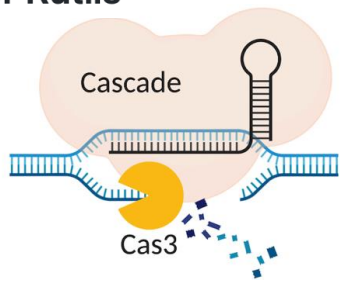
CHARACTERIZE CRISPR-CAS SYSTEMS

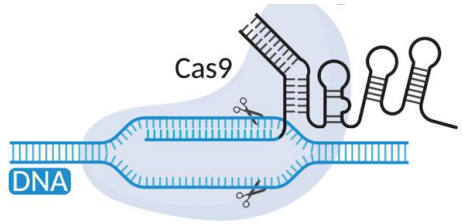


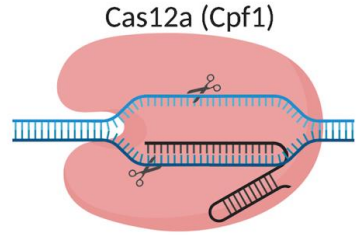
CRISPRdisco PAMFinder <CRISPRviz>
crDatabase CRISPR{classify} CRISPRutils



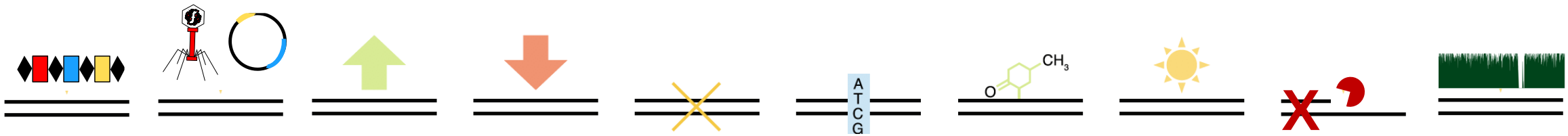
https://github.com/CRISPRlab





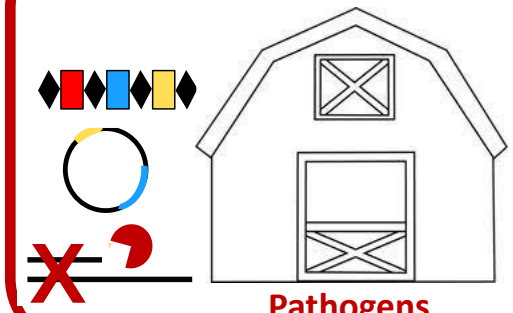


DEVELOP CRISPR-BASED TECHNOLOGIES

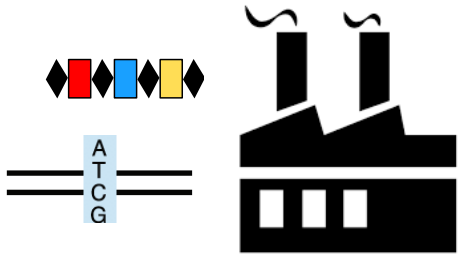


Typing* Immunity* Transcriptional control Genome Editing Epigenetics Imaging Killing* Screening*

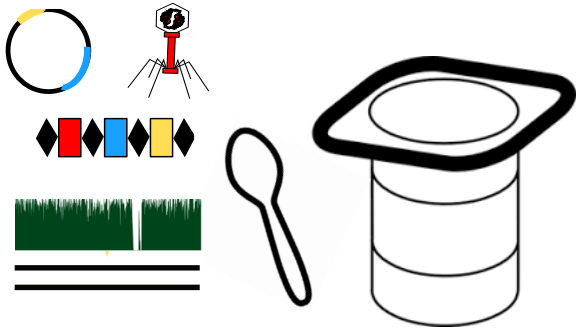
APPLY CRISPR-TECHNOLOGIES IN BACTERIA



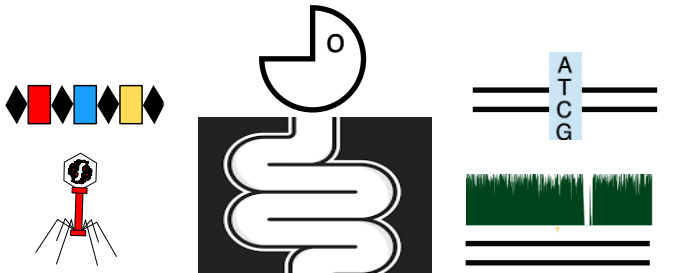
Pathogens



Spoilage & workhorses



Starter cultures*



Probiotics

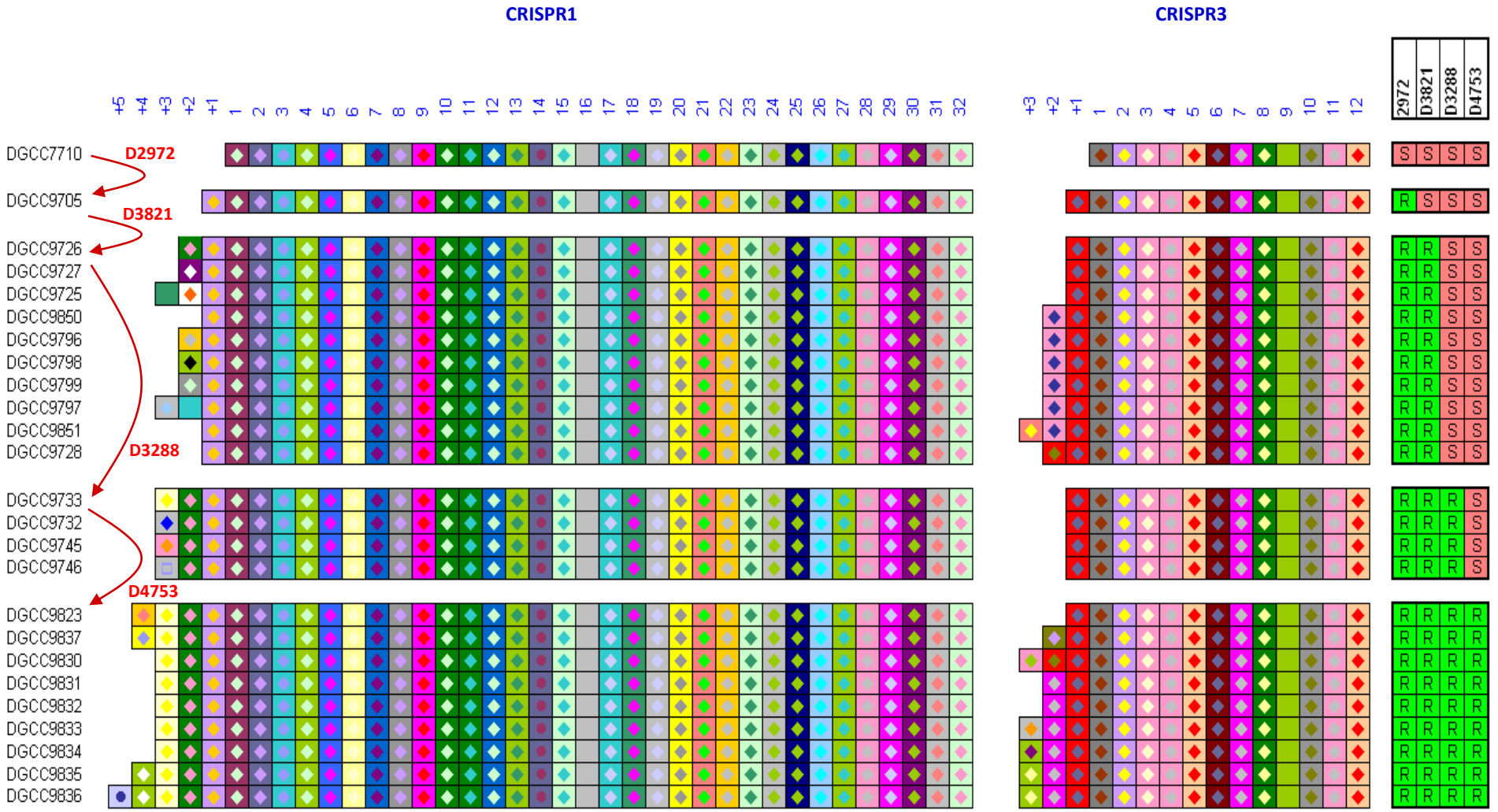
- Probiotics (Lga, Lcr, Lfe, Blo)
- Pathogens (Eco, Sal, Cdi)
- Dairy cultures (Sth)
- Spoilage organisms (Lbu)
- Microbiomes
- Metagenomics

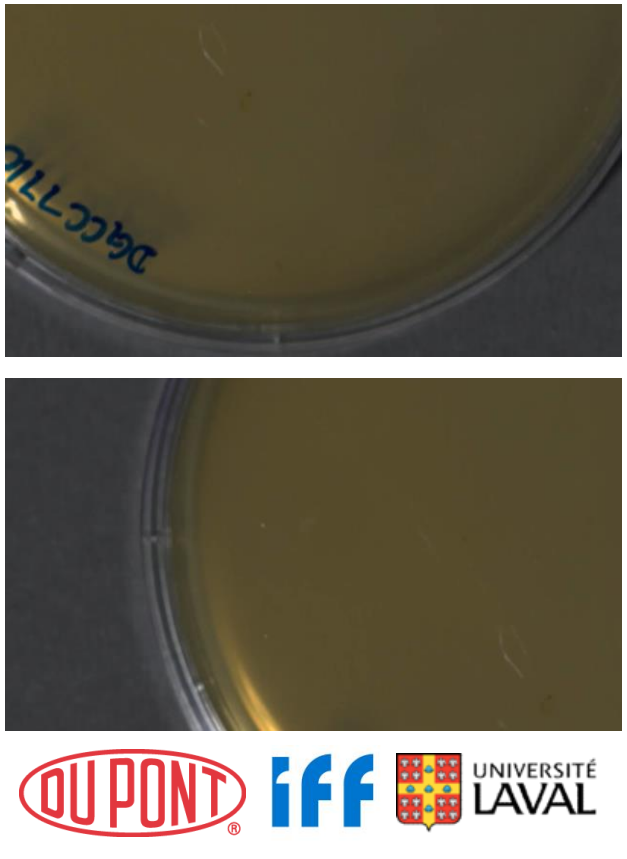
Genotyping

Organism	Type	Reference
Bifidobacterium animalis	Probiotic	Barrangou et al. 2009
Campylobacter jejuni	Pathogen	Kovanen et al. 2014
Clostridium difficile	Pathogen	Hargreaves et al. 2014
Corynebacterium diphtheriae	Pathogen	Mokrousov et al. 2009
Enterococcus faecalis	Pathogen	Lindenstrauss et al. 2011
Enterococcus faecium	Pathogen	Tremblay et al. 2013
Erwinia amylovora	Pathogen	Rezzonico et al. 2011
Escherichia coli	Pathogen	Yin et al. 2013
Lactobacillus buchneri	Spoilage	Briner & Barrangou 2014
Lactobacillus casei	Probiotic	Broadbent et al. 2012
Legionella pneumophila	Pathogen	D'Auria et al. 2010
Microcystis aeruginosa	Pathogen	Kuno et al. 2012
Mycobacterium tuberculosis	Pathogen	Groenen et al. 1993
Propionibacterium acnes	Pathogen	Brüggemann et al. 2012
Salmonella enterica	Pathogen	Shariat et al. 2014
Streptococcus agalactiae	Pathogen	Lier et al. 2015
Streptococcus thermophilus	Starter culture	Horvath et al. 2008
Staphylococcus aureus	Pathogen	Kinnevey et al. 2013
Vibrio parahemolyticus	Pathogen	Sun et al. 2015
Xanthomonas aeruginosa	Pathogen	Semenova et al. 2009
Yersinia pestis	Pathogen	Riehm et al. 2012

Genus, species, and strain name	Accession number(s) for chromosomal sequences	CRISPR/Cas systems	Number of repeats
Arthrobacter arilaitensis RE117	FQ311875	0	
Bifidobacterium adolescentis ATCC 15703	AP009256	1	86+3
Bifidobacterium animalis subsp. *lactis* AD011 (+ BB-12, CNCM I-2494, DSM 10140, V9)	CP001213 (+ CP001853, CP002915, CP001606, CP001892)	1	20+2
Bifidobacterium animalis subsp. *lactis* BI-04	CP001515	1	23+2
Bifidobacterium bifidum PRL2010	CP001840	0	
Bifidobacterium bifidum S17	CP002220	1	45
Bifidobacterium breve ACS-071-V-Sch8b	CP002743	1	16+12+37+2
Bifidobacterium breve UCC2003	CP000303	1	5+43+2
Bifidobacterium dentium Bd1	CP001750	2	18 81
Bifidobacterium longum DJO10A	CP000605	1	43
Bifidobacterium longum NCC2705	AE014295	0	
Bifidobacterium longum subsp. *infantis* 157F (+ ATCC 15697, JCM 1222)	AP010890 (+ CP001095, AP010889)	0	
Bifidobacterium longum subsp. *longum* BBMN68	CP002286	1	10+13+10
Bifidobacterium longum subsp. *longum* JCM 1217 (+ JDM301)	AP010888 (+ CP002010)	0	
Bifidobacterium longum subsp. *longum* KACC 91563	CP002794	1	33
Carnobacterium sp. 17-4	CP002563	0	
Lactobacillus acidophilus 30SC	CP002559	0	
Lactobacillus acidophilus NCFM	CP000033	1*	34
Lactobacillus amylovorus GRL 1112 (+ GRL 1118)	CP002338 (+ CP002609)	0	
Lactobacillus brevis ATCC 367	CP000416	1*	6+5+1
Lactobacillus sakei subsp. *sakei* 23K	CR936503	0	
Lactobacillus salivarius CECT 5713 (+ UCC118)	CP002034 (+CP000233)	1	30
Lactococcus lactis subsp. *cremoris* MG1363 (+ NZ9000, SK11)	AM406671 (+ CP002094, CP000425)	0	
Lactococcus lactis subsp. *lactis* CV56 (+ IL1403, KF147)	CP002365 (+ AE005176, CP001834)	0	
Leuconostoc citreum KM20	DQ489736	0	
Leuconostoc gasicomitatum LMG 18811	FN822744	0	
Leuconostoc kimchii IMSNU11154	CP001758	0	
Leuconostoc mesenteroides subsp. *mesenteroides* ATCC 8293	CP000414	0	
Oenococcus oeni PSU-1	CP000411	0	
Pediococcus pentosaceus ATCC 25745	CP000422	0	
Propionibacterium freudenreichii subsp. *shermanii* CRM-BIA1	FN806773	1	34
Staphylococcus carnosus subsp. *carnosus* TM300	AM295250	0	
Streptococcus thermophilus CNRZ1066	CP000024	1 + 1*	42
Streptococcus thermophilus JIM 8232	FR875178	2 + 1*	43 18 3
Streptococcus thermophilus LMD-9	CP000419	3	17 4 8
Streptococcus thermophilus LMG18311	CP000023	2	34 5
Streptococcus thermophilus ND03	CP002340	3	37 2 21
Lactobacillus buchneri NRRL B-30929	CP002652	1	26
Lactobacillus casei ATCC 334	CP000423	1	22
Lactobacillus casei BD-II (+ BL23, LC2W)	CP002618 (+ FM177140, CP002616)	1	22
Lactobacillus casei Zhang	CP001084	1	17
Lactobacillus crispatus ST1	FN692037	1	17+16+8
Lactobacillus delbrueckii subsp. *bulgaricus* 2038	CP000156	1	20
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 11842	CR954253	1	41
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC BAA-365	CP000412	1	21
Lactobacillus delbrueckii subsp. *bulgaricus* ND02	CP002341	1	66
Lactobacillus fermentum CECT 5716	CP002033	1	20+24
Lactobacillus fermentum IFO3956	AP008937	2	21+24 4
Lactobacillus gasseri ATCC 33323	CP000413	0	
Lactobacillus helveticus DPC 4571	CP000517	1 + 1*	23 2
Lactobacillus helveticus H10	CP002429	1	42
Lactobacillus johnsonii FI9785 (+ NCC 533)	FN298497 (+ AE017198)	0	
Lactobacillus kefiranofaciens ZW3	CP002764	1	5+4
Lactobacillus plantarum JDM1 (+ ST-III, WCFS1)	CP001617 (+ CP002222, AL935263)	0	
Lactobacillus reuteri DSM 20016 (+ JCM 1112, SD2112)	CP000705 (+ AP007281, CP002844)	0	
Lactobacillus rhamnosus ATCC 53103 (+ GG)	AP011548 (+ FM179322)	1	25
Lactobacillus rhamnosus Lc 705	FM179323	0	

Barrangou & Dudley 2016
Barrangou & Horvath 2012

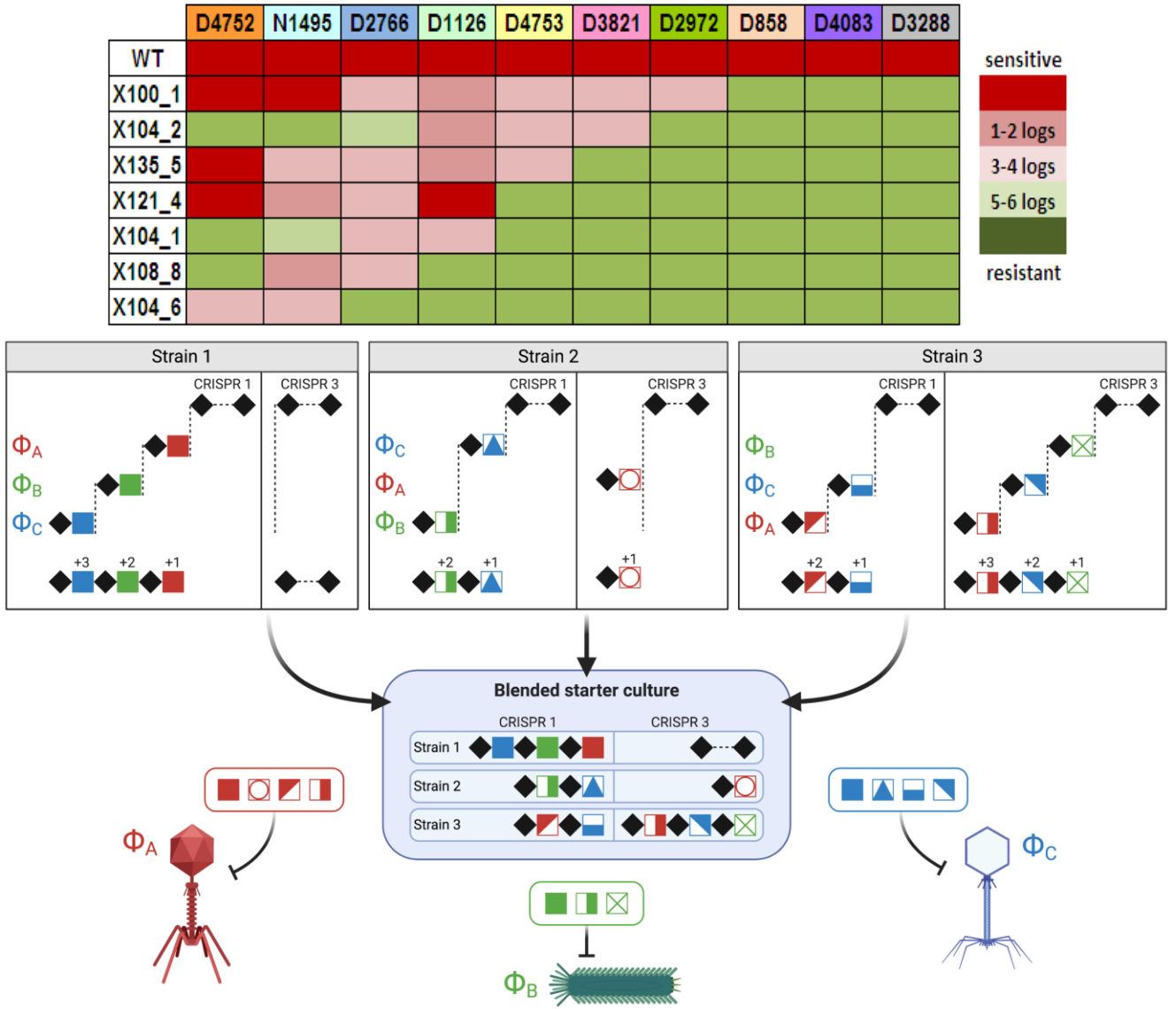




Σ +8 spacers +S5C1 +S3C3

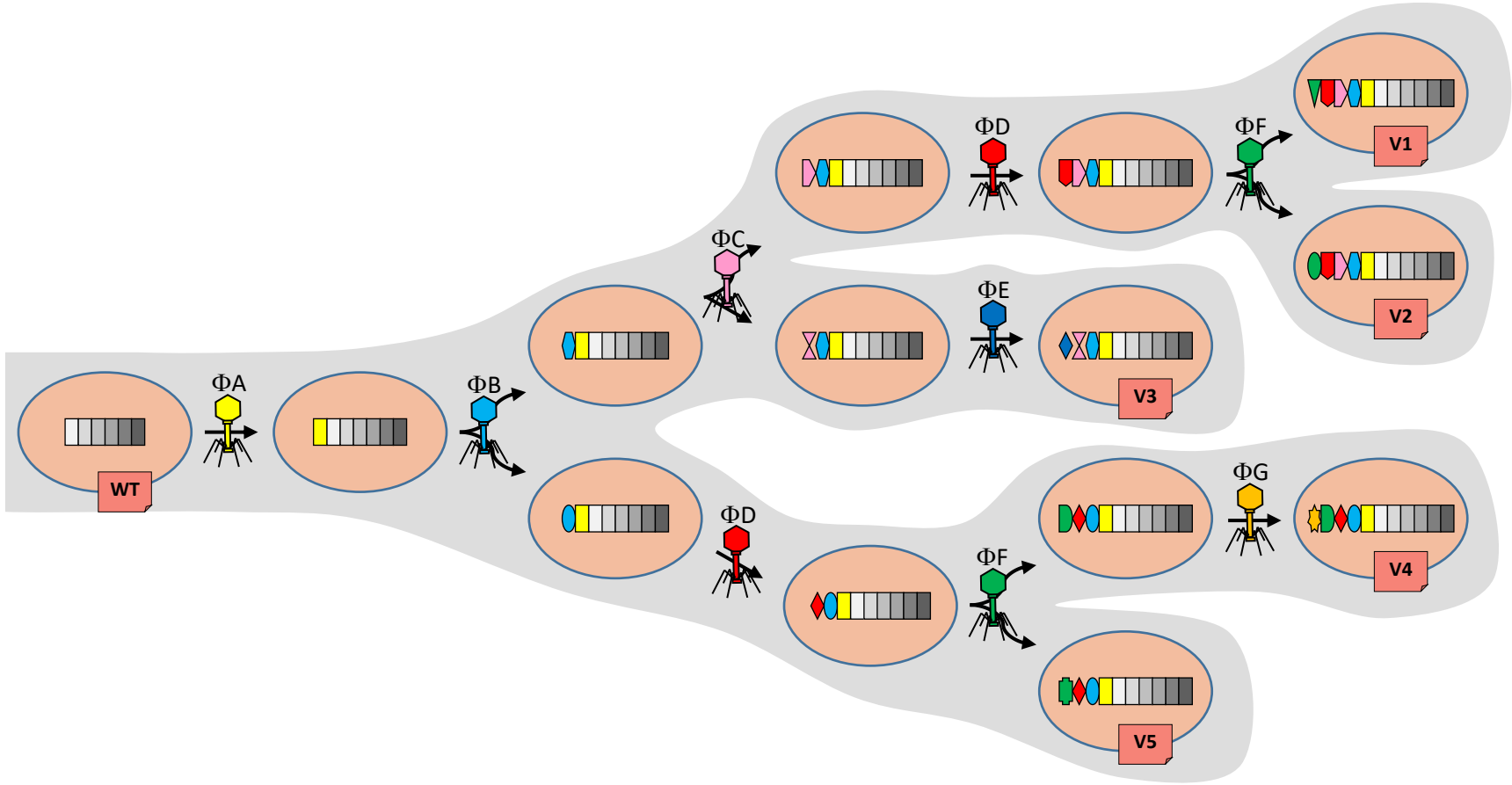
Natural vaccination of dairy starter cultures

Barrangou *et al.* 2013!



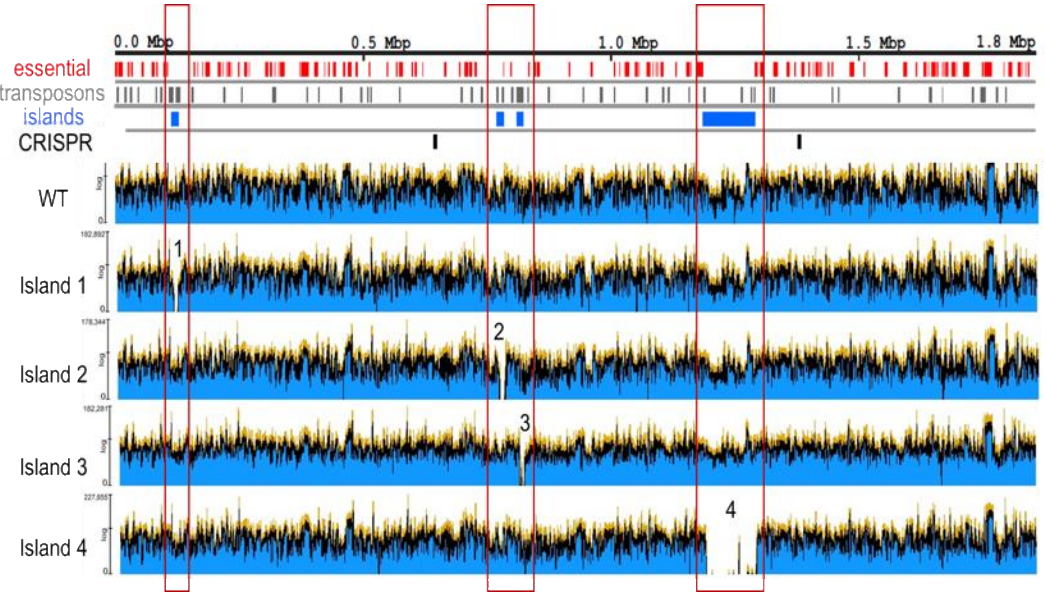
CRISPR phage resistance in blends

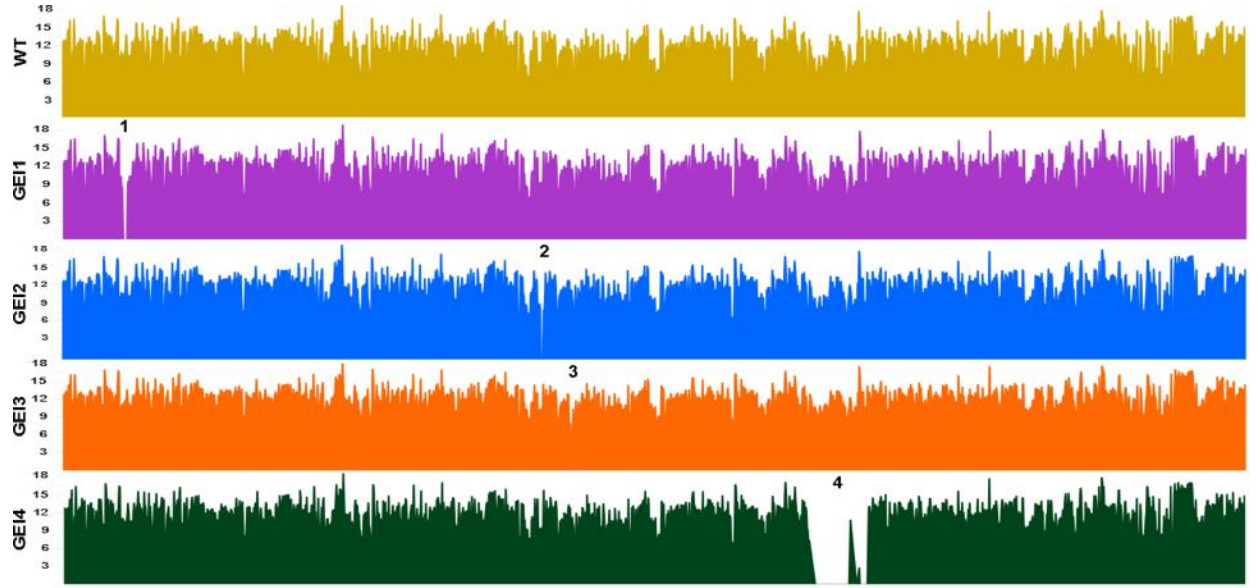
Roberts and Barrangou 2020



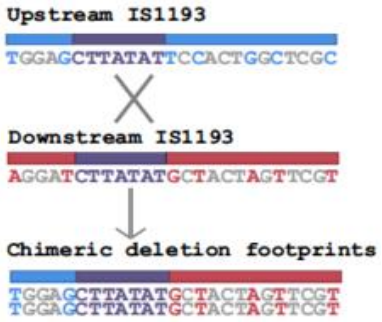
Natural genetic tagging

Barrangou *et al.* 2011

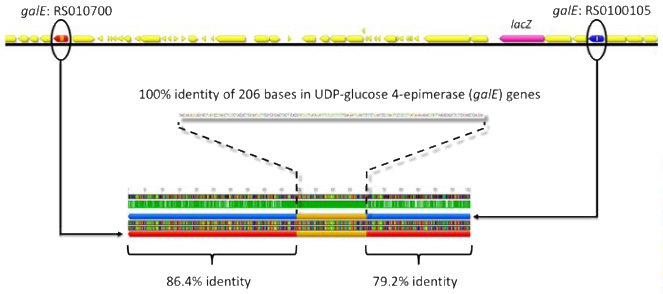


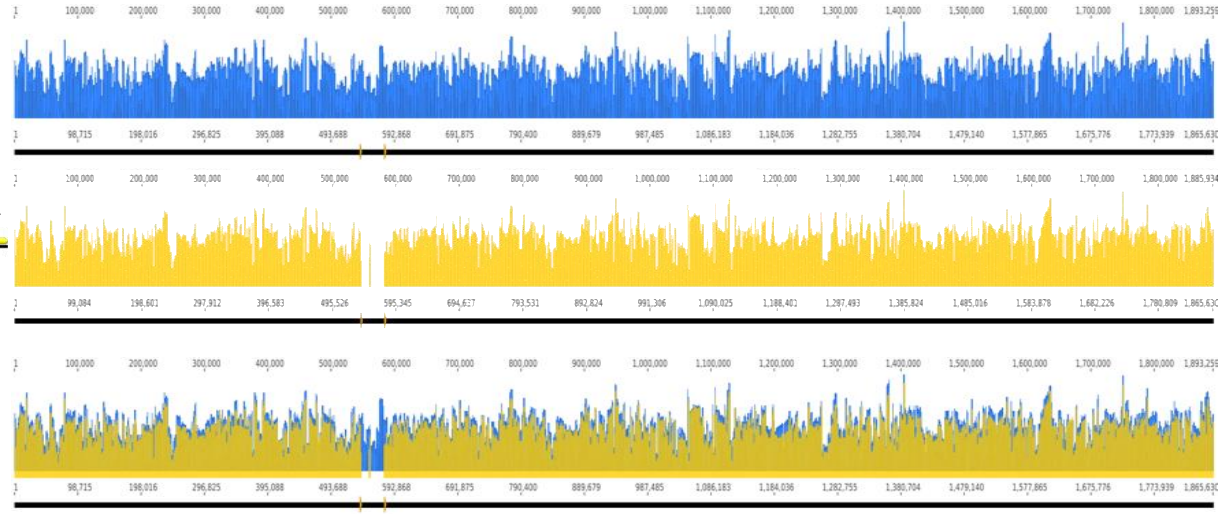


8-102 kb
7%
IS*1167*-driven
Pre-existing



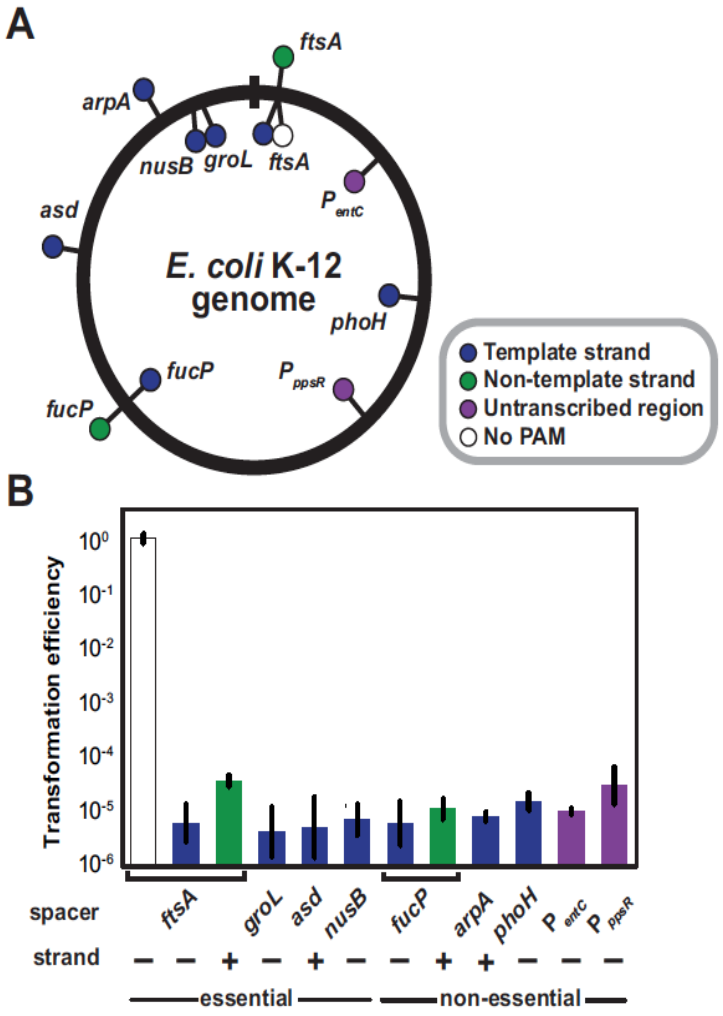
34 kb
Lactose-
Tn-independent



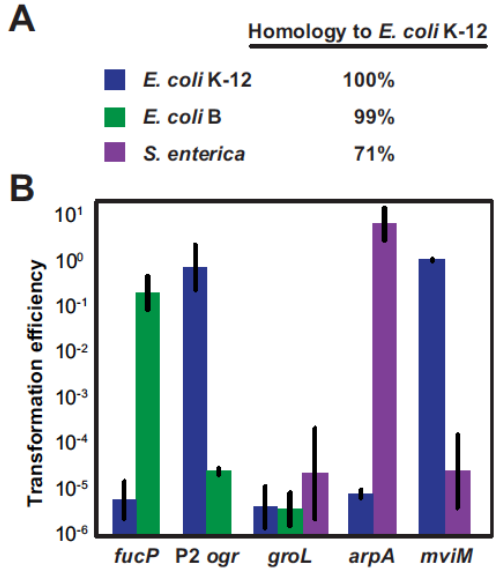


Natural screening

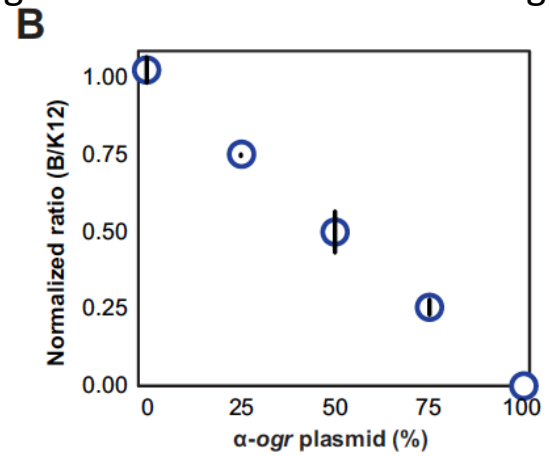
Selle *et al.* 2015
Canez *et al.* 2019



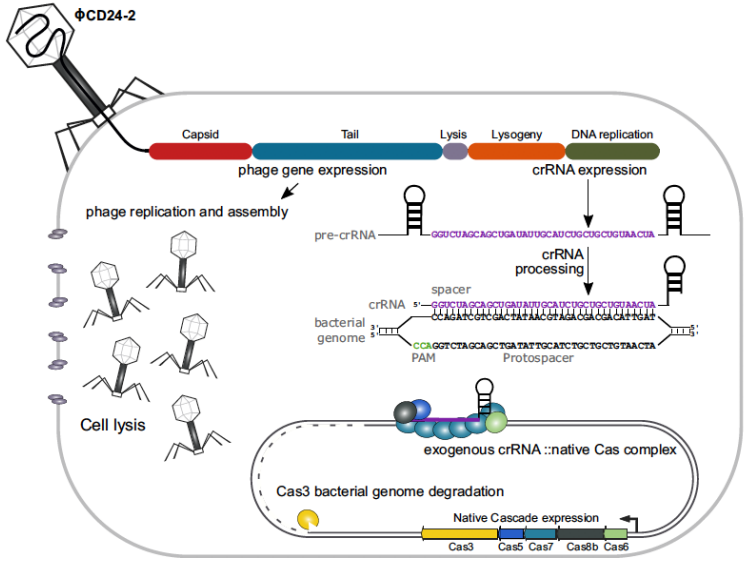
Lethal self targeting



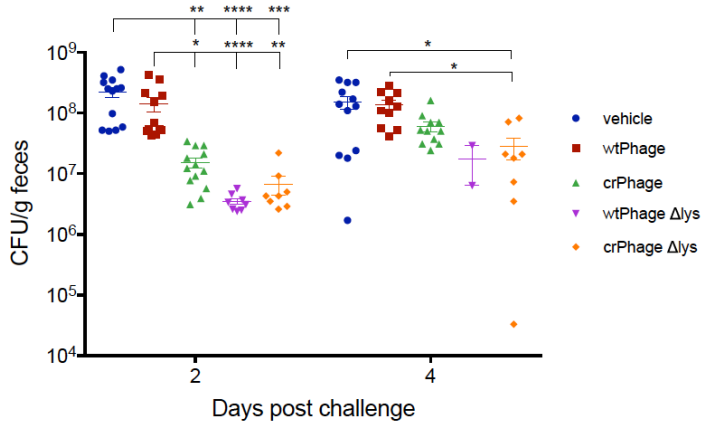
Programmable and selective killing



Dose-dependent community alteration



Engineering *C. diff* phages for self targeting



Lowering *C. diff* infection

N-gen antimicrobials

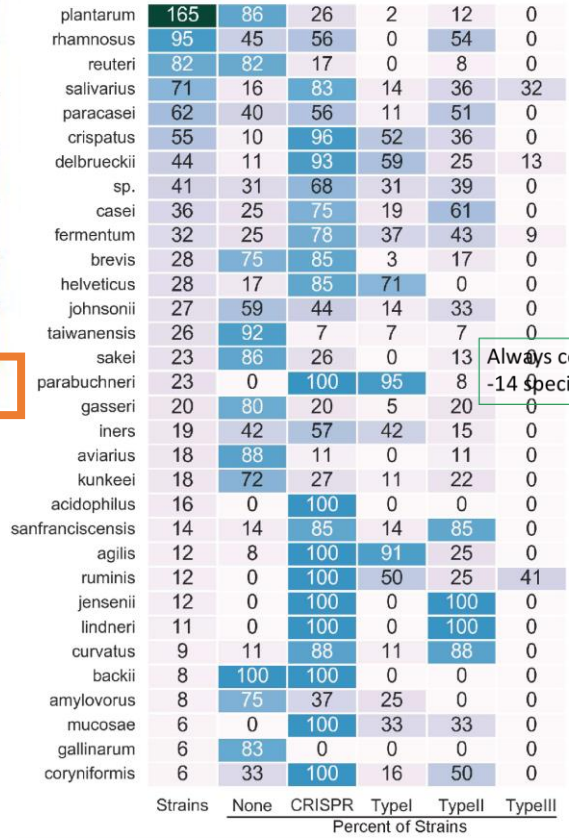
Gomaa *et al.* 2014
Selle *et al.* 2020

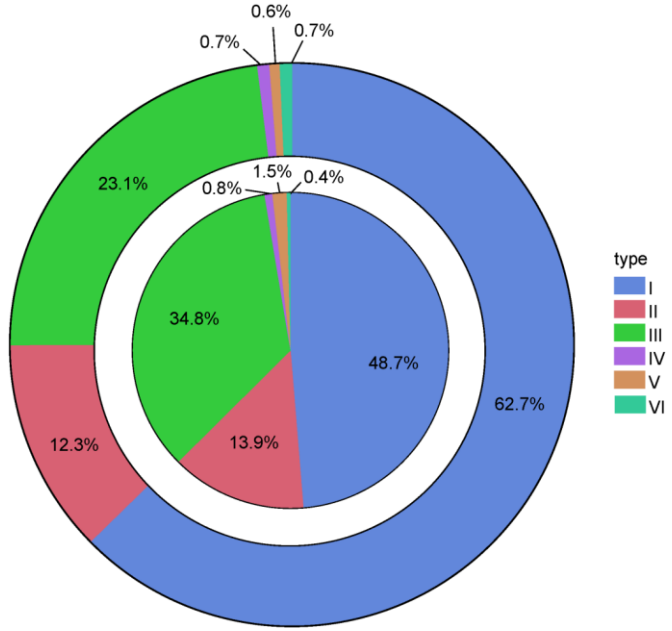
Total Counts by Taxonomic Class

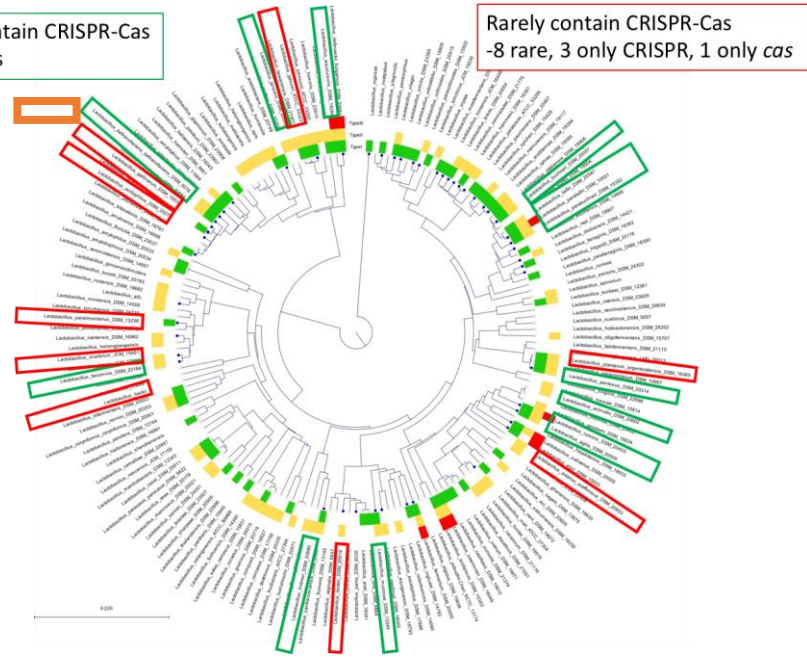
	Total Genomes	Complete Systems						Does Not Contain CRISPR	Contains CRISPR features
		Type I	Type II	Type III	Type IV	Type V	Type VI		
Archaea	144	46		32		1		18	121
Thermoprotei	40	21		13				2	38
Halobacteria	26	3						13	11
Methanomicrobia	22	6		4					17
Thermococci	16	6		6					16
Methanococci	15	7		4				1	14
Methanobacteria	10	1		1					10
Bacteria	2,450	346	116	86	4	4	6	899	1,391
Gammaproteobacteria	536	105	4	5		1		123	337
Bacilli	416	32	47	9			1	161	218

Technical needs

- PAM diversity (precision)
- Orthogonality (multiplexing)
- Efficiency and specificity?
- Toxicity and safe origin?

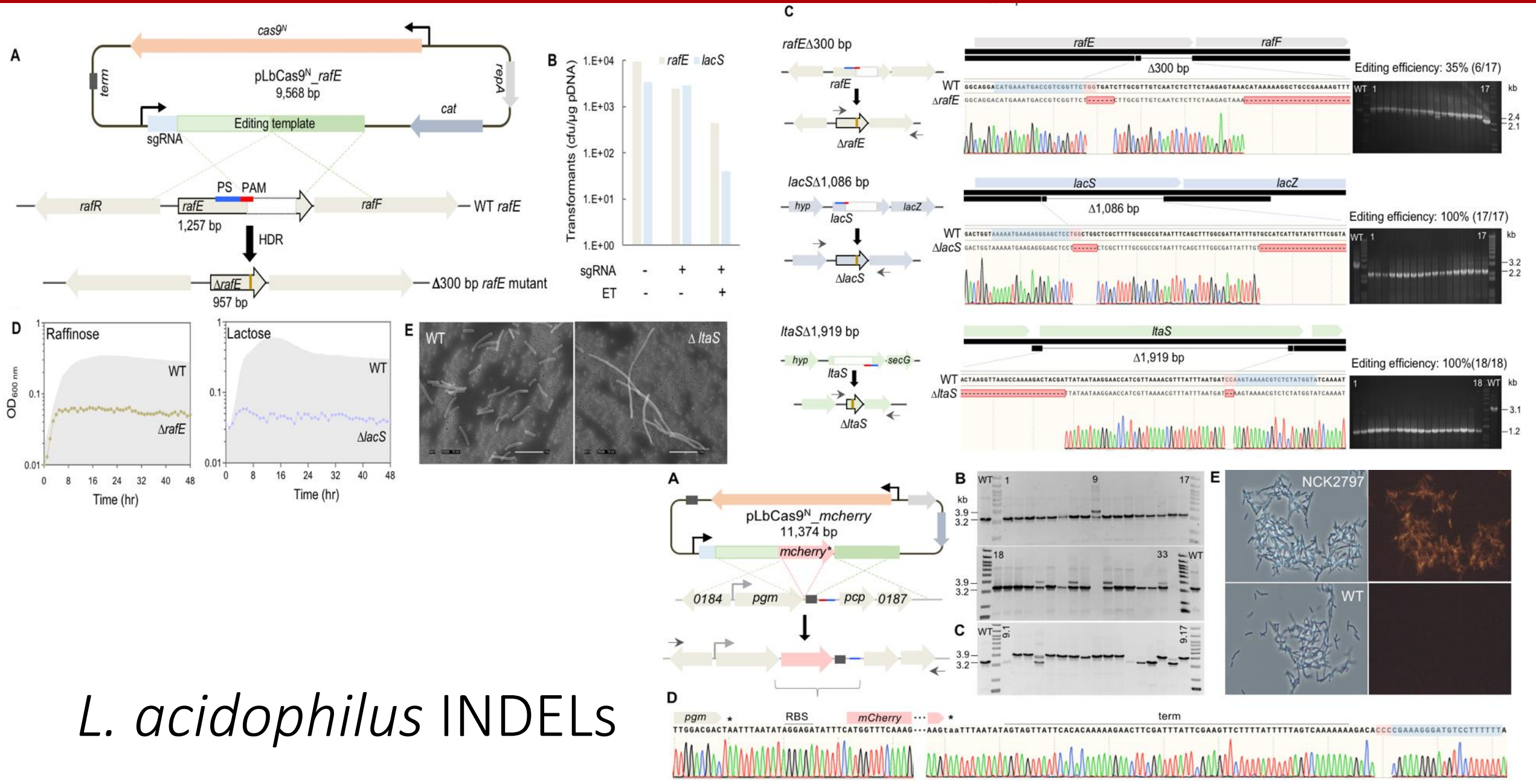






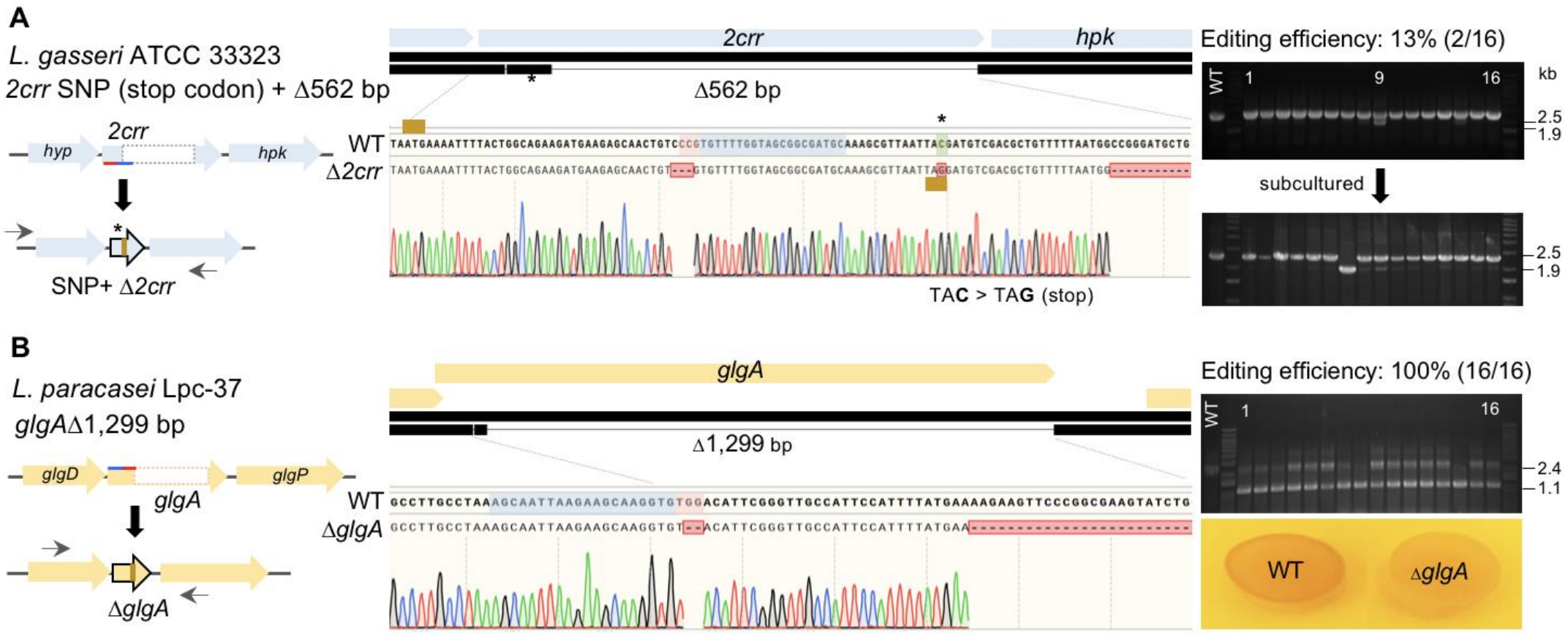
CRISPRdisco enrichment@Lb

Crawley *et al.* CRISPRj 2018



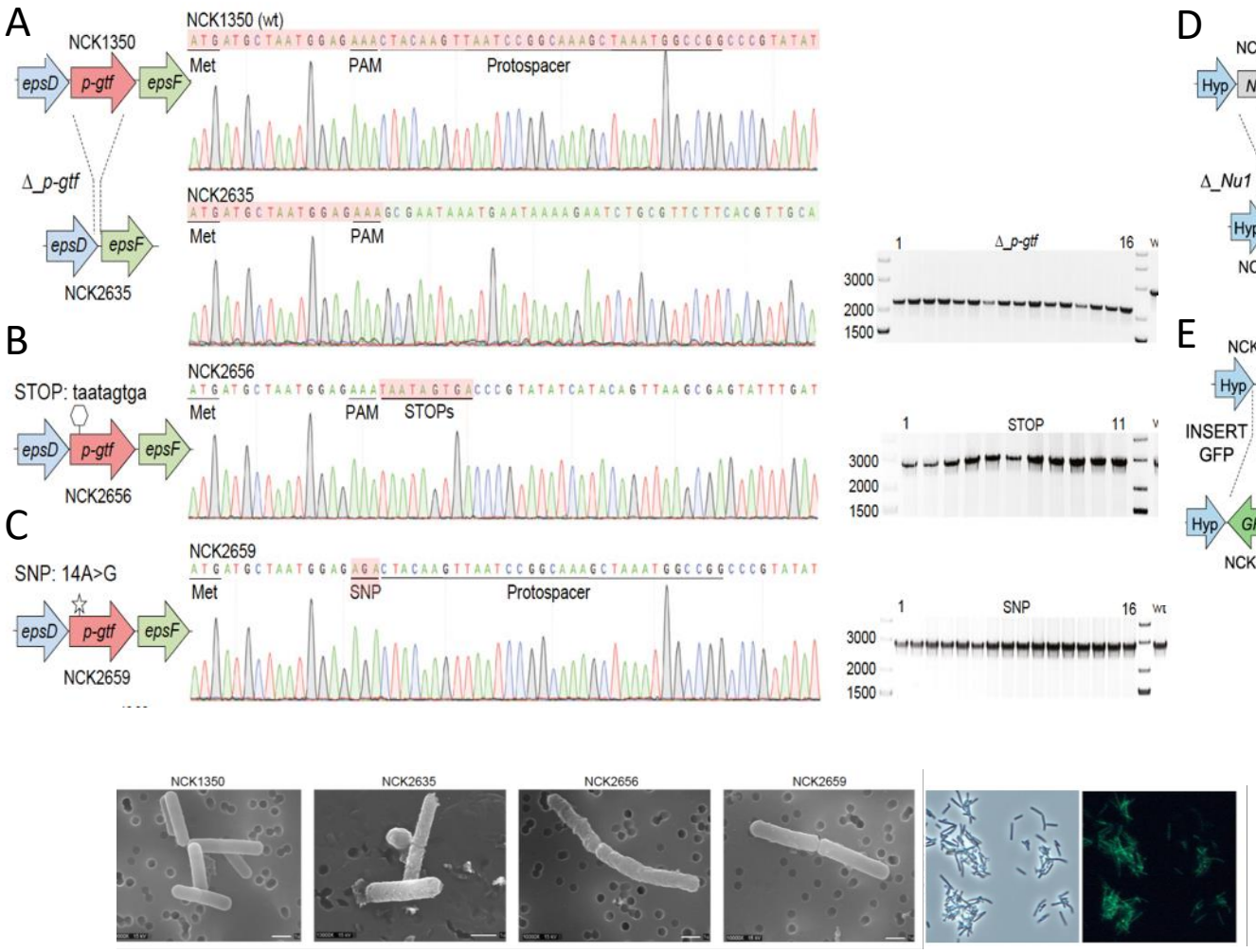
L. acidophilus INDELs

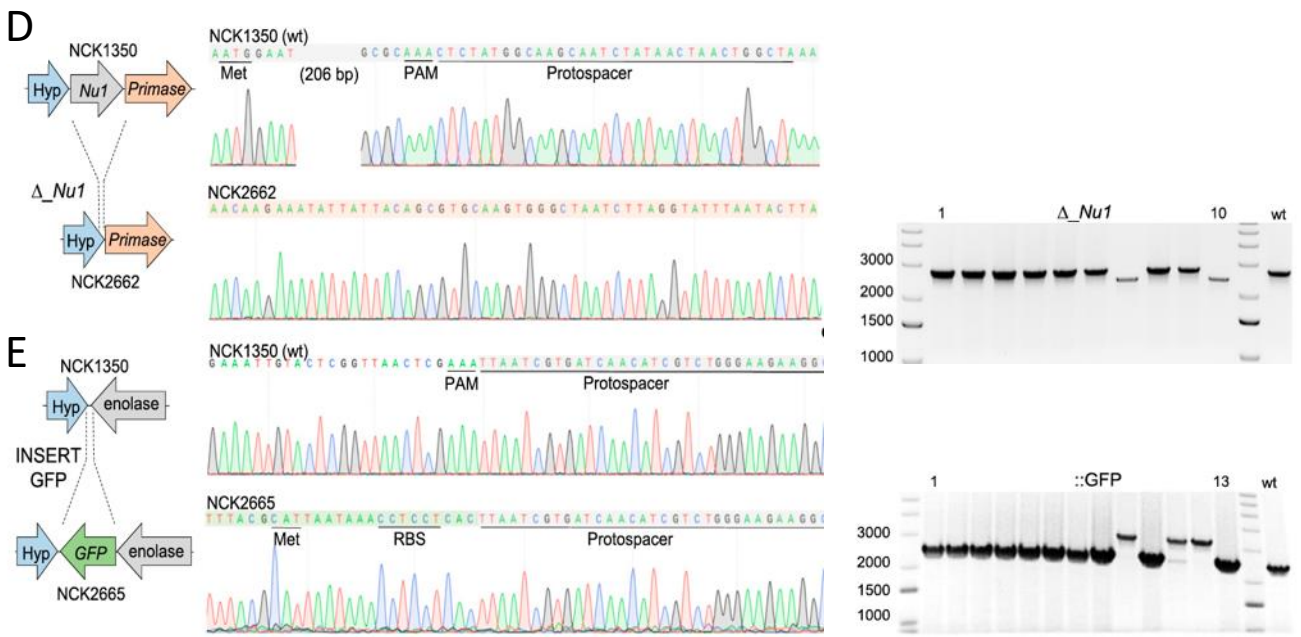
Goh & Barrangou 2021



Lactobacillus flexible genome editing

Goh & Barrangou 2021

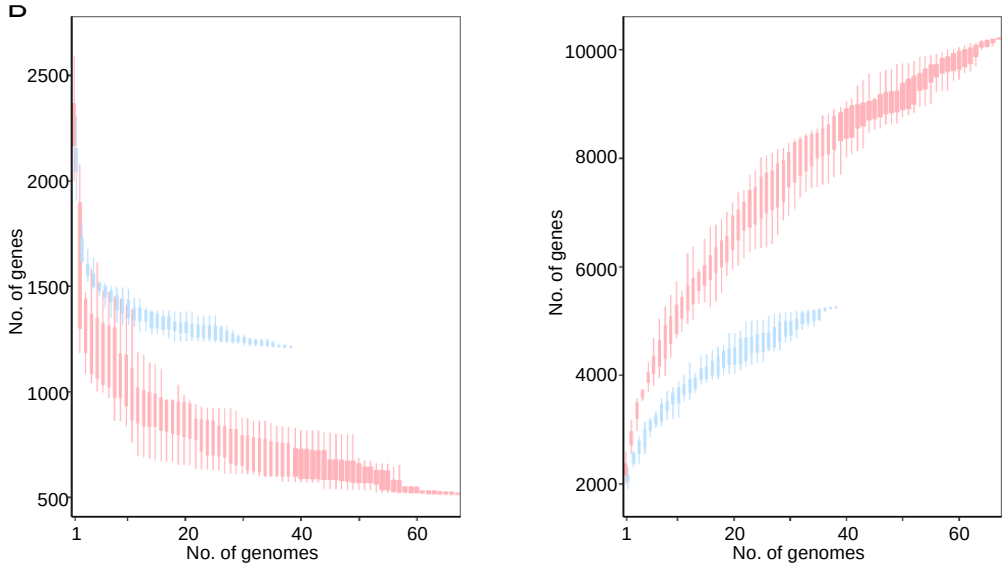


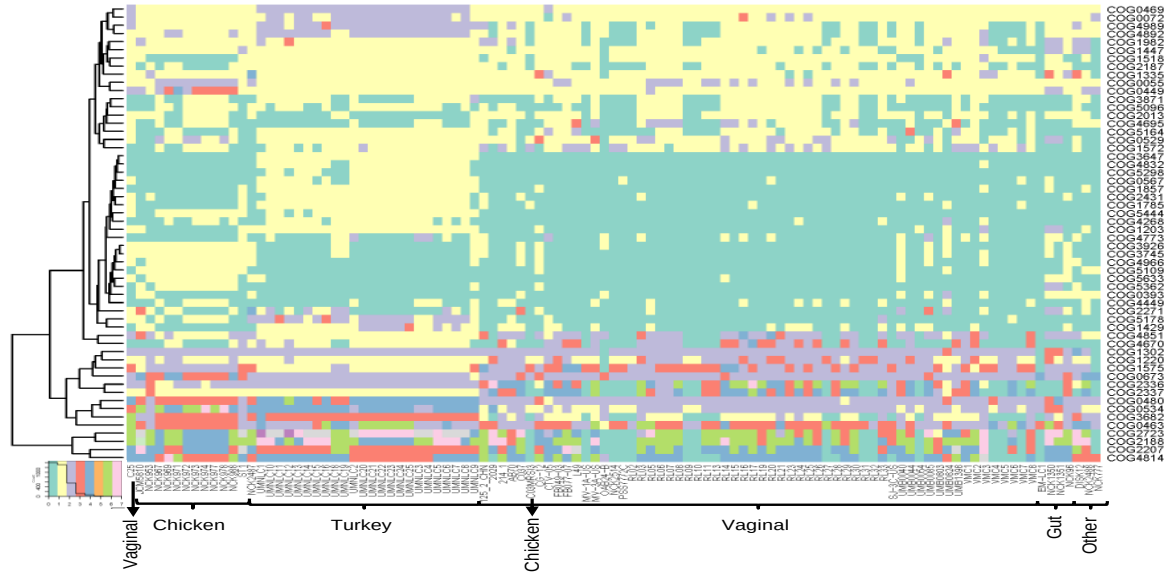


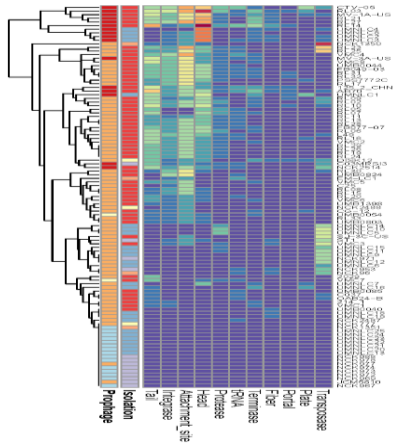
A	B	C	D	E
Δ*pgtf*	STOP*pgtf*	SNP*pgtf*	Δ*Nu1*	INS_GFP
100%	100%	100%	19%	30%
	Engineer EPS		Remove prophage	Add fluorescence

L. crispatus endoI-E genome editing

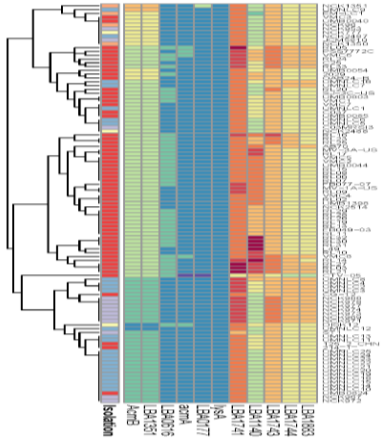
Hidalgo-Cantabrana *et al.* 2019



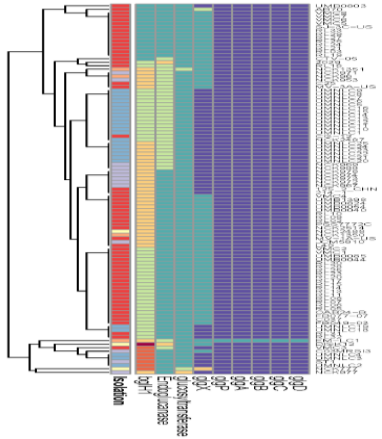




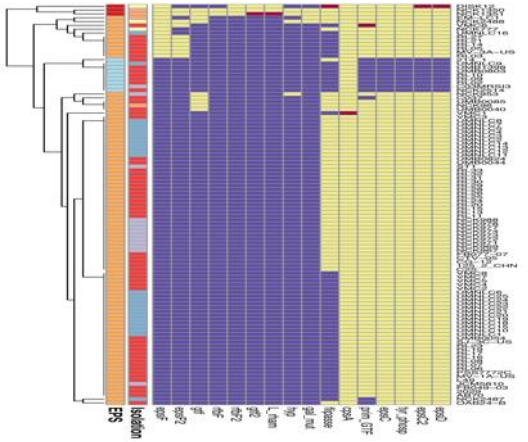
Prophage



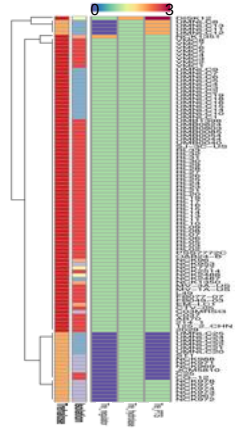
Autolysins



Glycogen cluster



EPS cluster

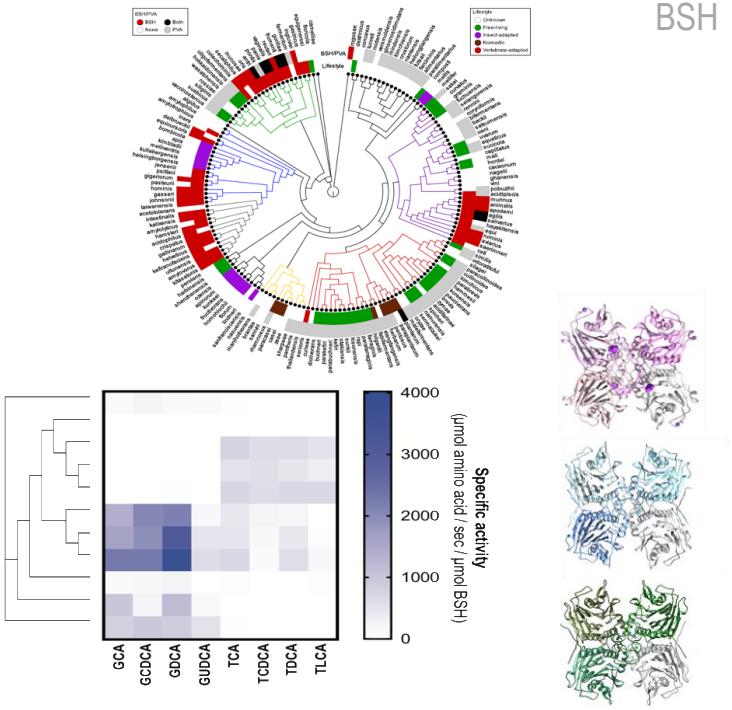


Trehalose cluster

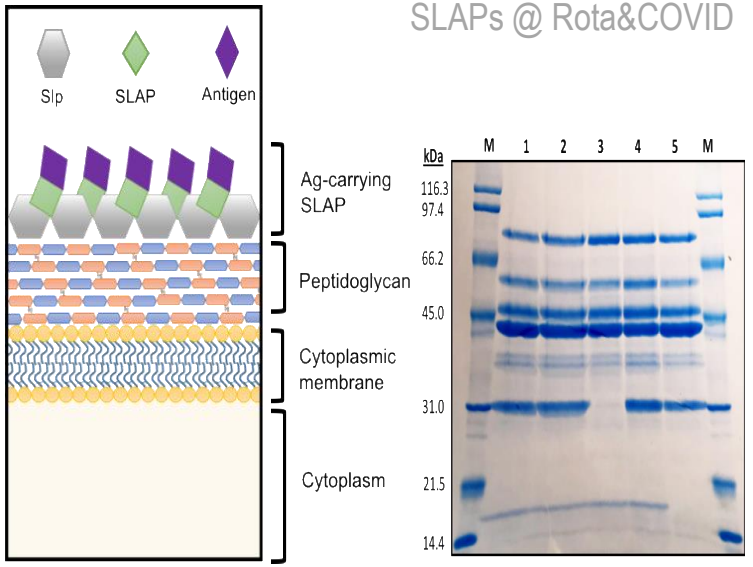
Lactobacillus crispatus features

Pan *et al.* 2020

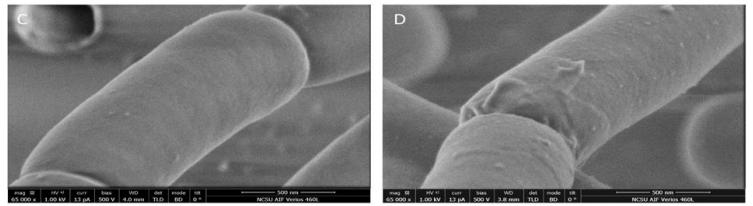
BSH



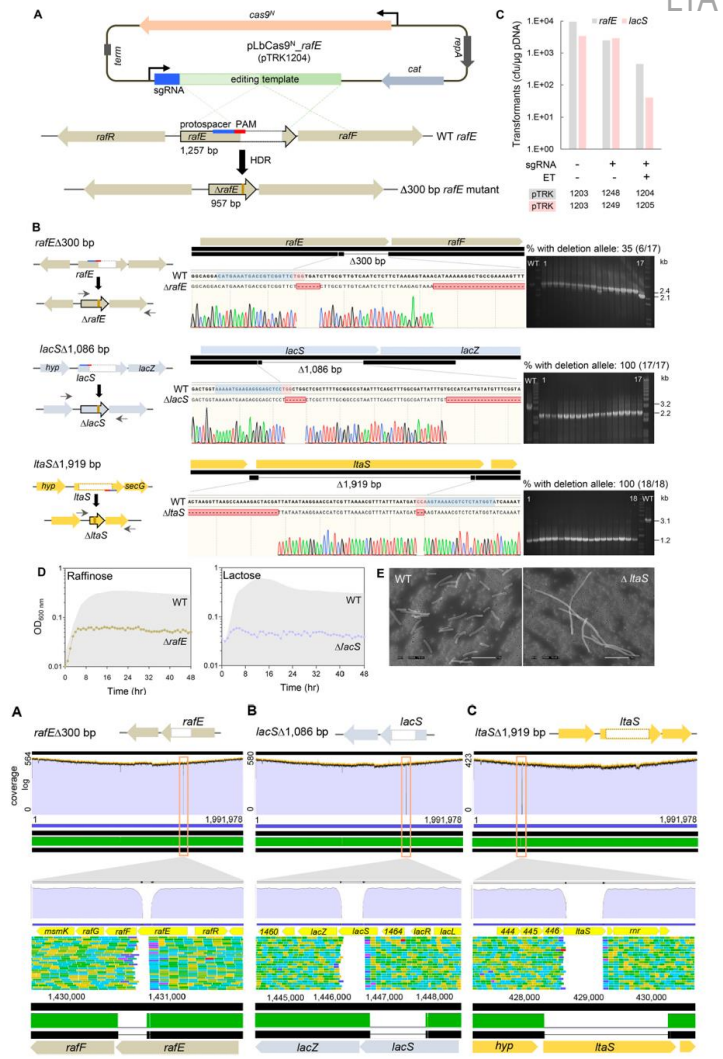
SLAPs @ Rota&COVID



Target	Species	Method	Reference
Colitis	*Lactobacillus gasseri*	SOD	Carroll et al Am J Physiol Gastrointest 2007
Anthrax	*Lactobacillus acidophilus*	Antigen	Mohamadzadeh et al. PNAS 2009
Anthrax	*Lactobacillus gasseri*	Antigen	Mohamadzadeh et al. Future Microbiol 2010
Colitis	*Lactobacillus acidophilus*	LTA deletion	Mohamadzadeh et al. PNAS 2011
Cancer polyps	*Lactobacillus acidophilus*	LTA deletion	Khazaie et al. PNAS 2012
Anthelmintic	*Lactococcus lactis*	Cry5B BT	Durmaz et al AEM 2015
HIV	*Lactobacillus acidophilus*	HIV epitope	Kajikawa et al PLoS One 2015
Botulism	*Lactobacillus acidophilus*	Antigen	O'Flaherty and Klaenhammer AEM 2016
Anthrax	*Lactobacillus acidophilus*	Antigen	O'Flaherty and Klaenhammer AEM 2016

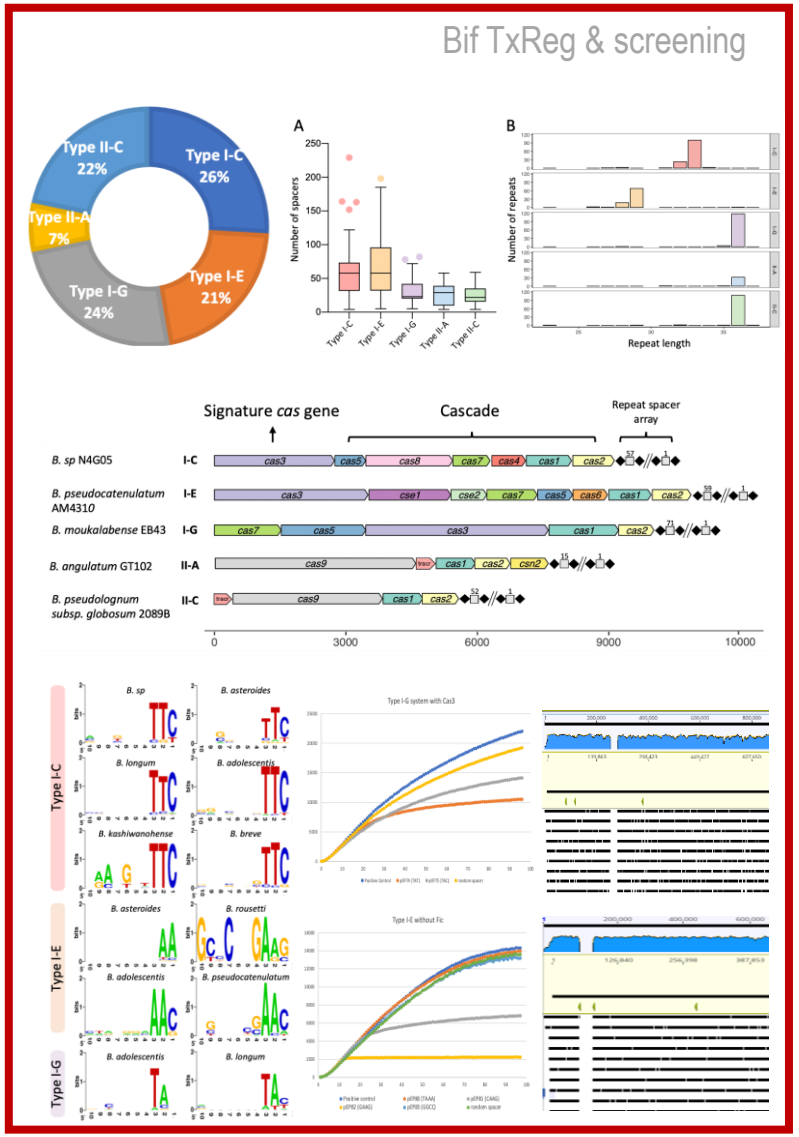


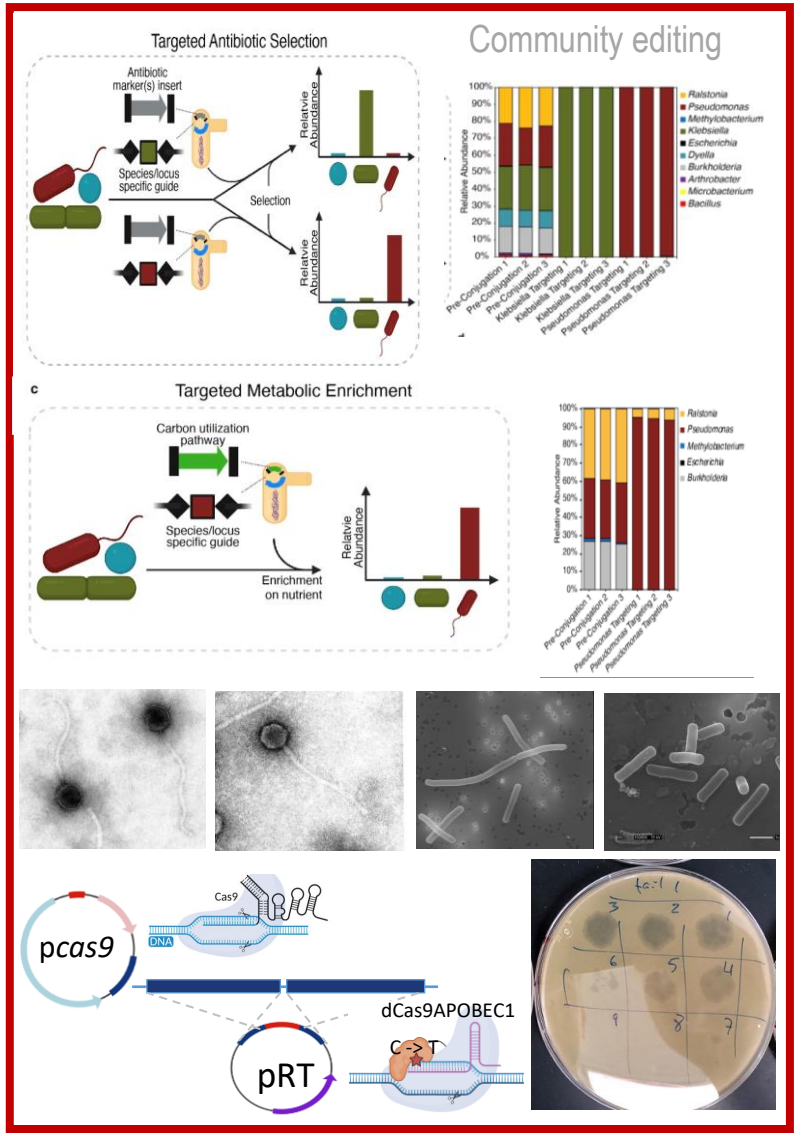
LTA

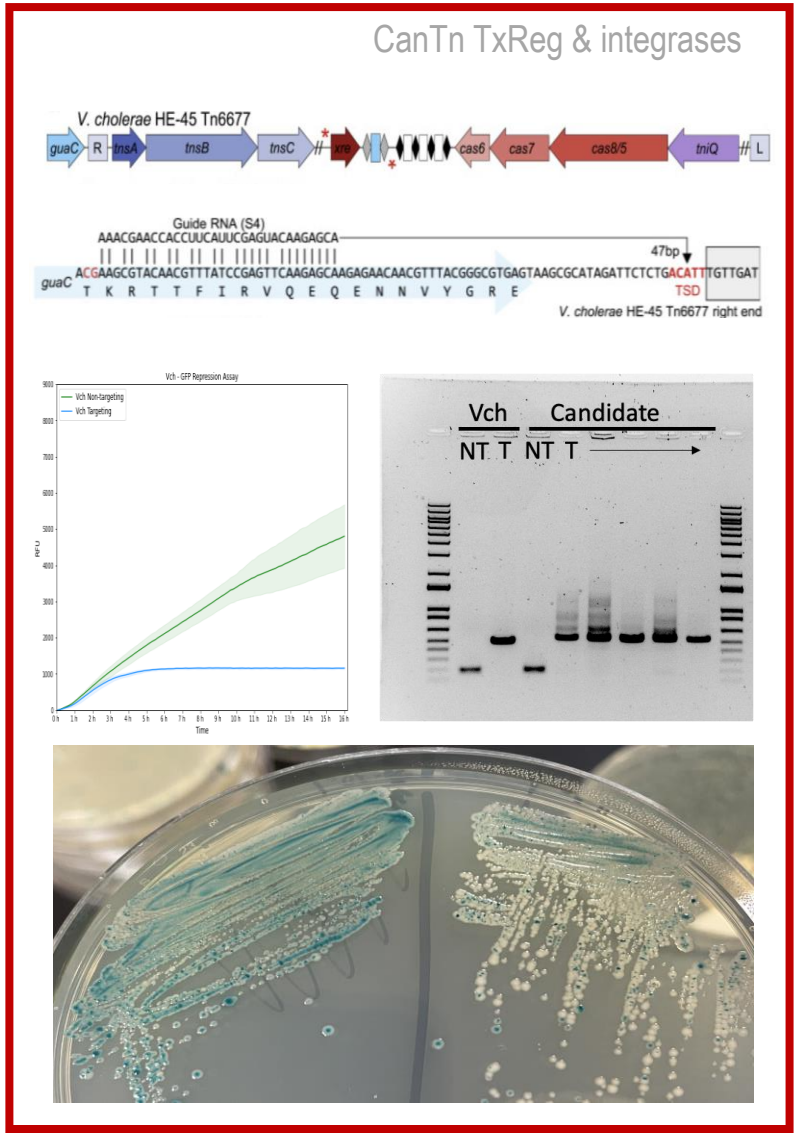


N-gen probiotic engineering

Klotz *et al.* 2020
Goh *et al.* 2021
Foley *et al.* 2021





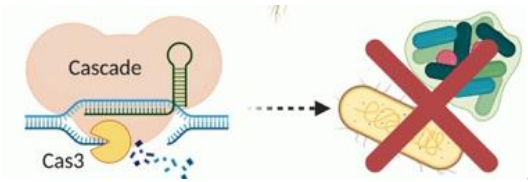


N-gen probiotic engineering

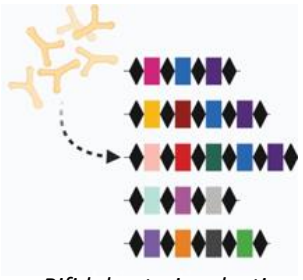
Pan *et al. in prep*

Nethery *et al. in prep*

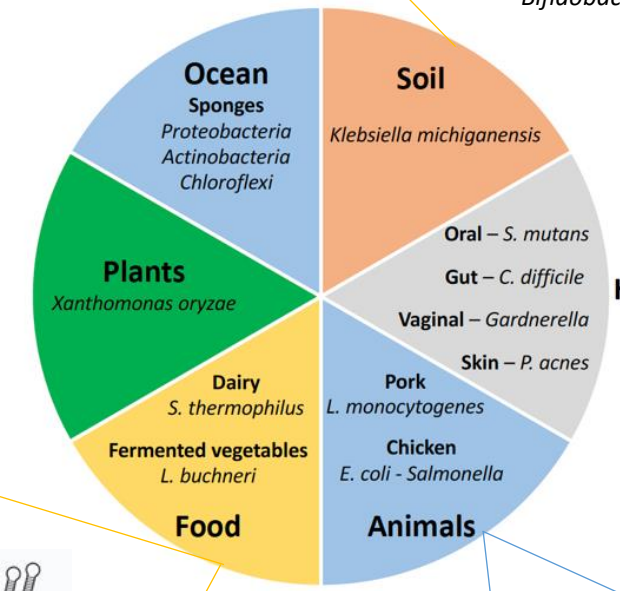
Roberts *et al. in prep*

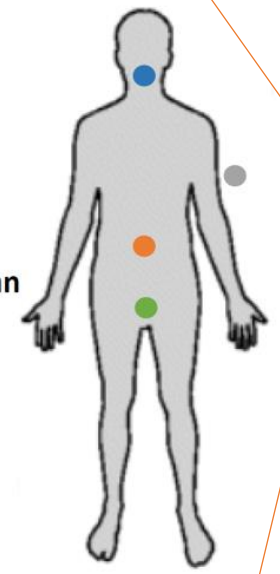


E. coli *Klebsiella michiganensis*

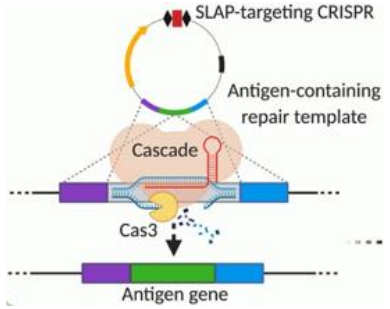


Bifidobacterium lactis



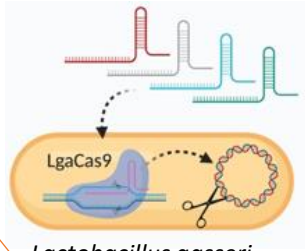


Oral
- *Streptococcaceae*
- *Rothia*
- *Neisseria*
- *Candida*
- *Veillonella*
- *Actinomyces*

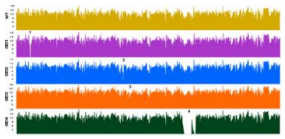


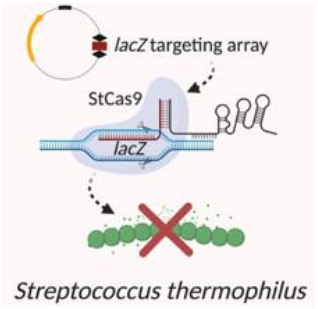
Lactobacillus acidophilus

Gut
- *Bacteroidaceae*
- *Clostridiaceae*
- *Prevotellaceae*
- *Eubacteriaceae*
- *Ruminococcaceae*
- *Bifidobacteriaceae*
- *Lactobacillaceae*
- *Enterobacteriaceae*
- *Saccharomycetaceae*
- *Methanobacteriaceae*

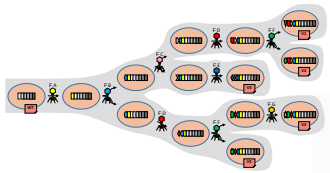


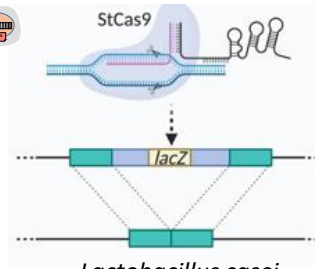
Lactobacillus gasseri





Streptococcus thermophilus



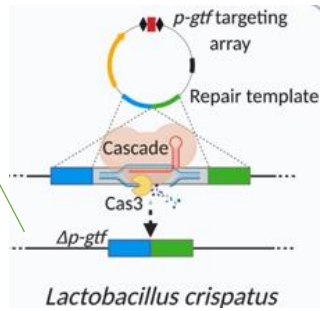


Lactobacillus casei

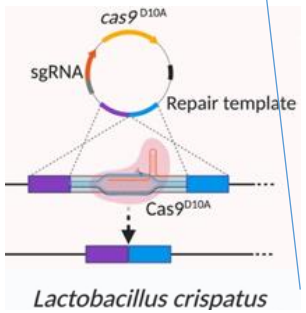
Lactobacillus crispatus

Vaginal
- *Lactobacillus*
- *Gardnerella*
- *Mycoplasma*

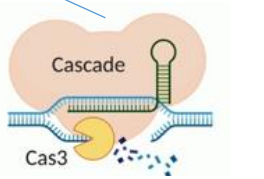
Skin
- *Propionibacterium*
- *Staphylococcus*
- *Corynebacterium*
- *Moraxella*
- *Malassezia*



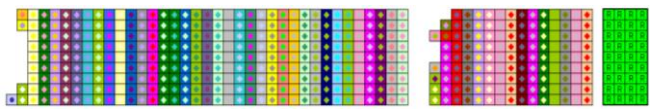
Lactobacillus crispatus



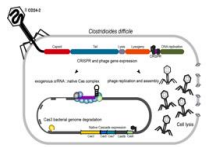
Lactobacillus crispatus

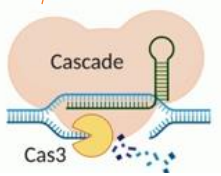


E. coli



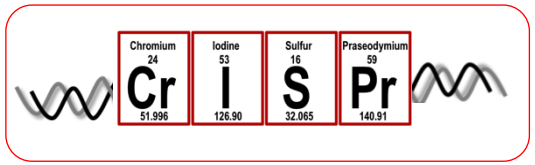
Streptococcus thermophilus & Clostridia



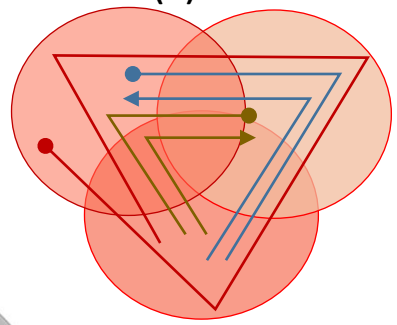


Clostridium difficile

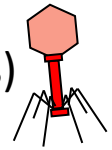
Cutibacterium acnes

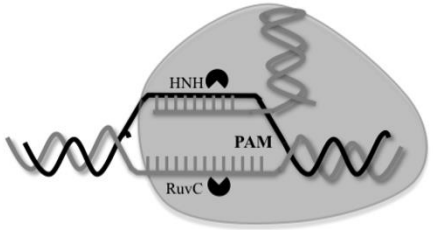


Microbiome(s)

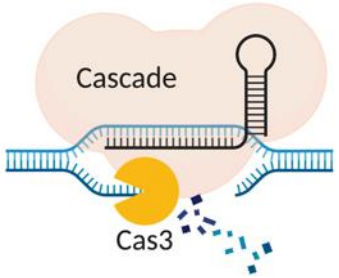


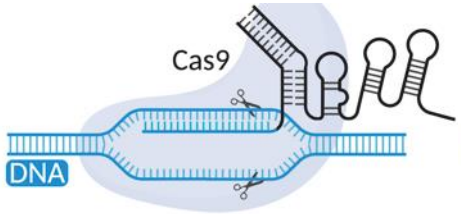
Virome(s)

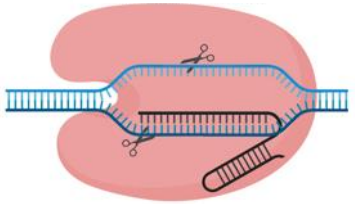


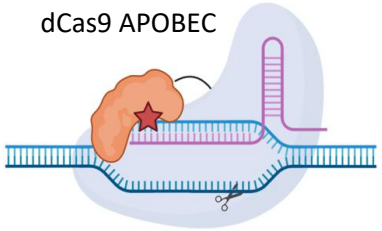


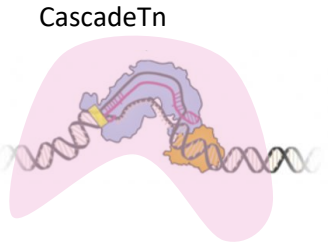
CRISPR

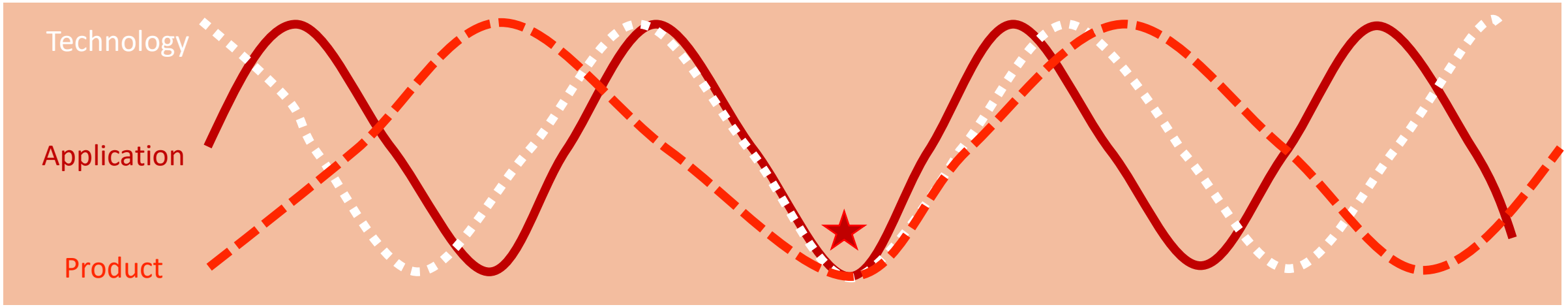




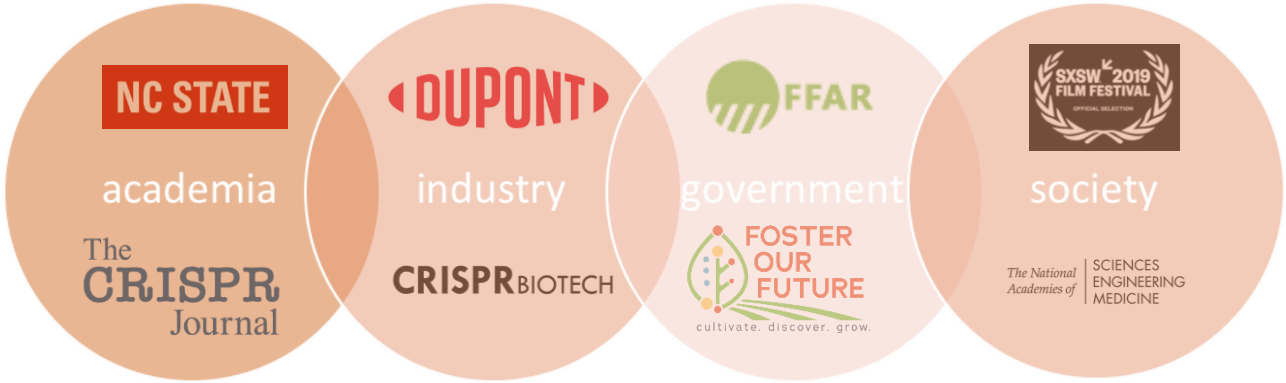








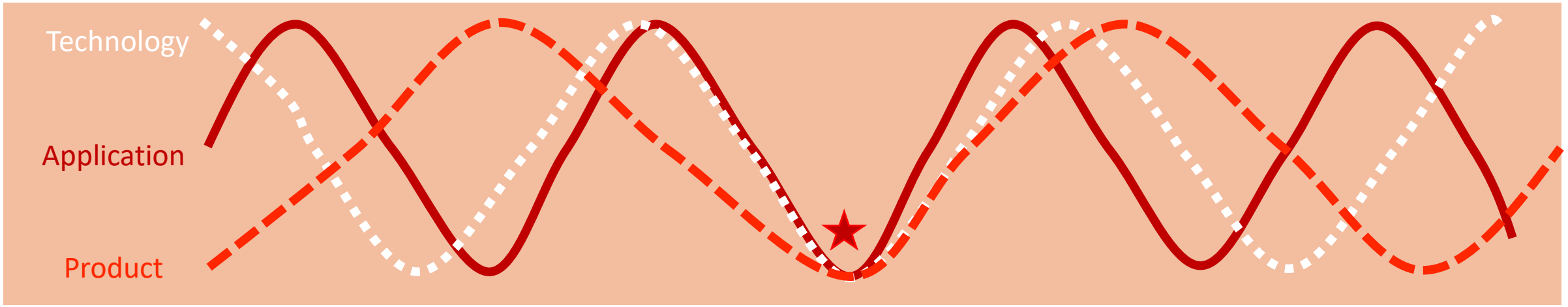
Science → Technology → Applications → Products → Acceptance



The innovator's dilemma

- Ethics
  - Editing technologies perception
  - Pace, scale and accessibility
  - Dialogue framework & moratorium
- Public relations
  - GMO concerns, skepticism
  - Public engagement and debates
  - Voices and stakeholders, RRI
  - Values, stewardship, benefits
- Regulatory Path(s)
  - Medicine vs. Ag
  - Risk:Benefit
  - DNA-free & non-GMO options

Societal implications



Science → Technology → Applications → Products → Acceptance

The applications & implications of CRISPR@LAB

Researcher pioneer@NAS

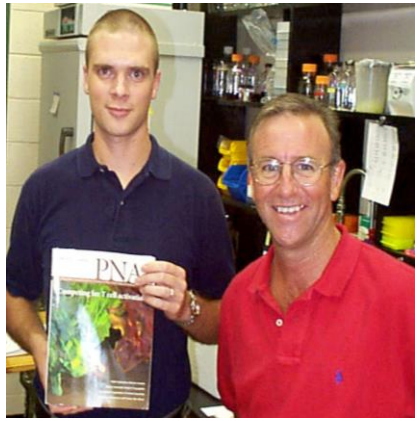
Teacher inspire@class&lab

Speaker keynotes&stories

Collaborator industry&academia

Mentor talent@lifelong

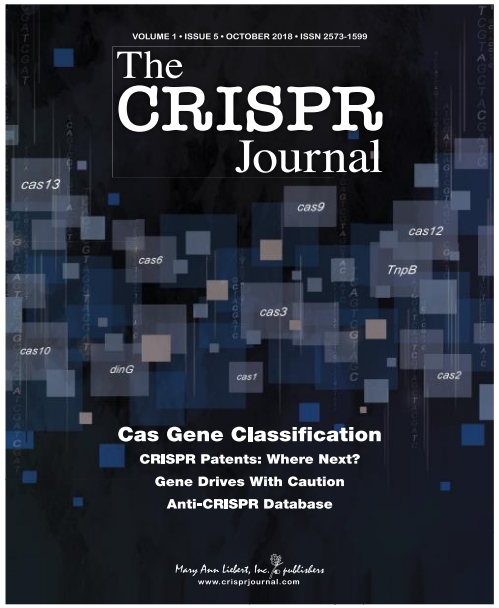
Competitor golf&rec.

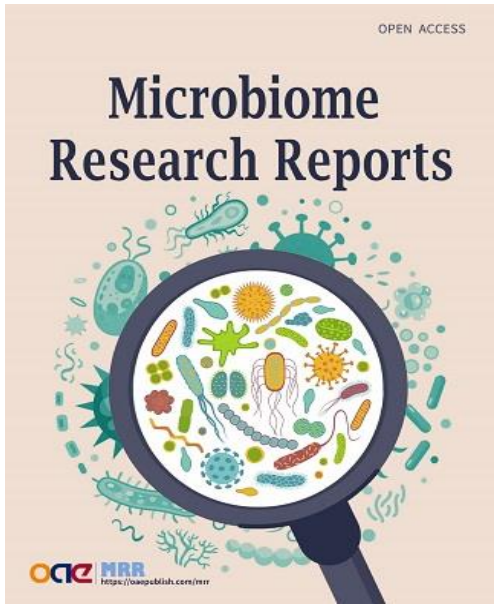


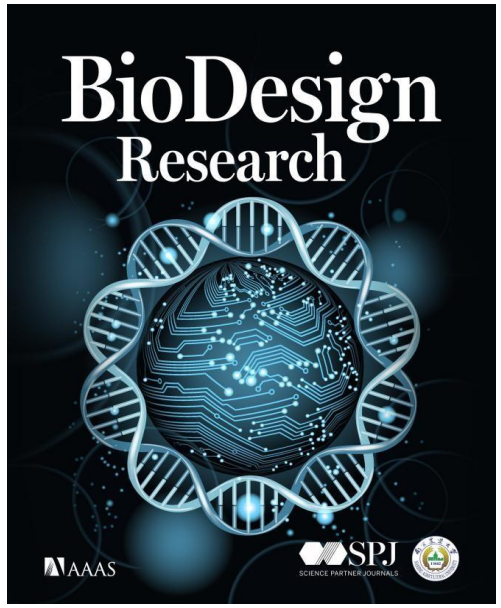
Writer LABpioneer&bar

Role model friend&more

Todd R. Klaenhammer, an inspirational food microbiologist who leaves a lasting legacy







13th International Symposium on Lactic Acid Bacteria

Funding & collaborations

DUPONT
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apc Microbiome Ireland
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THERIOT LAB
NC STATE

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