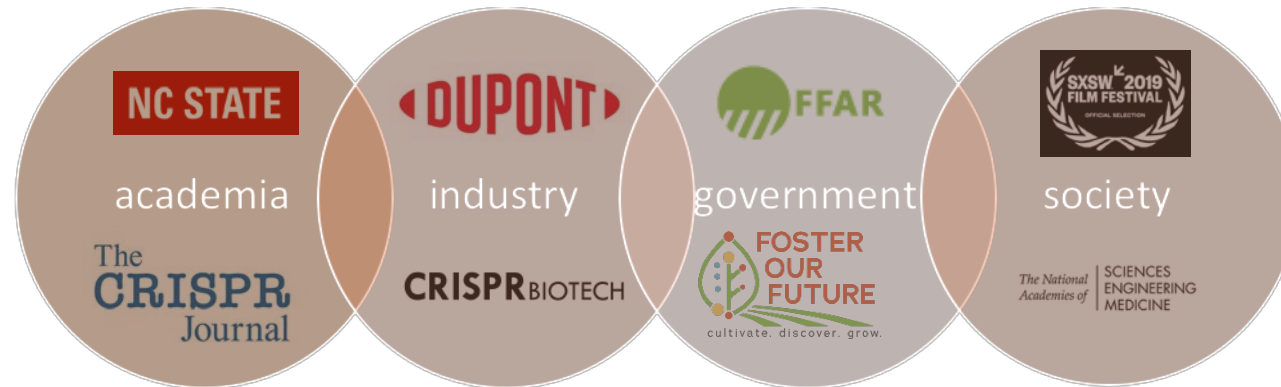
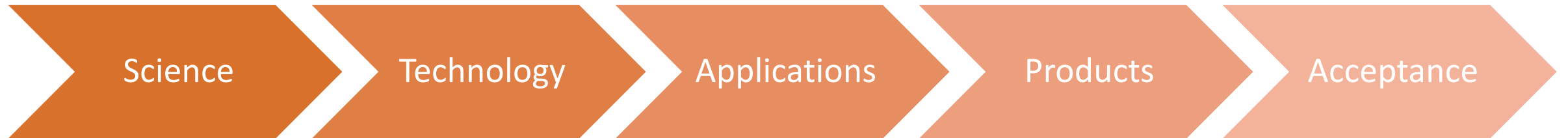
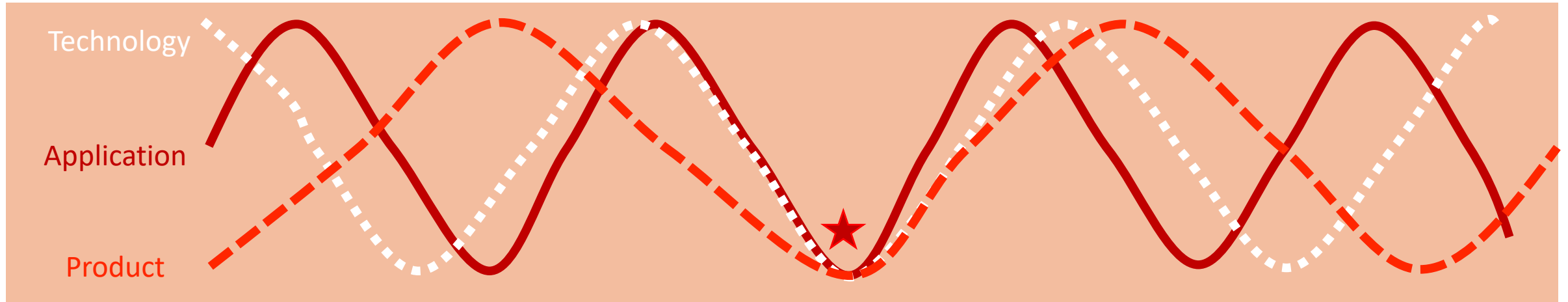


The CRISPR craze: managing disruptive genome editing technologies

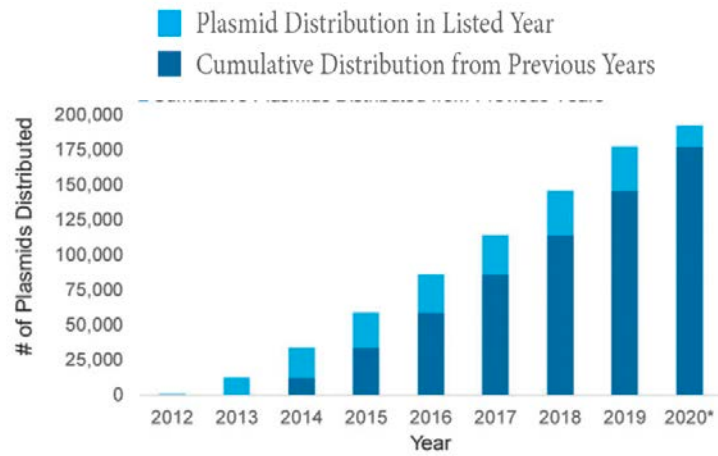
Rodolphe Barrangou | NC State | @CRISPRlab

The word "CRISPR" is rendered in a large, bold, red sans-serif font with a black outline. The letter "S" is replaced by the NC State logo, which consists of a red block letter "N" stacked on top of a red block letter "C", both with white outlines. A small registered trademark symbol (®) is located at the bottom right of the "S" logo.

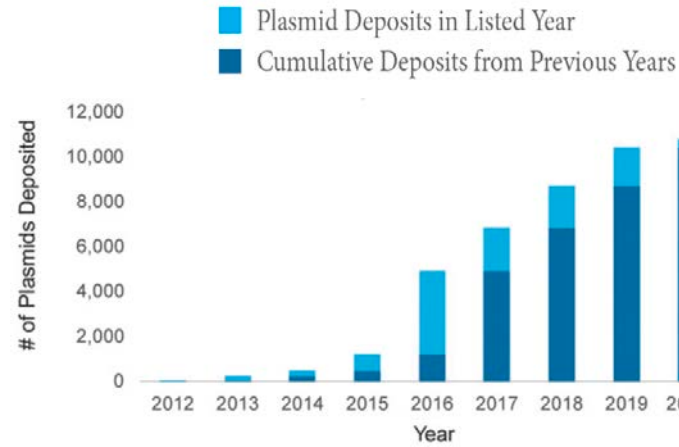


The Innovator's dilemma

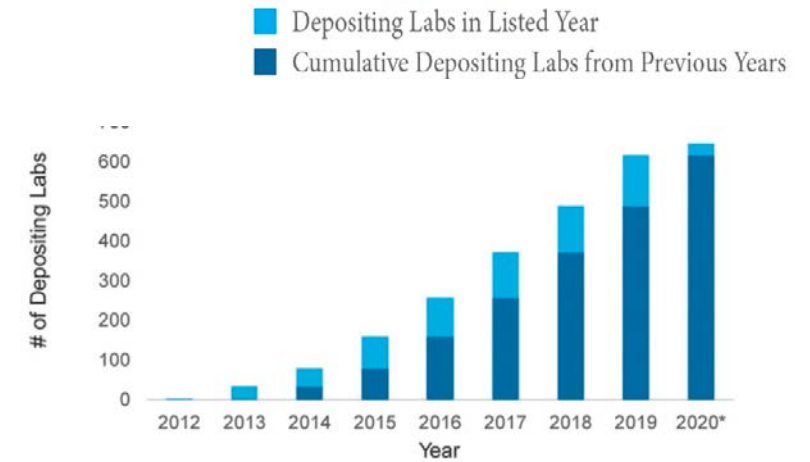
CRISPR Plasmid Distribution



Deposited CRISPR Plasmids



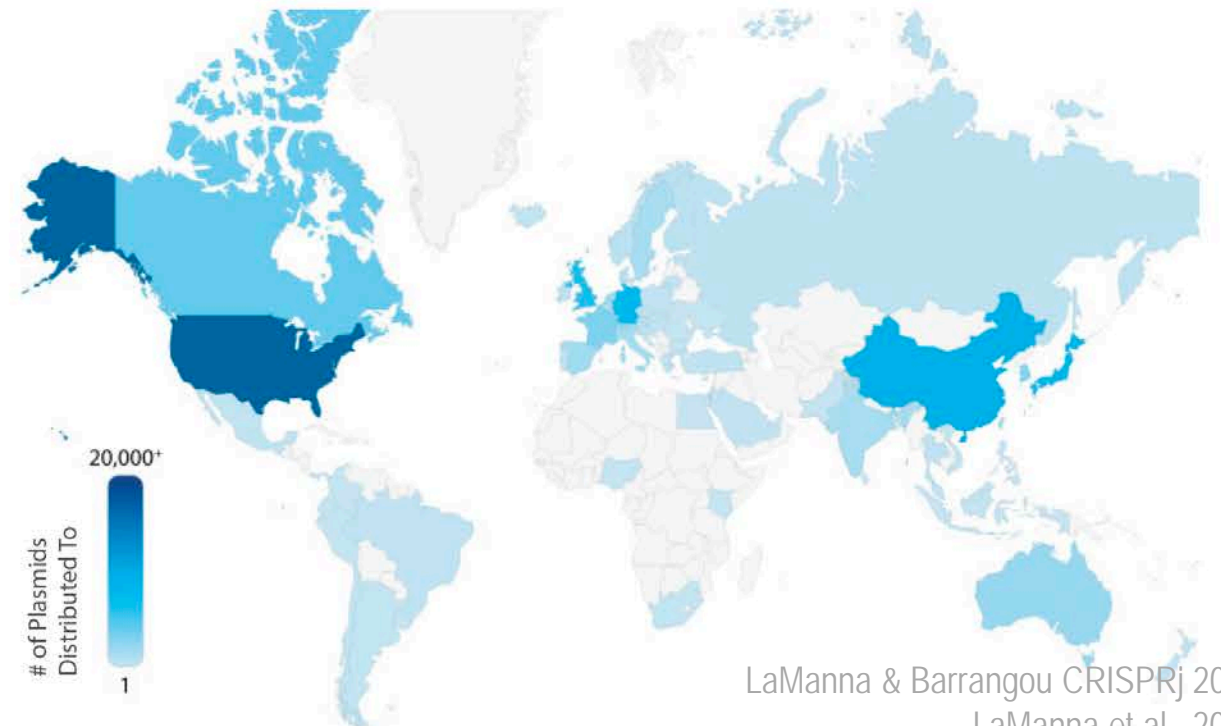
CRISPR Depositing Labs



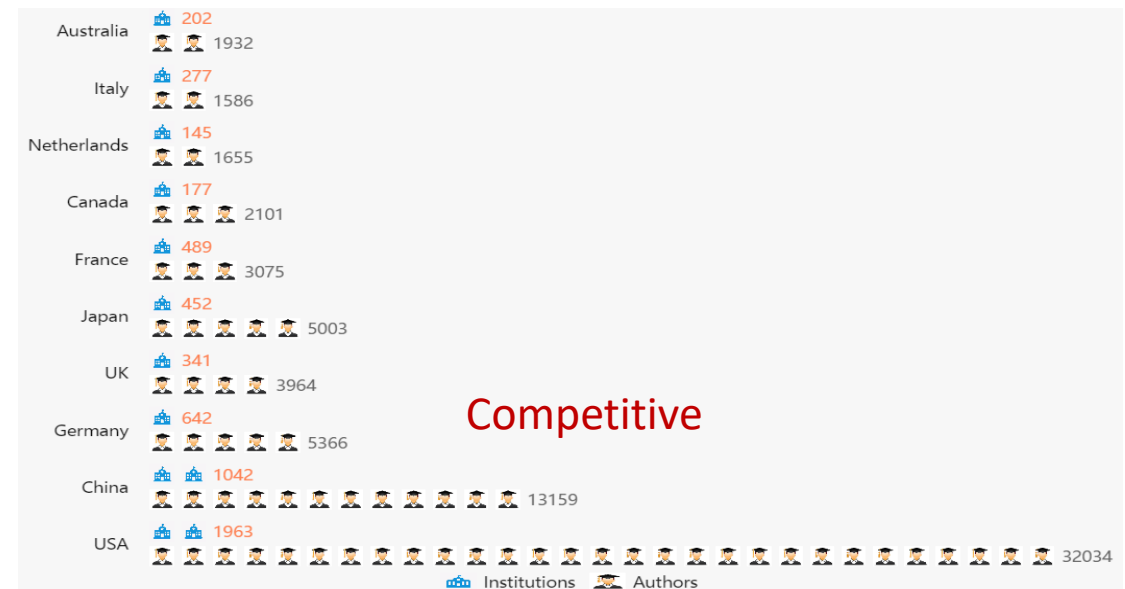
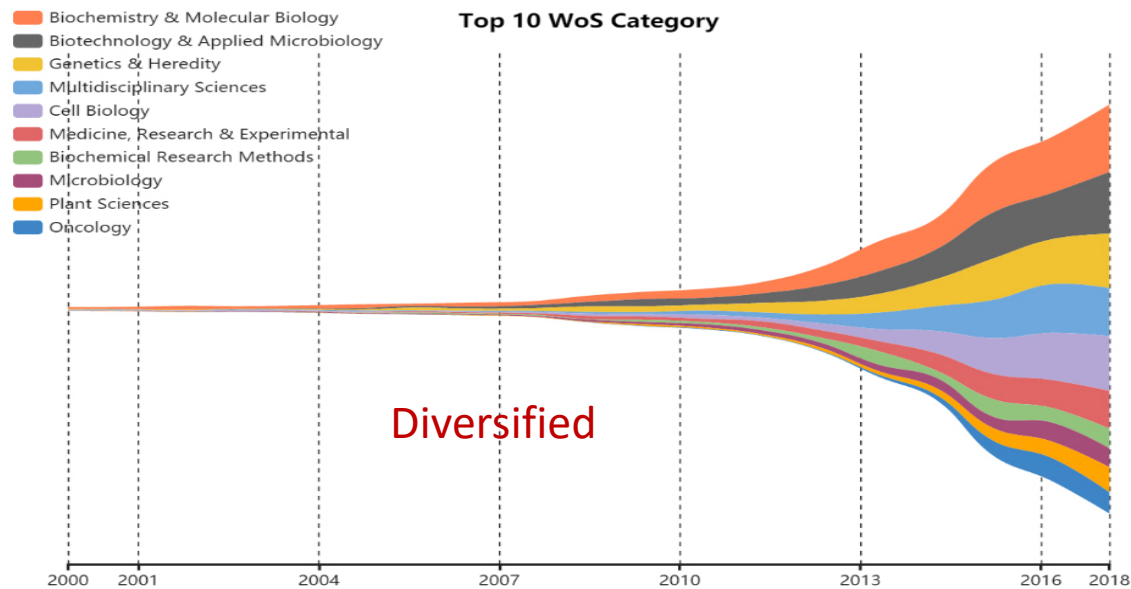
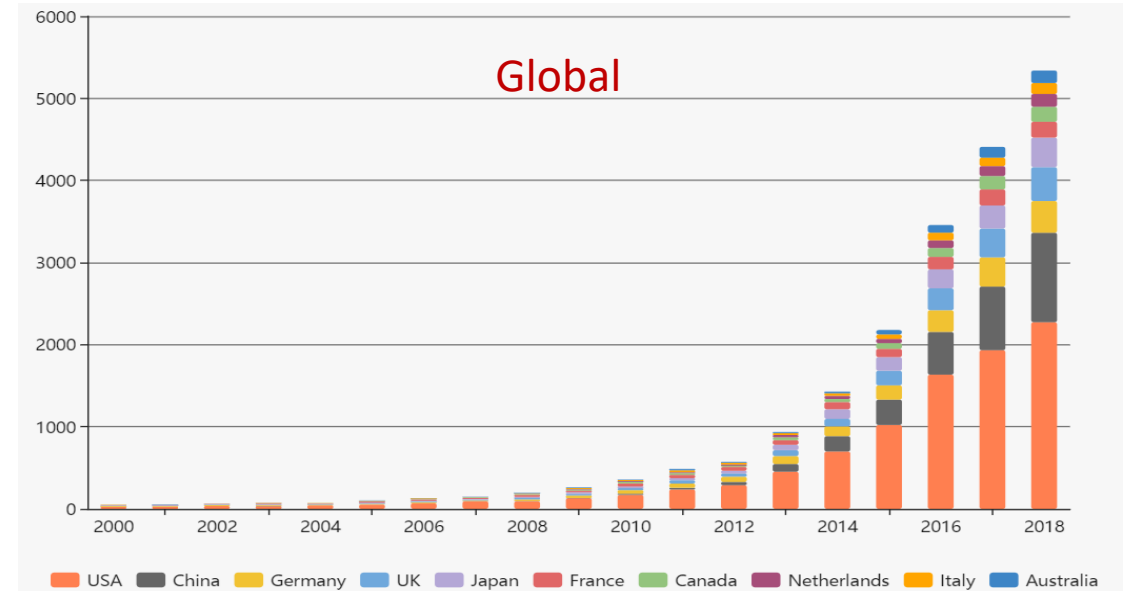
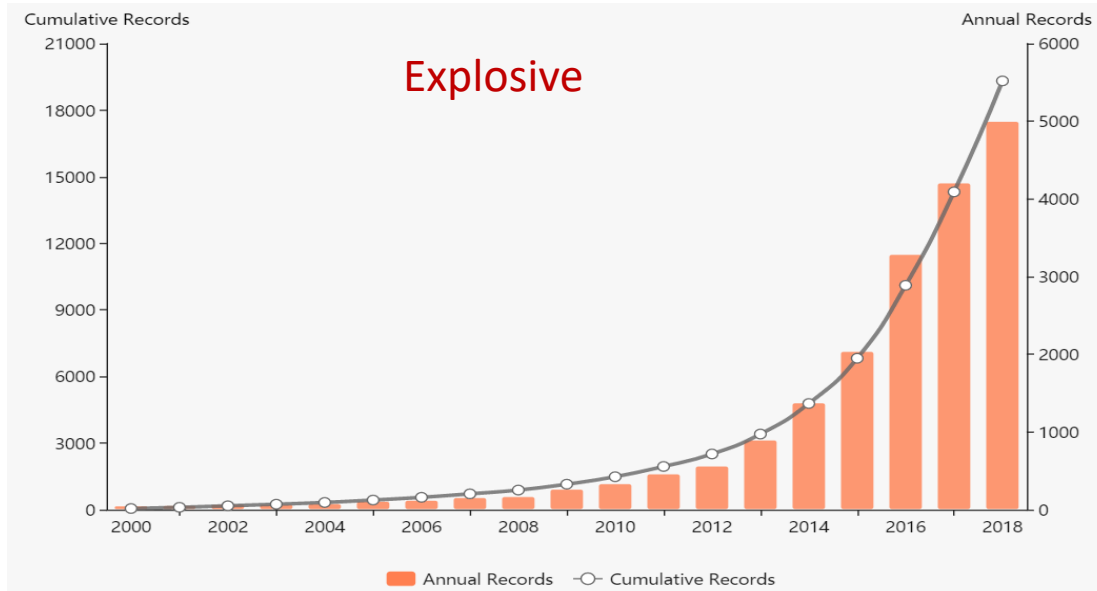
Where CRISPR Plasmids Were Deposited From



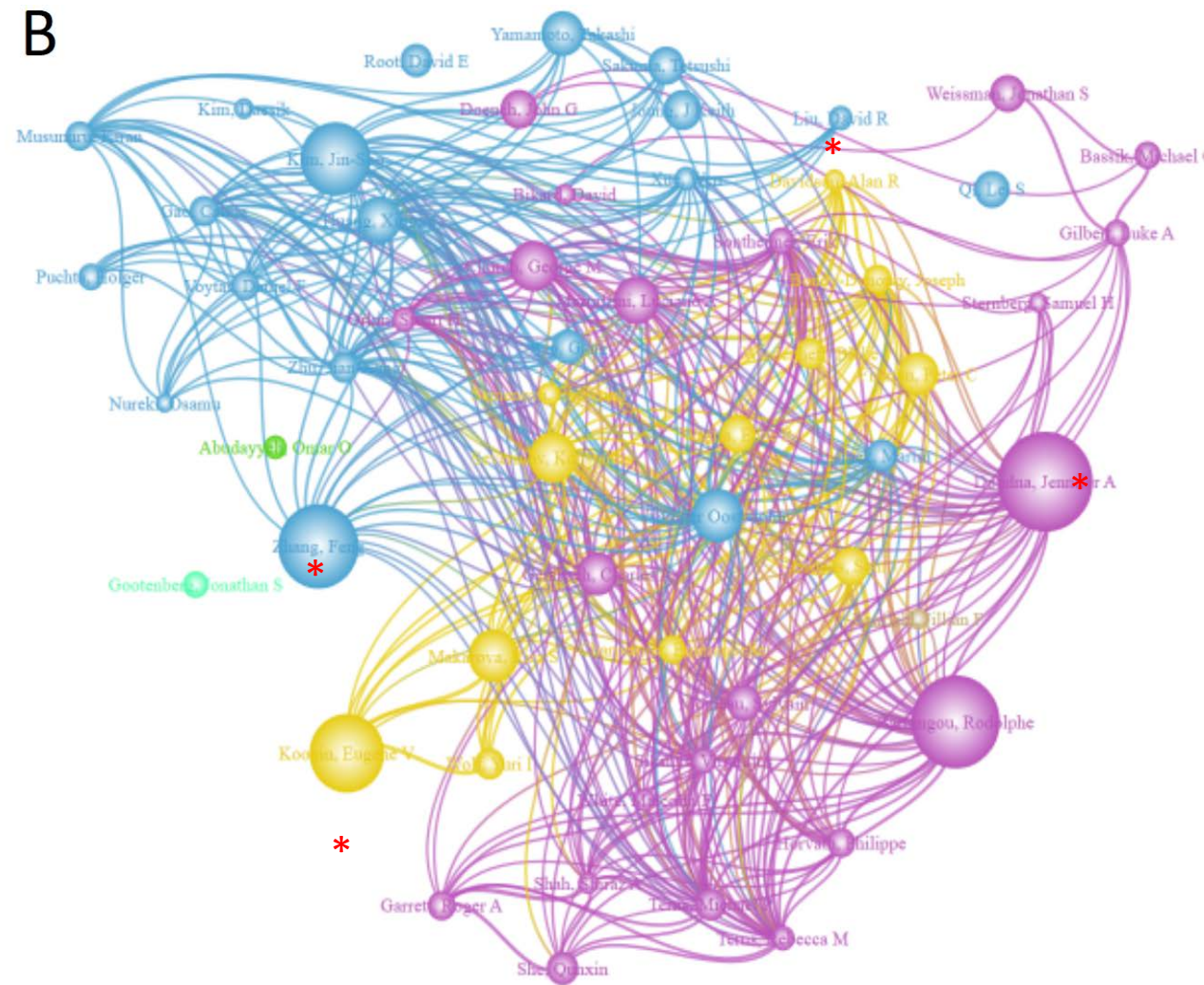
Where CRISPR Plasmids Were Distributed To



Living in a CRISPR world

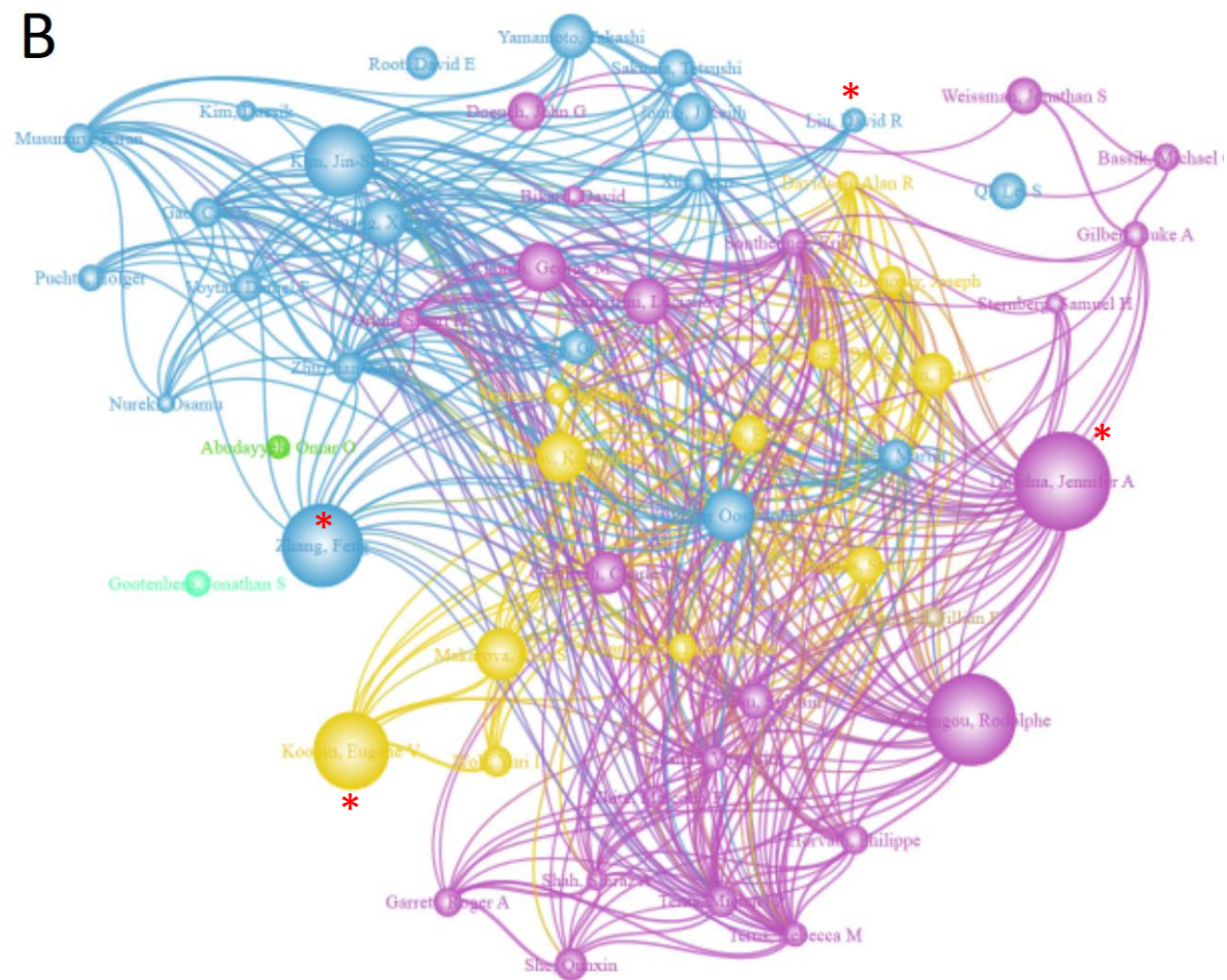


B

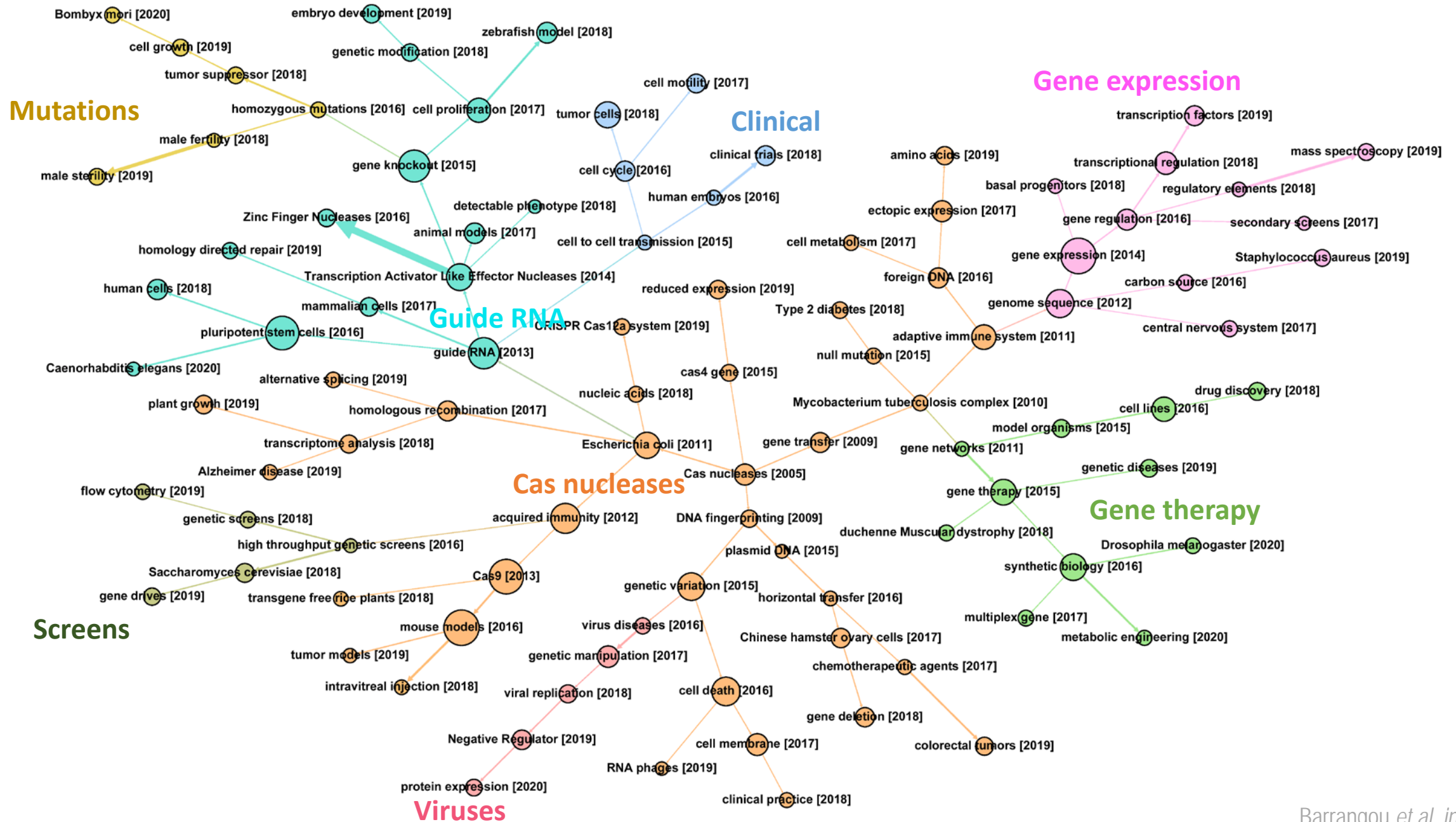


Co-authorships

B

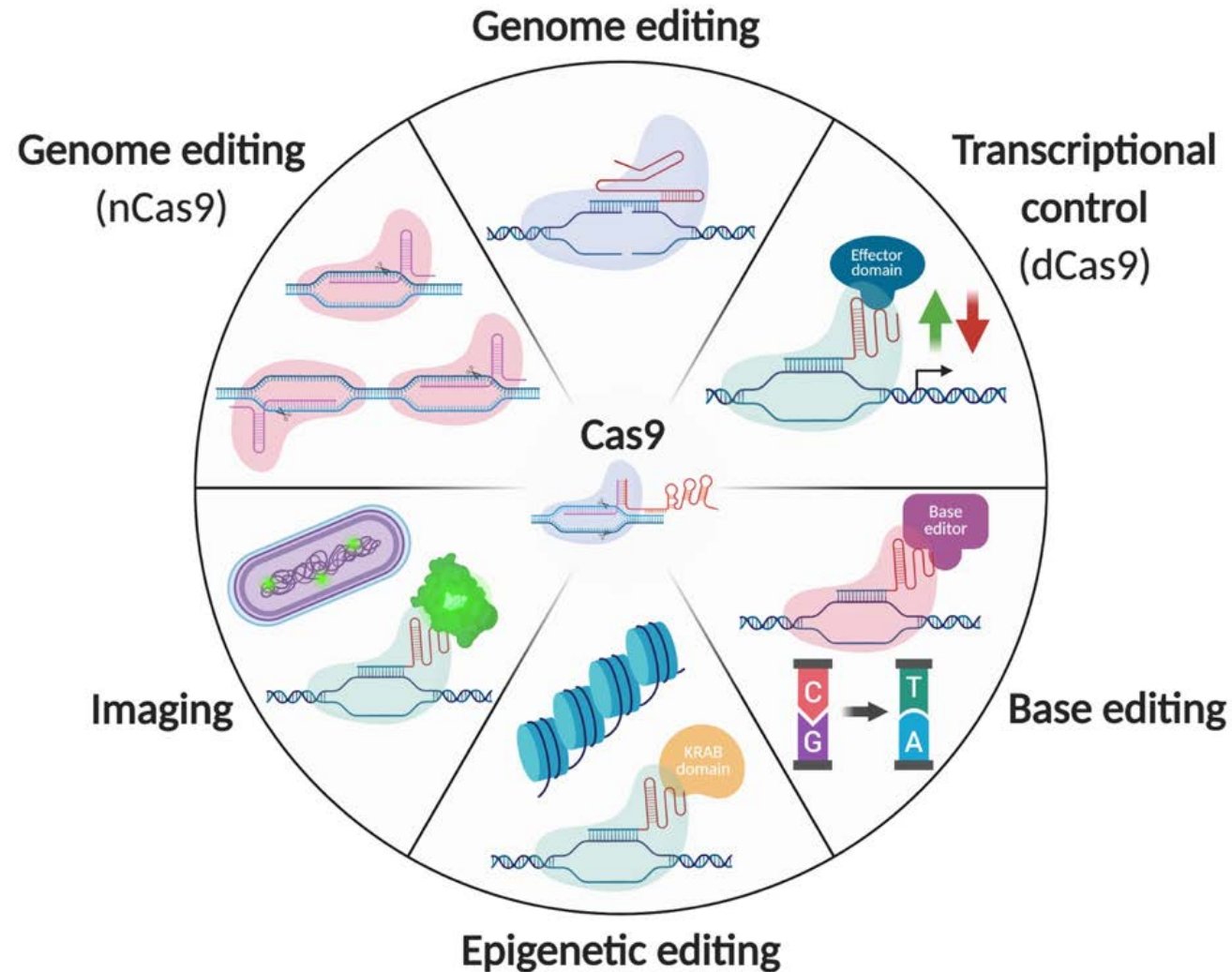


topics

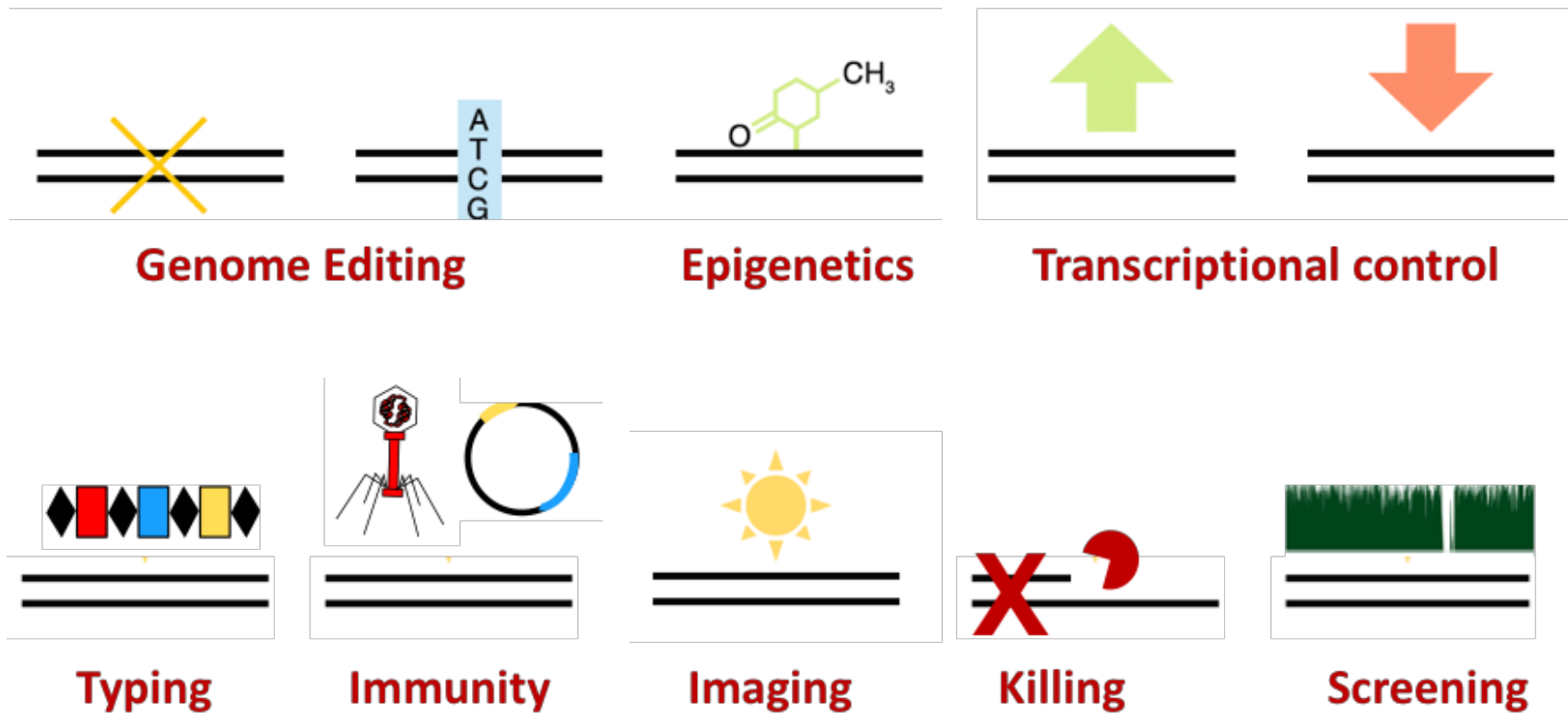




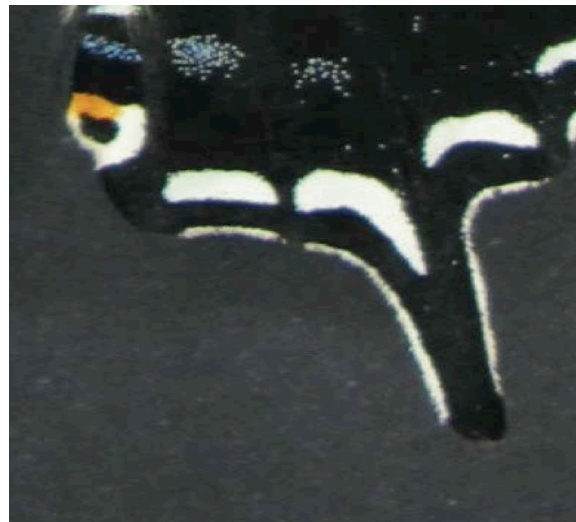
Genome editing



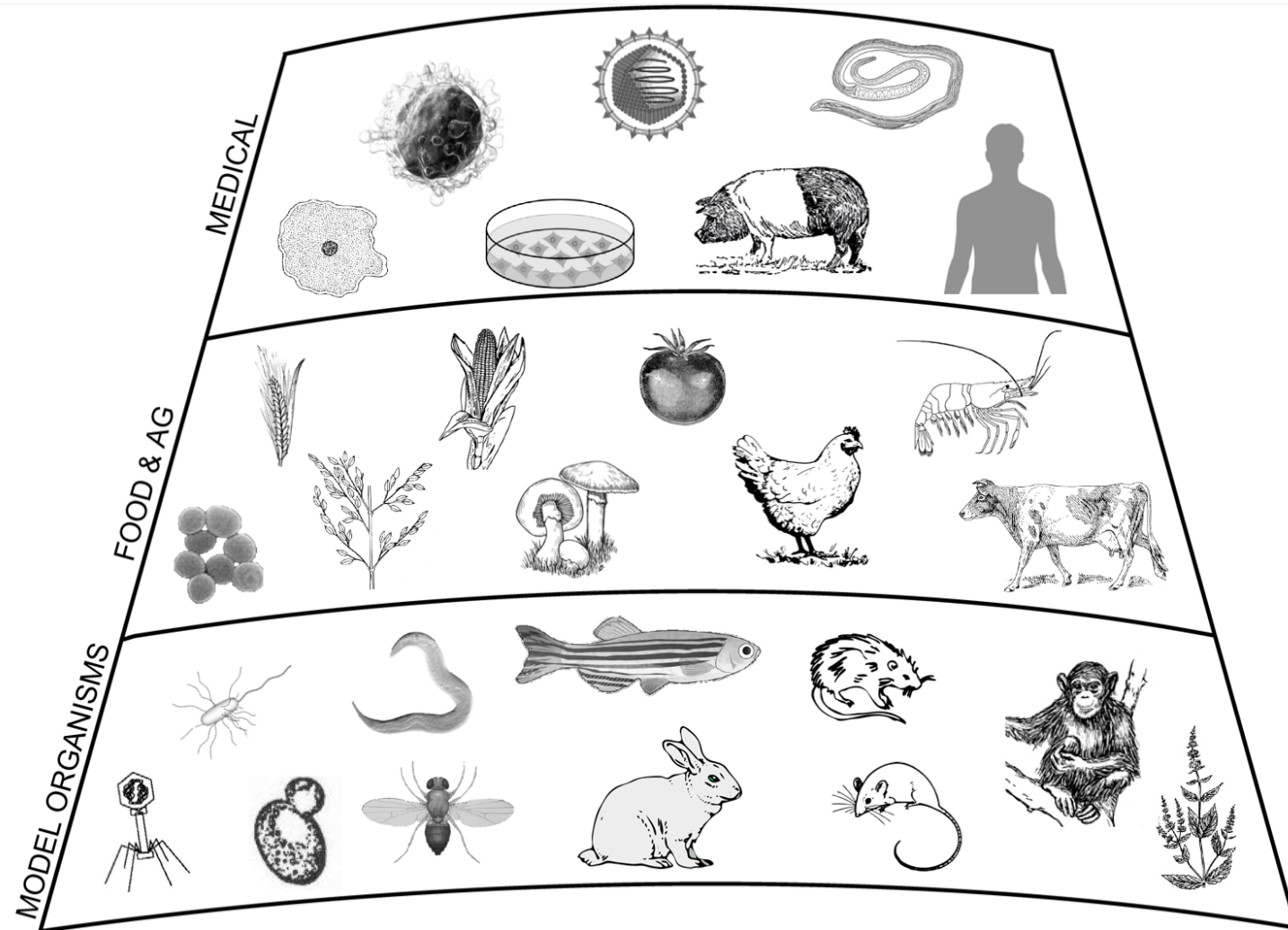
Genome Editing ^{2.0} Cas9, dCas9, nCas9



CRISPR^{2.0} * Cas3 Cas9 Cas12 Cas13 CasTn



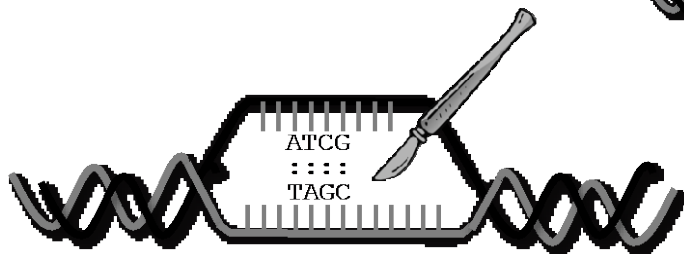
Illustration



The CRISPR zoo

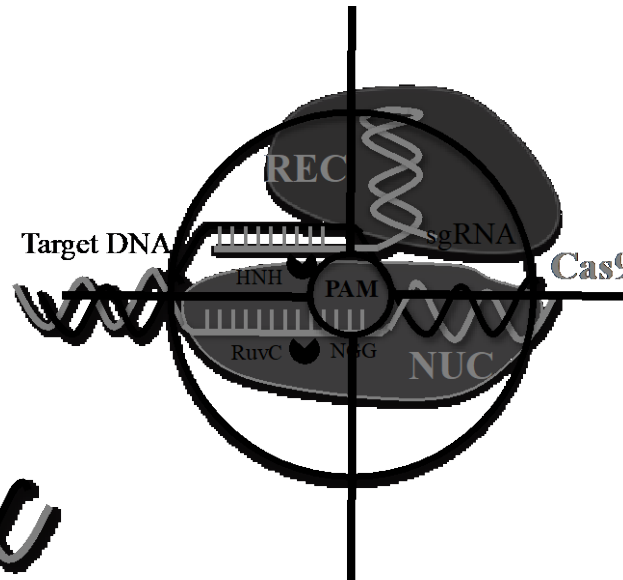
Advantages

Programmable
 Specific
 Transferable
 Efficient
 Precise
 Affordable
 Quick
 Multiplexable
 Scalable



Caveats

Large (package/deliver)
 PAM-dependent targeting
 PAM diversity
 Off target cleavage
 Various efficiencies



Opportunities

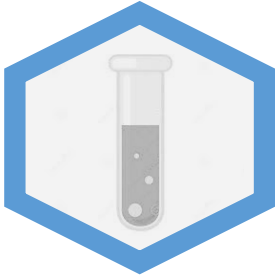
Guide design & composition
 Cas engineering
 Orthogonality
 Biodiversity (eff, act, saf, tox, siz)
 PAM diversity
DNA repair (cutting only front 50%)

Delivery

Electro/biolistic
 Peptides/RNPs/RNA
 Lipids/microinj.
 Viruses/phagemids
 Tissue/cell specific

CRISPR “technology” turns 7

RESEARCH



Tools
Guides
Enzymes
Software
Plasmids
Delivery
Kits
Primers
Cell lines

BIOTECHNOLOGY



Bacteria
Yeast
Algae

Food
Biotomanufacturing
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

Fields of use

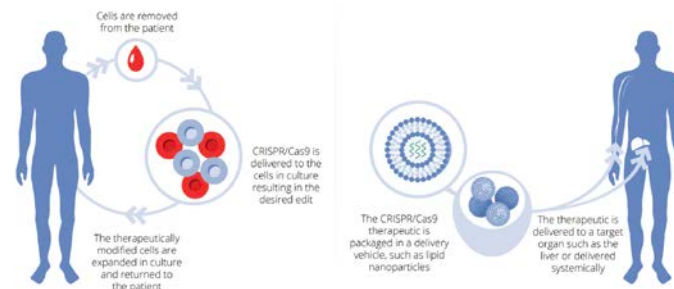
	Programs	Program Lead	Stage
In vivo	Genetic Disease		
	Transthyretin Amyloidosis	Inte:lia THERAPEUTICS REGENERON	Late Stage Preclinical
	Alpha-1 Antitrypsin Deficiency	Inte:lia THERAPEUTICS	Preclinical
	Primary Hyperoxaluria Type 1	Inte:lia THERAPEUTICS	Preclinical
ex vivo	Immunology		
	Acute Myeloid Leukemia	Inte:lia THERAPEUTICS	Preclinical
	Undisclosed	NOVARTIS	Preclinical
	Hematology		
	Sickle Cell Disease	NOVARTIS	Late Stage Preclinical
	Autoimmune Diseases		
	Undisclosed	Inte:lia THERAPEUTICS	Preclinical



Inte:lia THERAPEUTICS REGENERON	Inte:lia THERAPEUTICS
Initial Focus	Additional Exploration
<ul style="list-style-type: none"> Liver Diseases (LNP Delivery) 	<ul style="list-style-type: none"> Eye Muscle CNS

NOVARTIS	Inte:lia THERAPEUTICS
<ul style="list-style-type: none"> CAR T oncology HSC 	<ul style="list-style-type: none"> Non-CAR T oncology Autoimmune and inflammatory

Program	Editing approach	Research	IND-enabling	Ph I/II	Partner	Structure
Ex vivo: Hematopoietic						
CTX001: β -thalassemia	Disruption		CTA Approved	VERTEX	Collaboration	
CTX001: Sickle cell disease (SCD)	Disruption		CTA Approved	VERTEX	Collaboration	
Hurler syndrome (MPS-1)	Correction					Wholly-owned
Severe combined immunodeficiency (SCID)	Correction				CRISPR	Joint venture
Ex vivo: Immuno-oncology						
CTX110: Anti-CD19 allogeneic CAR-T	Various		IND filing YE18			Wholly-owned
CTX120: Anti-BCMA allogeneic CAR-T	Various					Wholly-owned
CTX130: Anti-CD70 allogeneic CAR-T	Various					Wholly-owned
Ex vivo: Regenerative Medicine						
Type 1 diabetes mellitus	Various				VIACYTE	Collaboration
In vivo: Liver						
Glycogen storage disease Ia (GSD Ia)	Correction					Wholly-owned
Hemophilia	Correction				CRISPR	Joint venture
In vivo: Other organs						
Duchenne muscular dystrophy (DMD)	Disruption					Wholly-owned
Cystic fibrosis (CF)	Correction				VERTEX	License option



Hemoglobinopathies

SICKLE CELL DISEASE (SCD) AND β -THALASSEMIABlood disorders caused by mutations in the β -globin gene

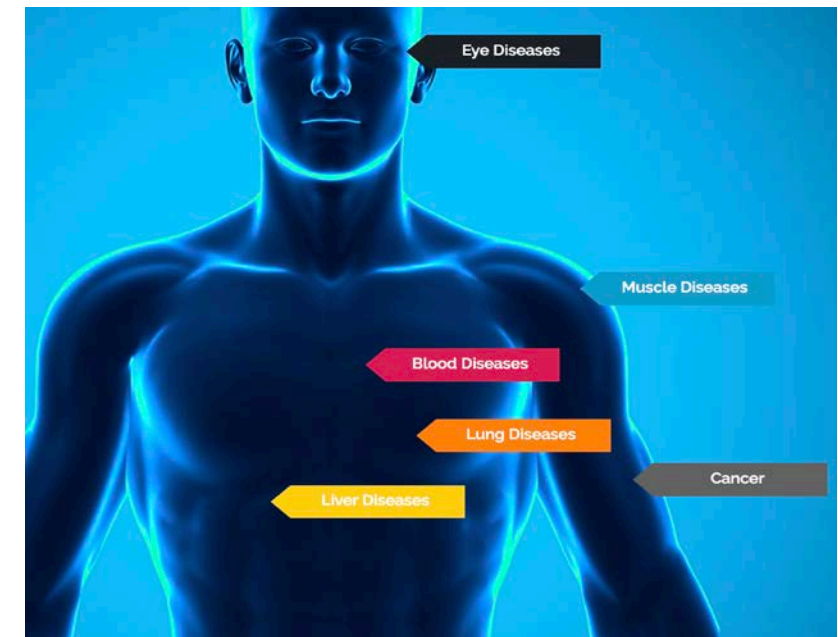
High morbidity and mortality

Significant worldwide burden
300,000 Annual births in SCD and β -thalassemia, respectively
60,000

Heavy burden of patient care



Our Programs	Editing Mechanism	Delivery Mode	Commercial Rights	Discovery
Eye Diseases				
Leber Congenital Amaurosis 10	NHEJ - Small Deletion	AAV local injection	editas	
Genetic and Infectious Disease(s) of Eye Usher Syndrome 2a, HSN-1	NHEJ	AAV local injection	editas	
Engineered T Cells				
Gene Editing in T Cells to Treat Cancer	NHEJ	RNP ex vivo	juno	
Additional Research Programs				
Non-Malignant Hematologic Diseases Beta Thalassemia, Sickle Cell	NHEJ & HDR	RNP ex vivo	editas	
Genetic Disease(s) of Muscle Duchenne Muscular Dystrophy	NHEJ - Small & Large Deletion	AAV or LNP	editas	
Genetic Disease(s) of Lung Cystic Fibrosis	NHEJ & HDR	AAV or LNP	editas	
Genetic and Infectious Disease(s) of Liver Alpha-1 Antitrypsin Deficiency	NHEJ & HDR	AAV or LNP	editas	



Bacteria

Species	Industrial relevance	Modification(s)	Refs
Bacteria			
<i>Bacillus smithii</i>	Moderate thermophile capable of C ₅ and C ₆ sugar metabolism	Recombination	[81]
<i>Bacillus subtilis</i>	Producer of industrial enzymes and valuable low-molecular-weight substances	Recombination	[82]
<i>Clostridium autoethanogenum</i>	Capable of fermenting CO, CO ₂ , and H ₂ into biofuel ethanol and 2,3-butanediol	Recombination	[83]
<i>Clostridium beijerinckii</i>	Production strain for biofuels and biochemical	Recombination and CRISPRi	[84,85]
<i>Clostridium cellulolyticum</i>	Capable of conversion of lignocellulosic biomass to valuable endproducts	Recombination	[86]
<i>Corynebacterium glutamicum</i>	Producer of amino acids	CRISPRi	[87]
<i>Clostridium ljungdahlii</i>	Capable of producing ethanol from synthesis gas	Recombination	[88]
<i>Clostridium pasteurianum</i>	Capable of converting waste glycerol to butanol	Recombination	[89]
<i>Escherichia coli</i>	Common production strain	Programmed antimicrobial, recombination, multiplex recombination, CRISPRi, multiplexed CRISPRi, gene circuit, RNA targeting	[5,6,12,26,51,52,55,71,75,90–94]
<i>Lactobacillus reuteri</i>	Probiotic strain and producer of biotherapeutics	Recombination	[95]
<i>Streptococcus thermophilus</i>	Probiotic and industrial fermentation strains	Engineered immunity	[3]
<i>Streptomyces albus</i>	Producer of heterologous secondary metabolites	Recombination	[96]
<i>Streptomyces coelicolor</i>	Source of pharmacologically active and industrially relevant secondary metabolites	Recombination and CRISPRi	[69,96]
<i>Streptomyces lividans</i>	Source of pharmacologically active and industrially relevant secondary metabolites	Recombination	[96]
<i>Streptomyces viridochromogenes</i>	Source of pharmacologically active and industrially relevant secondary metabolites	Recombination	[96]
<i>Tatumella citrea</i>	Producer of vitamin C precursor (2-keto-D-gluconic acid)	Recombination	[6]
Yeast			
<i>Candida albicans</i>	Common production strain, capable of phenol and formaldehyde catabolism	Recombination and multiplexed recombination	[62,97,98]
<i>Kluyveromyces fragilis</i>	Common production strain	Multiplexed recombination	[4]
<i>Pichia pastoris</i>	Common production strain	Multiplex mutagenesis and recombination	[99]
<i>Saccharomyces cerevisiae</i>	Common production strain	Donor-mediated gene disruption , multiplexed donor-mediated gene disruption, multiplexed recombination, CRISPRa, CRISPRi	[2,7,8,10,40,58,70,72,100–102]
<i>Ustilago maydis</i>	Natural producer of valuable biochemical; causative agent of corn smut	Mutagenesis	[103,104]
<i>Yarrowia lipolytica</i>	Natural producer of valuable biochemical	Multiplexed mutagenesis and recombination	[105,106]

Yeast

Fungi

Species	Industrial relevance	Modification(s)	Refs
Filamentous Fungi			
<i>Aspergillus aculeatus</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus brasiliensis</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus carbonarius</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus luchuensis</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus nidulans</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus niger</i>	Source of and producer of enzymes	Mutagenesis	[41]
<i>Aspergillus oryzae</i>	Production strain of enzymes and heterologous proteins; also an industrial fermentation strain	Mutagenesis	[107]
<i>Myceliophthora thermophila</i>	Thermophilic strain and producer of cellulases	Mutagenesis, recombination, multiplexed recombination	[108]
<i>Neurospora crassa</i>	Producer of industrially relevant lignocellulosic enzymes	Recombination	[64]
<i>Penicillium chrysogenum</i>	Producer of β -lactam antibiotics	Recombination	[66]
<i>Trichoderma reesei</i>	Common production strain	Mutagenesis and recombination	[59]

RESEARCH



Enzymes
Proteins

BIOTECHNOLOGY



Enzymes
Biofuels
Biomass
Vitamins

AGRICULTURE



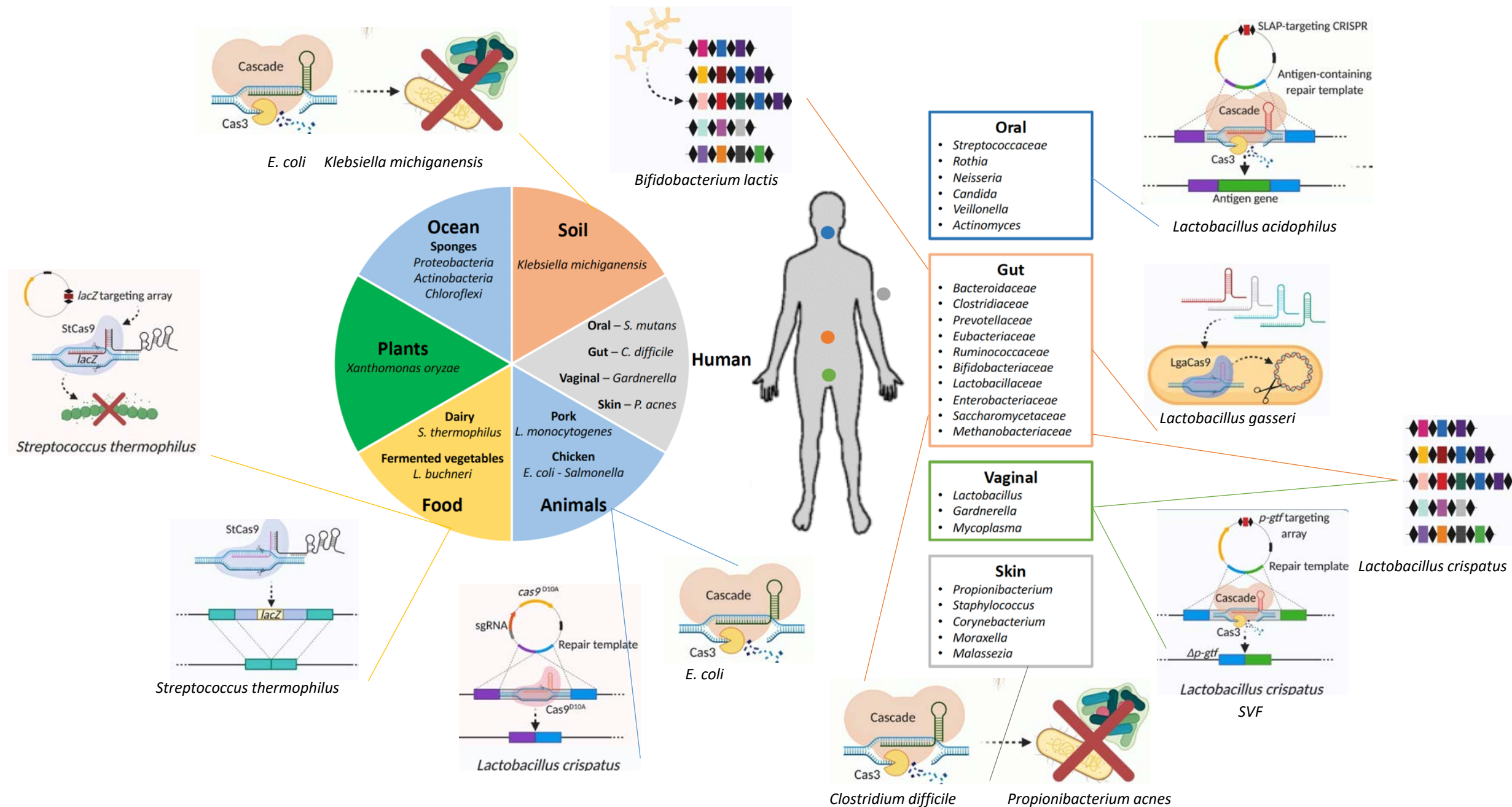
Cheese
Yoghurt

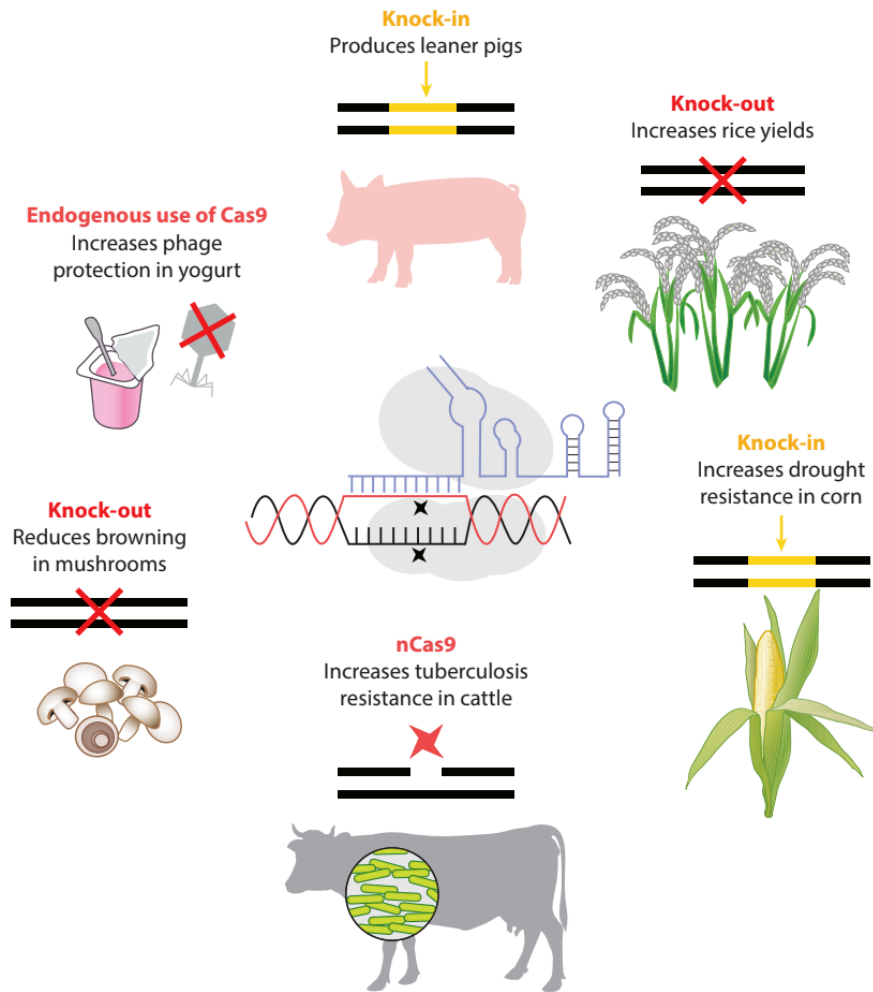
THERAPEUTICS



Probiotics
Antibiotics
Insulin

Industrial Biotechnology





Ag diversification

Corn
Wheat
Soy

Crops

Tomatoes
Mushrooms

Fruits & vegetables

Tobacco / cotton / hemp
Trees
Cellulose

Non-food crops
Forestry
Bio-energy



Bacteria



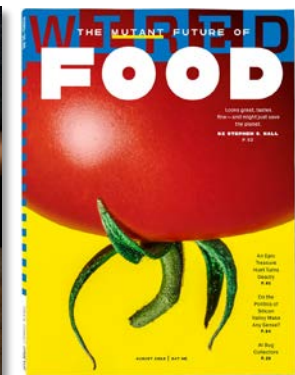
Plants



Animals



Yeast





System Initiative on Shaping the Future of Food Security and Agriculture







Innovation with a Purpose: The role of technology innovation in accelerating food systems transformation





CRISPR is a breeding game changer for Ag

Figure 3: Combinations of 4IR technologies can enable innovation to solve challenges faced in food systems





Digital building blocks

-  New computing technologies
-  Big data and advanced analytics
-  The Internet of Things (IoT)
-  Artificial intelligence and machine learning
-  Blockchain
-  Virtual reality and augmented reality

Advances in science

-  Next-generation biotechnologies and genomics
-  Energy creation, capture, storage and transmission

Reforming the physical

-  Autonomous and near-autonomous vehicles
-  Advanced, smart robotics
-  Additive manufacturing and multidimensional printing
-  Advanced materials and nanotechnologies

Creating effective production systems

PRECISION AGRICULTURE FOR INPUT AND WATER USE OPTIMIZATION



- Reduce farmers' costs by up to \$100 billion
- Increase production by up to 300 million tonnes
- Reduce freshwater withdrawals by up to 180 billion cubic metres



BIOLOGICAL-BASED CROP PROTECTION AND MICRONUTRIENTS FOR SOIL MANAGEMENT

- Increase production by up to 50 million tonnes
- Reduce GhG emissions by up to 5 megatonnes of CO₂ eq.



GENE-EDITING FOR MULTI-TRAIT SEED IMPROVEMENTS

- Generate up to \$100 billion in additional farmer income
- Increase production by up to 400 million tonnes
- Reduce the number of micronutrient deficient by up to \$100 million

MICROBIOME TECHNOLOGIES TO ENHANCE CROP RESILIENCE



- Generate up to \$100 billion in additional farmer income
- Increase production by up to 250 million tonnes
- Reduce GhG emissions by up to 30 megatonnes of CO₂ eq.

OFF-GRID RENEWABLE ENERGY GENERATION AND STORAGE FOR ACCESS TO ELECTRICITY



- Generate up to \$100 billion in additional farmer income
- Increase production by up to 530 million tonnes
- Reduce freshwater withdrawals by up to 250 billion cubic metres

Advances in science



Next-generation biotechnologies and genomics



GENE-EDITING FOR MULTI-TRAIT SEED IMPROVEMENTS

- Generate up to \$100 billion in additional farmer income
- Increase production by up to 400 million tonnes
- Reduce the number of micronutrient deficient by up to \$100 million

CRISPR is a breeding game changer for Ag

Creating effective production systems



**PRECISION
AGRICULTURE
FOR INPUT AND
WATER USE
OPTIMIZATION**



**GENE-EDITING
FOR MULTI-
TRAIT SEED
IMPROVEMENTS**



**MICROBIOME
TECHNOLOGIES
TO ENHANCE
CROP
RESILIENCE**



**BIOLOGICAL-BASED
CROP PROTECTION
AND MICRONUTRIENTS
FOR SOIL
MANAGEMENT**



**OFF-GRID RENEWABLE
ENERGY GENERATION
AND STORAGE
FOR ACCESS TO
ELECTRICITY**

**GENE-EDITING FOR
MULTI-TRAIT SEED
IMPROVEMENTS**



10-15% of
farms (60-100
million farms)
chose to use
gene-edited
seeds by 2030

Increased income
Billions of dollars

40-100

1-2% of the total
agricultural production
value⁴⁹

Increased yield, and reduced
loss from drought, pest and
disease

Increased yields
Millions of tonnes

100-400

1-5% of the total
agricultural production⁴⁶

Increased yield from improved
seed genotype

Reduced food loss
Millions of tonnes

5-20

1-2% of total food
losses⁵⁰

Increased crop resilience to pest,
disease and drought

**Reduced micronutrient
def. Million people**

20-100

1-5% of total people in a
state of malnutrition⁵¹

Ability to biofortify seeds and
crops

CRISPR is a breeding game changer for Ag



BBC

WIRED

The Washington Post
Democracy Dies in Darkness

npr



THE NEW YORKER

TIME

REUTERS

The New York Times

MIT
Technology
Review

HUMAN NATURE

CARIBOU
BIOSCIENCESIntelia
THERAPEUTICSeditas
MEDICINElers
GENOMICSCRISPR
THERAPEUTICSBeam
THERAPEUTICS

MammothBiosciences

CASEBIA
THERAPEUTICSSHERLOCK
BIOSCIENCES

TESSERA

eGenesis
ENGINEERING LIFE

GRAPHITE BIO

αCRIGEN
BIOSCIENCES

METAGENOMI

LOCUS BIOSCIENCES

ELIGO
BIOSCIENCE

NEXTBOTICS

SNIPRBIOME
A CRISPR COMPANY

LifeEDIT

TWELVE.BIO

arbor
biotechnologiesCOSPR
ANTICRISPR

CASZYME

pairwise

FREECO

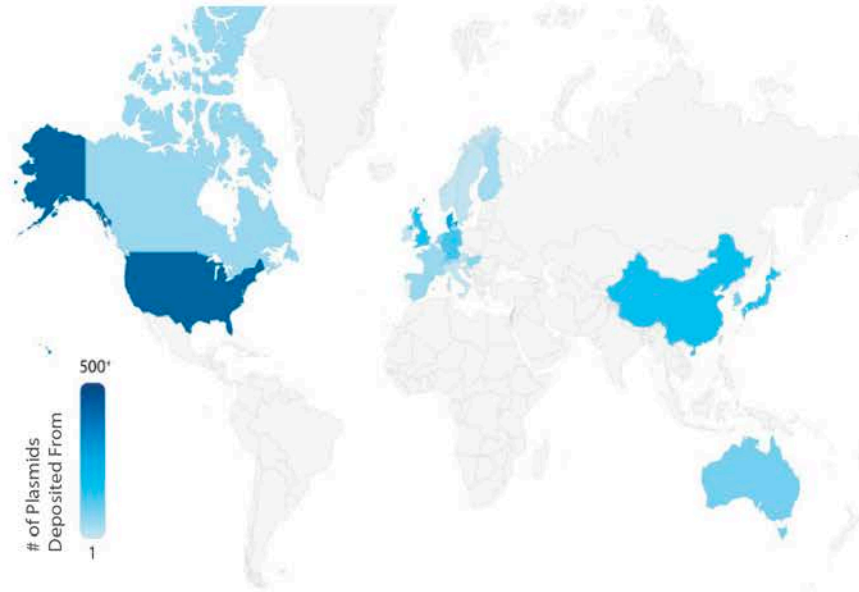
CasTAG
BioSciencesINARI
A Flagship Promising Company

CRISPRBIOTECH

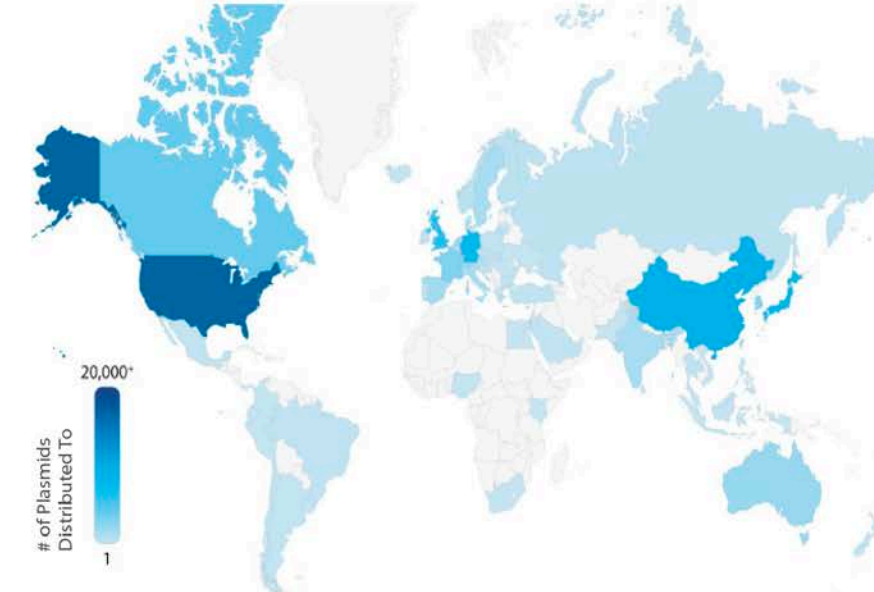
The CRISPR craze: fueling the bio-economy



Where CRISPR Plasmids Were Deposited From

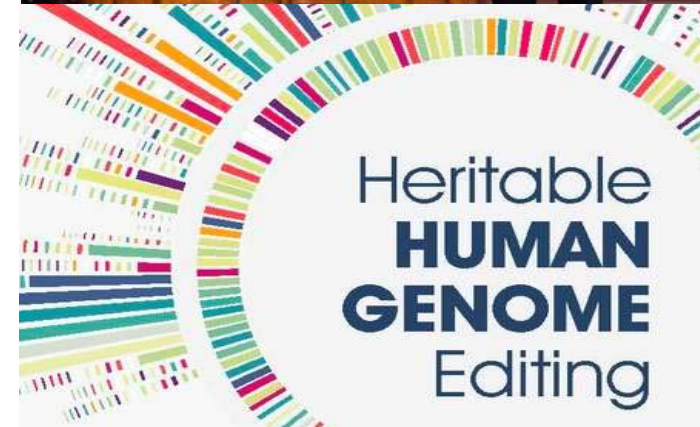


Where CRISPR Plasmids Were Distributed To



- **Ethics**
 - Editing the human germline
 - Pace and scale and accessibility
 - Dialogue framework and moratorium
- **Public relations**
 - GMO concerns
 - Oxford debate
 - Voices and stakeholders
- **Regulatory Path(s)**
 - Medicine vs. Ag
 - Risk:Benefit
 - DNA-free, non-GMO options

Societal implications



THE HUMAN GAME

N SERIES unnatural selection

HUMAN NATURE



Narratives and dialogues

HUMAN NATURE

DAVID BALTIMORE
Cal Tech

JILL BANFIELD
UC Berkeley

RODOLPHE BARRANGOU
NC State University

ALTA CHARO
U of Wisconsin – Madison

EMMANUELLE CHARPENTIER
Max Planck Institute

GEORGE CHURCH
Harvard University

GEORGE DALEY
Harvard Medical School

JENNIFER DOUDNA
UC Berkeley

HANK GREELY
Stanford University

IAN HODDER
Stanford University

STEPHEN HSU
Genomic Prediction

KELSEY MCCLELLAND
DNA Dialogue

FRANCISCO MOJICA
University of Alicante

RYAN PHELAN
Revive and Restore

MATT PORTEUS
Stanford University

ANTONIO REGALADO
MIT Technology Review

DAVID SANCHEZ
Sickle cell patient

SYNTHEGO
RNA synthesis company

FYODOR URNOV
Altius and IGI

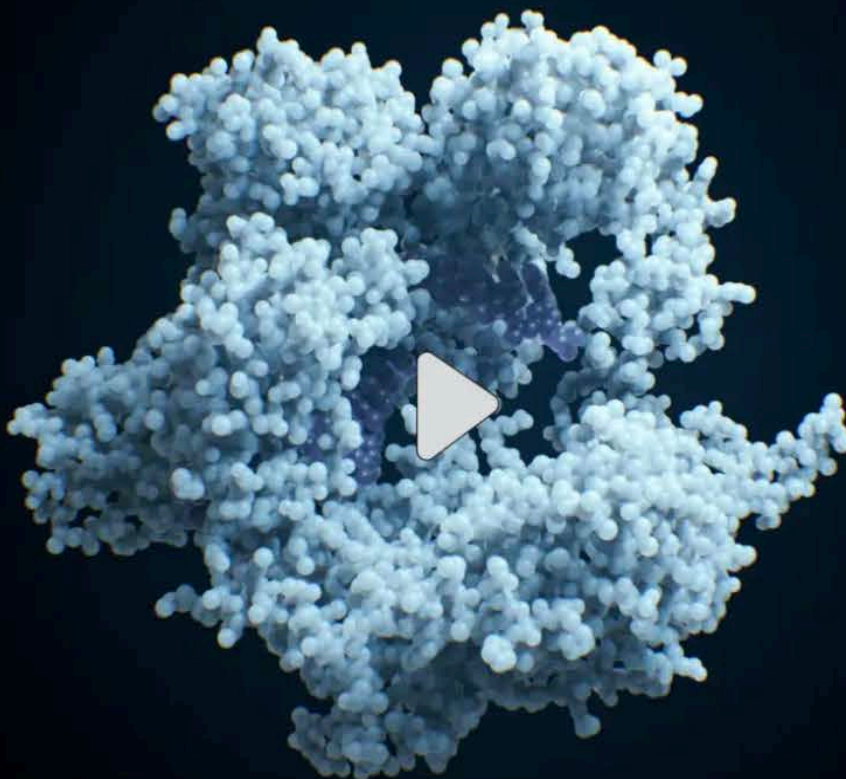
ETHAN WEISS
Ruthie's father

PALMER WEISS
Ruthie's mother

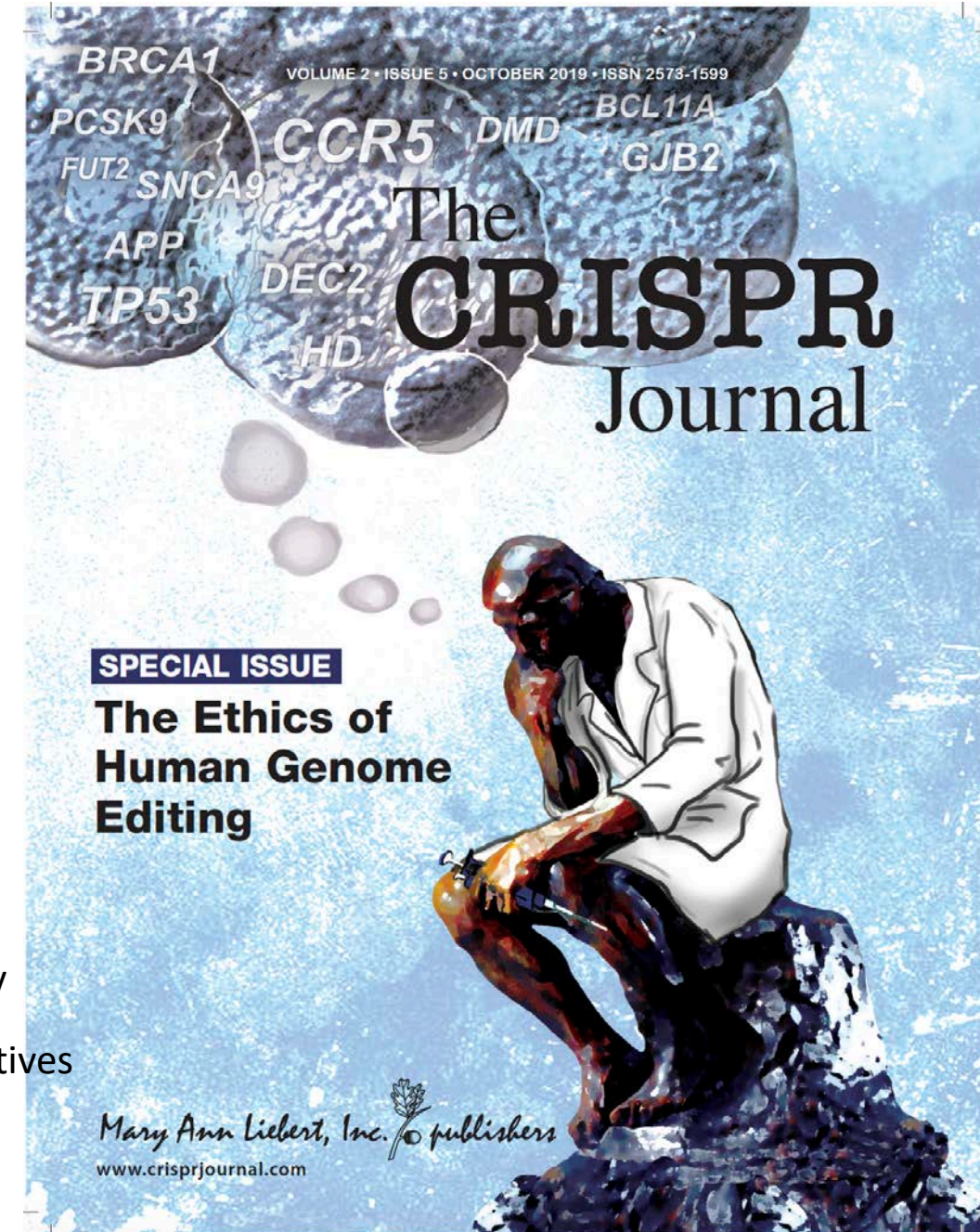
RUTHIE WEISS
5th grader

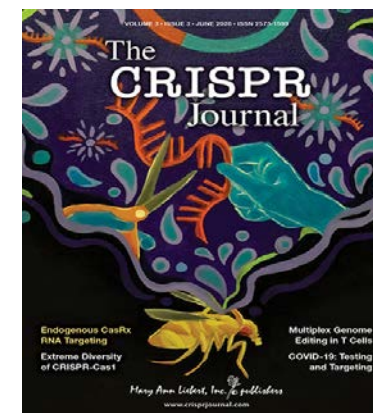
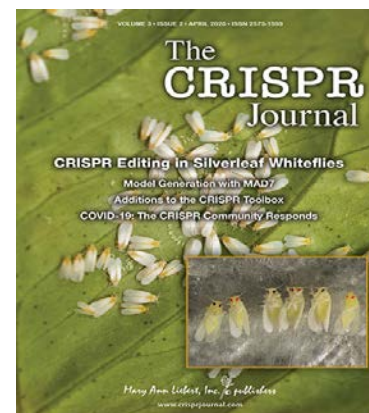
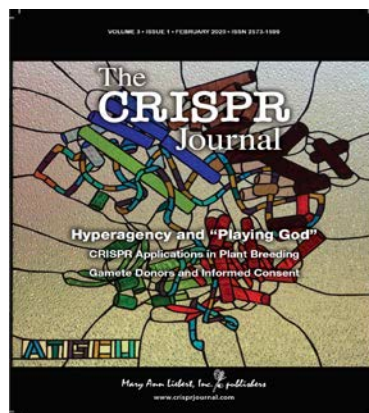
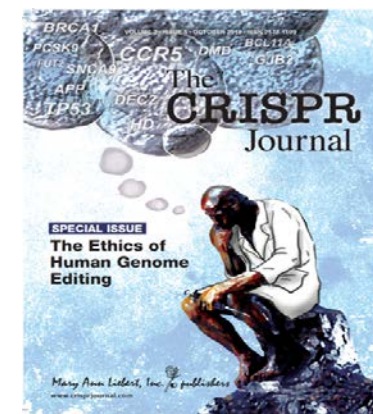
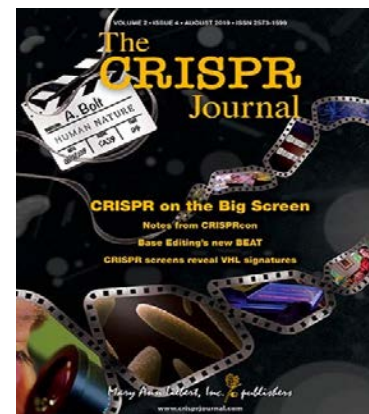
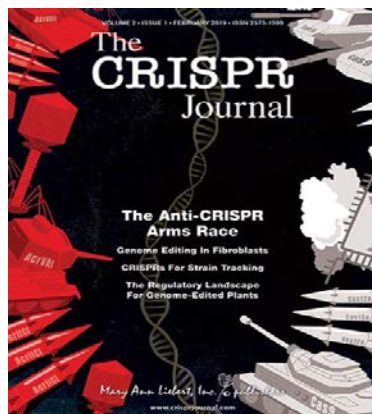
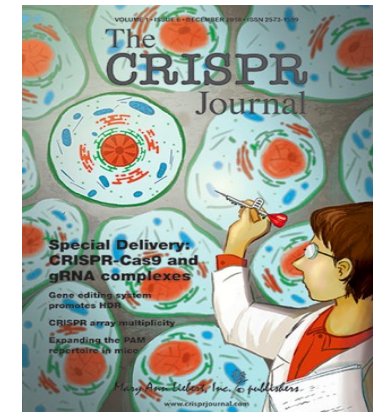
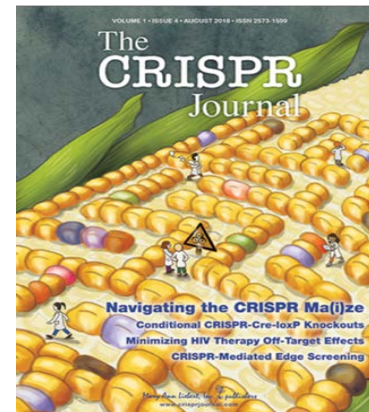
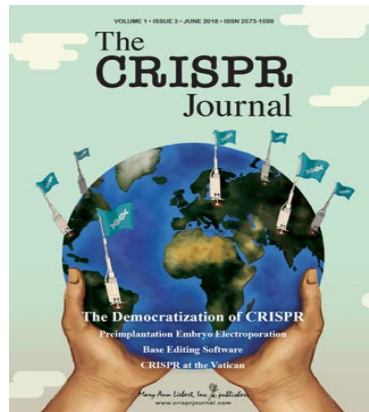
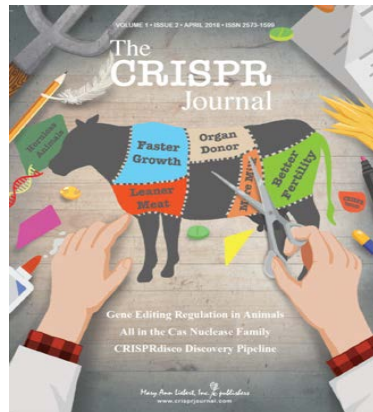
LUHAN YANG
e-Genesis

FENG ZHANG
Broad Institute



- Greely: ethically defensible, now new, not soon
 - Hurlbut: 6 principles for governance*
 - McIntosh: the downside of a global moratorium
 - Carroll: the daunting economics of gene therapies
 - Knoppers: future children rights to health
 - Alpern: human rights impact assessment
 - Sherkow: regulating through law and legal regimes
1. Make room for greater diversity in posing and framing questions
 2. Ask about the purposes of research before next steps are taken
 3. Do not champion self governance by scientists
 4. Reflect on the global nature of human values, especially human integrity
 5. Rein in the language of “running ahead” to account for broader perspectives
 6. Consider researchers’ intentions along with their practices





The CRISPR J

- Values (stewardship, safety, efficacy)
- Trustworthy voices (farmers, scientists)
- Environmental benefits (sustainability, resilience)
- Consumer benefits

GeneEditing.FoodIntegrity.org

WHO DO
CONSUMERS
TRUST
FOR INFO
ON GENE
EDITING

TRUST

HIGH

LOW

SCIENCE
LEADERS

SCIENTISTS

ACADEMIC
INSTITUTIONS

REGULATORY
AUTHORITIES

FARMERS

NUTRITIONISTS

MEDICAL
CONTACTS

AG COMPANIES
THAT DEVELOP
PRODUCTS

COMPANIES/
RETAILERS THAT
SELL FOOD

WHICH
OF THE
FOLLOWING
BENEFITS
ARE MOST
IMPORTANT
FOR
AGRICULTURE
TO DELIVER
THROUGH
GENE
EDITING?

IMPORTANCE

MOST

LEAST

ENVIRONMENTAL
STEWARDSHIP

DISEASE
RESISTANCE

NUTRITIONAL
BENEFITS

TASTE

SHELF LIFE

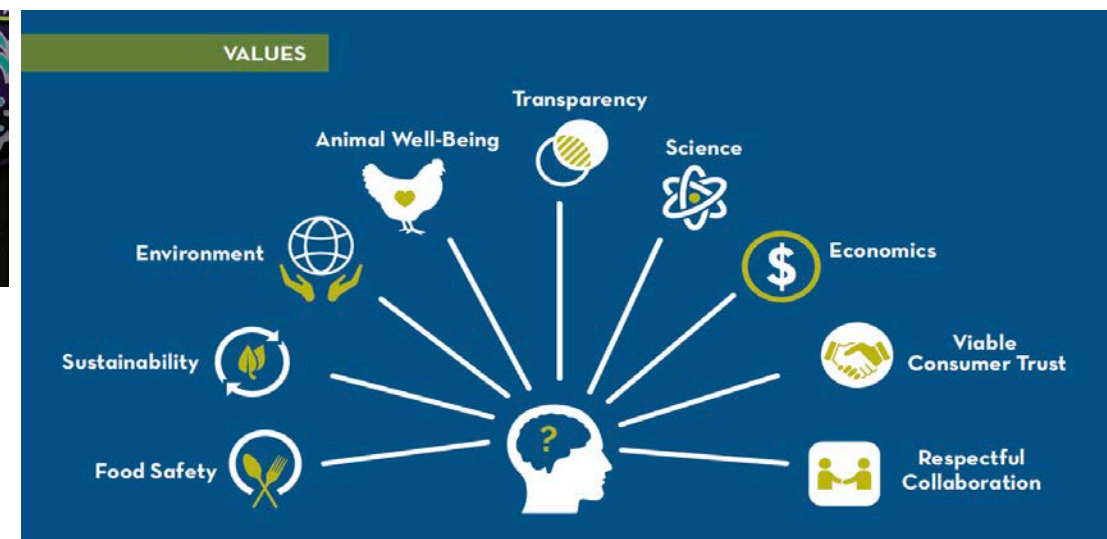
CHOICE
& VARIETY

"COSMETIC"
CHANGES

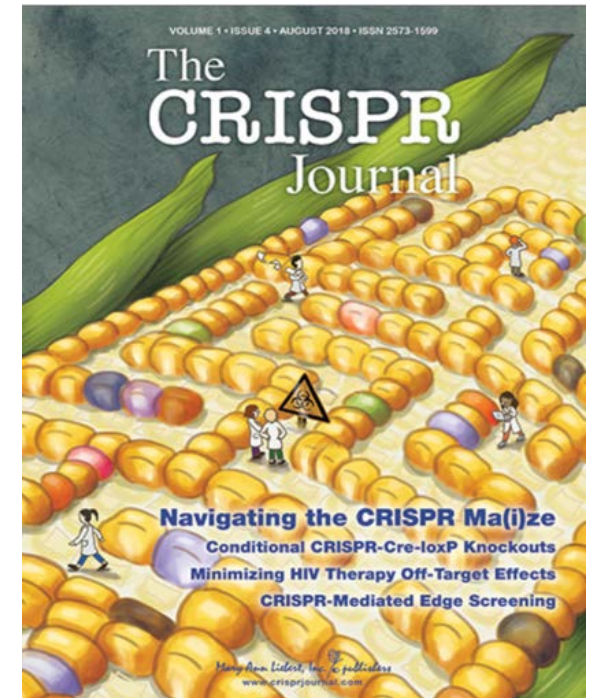
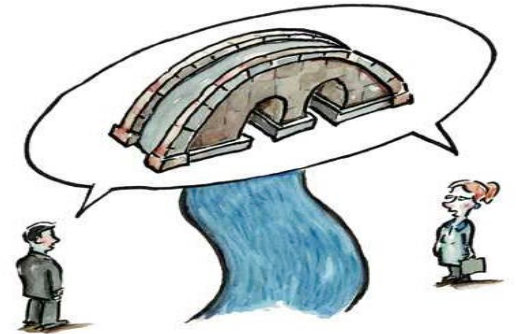
FEEDING
THE WORLD



Framing the dialogue



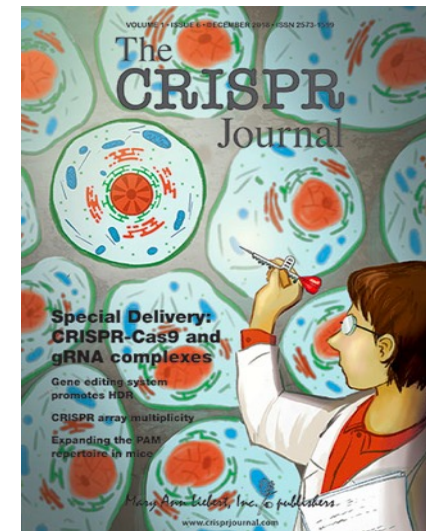
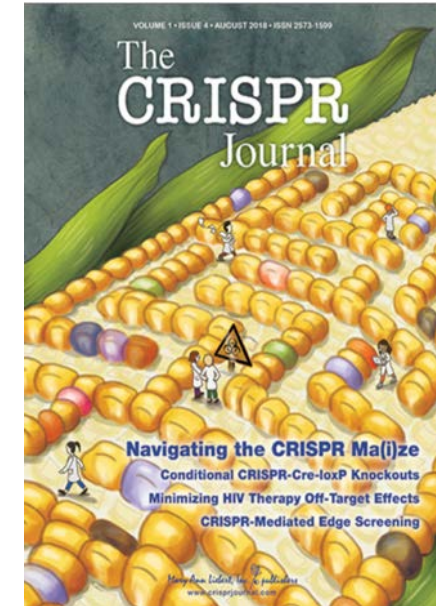
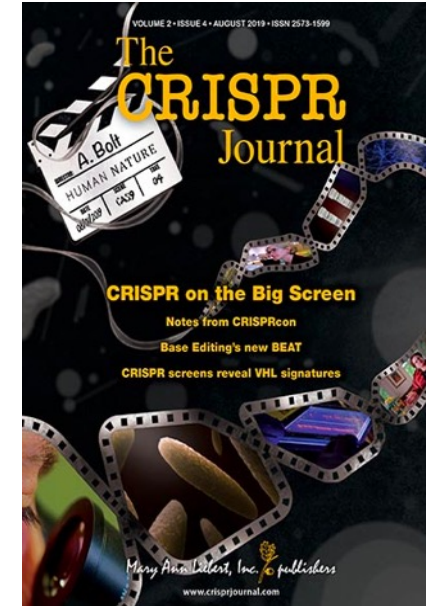
- Ethical issues focused on human germline
- IP wars focused on human therapies; cross licensing pool
- Technical access and implementation feasibility (delivery)
- Recapitulation of natural diverse genotypes
- Early regulatory indications (USDA vs. ECJ, SECURE)
- Use of RNPs and non-foreign DNA implications (DNA-free)
- Non-DNA editing alternatives (CRISPRi|a|e|epi)
- Ability to screen (select phenotype, screen genotype)
- Breadth of applications (foods vs. animals, vs. plants vs. trees)
- Speed of execution, costs, and process timeline
- No concerns about toxicity and immune response



Why Ag is poised to win the CRISPR race

- Clinical success (PR opportunity)
- Public enthusiasm (tech acceptance)
- Europe catches up (rebalancing and shift)
- Put tools to use (industry fuel)
- Beyond Tx (Ag consolidation and diversification)
- Cooler heads prevail (IP and beyond)
- Business deals, M&A
- Responsible guidelines (SECURE rule)
- CRISPR fatigue (tech to products)
- Global geopolitical games (US vs CN)

**Foresight is 2020:
Ten Bold Predictions for the New CRISPR Year**



- Redistribution
- Consolidation
- Communication
- Diversification



USA to EU > USA = CN

big 6 down to 4

re-branding

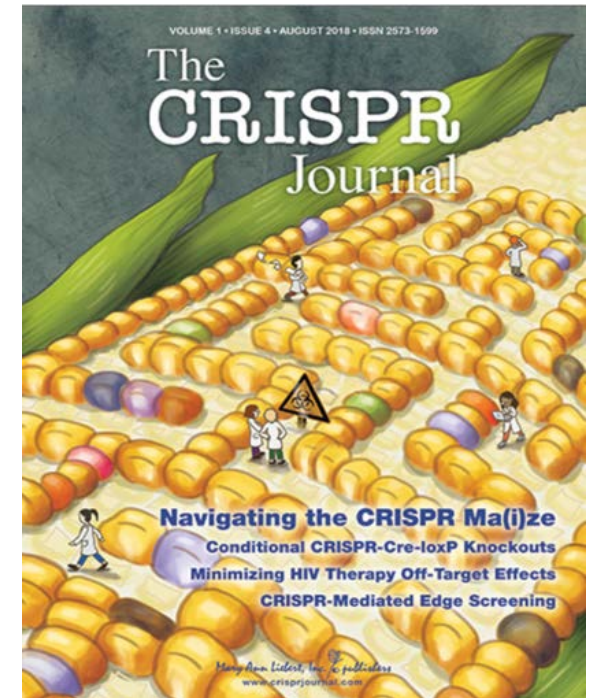
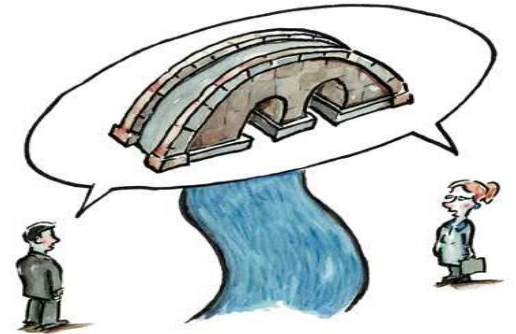
GM traits vs breeding

chemicals vs biologicals

big vs nimble

dominant vs strategic

new ventures & partners



The shifting Ag landscape

To Know

- Beyond Tx
- Disruptive tech
- Democratized
- Tech push & market pull
- 2020 is pivotal

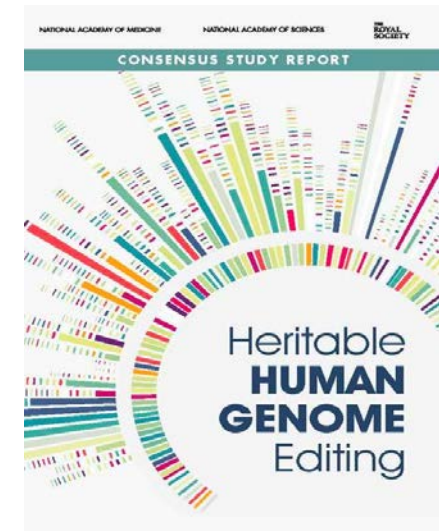
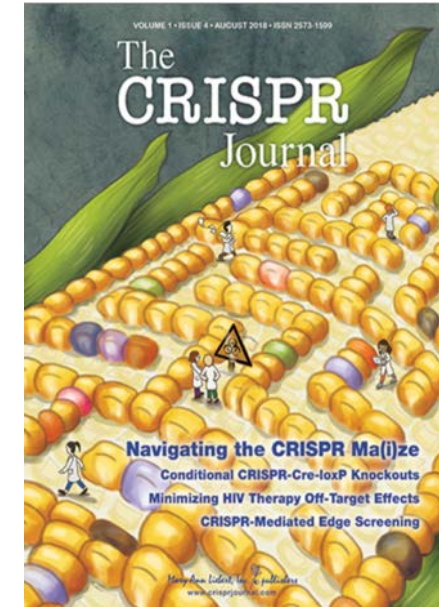
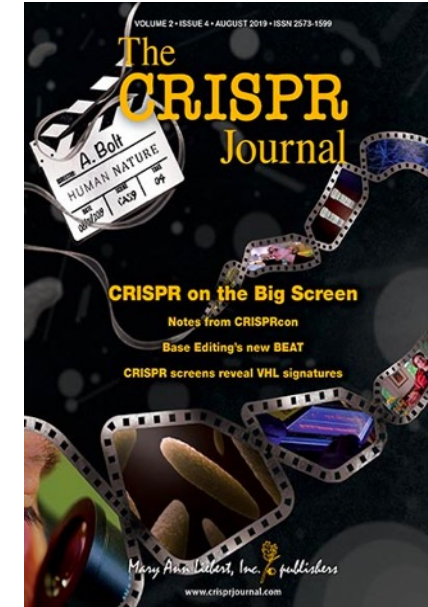
To Ponder

- Acceptance bottleneck
- Tip of the iceberg
- Navigate the nexus
- Science comm. 2.0
- Highway of science

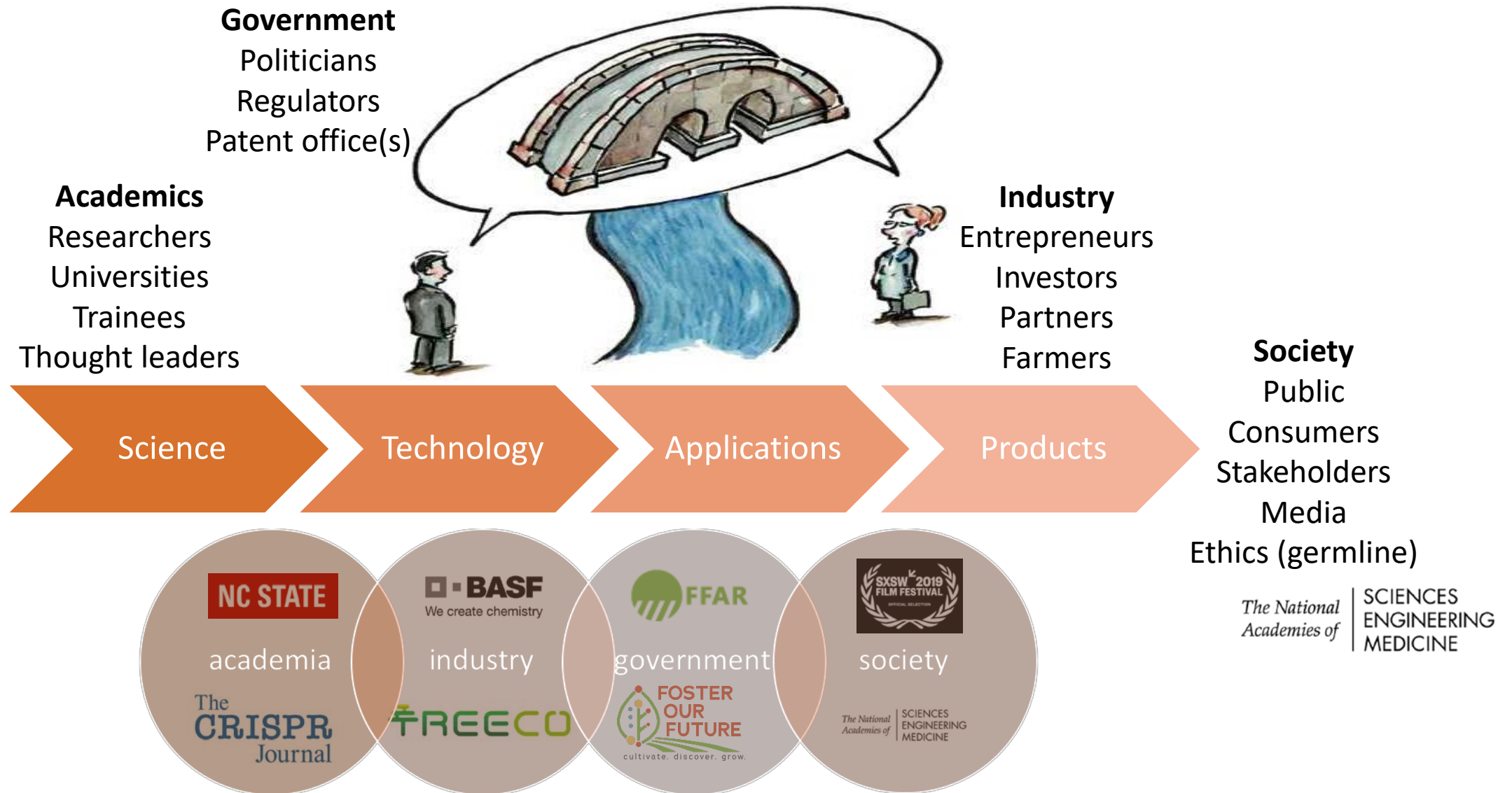
Sci Comm

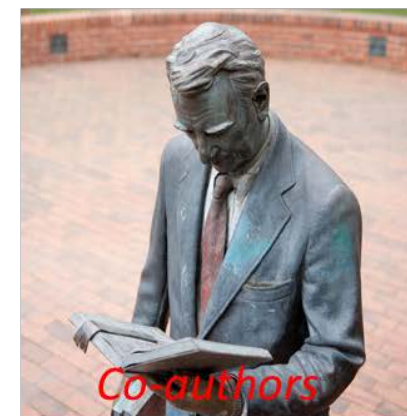
- Skepticism & Trust
- Transparency & RRI

Thinking about CRISPR



Enabling a competitive bio-economy



Researchers*Inventors ORC IP**Teachers**Talent developers**Speakers**PR-ambassadors**Entrepreneurs**Founders**Learners**Recruits & alumni**Coaches**Advisors**Writers**Co-authors**Administrators**Advocates KOLs*

Challenging times & inclusive excellence



Funding & collaborations



NC Agricultural Foundation, Inc.



COIs

