

Precision Genome Engineering for a Bright Food Future

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Genomics**

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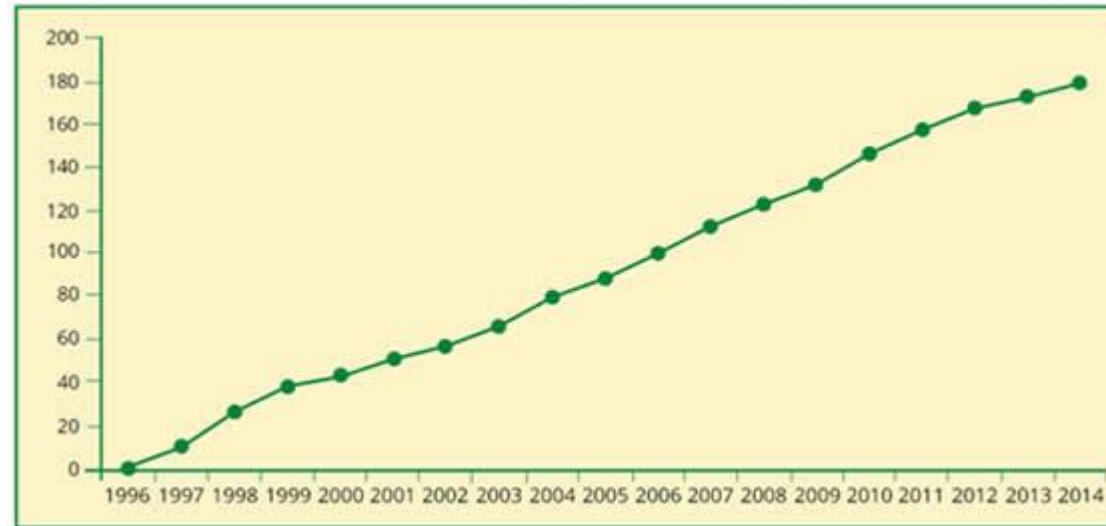
1976



2016

Genetic modification is one of the most rapidly adopted technologies in the history of agriculture

Global Area of Biotech Crops, 1996 to 2014 (Million Hectares)



Source: Clive James, 2014/ISAAA



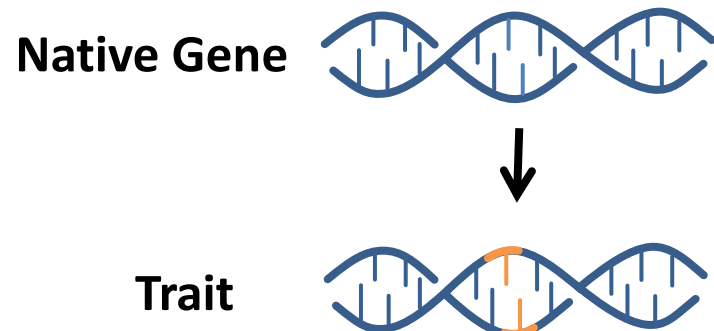
However, GM crops are still grown on only slightly more than 10% of cultivated land



Genome editing is the direct engineering of the plant's own genes

ARCUS Precision Breeding

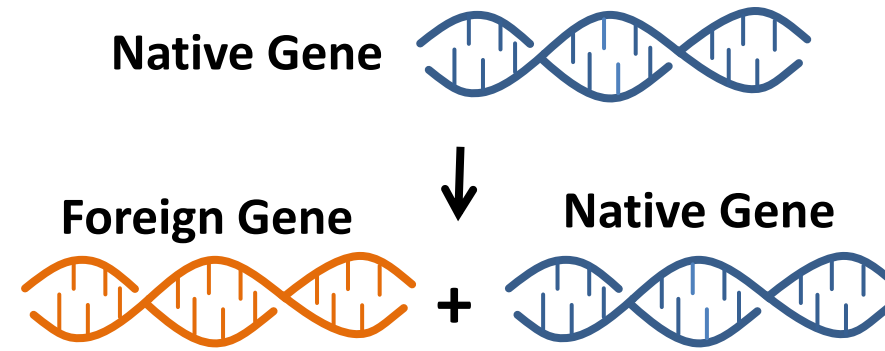
6 – 8 years, <\$10M



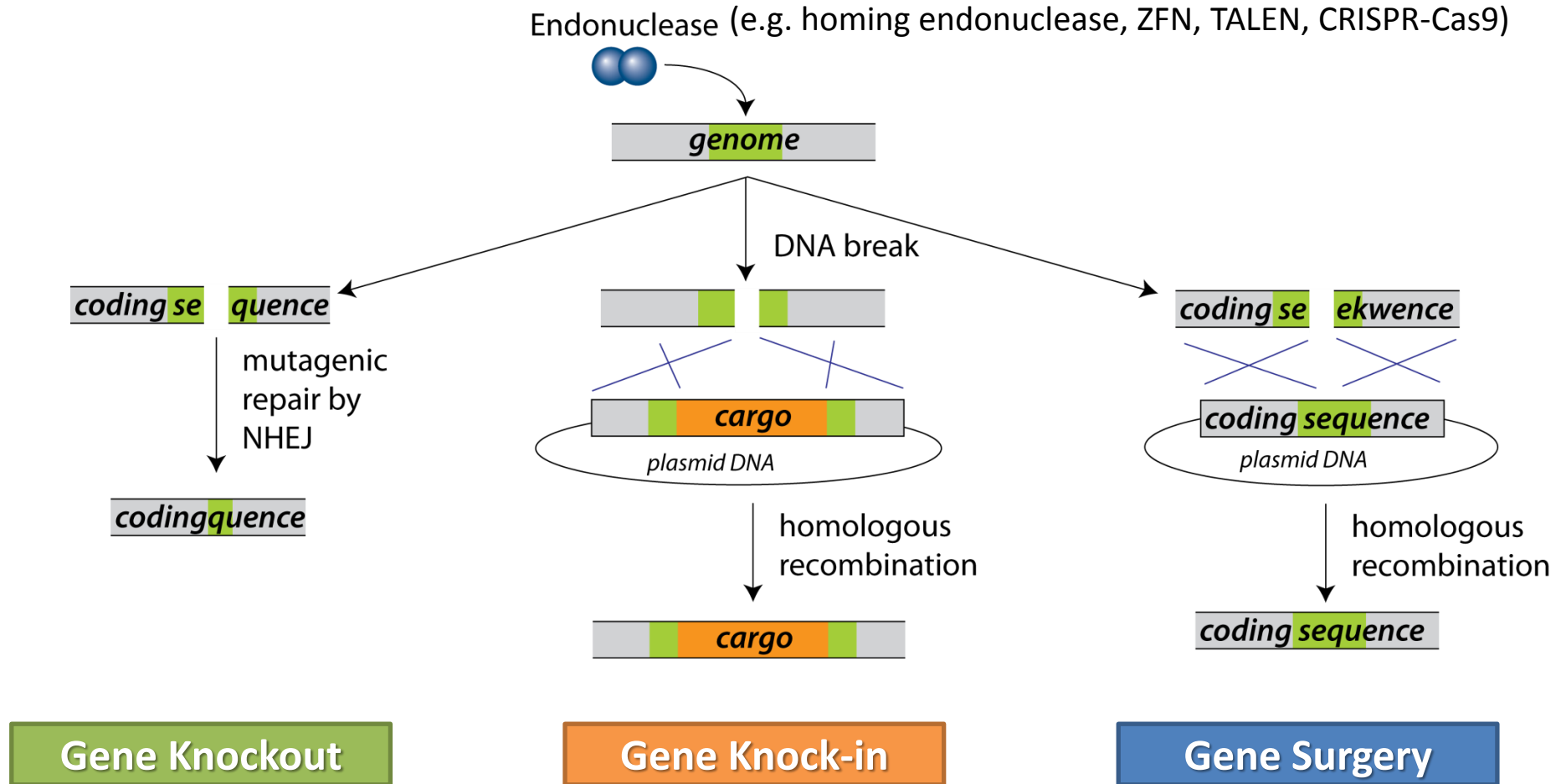
- ✓ Fast
- ✓ Precise
- ✓ Cost effective
- ✓ Non-GMO

Traditional Transgenic

12+ years to market, >\$100M

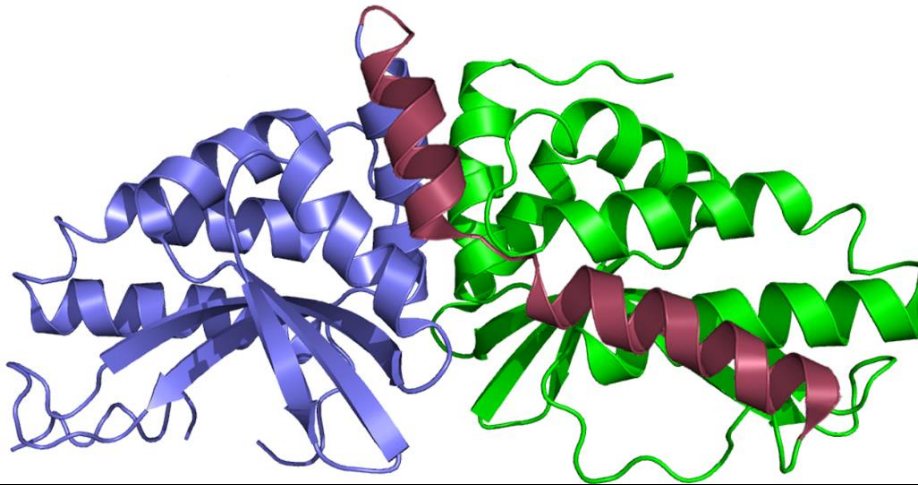


- Lengthy
- Random
- Expensive
- Public concern



ARCUS Genome Editing

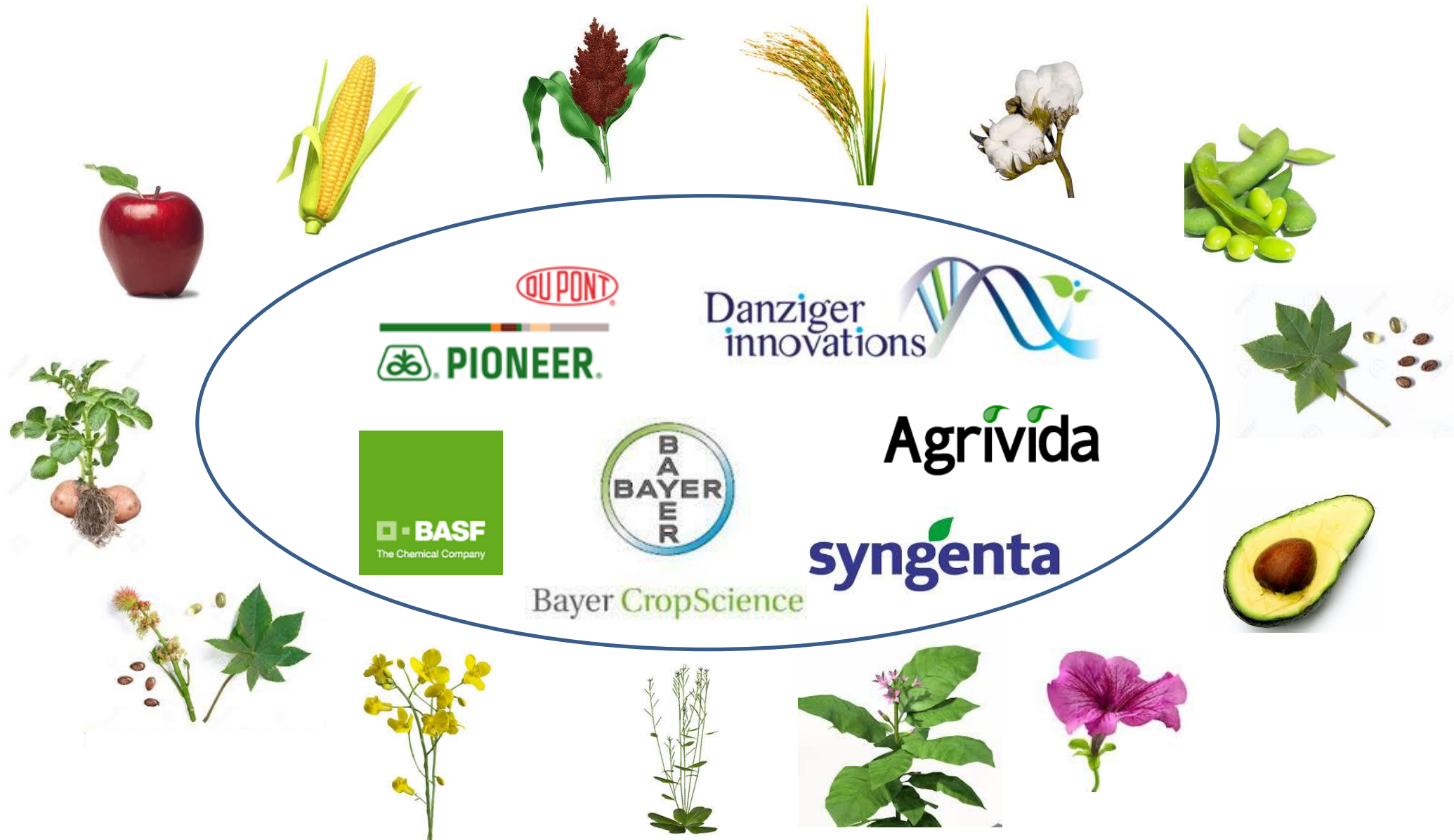
ARC Nuclease



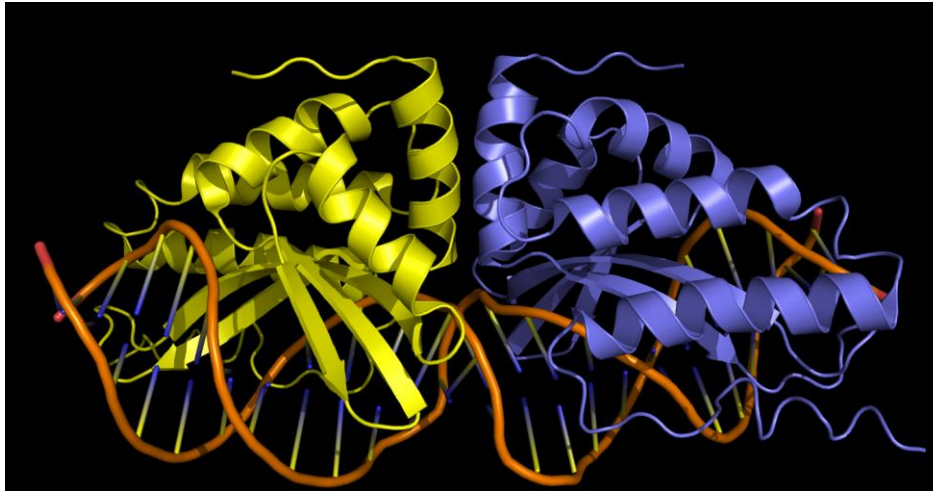
Advantages to ARCUS Genome Editing

- ✓ The only proprietary, wholly owned gene editing platform in the industry
- ✓ Unmatched selectivity, minimizing off target cutting and effects
- ✓ Delivered as a single <1kb gene, allowing for multiplex applications
- ✓ Promotes homologous recombination, enabling efficient targeted gene insertion

10-Year Track Record of Successful Partnerships



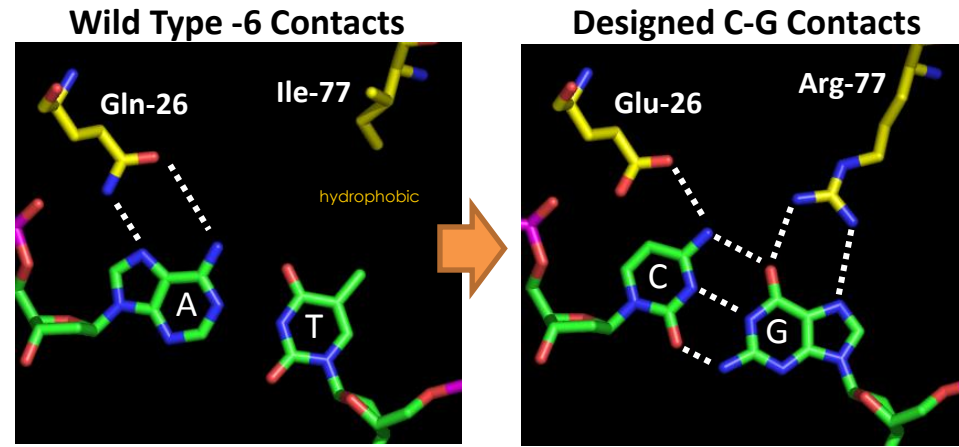
I-Crel Serves As Basis For The ARCUS Platform



CAGGCTGTC GTAC GACAGCCTG
GTCCGACAG CATG CTGTCGGAC

- Homing endonuclease from *C. reinhardtii*; serves as basis for ARCUS design platform
- Recognizes a 22 bp target site
- Protein monomers recognize individual half-sites
- Customizable redesign to target new 22-mers
- **Extremely high level of specificity** to prevent off-target genome cutting
- Generates a 3' overhang, facilitating homologous recombination

in silico design (2004-2012)



A unique asset 8 years in the making: Precision's design database

Number of models: **5749**

Number tested experimentally: **3220**

Number delivered to partners: **67**

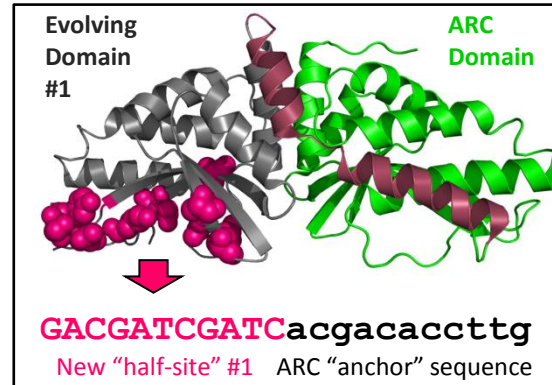
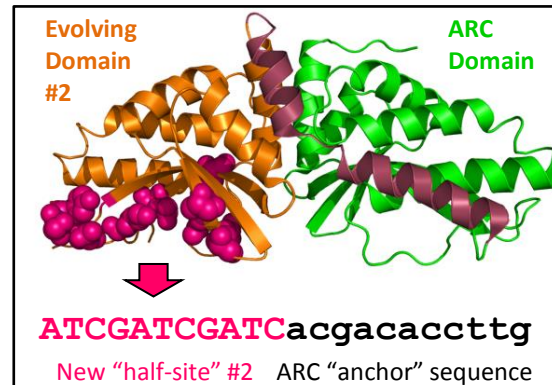
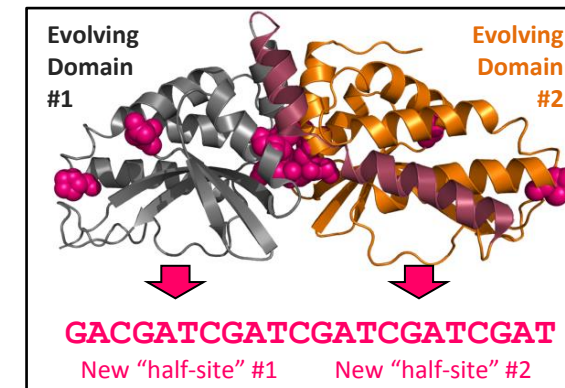
Historically:

- I-CreI variants modelled on the computer and then cloned and tested experimentally
- Established a market with technology developers in food/ag
- Up to 6 months and \$100K to make one meganuclease
- Limited range of DNA sequences that could be targeted

ACCATCGACGATCGATCGATCGATCGATCGA
TCGATGCATCGATCGATCGATCGACGATCGA
TCGACCGTAGCTAGCACGATGCATCGATCGA
TCGATCGATCCGTAGCTAGCACGTAGTGTAG
CTGATCGTACAGCTGTAGTGTGATCACGGGG
ACGAGTCGTATTGCTAGTGTAGCTAGTCTA
GTCGGATGTCACGATGCTAGGCTGATGCGCT
ACCATCGACGATCGATCGATCGATCGATCGA
TCGATGCATCGATCGATCGATCGACGATCGA
TCGACCGTAGCTAGCACGATGCATCGATCGA



1. **GACGATCGATC GATCGATCGAT**
2. CGATCGATGCATCGATCGATCG
3. ATCGACGATCGATCGACCGTAG
4. CTAGCACGATGCATCGATCGAT
5. CGATCGATCCGTAGCTAGCACG
6. TAGTGTAGCTGATCGTACAGCT
7. GTAGTGTGATCACGGGGACGAG
8. TCGTATTGCTAGTGTAGCTAG



Validation of two-domain nuclease

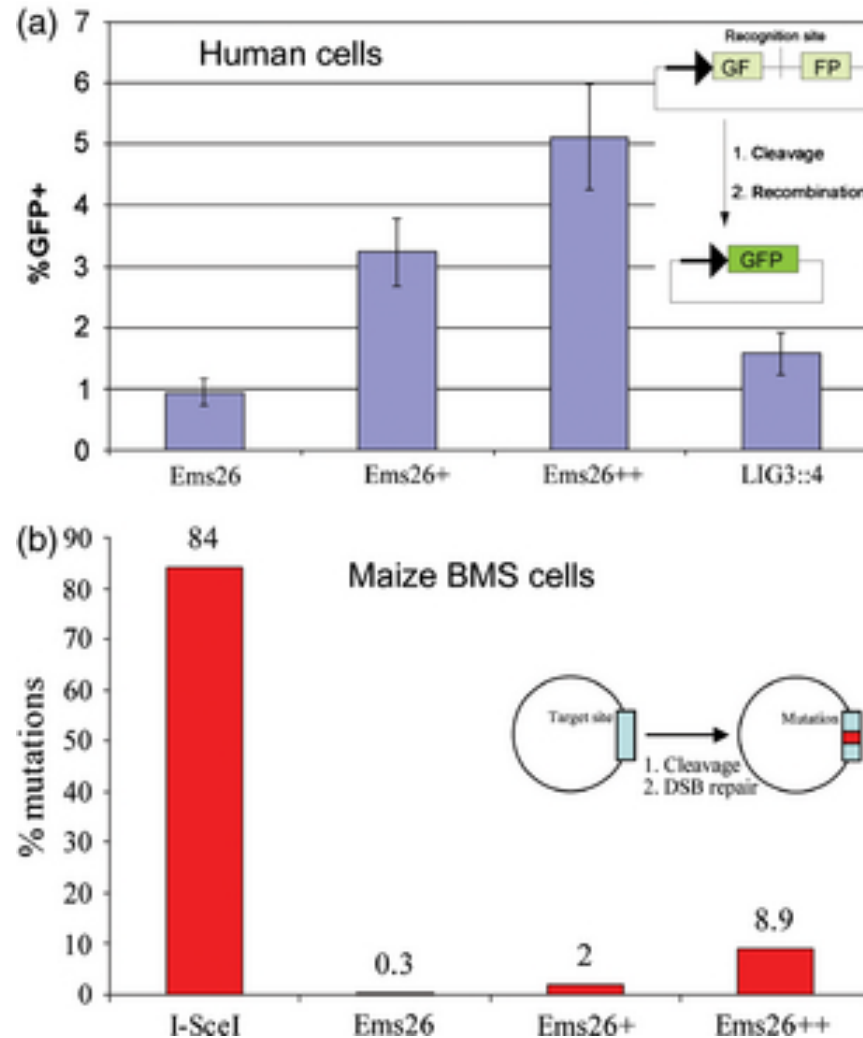
① Select target sites

② Evolve nuclease domains

③ Validate nuclease

Nucleases are designed using a bacterial selection scheme

Nuclease Activities Are Validated *in vivo*



- Human cell line carrying a split GFP gene flanking the nuclease target site
 - Nuclease transfection reconstructs the GFP
-
- Nucleases expressed in a plant cell line harboring a target site plasmid
 - Mutation frequency determined by sequencing



Medford, MA

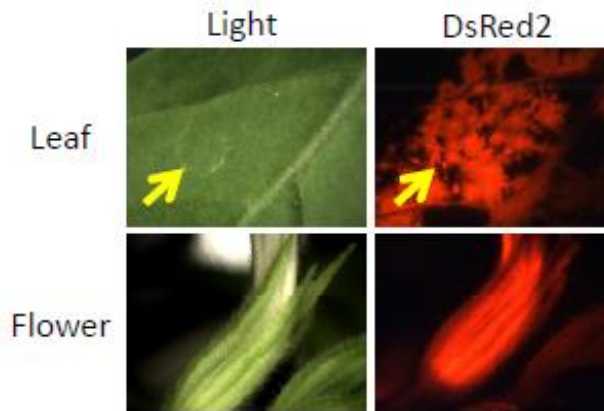
Focus: Plant gene expression technology for animal nutrition

Goal: Increase corn leaf starch content

- Designed two ARCUS meganucleases to knock out a starch pathway regulatory gene
- Transformed embryogenic callus using Agro and recovered T0 plants
- Recovered both mono- and bi-allelic mutations at high frequency

Corn Events	Wild-type	Hemizygous KO	Homozygous KO	# of Unique Mutations
88	31	14	43	26
Leaf Starch Content →	0.5%	3%	20%	

Transient Delivery of ARCUS Nucleases



- Targeted one of two DFR reductase genes (leucoanthocyanadin pathway) in *N. alata*
- Expressed nuclease + DsRed from tobacco rattle virus vector
- Monitored viral spread with DsRed



Wild-type

Knock-out

- DFR indels were observed in all inoculated plants
- Flowers showed a 30% reduction in anthocyanin content
- TRV not passed thru meiosis

PRECISION
BIOSCIENCES



Target line (GHB119)

Dralll ↓ 1561bp 2072bp RB cry2Ae bar LB ↓ Stul

IB616 2992 bp IB527

4.5 kb

Repair DNA (pCV211)

COT-5/6 + 1561bp epsps hppd 2072bp

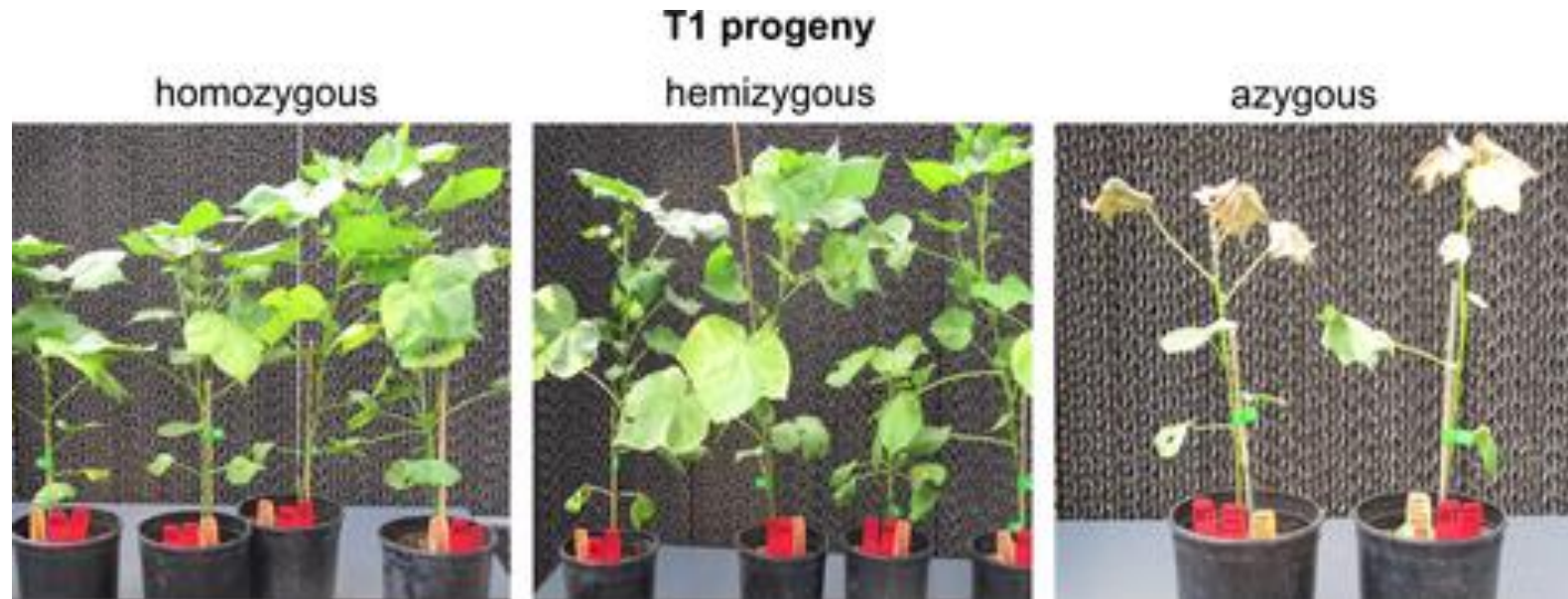
Stacked event

Dralll ↓ 1561bp epsps hppd 2072bp RB cry2Ae bar LB ↓ Stul

IB616 9639 bp IB617 IB527

VDS406 2731 bp IB589 3893 bp

11 kb



- Mendelian segregation of the molecular stack was demonstrated in T1 generation
- The engineered events displayed tolerance to 200g/Ha tembotrione (HPPD inhibitor)
- Most events showed no evidence for additional, non-targeted DNA insertions

Funding

- 2015: \$25.6M Series A financing: venBio, F-Prime, Amgen, Baxalta, Osage, others
- 2016: **Baxalta** collaboration in cancer immunotherapy
\$105M upfront with up to \$1.6B in option fees, milestones + royalties
- Precision PlantSciences: Multiple revenue-generating partnerships

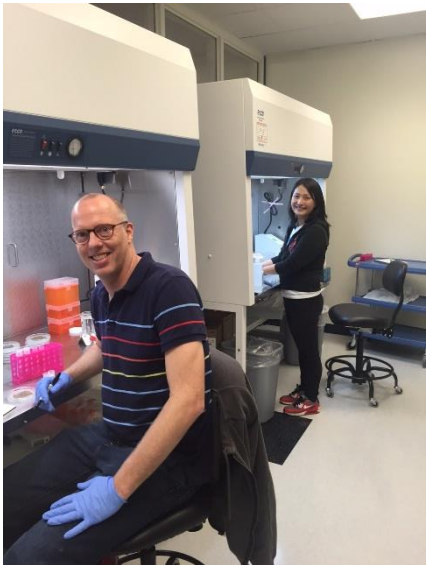
Industry Leader

- Early innovators in plant genome engineering, from 2006
- Team of thirty five, doubling in 2016
- Dominant IP position with clear and tested freedom to operate



Focus

- Open to all crops, traits, markets
- Areas of interest
 - Consumer traits
 - Specialty crops
 - Animal production and animal health
 - Adjacencies: bioinformatics, phenotyping, agronomy

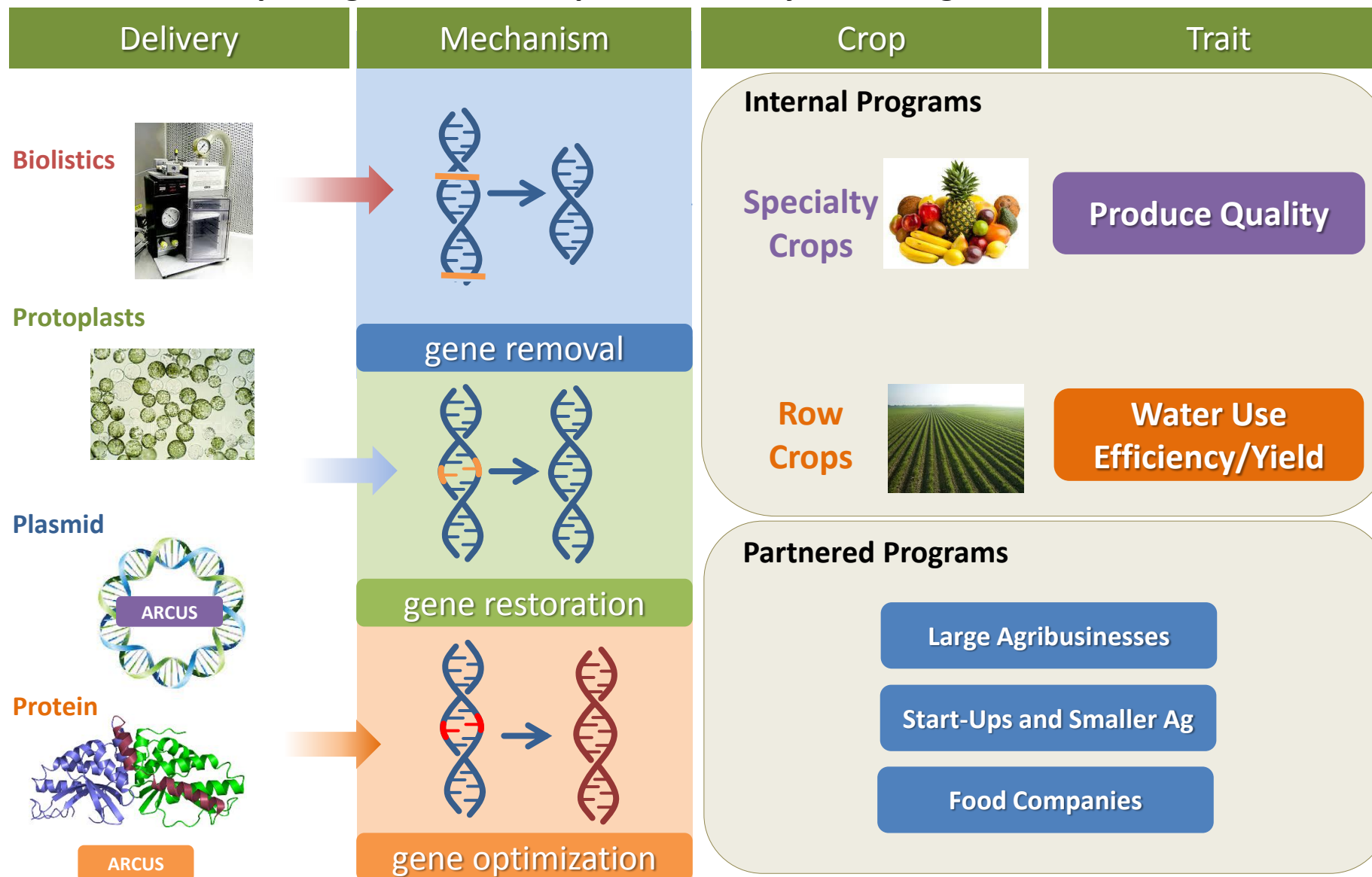


	Target Gene	Nuclease Design	Event Generation	Event Selection	Commercial-ization
Traditional	Partner		Partner	Partner	Partner
New	Partner			Partner or 	Partner

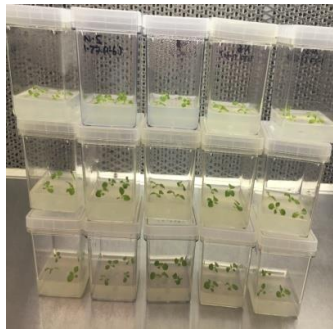
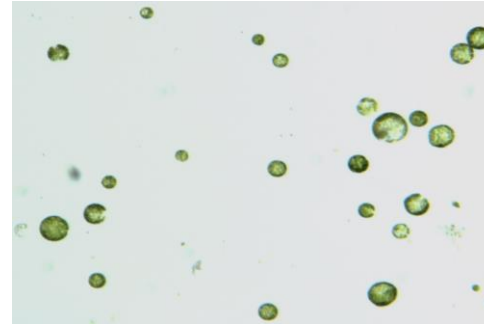
ARCUS Precision Breeding for Food and Ag



R&D focused on pairing ARCUS with optimal delivery technologies



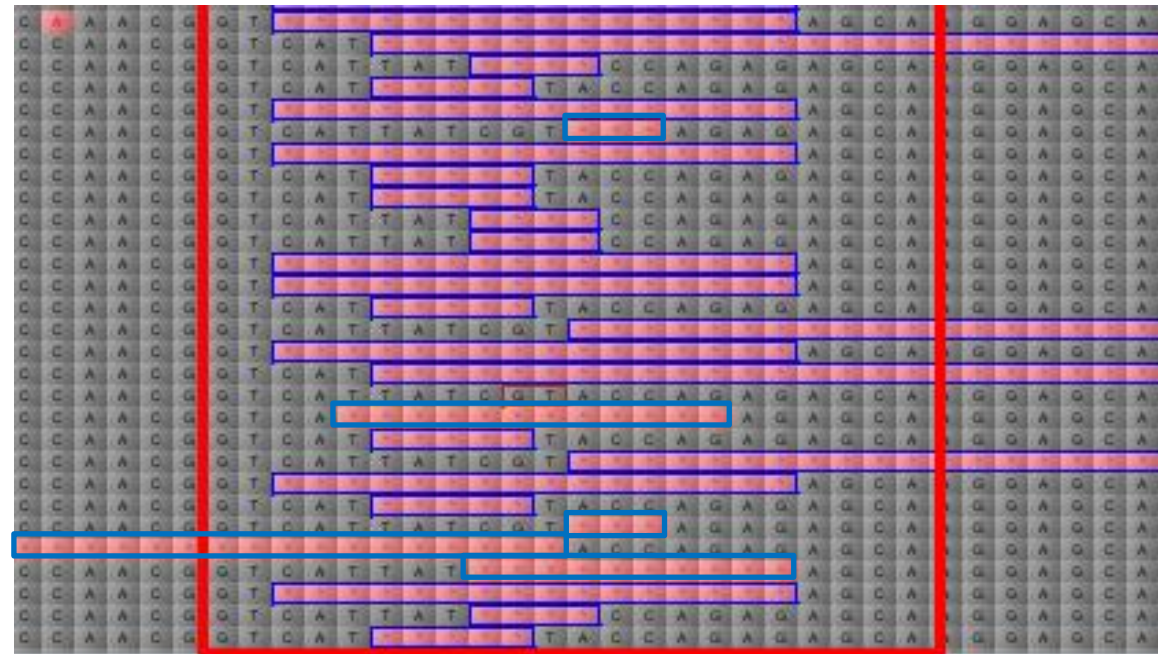
Editing the Tobacco Nicotine Pathway



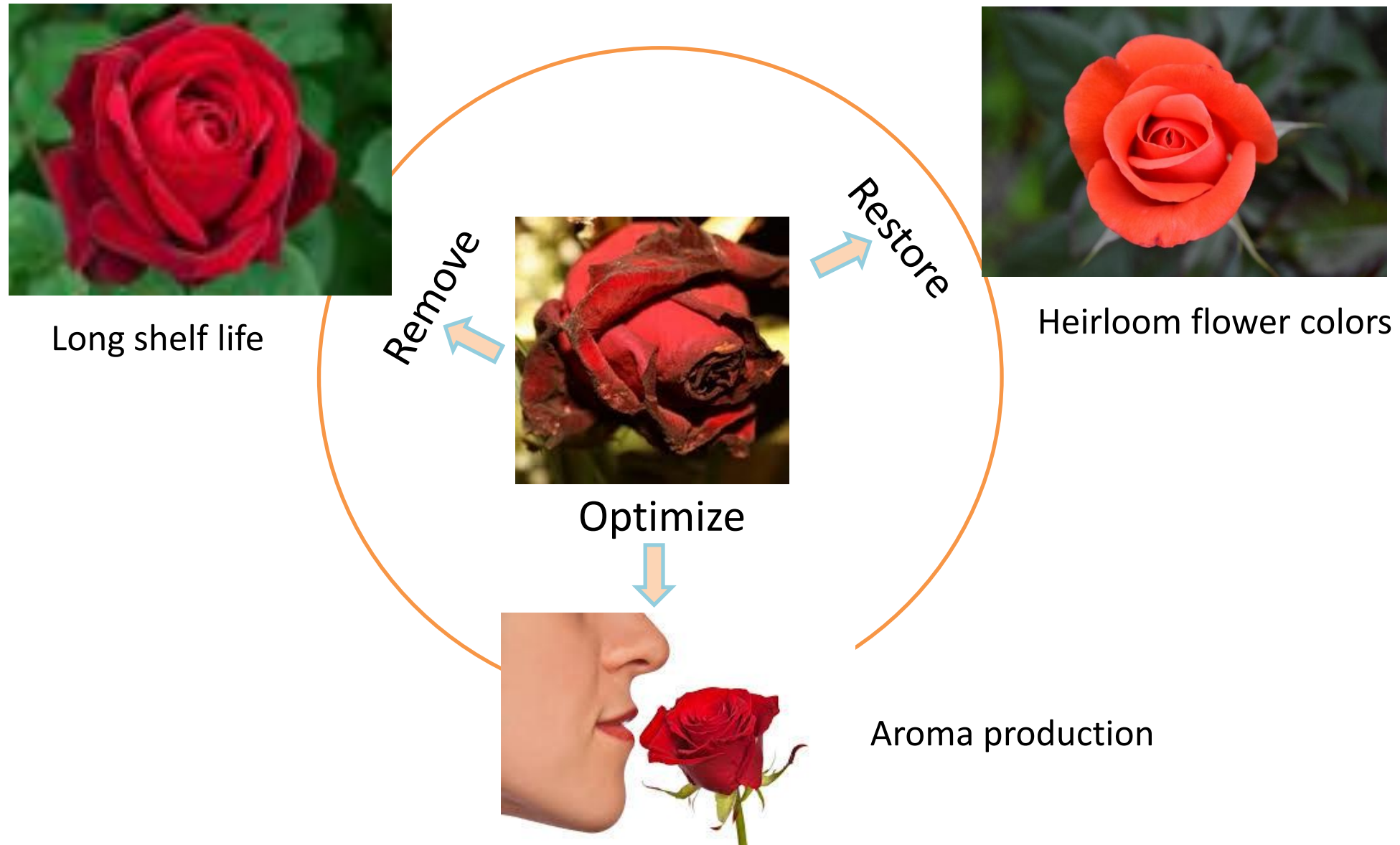
Introduce
ARCUS
Nuclease



Deletions (indicated
by pink boxes) in the
BBL-1 gene recovered



Gene Editing Opens New Product Opportunities





www.precisionbiosciences.com