Transgene (DNA)-Free Genome Editing

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Generation of Biotechnologies

Refers to different eras in biotechnological techniques & applications

First Generation of Biotechnology

Initial phase in the use of biotechnological techniques

Key aspects

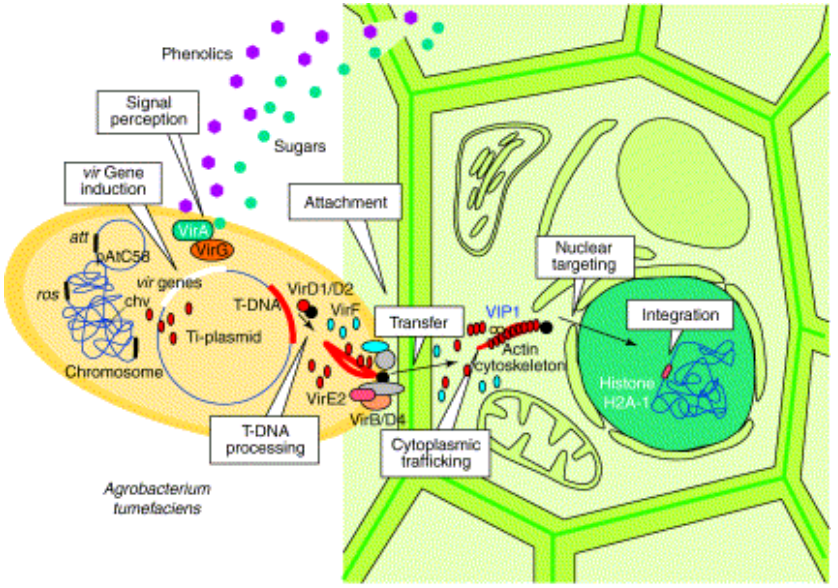
- ❖ Application of traditional breeding methods combined with emerging molecular biology techniques

- ❖ Development of Tissue Culture Techniques

Early Genetic Engineering

- •Genetic Transformation
- •Development of Transgenic Plants

www.plantcelltechnology.com



Gelvin, 2003

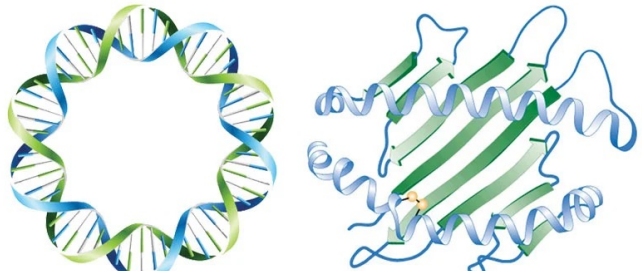
Second Generation of Biotechnology

Represents more sophisticated techniques and applications, particularly in genetic engineering and molecular biology.

Azevedo & Arruda, 2010

Key Aspects

Advanced Genetic Engineering- Creation of genetically modified organisms (GMOs) with specific traits and more complex manipulations of DNA, like gene silencing & gene editing



microbenotes.com

Proteomics and Genomics- Study and manipulation of entire genomes and proteomes for understanding complex biological processes and finding novel traits to control diseases and pests

https://cb.iiitd.ac.in/

Bioinformatics and Computational Biology- Using computational tools to manage and analyze biological genomics and metabolomics data and understanding biology

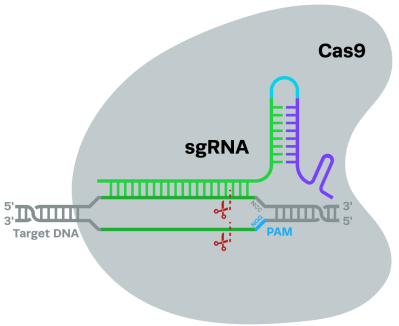
Third Generation of Biotechnology

Represents an even more advanced phase in the evolution of biotechnological applications, with cutting-edge techniques and innovative approaches.

Key aspects

Advanced Gene Editing Technologies

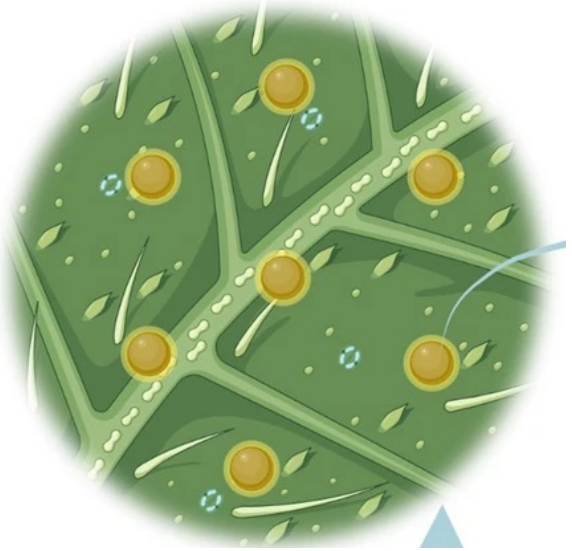
CRISPR-Cas9 become more refined, allowing for targeted gene editing with higher accuracy and fewer off-target effects.



https://www.synthego.com/

Biofortification

Metabolic Engineering

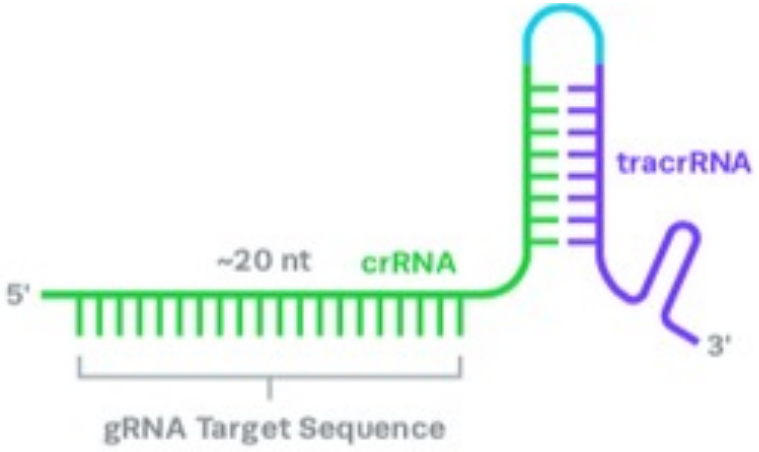


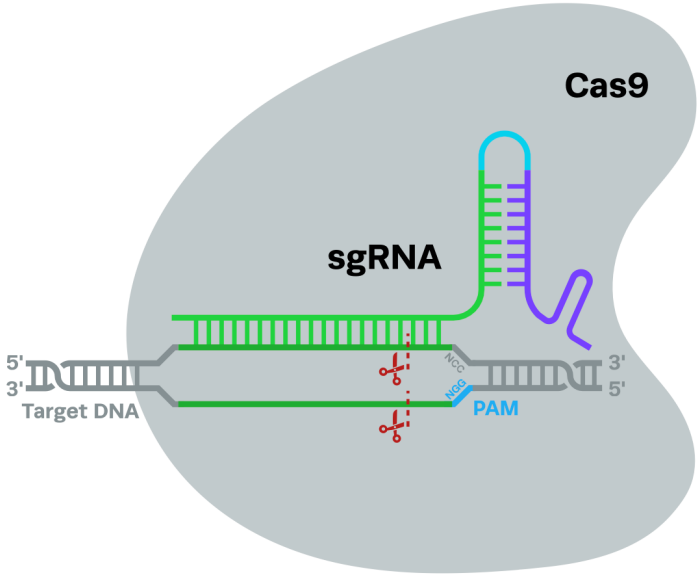
Jiang et al. 2021

Use of NanoPhytoBiotechnology

New Breeding Techniques (NBTs)-Targeted Gene Editing

- Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein-9 (CRISPR-Cas9)
- Directing a guide RNA (gRNA) complementary to a defined target, together with a CRISPR-associated endonuclease, Cas9, to create double-stranded DNA cleavage
- gRNA: crispr RNA (crRNA), a 17-20 nucleotide sequence complementary to the target DNA, and a tracrRNA, which serves as a binding scaffold for the Cas nuclease





https://www.synthego.com/

Where to start……

Method

Crop?

Tissue culture?

Gene Information and gRNA Design?

Mutant screening?

Regulation?

Rapeseed

- Third-largest source of vegetable oil after soybean and palm oil
- The terms “rapeseed”, “canola”, “mustard” interchangeably used in different continents
- In Europe, it is known as rape and oilseed rape
- In India, use of rapeseed oil in lamps since 2000 BC
- In Canada, lubricant for war ships (1936)
- In Europe, making food and soap since 13th century
- Rapeseed (traditional name) - group of oilseed crops in the Brassicaceae family, and naturally contains high amounts of erucic acid (>45%) and glucosinolates
- ‘Canola’ refers to the edible oil crop - low erucic acid (less than 2%) and <30 µmol/g of dried defatted meal of glucosinolates -developed via traditional crossbreeding

Genome organization

- Rapeseed, *Brassica napus* subspecies, *napus*, is a large winter or spring annual oil crop
- Rapeseed is related to Arabidopsis, mustard, cabbage, broccoli, cauliflower and turnip
- Many important crops are polyploid, and rapeseed is one among them
- Polyploid plants are generated by evolutionary processes and/or crop domestication
- Doubling of genomes at least once in evolutionary history, resulting in polyploidy
- Autopolyploid- whole-genome duplication event
- Allopolyploid- interspecific or intergeneric hybridization event followed by chromosome duplication

https://www.canolacouncil.org/

Rapeseed

Nutrient Composition

Fats **40-45%**

MUFA **Oleic acid** (ω-9) 60-65% Stable

PUFA

linoleic acid (ω-6) 20%

α-linolenic acid (ω-3) 9-11%

1:1

SFA 7-8%

Erucic acid < 2%

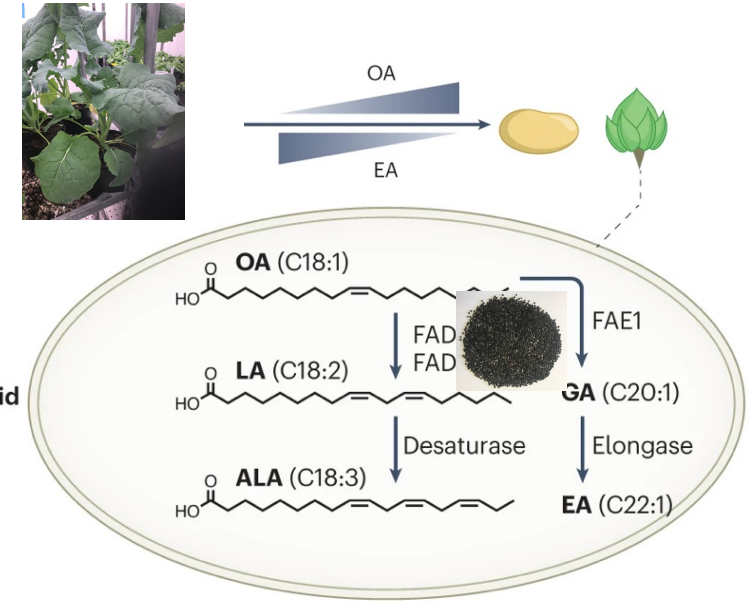
Protein ~ 20%

Fiber ~14%

Carbohydrates < 10-15%

Vitamins E & K

Minerals



Tuncel et al. 2023 Nature Reviews Bioengineering

Genetic Improvement in Rapeseed

- Rapeseed (AACC, 2n = 38)
- Interspecific hybridization - *B. oleracea* (CC, 2n = 18) and *B. rapa* (AA, 2n = 20)

Major Quality issues/Challenges: Erucic acid and Glucosinolates

- 1970 -Intensive breeding programs
- Back-cross with high erucic acid line to high erucic acid cultivars for non-food purposes
- Back-cross with low erucic acid line and low glucosinolate line to develop low erucic-acid and glucosinolate cultivars

Agronomic traits and oil quality

By-products of oil industry- Oilseed cake or meal?

- Second-largest source of protein meal (heat and press)/cake (solvent)
- Source of protein, energy, carbohydrates and mineral contents

Challenges

- ❖ Highly complex and repetitive nature
- ❖ More copies of each gene that are identical to each other
- ❖ Chromosomal rearrangements and epigenetic shifts
- ❖ Activation of transposable elements,
- ❖ Neo- and subfunctionalization events after gene duplication
- ❖ Co-editing of multiple alleles/genes

Advantages

- ○ Generation of a range of functionally differing phenotypes by partial loss of expression
- ○ Complete loss-of-function may impair plant performance

Antinutrient factors (ANF) in rapeseed

Seedcake/meal
Protein: 35-45%
Rich in Tryp & Threonine
Good amounts of Lys, Met & Cys
Fiber: 10-12%
Fat: 3-5%

Photo: Anja Persson

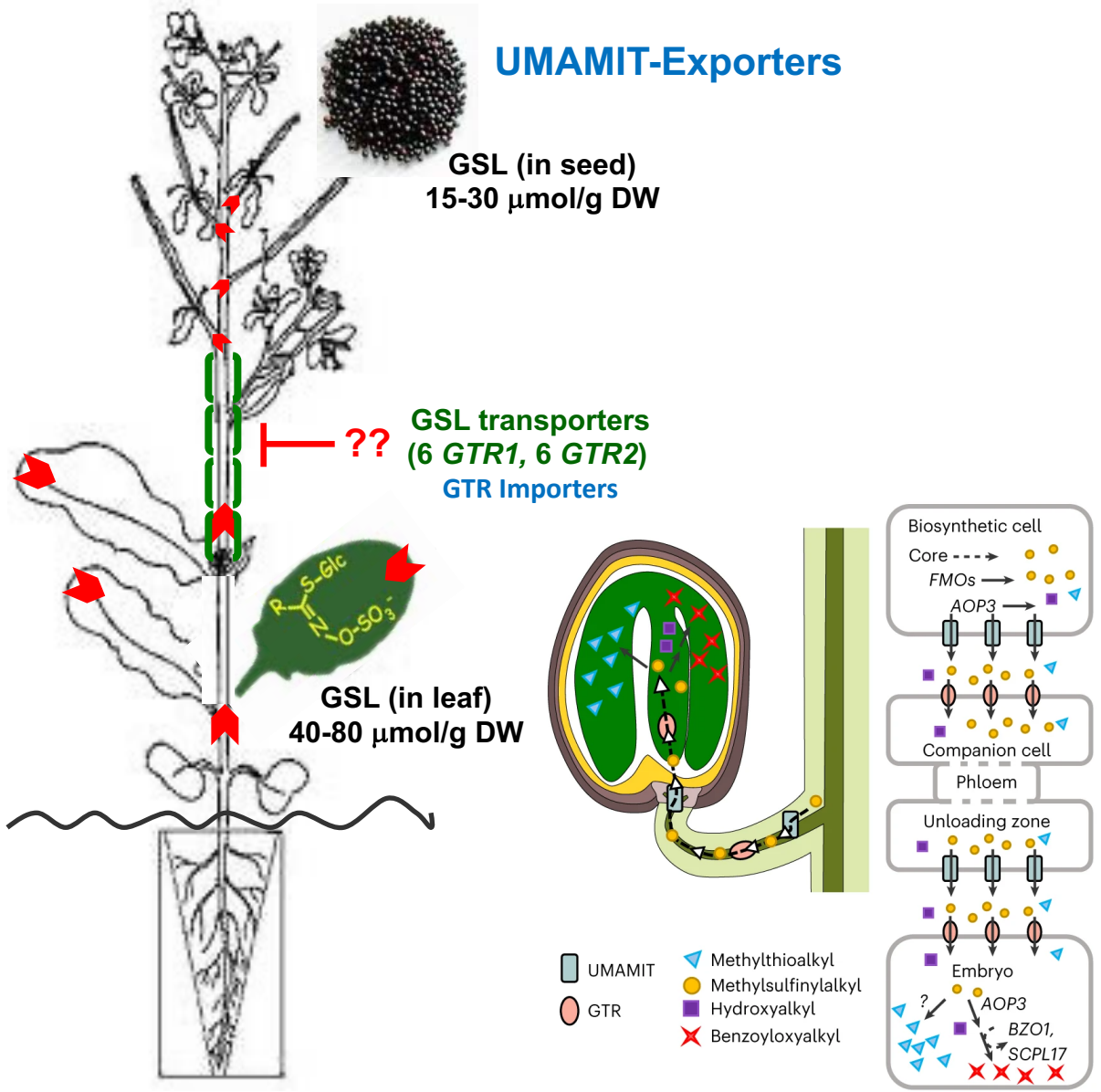
Anti-Nutritional Factors

1.Erucic Acid: Harmful to heart health

2.Glucosinolates: Sulfur-containing compounds-bitter-interfere with thyroid function

3.Sinapine: A phenolic compound - bitter taste- binds to minerals and digestive enzymes

4. Phytic Acid: Binds to minerals, iron and zinc



Long-distance glucosinolate transport

Sanden et al. 2024

Glucosinolates

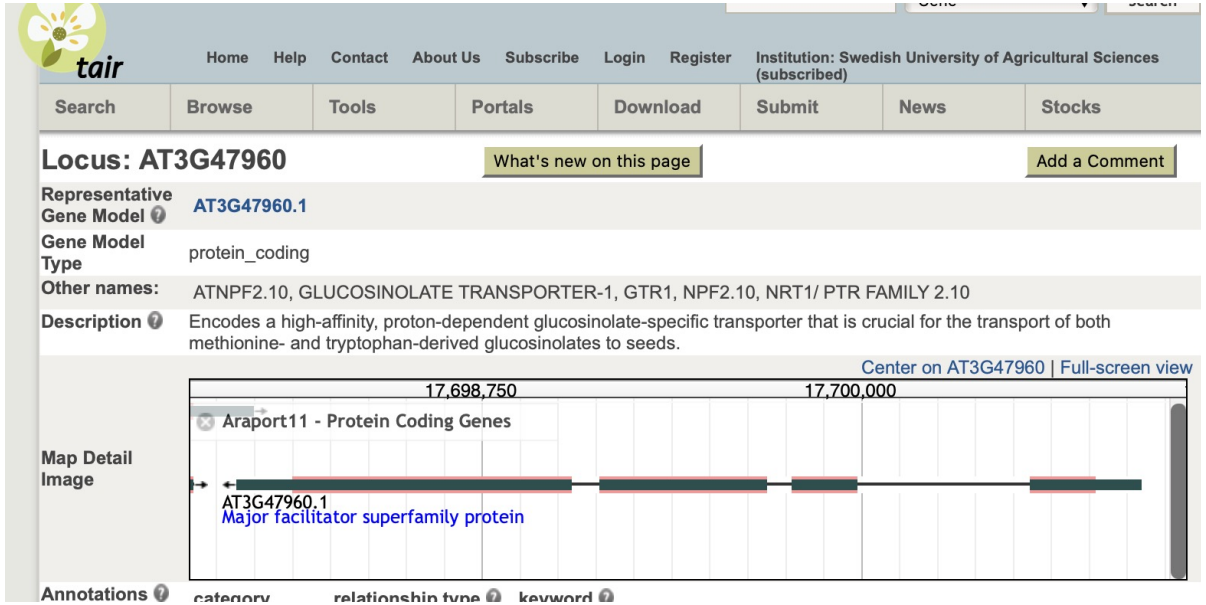
- Synthesized in vegetative tissues and transported to seeds which is mainly regulated by glucosinolate transporter (*GTR*) genes (*GTR1* and *GTR2*)
- Glucosinolates-sulfur- and/or nitrogenous secondary metabolites
- Glucosinolates-defense compounds against pests
- Antinutritional and negative physiological effects
- Pungent flavor in rapeseed is imparted by glucosinolates

Constraints in traditional breeding and MAS

- Multiple homoeologous copies (alleles) of each gene
- Challenges in mutation breeding
- Extensive backcrossing or introgression

Gene identification

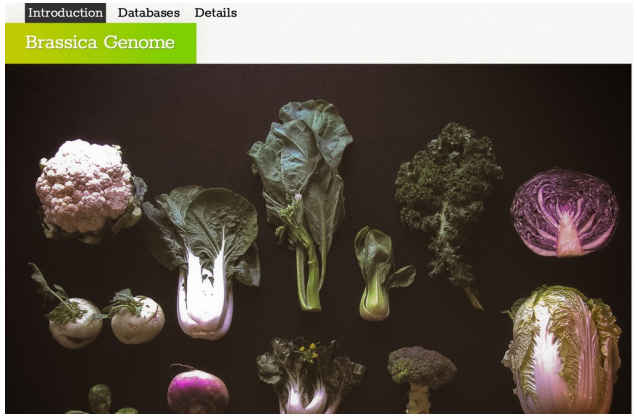
Functionally characterized genes from Arabidopsis

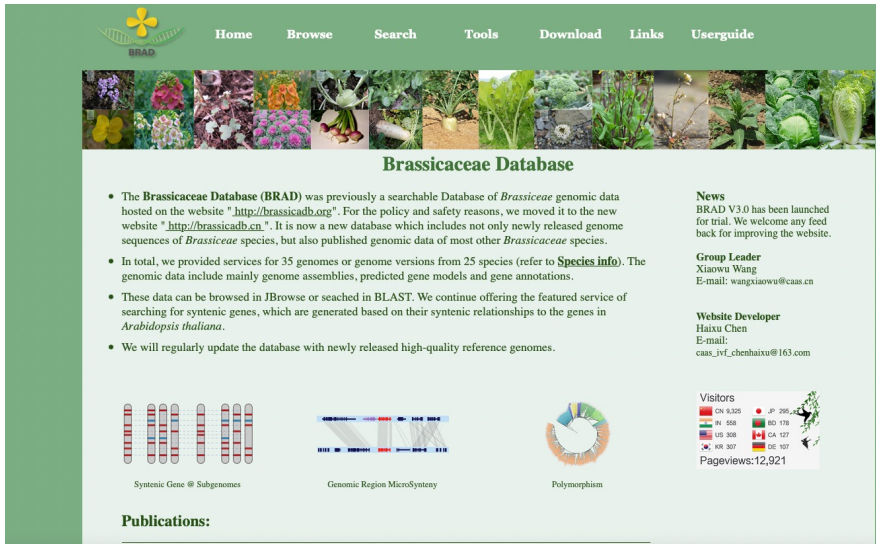


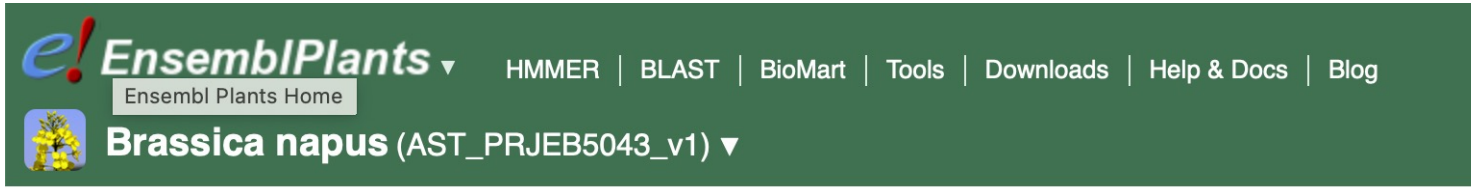
Arabidopsis database

GTR1 gene

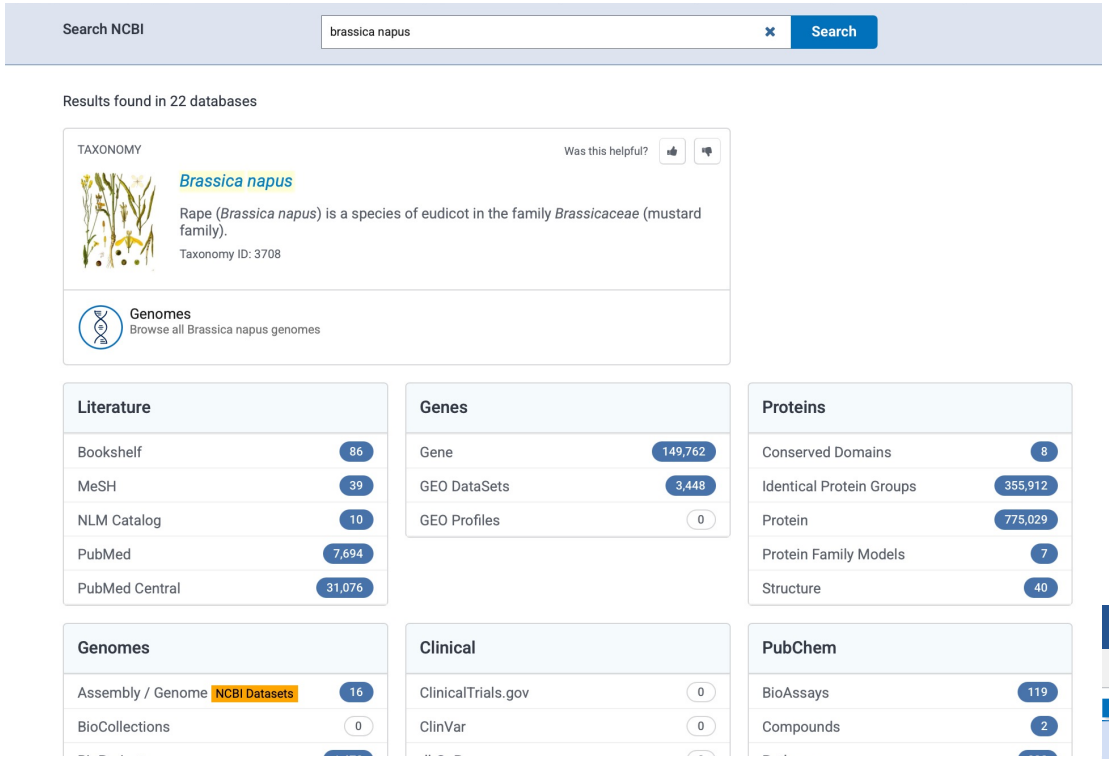
Brassica napus databases

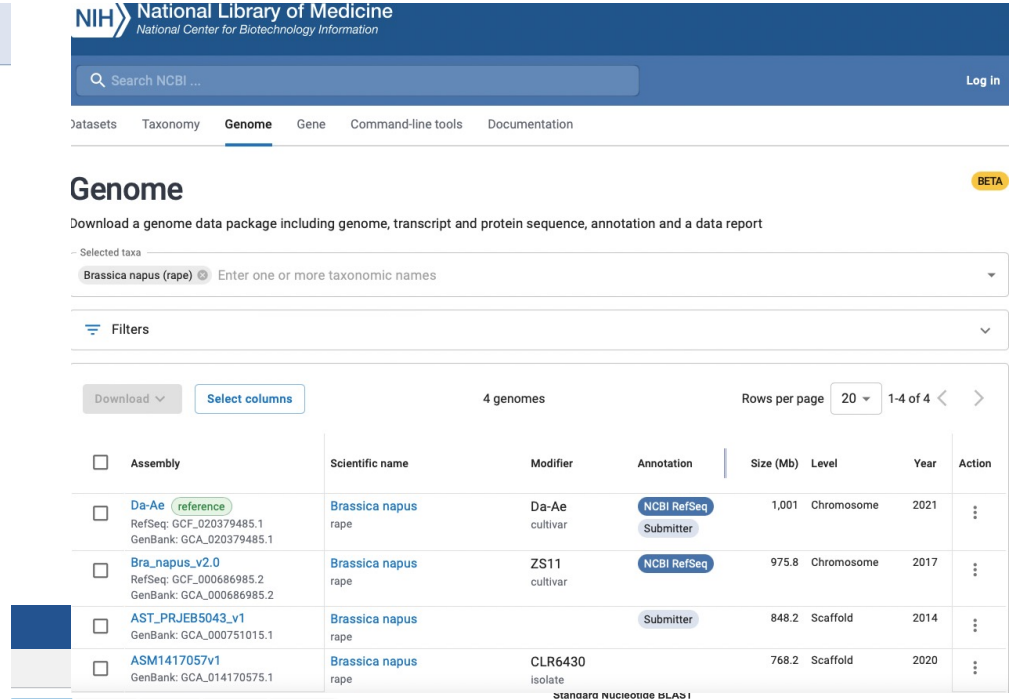


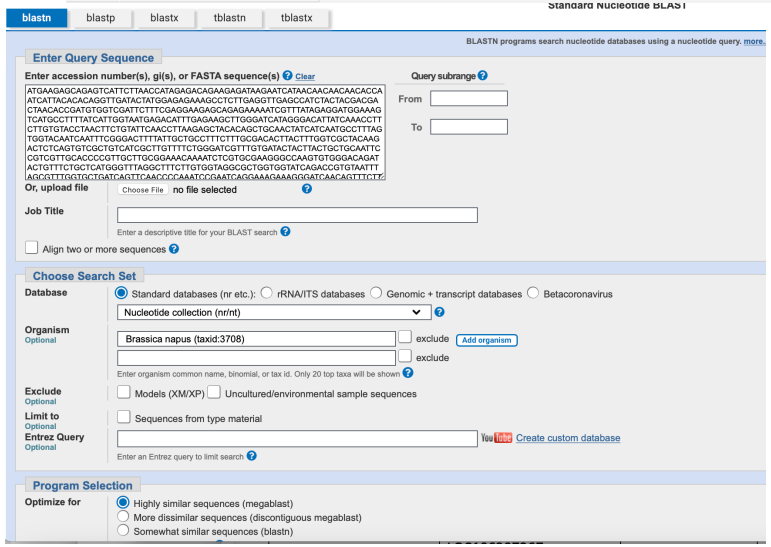




NCBI databases for *B. napus*







NCBI database for *B. napus*

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download | New Select columns | Show 10

select all *10 sequences selected* GenBank Graphics Distance tree of results New MSA Viewer

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC106397267), mRNA	Brassica napus	rape	3708	1912	1912	90%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC106410496), misc_RNA	Brassica napus	rape	3708	1912	1912	90%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10 (LOC106445255), mRNA	Brassica napus	rape	3708	1906	1906	90%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC106408997), mRNA	Brassica napus	rape	3708	1890	1890	90%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC111202315), mRNA	Brassica napus	rape	3708	1873	1873	91%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC106414122), mRNA	Brassica napus	rape	3708	1845	1845	91%
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PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.11-like (LOC106366160), transcript variant X2, misc_RNA	Brassica napus	rape	3708	1040	1040	87%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.11-like (LOC106366160), transcript variant X1, mRNA	Brassica napus	rape	3708	1040	1040	87%
PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.11-like (LOC106366161), mRNA	Brassica napus	rape	3708	1035	1035	87%

Download GenBank Graphics ▼Next ▲Previous ◀◀Descriptions

PREDICTED: Brassica napus protein NRT1/ PTR FAMILY 2.10-like (LOC106397267), mRNA

Sequence ID: XM_013837850.2 Length: 2254 Number of Matches: 1

Range 1: 370 to 2106 GenBank Graphics ▼Next Match ▲Previous Match

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Sbjct  670  GATACTACTGACGGCTGCAGTTCCAGGATTGCACCCCATTCCTTGTGGAACACAAAGT-T  728
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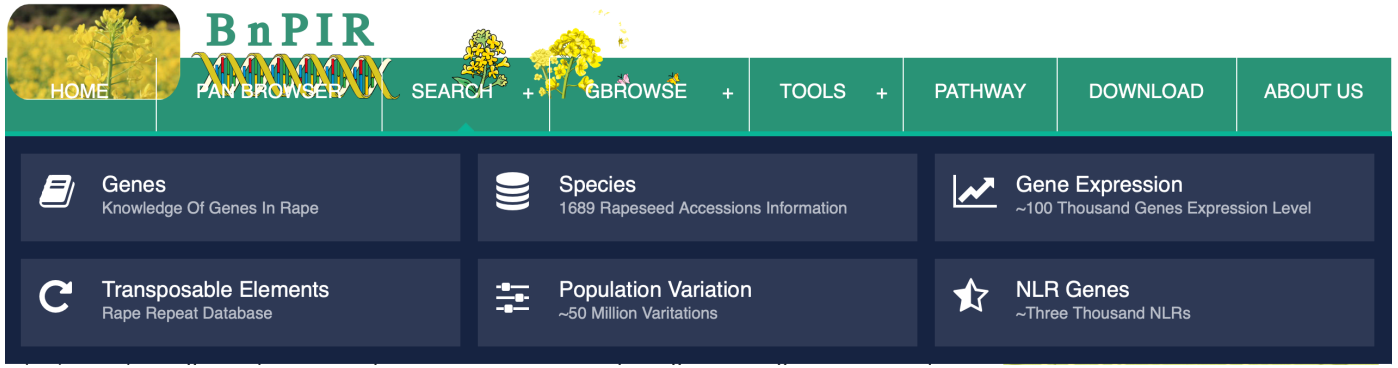
Related Information

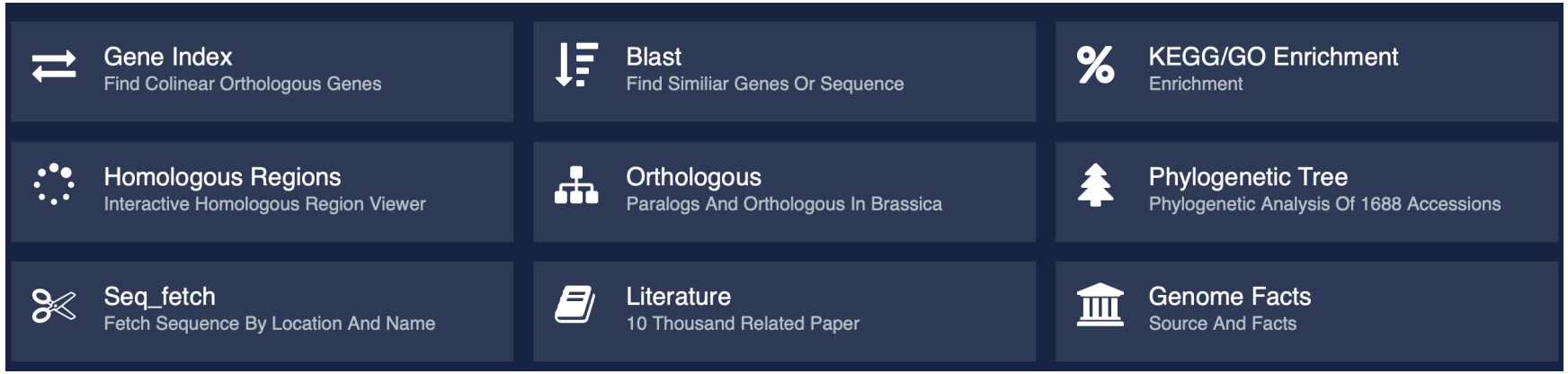
Gene - associated gene details

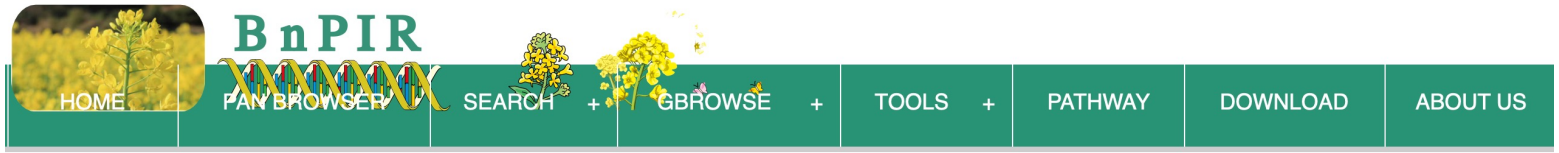
Genome Data Viewer - aligned genomic context

NCBI genome database for *B. napus*

B. napus pan-genomic database







Rapeseed Genome Facts

BnPIR Rape Genome Annotations, release v0:

Accessions	ZS11	Westar	No2127	Zheyou7	Gangan	Shengli	Tapidor	Quinta
Gene Number	100,919	97,514	95,385	96,209	96,843	94,586	96,117	95,492

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases (B. rape, B. oleracea, and eight B. napus accessions) and calculates the statistical significance.

BLAST program BLASTN: NT query, NT db

Organism: ◉ zs11 ○ westar ○ tapidor ○ no2127 ○ darmor ○ Bra ○ Bol

Database zs11_CDS(-UTR-intron)(NT)

Query sequence

```
ATGAAGAGCAGAGTCATCCACAGCCATAGAGAGAGAAGAGATAAGCATAATTACACACAGATTGAAACAATGGAG
AGAAAGCCCTTTGATGTTGAGACAACTGAGGATCACAAACCCTACTCCTCCGTCGATGGCGGTGGTTCTGATTC
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TACTACTGACGGCTGCAGTTCCAGGATTGCACCCCATTCCTTGTGGAACACAAAGTTCTTGCCAAGGCCCAAGC
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Descriptions

Description	Max score	Total score	Query cover	E value	Ident
BnaA06T0259000NO	3519	3519	100%	0	100%
BnaC03T0484400NO	3342	3342	100%	0	98%
BnaA06T0166500NO	2243	2243	93%	0	89%
BnaC03T0534600NO	2237	2237	96%	0	89%
BnaA01T0192000NO	2097	2097	95%	0	88%
BnaC05T0292100NO	2075	2075	95%	0	87%
BnaC03T0462800NO	1129	1129	88%	0	79%
BnaA06T0281700NO	1118	1118	88%	0	79%
BnaC02T0484300NO	1111	1111	89%	0	79%
BnaA02T0392300NO	1091	1091	94%	0	78%
BnaC09T0042300NO	1067	1067	88%	0	78%

Alignments

>BnaA06T0259000NO locus=no2127v0_A06:32588651:32591193:+ Length: 1905 Number of Matches: 1

Range 1: 1 to 1905

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Query 61    ATTGAAACAATGGAGAGAAAGCCCTTTGATGTTGAGACAACTGAGGATCACAAACTCTAC  120
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Sbjct 61    ATTGAAACAATGGAGAGAAAGCCCTTTGATGTTGAGACAACTGAGGATCACAAACTCTAC  120

Query 121   CCCTCCGTCGATGGCGGTGGTTCTGATTCGGTCGATTCATTTGAAGACGAGCAGAAAAAG  180
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Query 181   CTCGTTTATAGAGGCTGGAAAGTCATGCCCTTTATCATTGGTAATGAGACATTTGAGAAG  240
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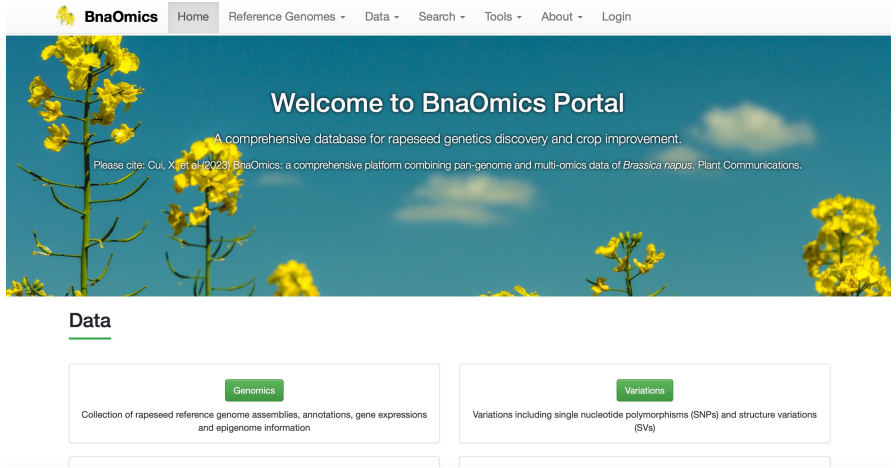


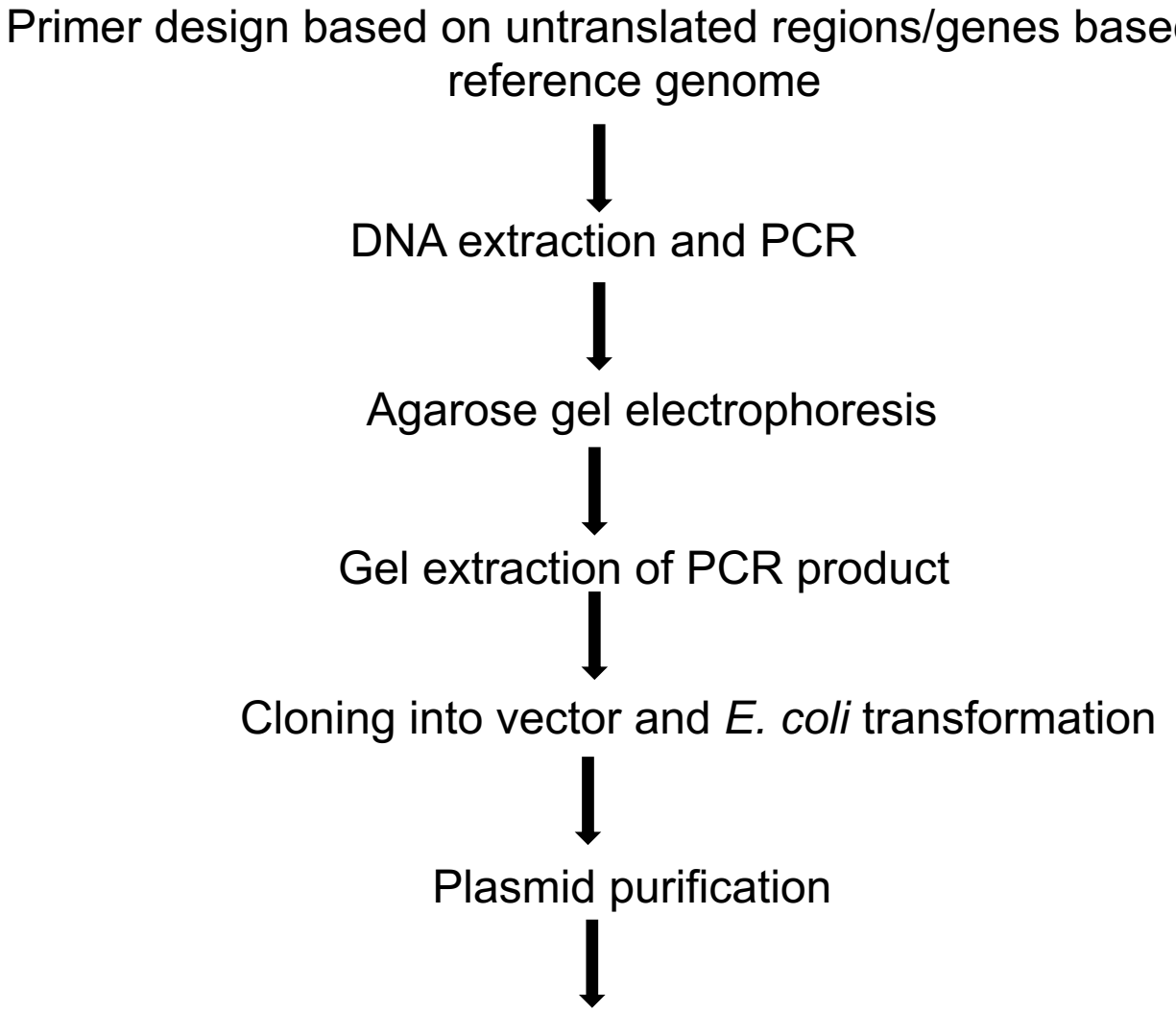
Database Name	Source
Brassica napus pan-genome v3	
Brassica napus Westar genome	
Brassica napus Kale genome	
Brassica napus Mendel genome	
Brassica napus LT genome	
Brassica napus ZS11 genome FAFU	Chen et al. 2020
Brassica napus ZS11 genome HZAU	Song et al. 2020
Brassica napus ZS11 genome v201608	Sun et al. 2017
Brassica napus Darmor genome v4.1	Chalhoub et al. 2014
Brassica napus Darmor genome v10	Rousseau-Gueutin et al. 2020
Brassica napus Express617 genome v1	Lee et al. 2020
Brassica napus NY7 genome v2	Zou et al. 2019
Brassica napus ganganF73 genome	Song et al. 2020
Brassica napus no2127 genome	Song et al. 2020
Brassica napus quintaA genome	Song et al. 2020
Brassica napus shengli3 genome	Song et al. 2020
Brassica napus tapidor3 genome	Song et al. 2020
Brassica napus westar genome HZAU	Song et al. 2020
Brassica napus zheyou73 genome	Song et al. 2020

Whole Genome Sequencing

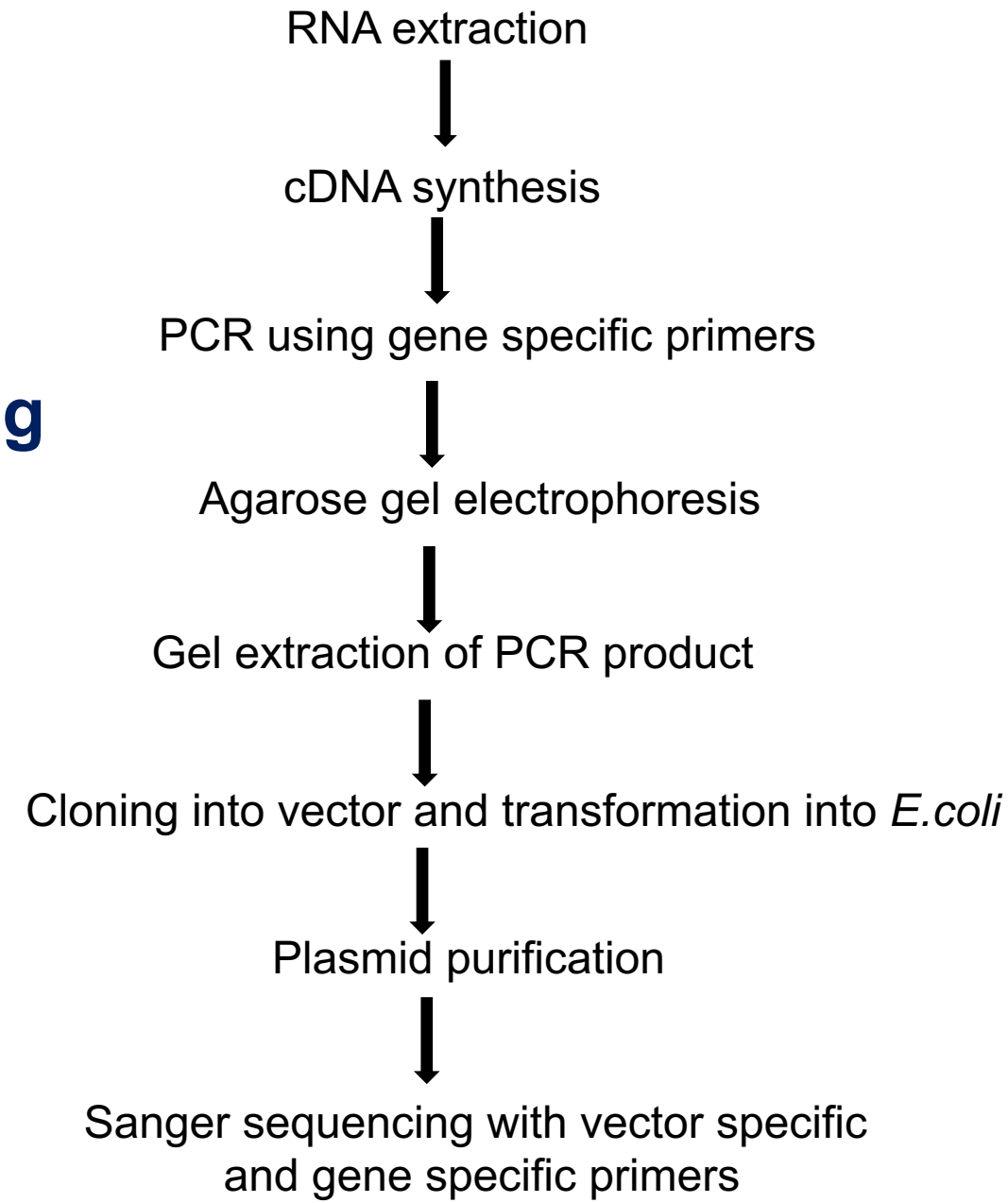
Bottlenecks in the gene identification

- DNA sequence of genes is needed for gene editing
- Chromosomal-level gene assemblies are needed
- Identical copies of genes in the same chromosome

General workflow for cloning of a target genes



General workflow for cloning of a target gene from cDNA



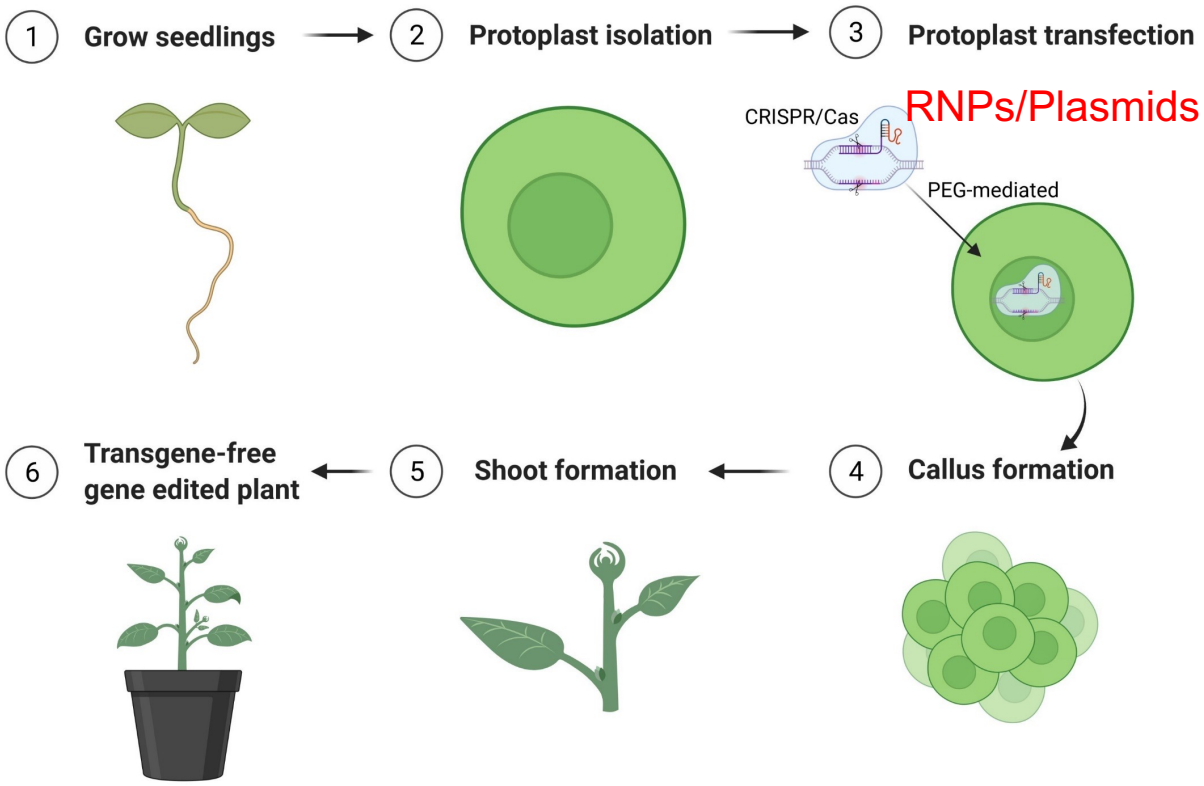
Nucleotide similarity of *GTR1* genes in *B. napus*

	LOC106397267	LOC106408997	LOC106410496	LOC106414122	LOC106445255	LOC111202315
LOC106397267		87.32	87.32	86.41	**98.16**	86.35
LOC106408997	87.32		**98.48**	85.88	87.21	85.56
LOC106410496	87.32	**98.48**		85.99	87.10	85.67
LOC106414122	86.41	85.88	85.99		86.51	**97.84**
LOC106445255	**98.16**	87.21	87.10	86.51		86.46
LOC111202315	86.35	85.56	85.67	**97.84**	86.46	

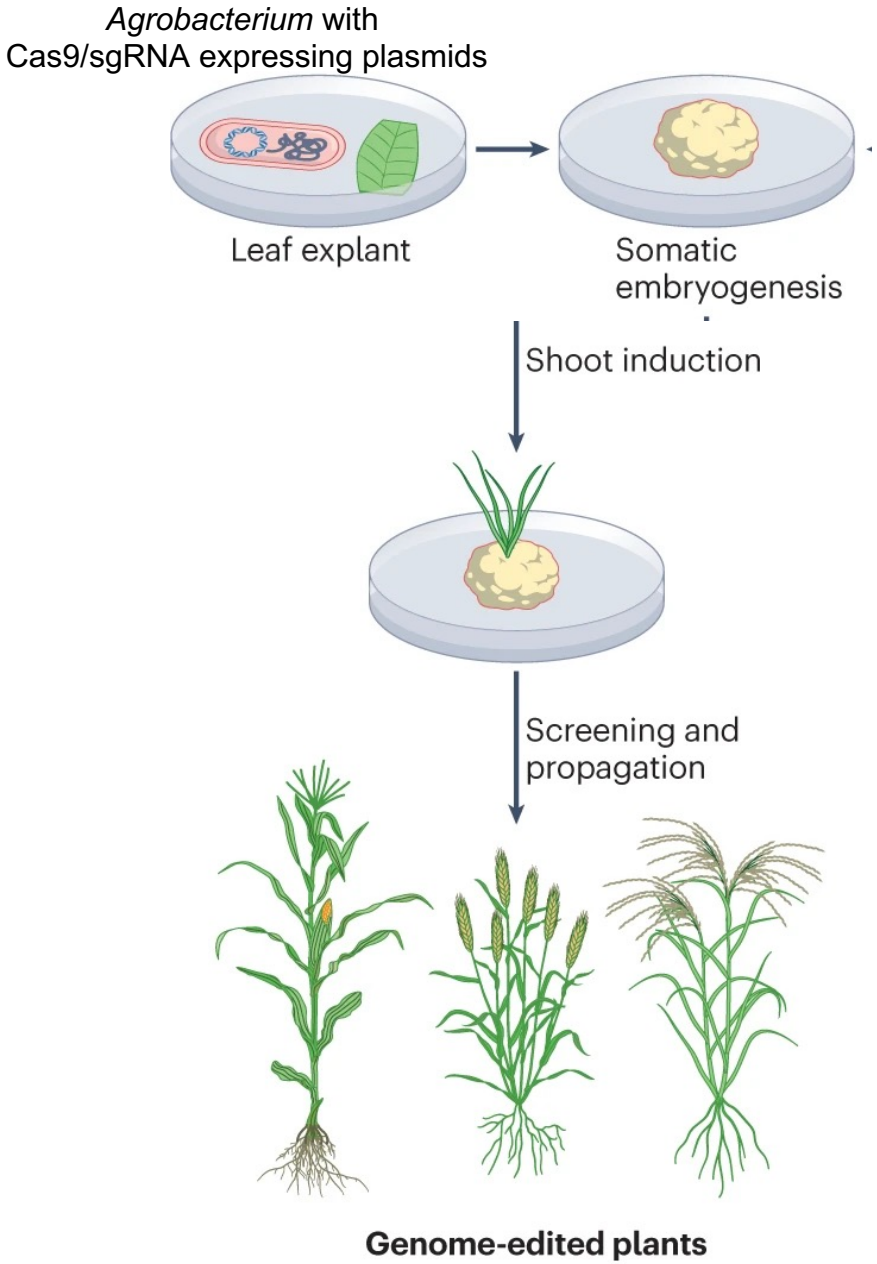
Protein identity of *GTR1* genes in *B. napus*

	LOC106397267	LOC106408997	LOC106410496	LOC106414122	LOC106445255	LOC111202315
LOC106397267		88.94	89.27	85.37	**98.58**	85.04
LOC106408997	88.94		**99.19**	86.41	89.11	85.76
LOC106410496	89.27	**99.19**		86.57	89.43	86.08
LOC106414122	85.37	86.41	86.57		85.53	**97.56**
LOC106445255	**98.58**	89.11	89.43	85.53		85.20
LOC111202315	85.04	85.76	86.08	**97.56**	85.20	

Transformation Techniques (Tissue culture dependent)

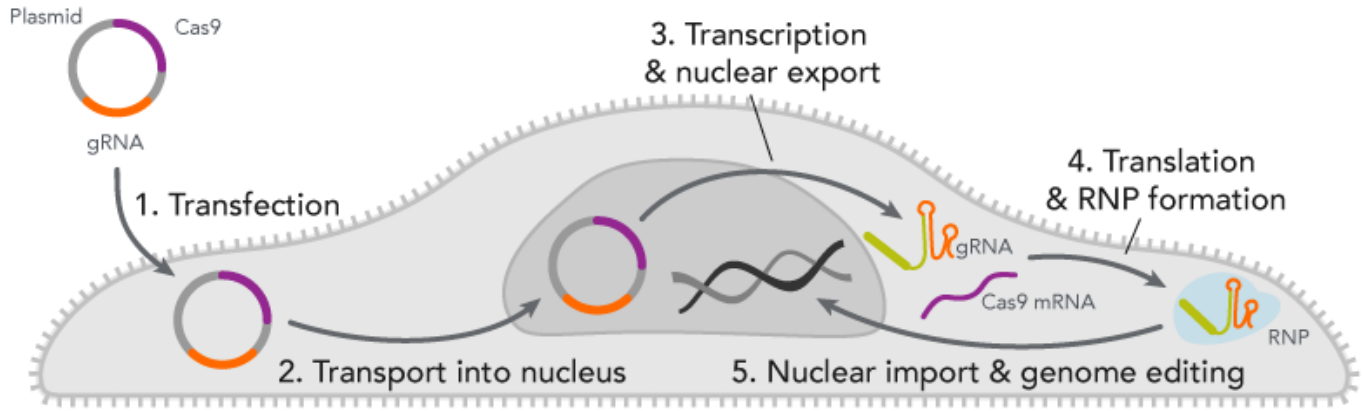


Genome editing using protoplasts
Homozygotic transgene-free mutants at T0 generation

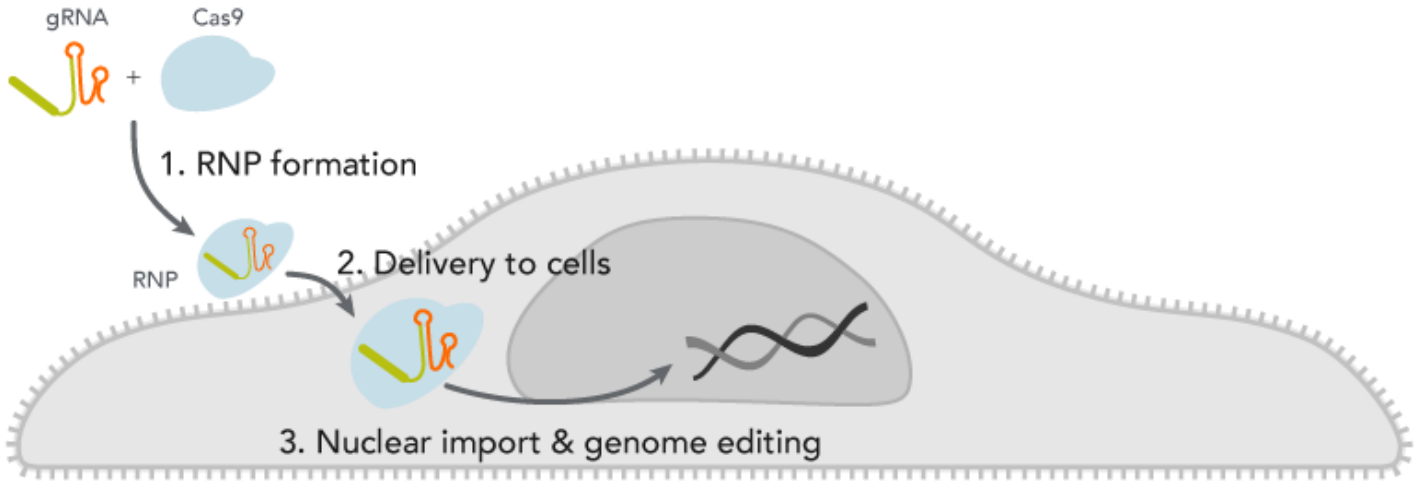


Tuncel et al. 2023 Nature Reviews Bioengineering

Plasmid-based editing



RNP-based editing



https://eu.idtdna.com

Plasmid-based editing

Adaptors primers design

↓

Cloning of sgRNA expression cassettes into vector

↓

Golden Gate cloning

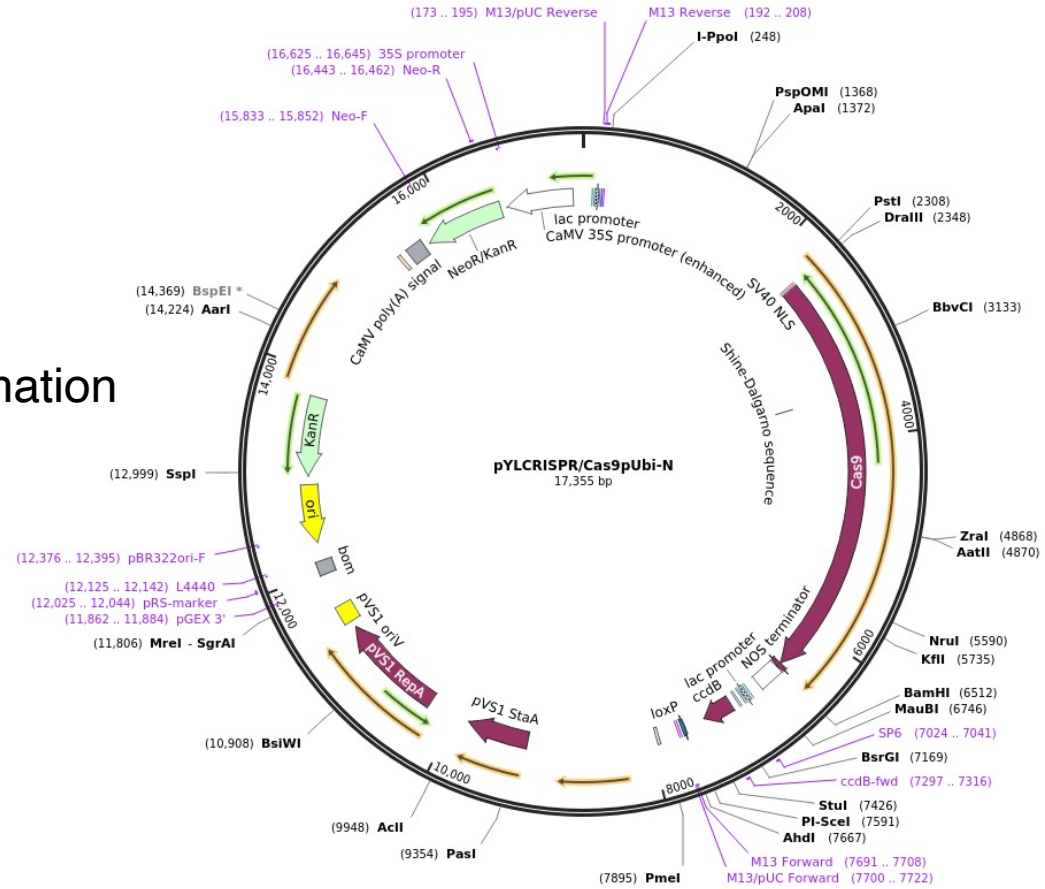
↓

E. coli and/or *Agrobacterium* transformation

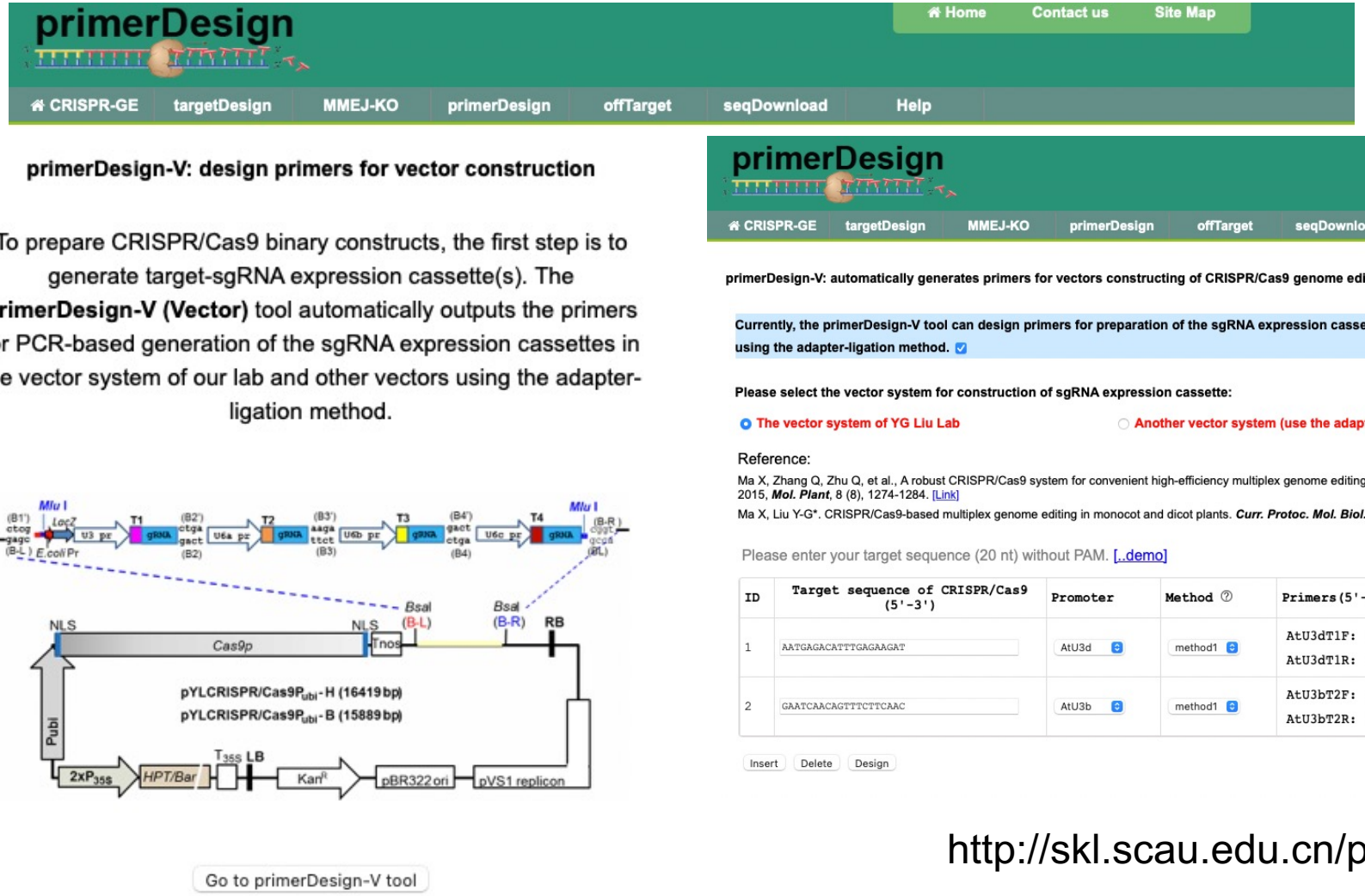
↓

Transfection/Transformation

Vector Map



Adaptors primers design



http://skl.scau.edu.cn/primerdesign/vector/

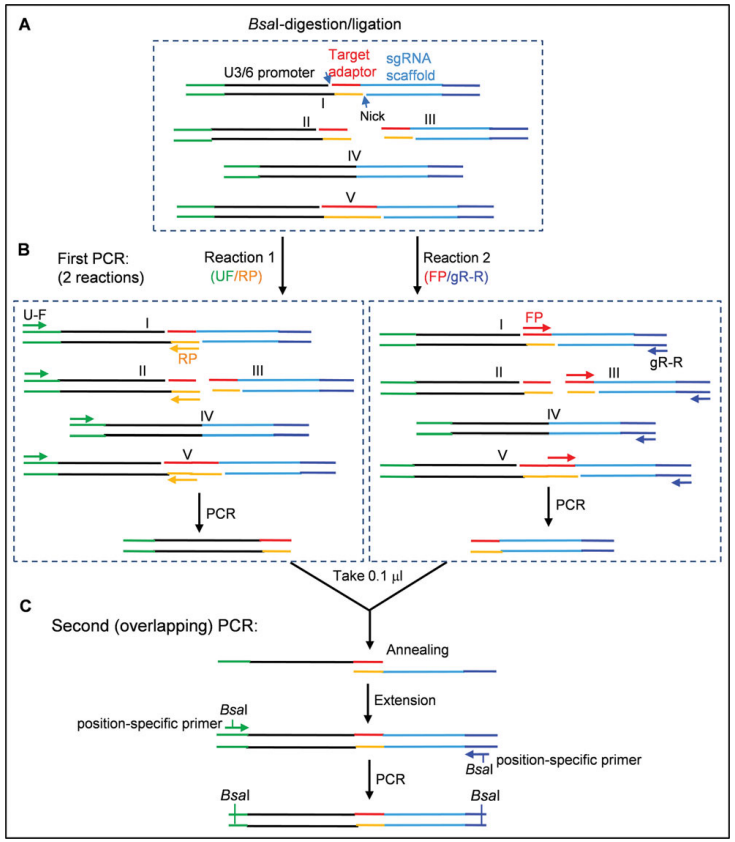
Ma, X. and Liu, Y.-G. 2016. CRISPR/Cas9-based multiplex genome editing in monocot and dicot plants. Curr. Protoc. Mol. Biol. 115:31.6.1-31.6.21. doi: 10.1002/cpmb.10

Assembly of sgRNA expression cassettes into a CRISPR/Cas9 construct

- Preparation of sgRNA expression cassette templates
- Arrangement of sgRNA cassettes in a pYLCRISPR/Cas9 vector

1 target: LacZ-AtU3d
2 targets: LacZ-AtU3d—AtU3b
3 targets: LacZ-AtU3d—AtU3b—AtU6-1
4 targets: LacZ-AtU3d—AtU3b—AtU6-1—AtU6-29

- *E. coli* and/or *Agrobacterium* transformation
- Transfection/Transformation



A strategy for preparation of sgRNA expression cassettes

Ma, X. and Liu, Y.-G. 2016. CRISPR/Cas9-based multiplex genome editing in monocot and dicot plants. Curr. Protoc. Mol. Biol. 115:31.6.1-31.6.21. doi: 10.1002/cpmb.10

gRNA Design

CRISPR-P 2.0

CRISPOR

CRISPOR (citation) is a program that helps design, evaluate and clone guide sequences for the CRISPR/Cas9 system. CRISPOR Manual

July 2023: A faster server has arrived and should come online within 2-3 months. Python3 upgrade on hold, the ML models output different scores on Py3. Full list of changes

Step 1

Planning a lentiviral gene knockout screen? Use CRISPOR Batch

Sequence name (optional):

Enter a single genomic sequence, < 2300 bp, typically an exon

Clear Box - Reset to default

cttcctttgtccccaatctgggcgcgcgccggcgcccctggcggcctaaggactcggcgcgccggaagtggccagggcgggggcgacctcggctcaca gcgcgcccggctattctcgcagctcaccatgGATGATGATATCGCCGCGCTCGTCGTCGACAACGGCTCCGGCATGTGCAAGGCCG GCTTCGCGGGCGACGATGCCCCCGGGCCGTCTTCCCCTCCATCGTGGGGCGCC

ıse is preserved, e.g. you can mark ATGs with lowercase.
ł of a sequence, you can paste a chromosome range, e.g. chr1:11,130,540-11,130,751

Step 2

Select a genome

Solanum tuberosum - potatoes - NCBI GCA_010127505.1 (ASM1012750v1)

We have 1029 genomes, but not yours? Search NCBI assembly and send a GCF_/GCA_ ID to CRISPOR support.

Step 3

Select a Protospacer Adjacent Motif (PAM)

20bp-NGG - Sp Cas9, SpCas9-HF1, eSpCas9 1.1

See notes on enzymes in the manual.

SUBMIT

Version 5.01 - Documentation - Contact us - Downloads/local installation - Citation - License

CCTop - CRISPR/Cas9 target online predictor

CENTRE FOR ORGANISMAL STUDIES

- CCTop
- ACEofBASEs
- Help
- Supported species
- CCTop Q/A forum
- CCTop standalone

name: Pea

single query batch mode

query sequence: (plain nucleotide sequence, max 500 bases) (multi-)fasta file Choose File no file selected

be notified by email your@email.com

PAM type : NGG (Streptococcus pyogenes)

Target selection **Off-target prediction**

5' limitation PAM
5' NNNNNNNNNNNNNNNNNNNNNGG
3' limitation

max 4 MM PAM
5' NNNNNNNNNNNNNNNNNGG
max 2 MM core

target site length : 20
target site 5' limitation : NN
target site 3' limitation : NN

max. total mismatches : 4

core length : 12

max. core mismatches : 2

In vitro transcription
T7 U6 Custom
fwd overhang: TAGG
rev overhang: AAAC

species : Search

- Petunia (Petunia axillaris)
- Pisum sativum (pea)
- Populus alba (sPta717 v2)
- Populus tremula (sPta717 v2)
- Populus trichocarpa (JGI v3.1)
- Potato (Solanum tuberosum DM1-3 v4.04)

Reset Submit

Supported by:

CRISPR MultiTargeter

Search for common and unique CRISPR guide RNA targets in multiple similar sequences

5' dinucleotide: NN

Target length: 20

PAM sequence orientation: 5' 3'

Allow a mismatch in the first 8 nucleotides? Yes No

PAM sequence: NGG (scoring is available*) Your own PAM sequence

*Scoring is available for 20-nt type II sgRNAs. See Doench et al., 2014 for details. High scores may indicate more potent sgRNAs. However, some potent sgRNAs have scores < 0.1 and a number of successful sgRNAs had scores between 0.1 and 0.2. Therefore, potential experimental utility and subsequent experimental verification should guide the choice of sgRNAs.

Off-target analysis parameters:
GT-Scan: No restrictions on PAM sequence or target length. Adjust the target rule on the submission form.
Cas-OFFinder: Length should be 20bp for SpCas9 (PAM = 'NGG'), 18bp for StCas9 (PAM = 'NNAGAAW'), and 24bp for NmCas9 (PAM = 'NNNNGMTT').

Input sequence(s)*

*Multiple sequences must be submitted as a FASTA text. The size of any sequence must be smaller 50 kb.

OR Upload a file with sequences: Choose File no file selected

Demo input

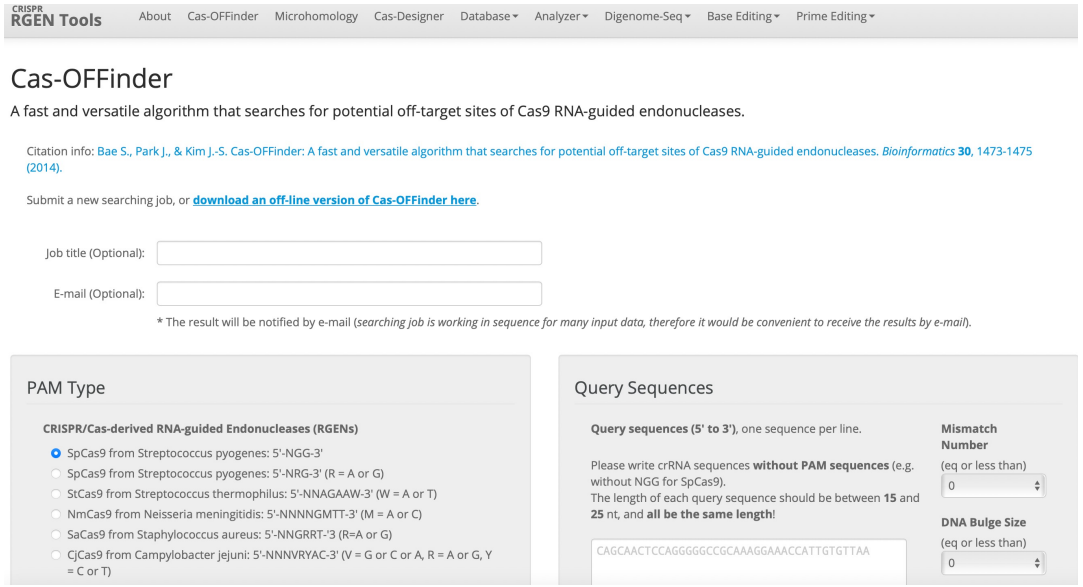
Submit

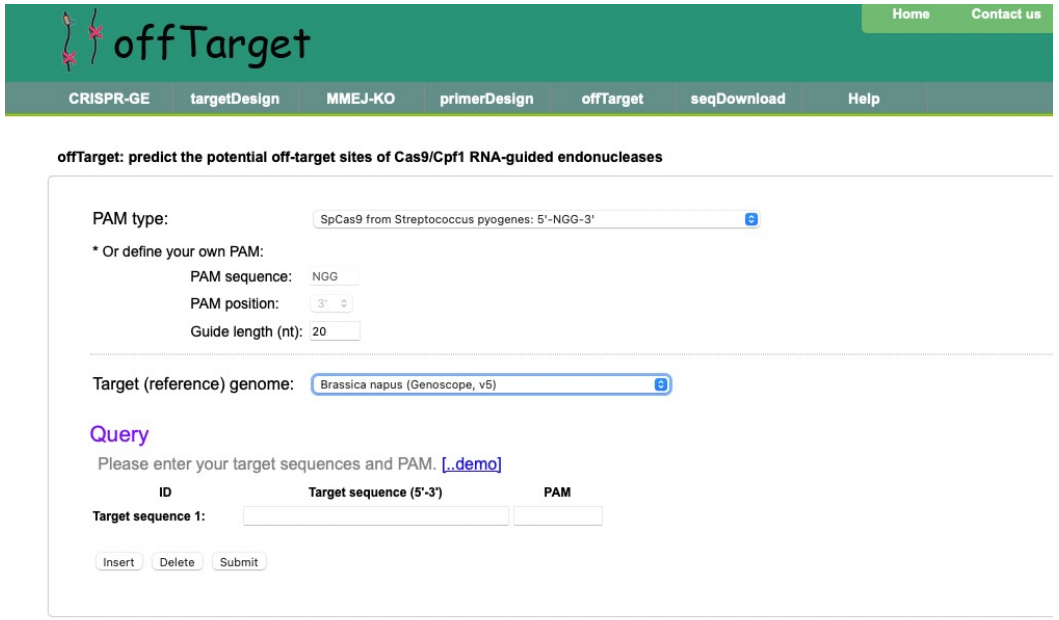
gRNAs targeting Multisequence

https://multicrispr.net/multalign_input.html

Off Target Prediction

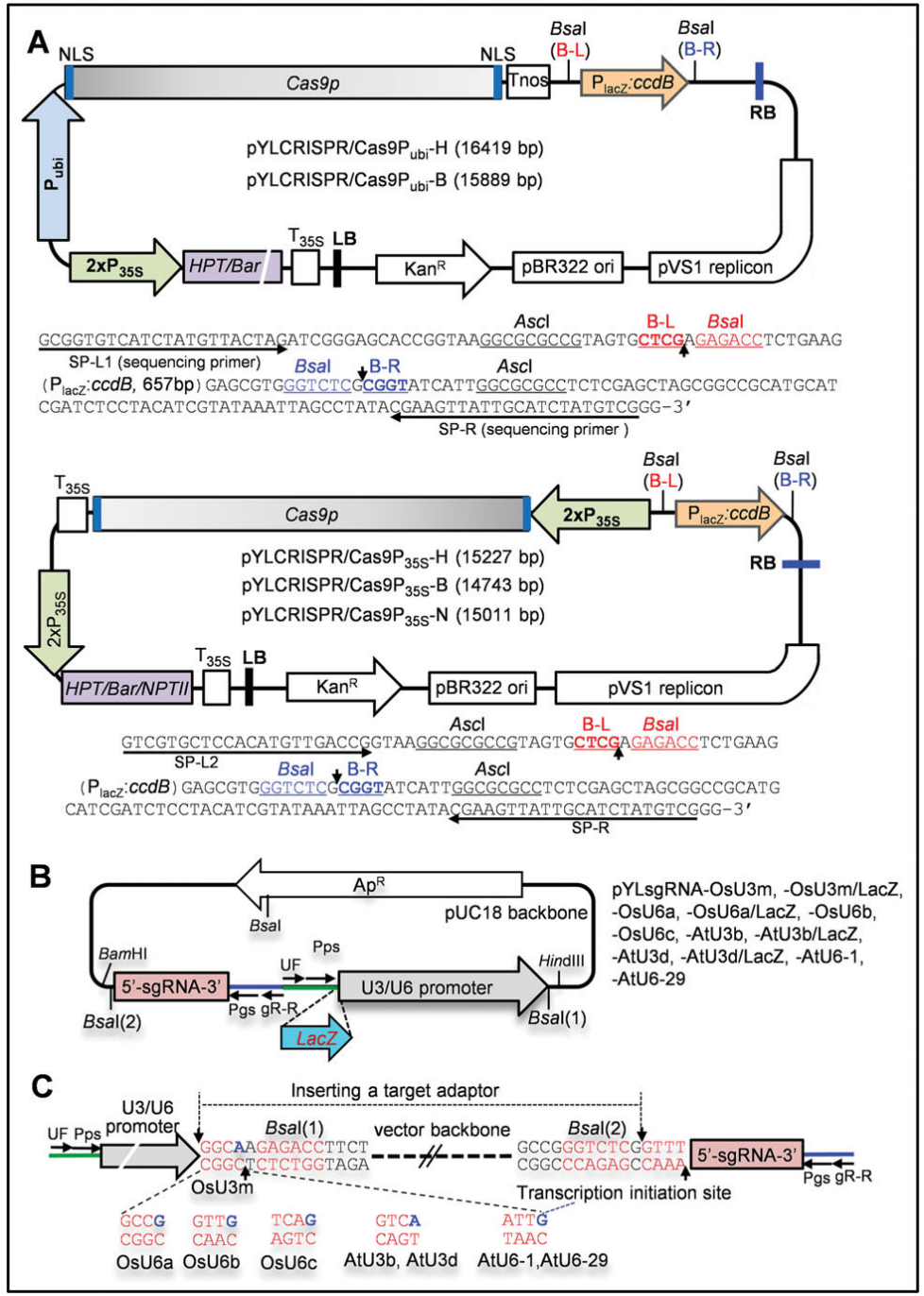
http://www.rgenome.net/cas-offinder/





http://skl.scau.edu.cn/offtarget/

Vectors and intermediatory Plasmids



RNP-based editing

- CRISPR relies on the nuclease activity of Cas protein and their specific binding to the genome directed by guide RNAs (gRNAs)

CRISPR-endonuclease production

↓

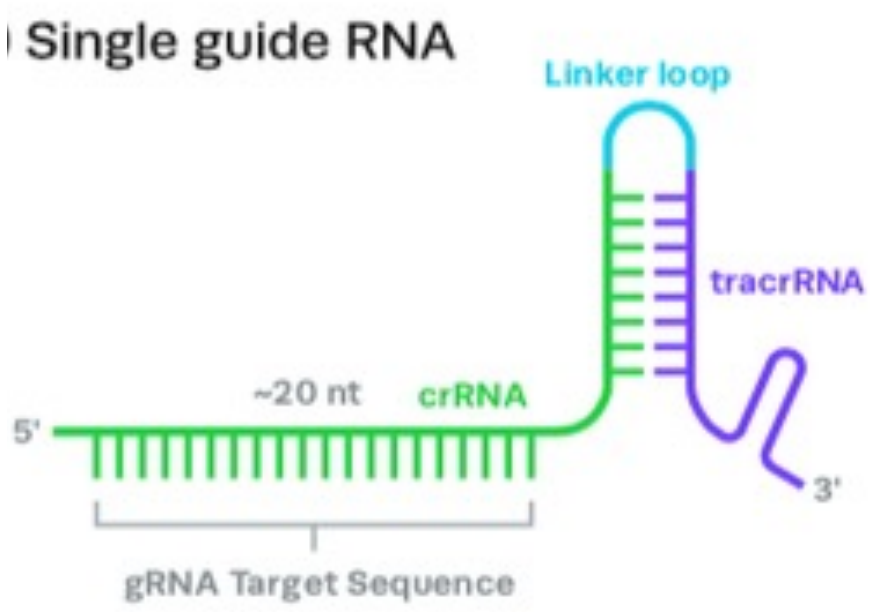
sgRNA production

↓

RNP assembly

↓

Transfection to protoplasts



RNP-based editing

Why it is needed

- Transgenes can be segregated through breeding; the process is usually time- and labor-intensive
- Not a viable option for species having a lengthy juvenile growth period or vegetatively propagated plants
- Self-incompatible plants
- If genomic DNA is continuously exposed to CRISPR construct, there is the possibility of off-target mutagenesis and chimeric mutants

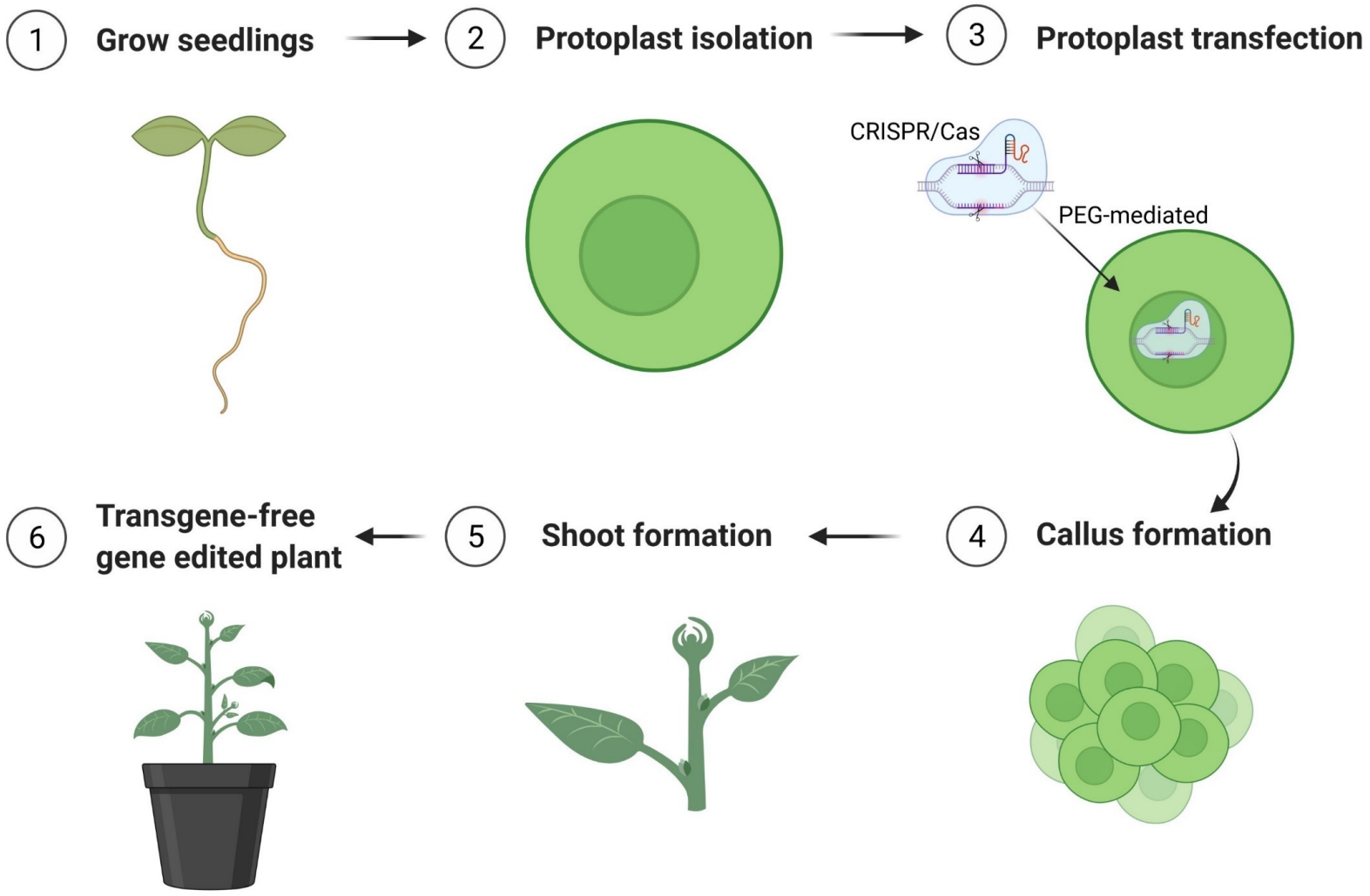
Advantages

- Enables generation of transgene-free gene-edited lines
- Minimal off-target effects
- Reduced toxicity due to the rapid degradation of RNPs
- Ability to titrate their dosage while maintaining high editing efficiency

Disadvantages

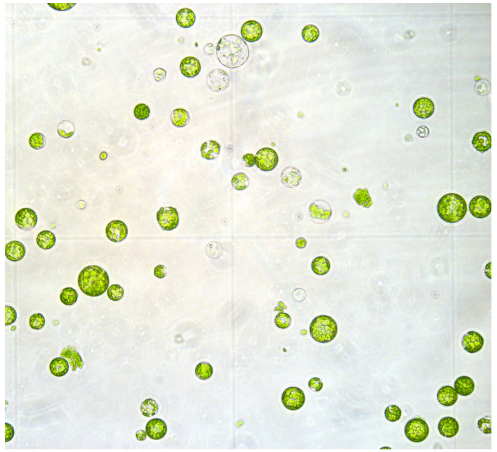
- Regeneration of plants from protoplasts is a major bottleneck in most crop species
- Somaclonal Variation and Genomic Instability

Workflow for genome editing using protoplasts

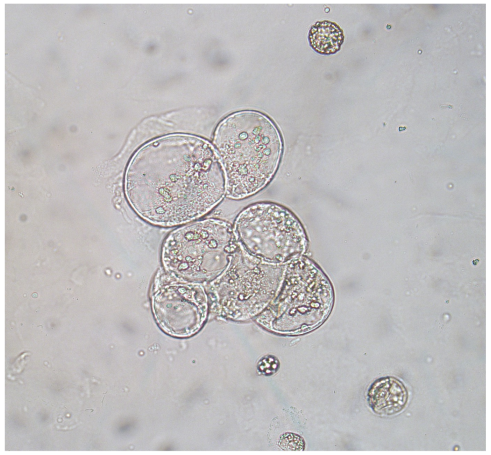


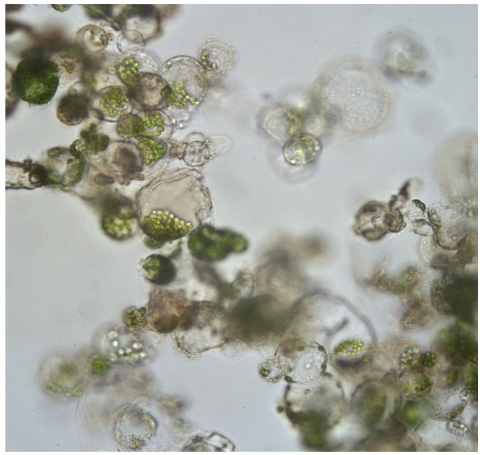
Courtesy: Sjur Sandgrind

PEG-mediated protoplast transfection/regeneration



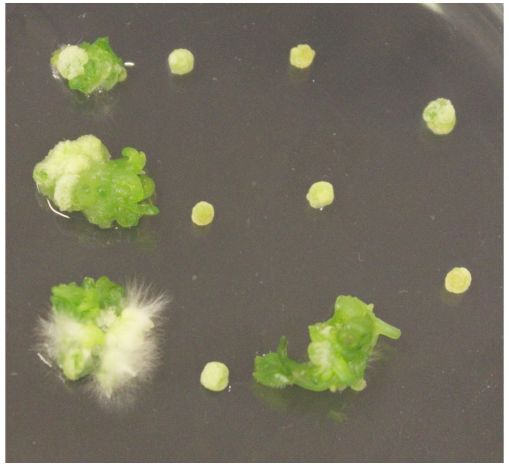
Freshly isolated protoplasts





Protoplasts undergoing cell divisions and multiplication

Protoplast colonies

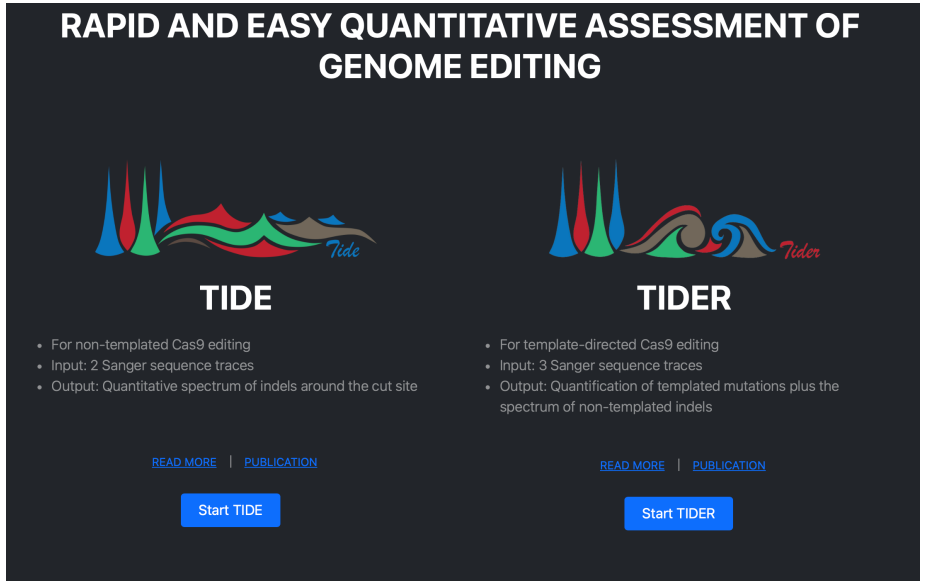


Shoot regeneration from protoplast colonies

Molecular characterization of editing events

Medium-throughput assays

- T7 Endonuclease 1 (T7E1) Assay
- HRFA



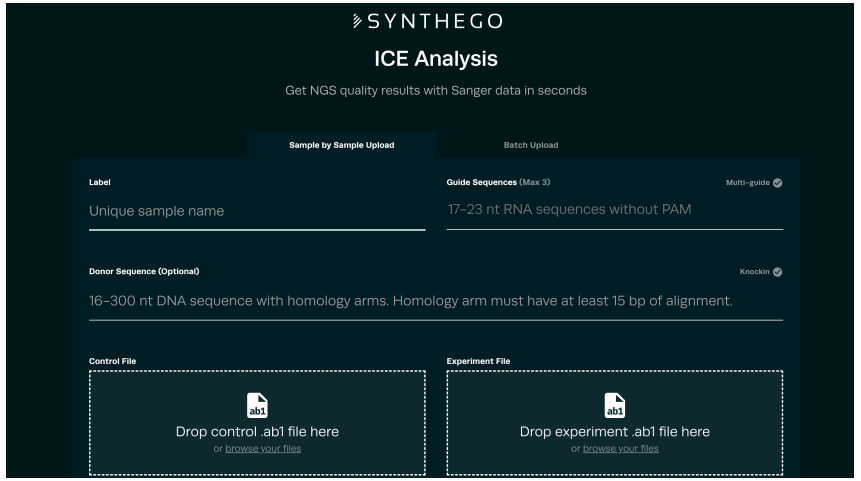
High-throughput assays

Sanger Sequencing

Tracking of Indels by Decomposition (TIDE)

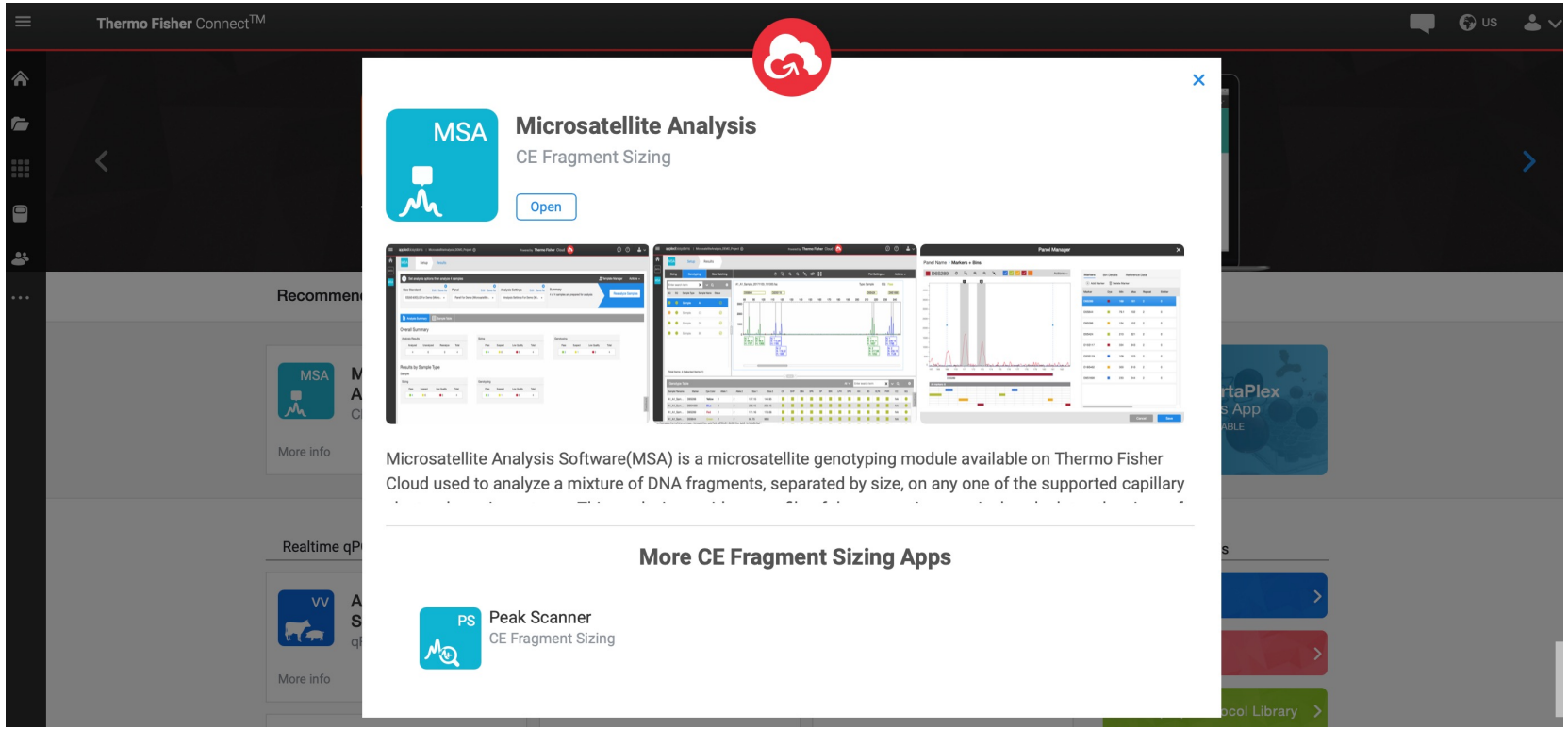
ICE (inference of CRISPR edits)

NGS



High Resolution Fragment Analysis

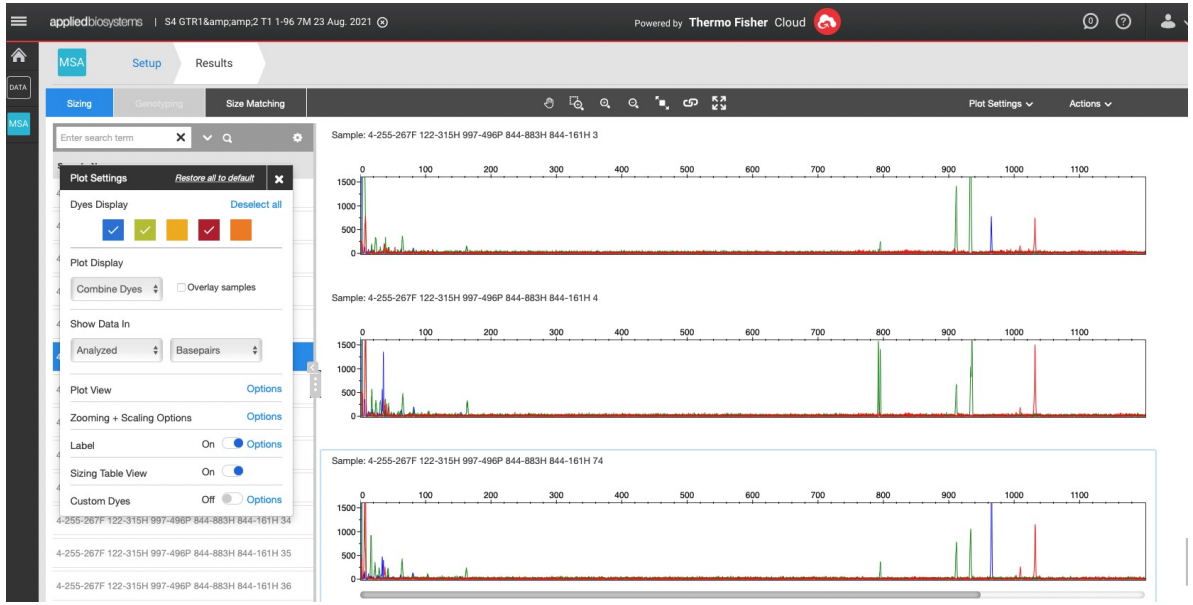
- 3500 Genetic analyzer
- PCR with one of the primer attached Florescent dyes
- 6 different dyes at the same time
- GeneMapper™ analysis
- Thermo Fisher Connect™

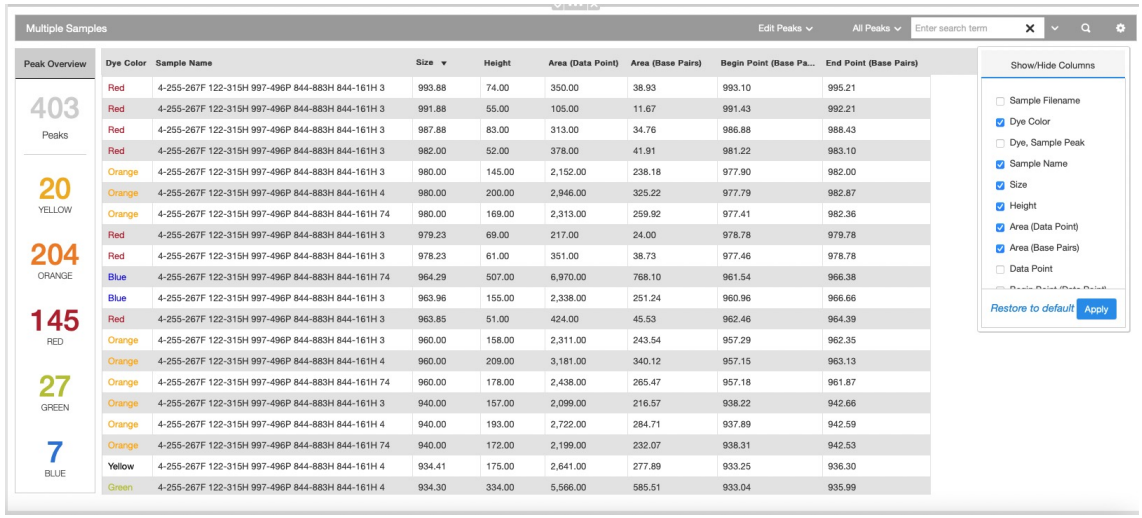


https://apps.thermofisher.com/apps/spa/#/dashboard

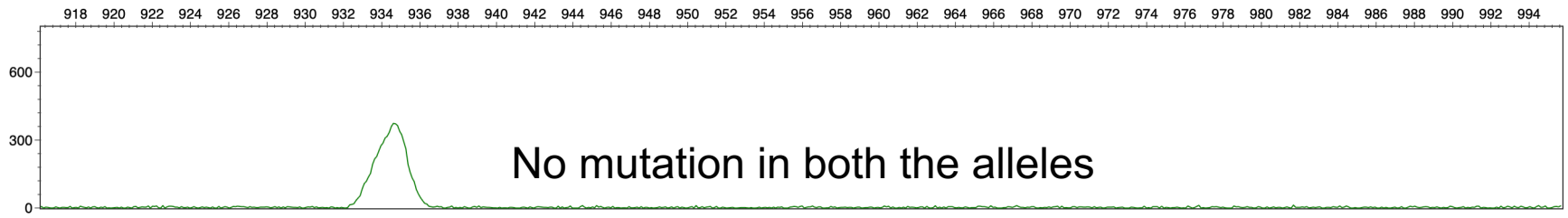
High Resolution Fragment Analysis

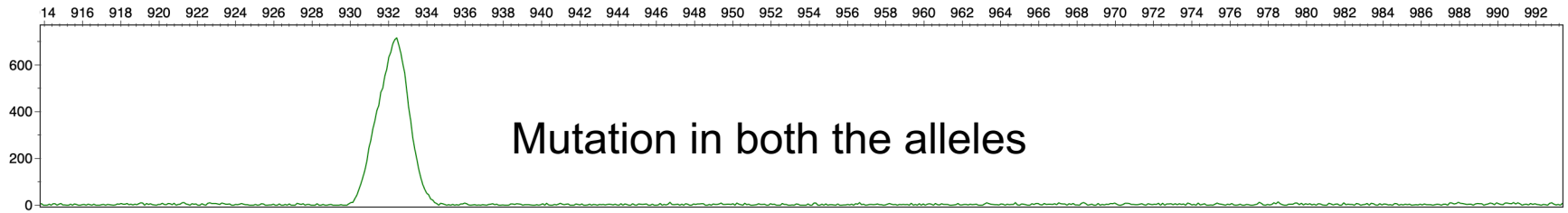
Fragment analysis using Thermo Fisher Connect™

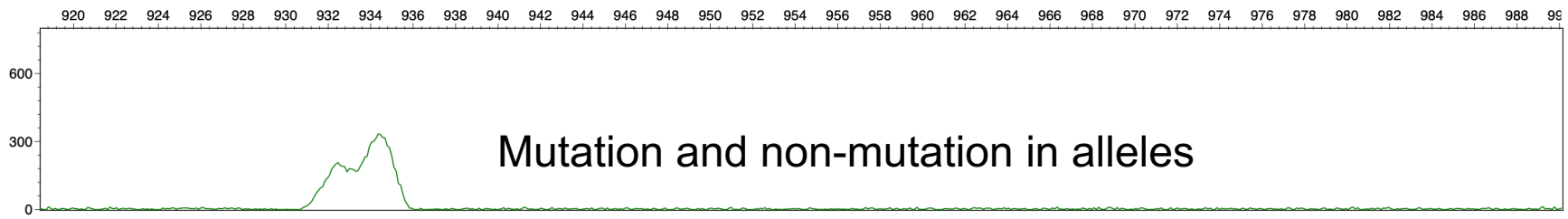




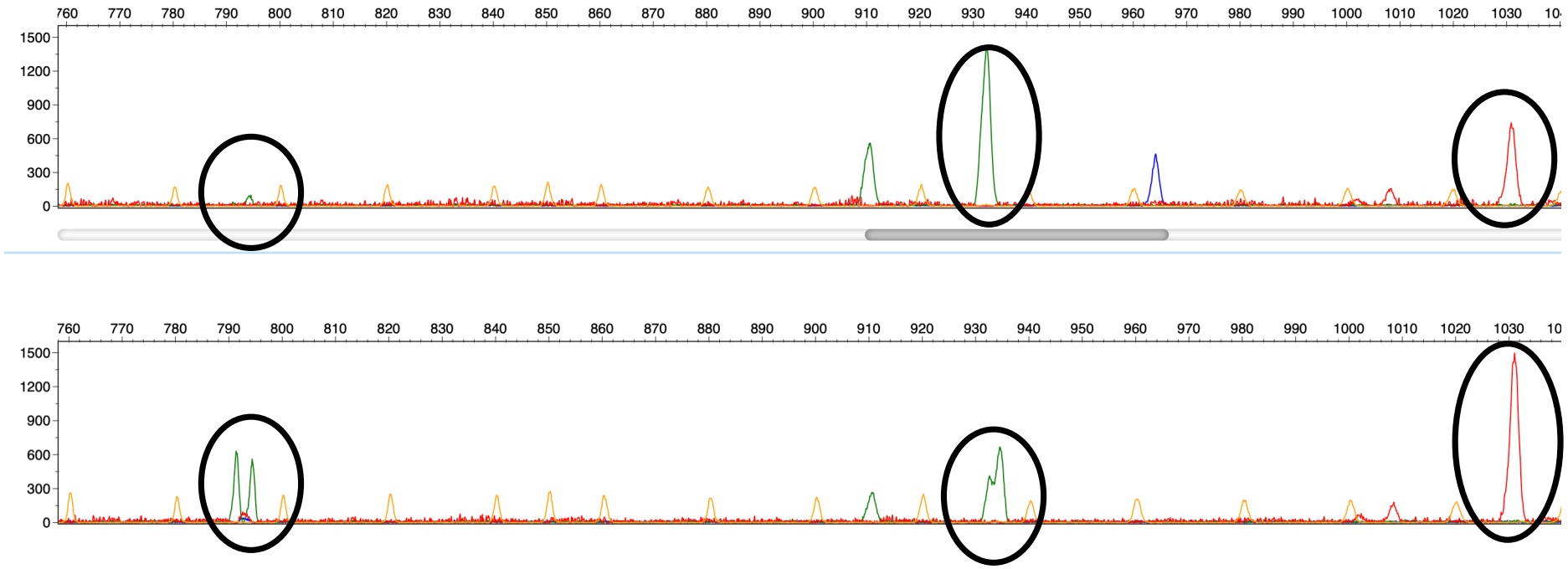
High Resolution Fragment Analysis







High Resolution Fragment Analysis

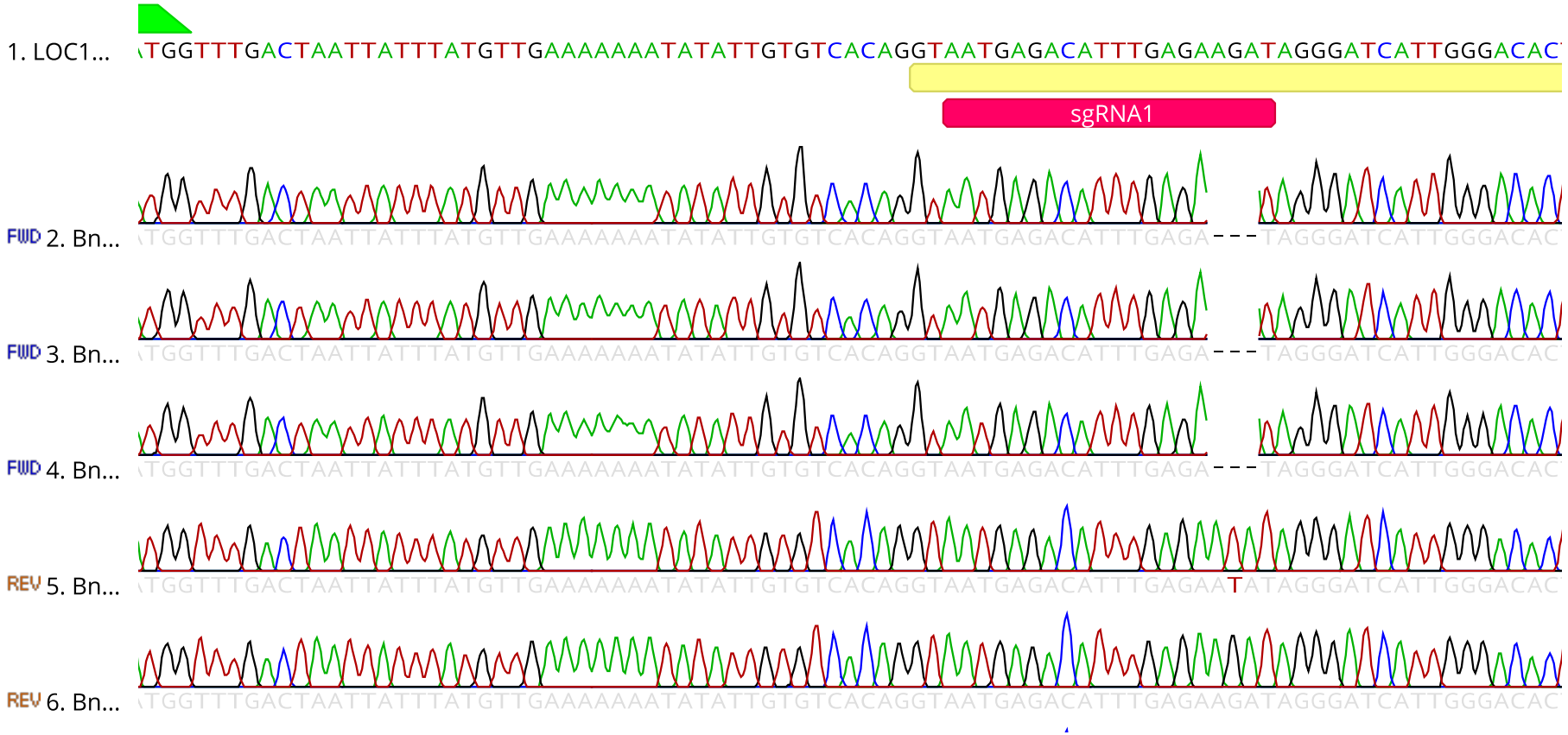


Multiple PCR products in one sample

Sanger Sequencing Result Analysis

PCR with gene-specific primers amplifying gRNA targeting DNA

Cloning and transformation



Molecular characterization of editing events

NGS-Illumina-Next Generation Sequencing Technologies

Amplicon sequencing Analysis

CRISPResso2

Analysis of genome editing outcomes from deep sequencing data

CRISPResso2

geneious prime

Cas-Analyzer

A JavaScript-based instant assessment tool for high-throughput sequencing data for genome edited cells.

Thanks to the improvements in the newest JavaScript engines in the most recent web browsers, the JavaScript based internal algorithm of Cas-Analyzer completely runs on the client-side so that large amounts of sequencing data do not need to be uploaded to the server. Currently, Cas-Analyzer supports various single nucleases (SpCas9, StCas9, NmCas9, SaCas9, CjCas9, and AsCpf1/LbCpf1) and paired nucleases (ZFNs, TALENs, Cas9 nickases, and dCas9-FokI nucleases).

Citation info: Park J. *et al*. Cas-Analyzer: an online tool for assessing genome editing results using NGS data. *Bioinformatics* **33**, 286-288 (2017).

For the ones who would like to clarify the errors derived from DNA polymerase during PCR or sequencing process, comparison of treated sample and negative control (e.g. untreated sample) is recommended.

Please input your data in below form, or download an example data here.

Sequencing Data

File Type: Paired-end reads

Read 1 (fastq or gzipped fastq): Choose File no file selected

Read 2 (fastq or gzipped fastq): Choose File no file selected

Basic Information

Full reference sequence (5' to 3'):

Nuclease Type: Single nuclease

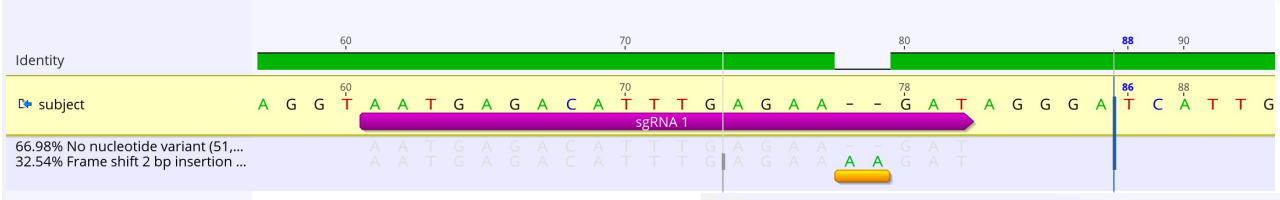
Select Nuclease:

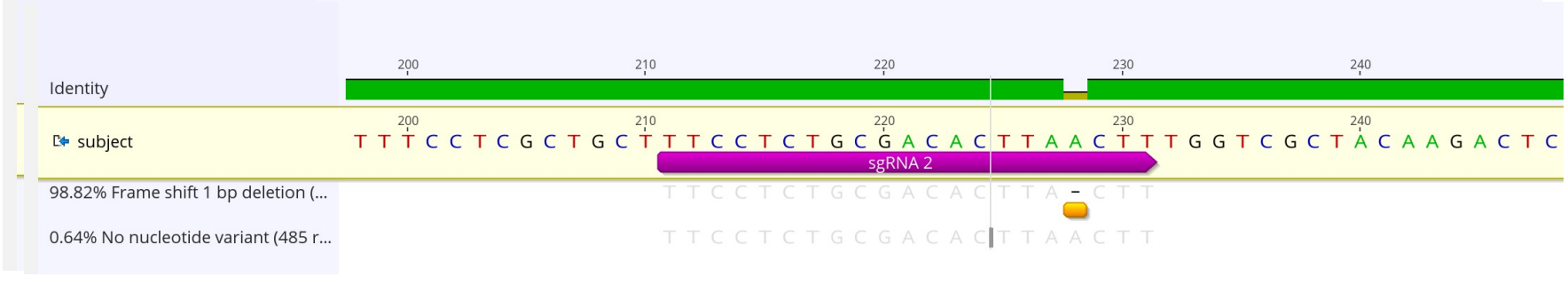
Analysis Parameters

Comparison range (R) [?] 70 or use both ends

Minimum frequency (n) [?] 1

(Optional) WT marker (r) [?] 5





Regulatory Landscape of Genome Editing

- Aim of plant breeding is to find or generate new genetic variation by searching for different alleles in germplasms or induced mutagenesis using chemicals or physical mutagens

- Regulation in North America, South America, and Asia-Pacific region rapidly approving gene-edited crops, especially crops without external DNA having no off-target edits

- On July 5, 2023, the EU adopted a proposal to deregulate the approval of gene-edited crops under New Genomic Technologies (NGT)

- NGTs are currently under debate in EU parliament with some specifications, such as
  - Up to 20 different independent genetic changes
  - Substitution or insertion of no more than 20 nucleotides
  - Deletion of any number of nucleotides
  - Modification does not interrupt an endogenous gene, etc.
  - No risk assessments, mandatory traceability, or labelling on the end product as compared to transgenic crops developed in earlier biotechnology techniques
  - Gene-editing crops can not patentable

Conclusion

- Limited genetic diversity in available elite cultivars/germplasms
- CRISPR/Cas9 could be a game-changer in modern plant breeding
- Targeted gene modifications can be obtained more precisely and faster than conventional plant breeding techniques
- Need to find out the novel traits and genes for biofortification to address the hidden hunger
- While challenges in genome editing in many crops, and advancements in nutritional biotechnology are paving the way

Questions?

selvaraju.kanagarajan@slu.se