

Genomic editing

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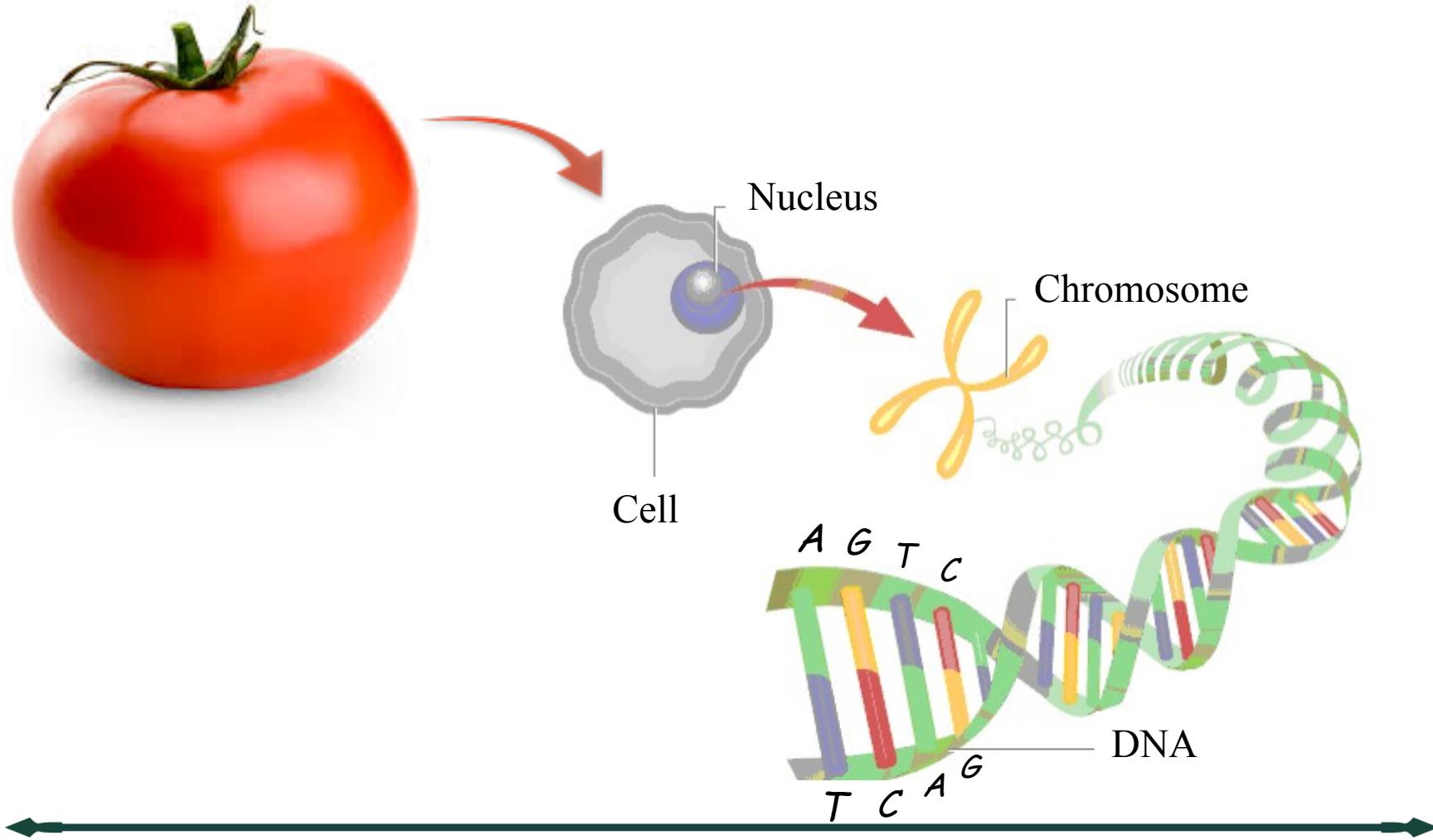
Outline

- Background information
- Objective
- Preliminary results
- Future steps



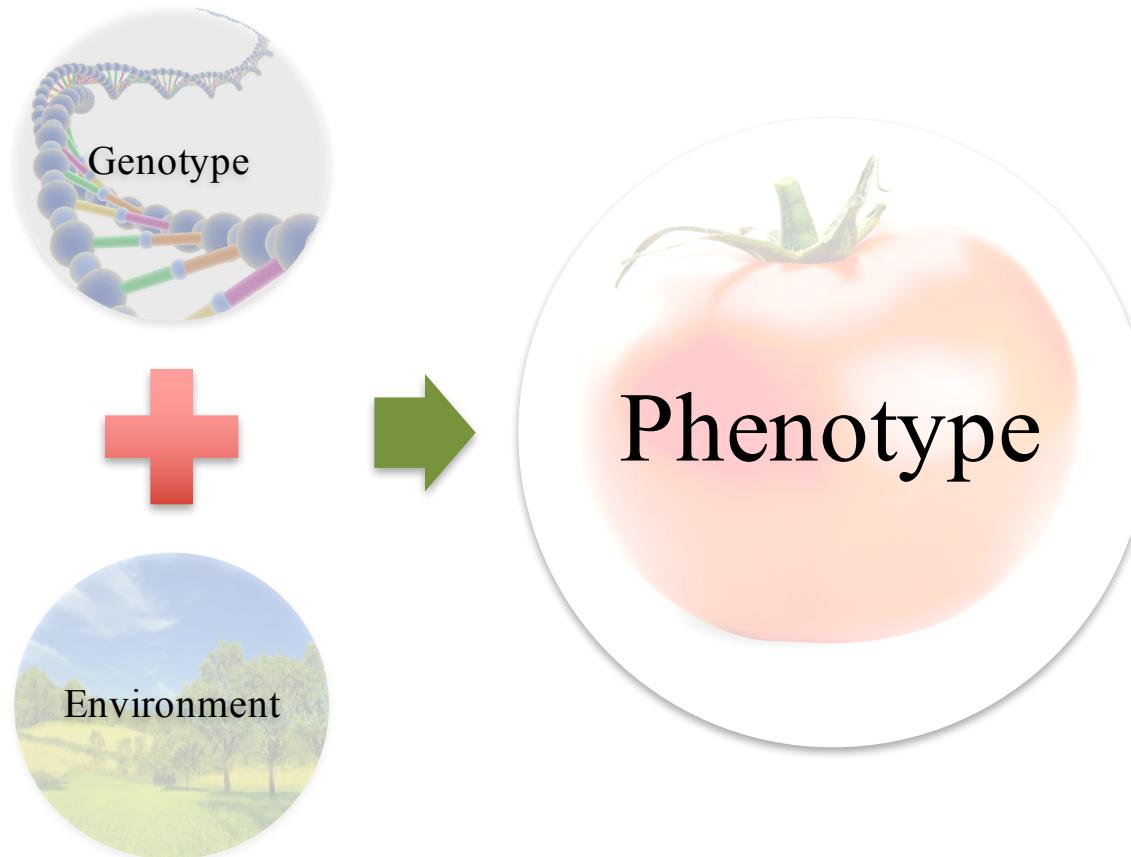
Background

Genetic information



Background

Genetic diversity

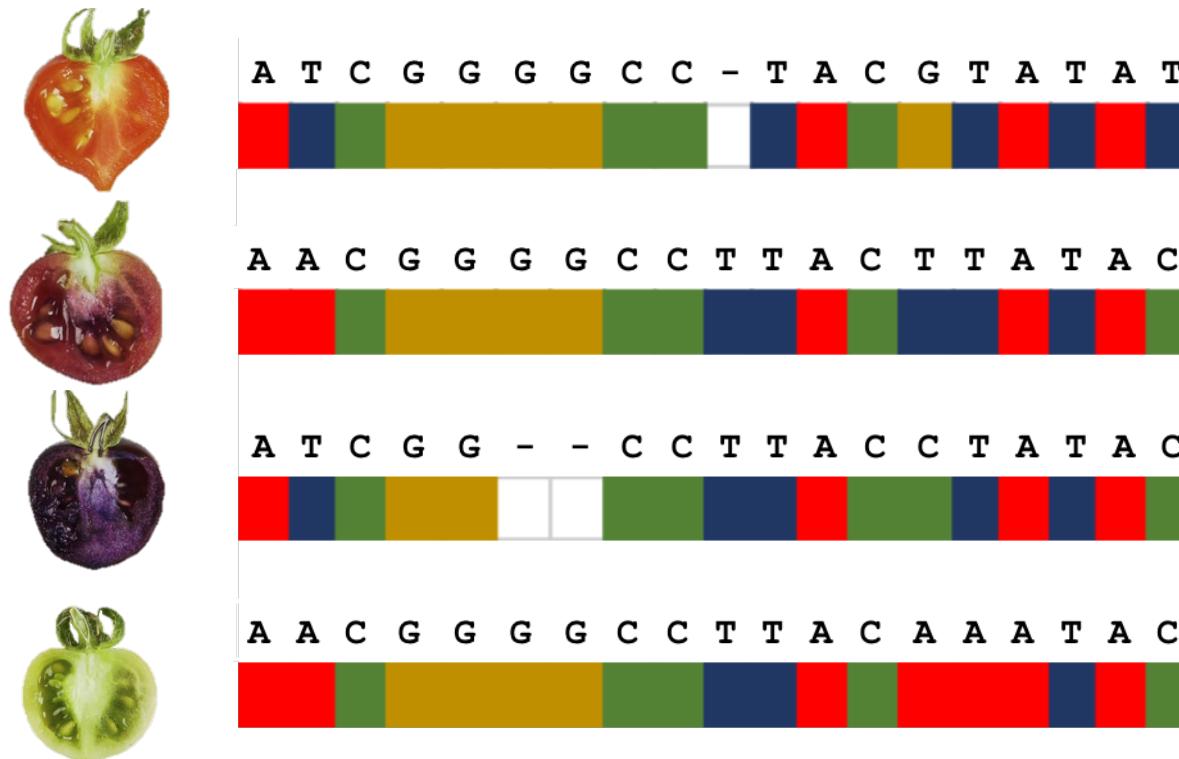


Variations in nucleotide order and composition

Background

Genetic diversity

Species: capacity to evolve rapidly under the pressure of their environment

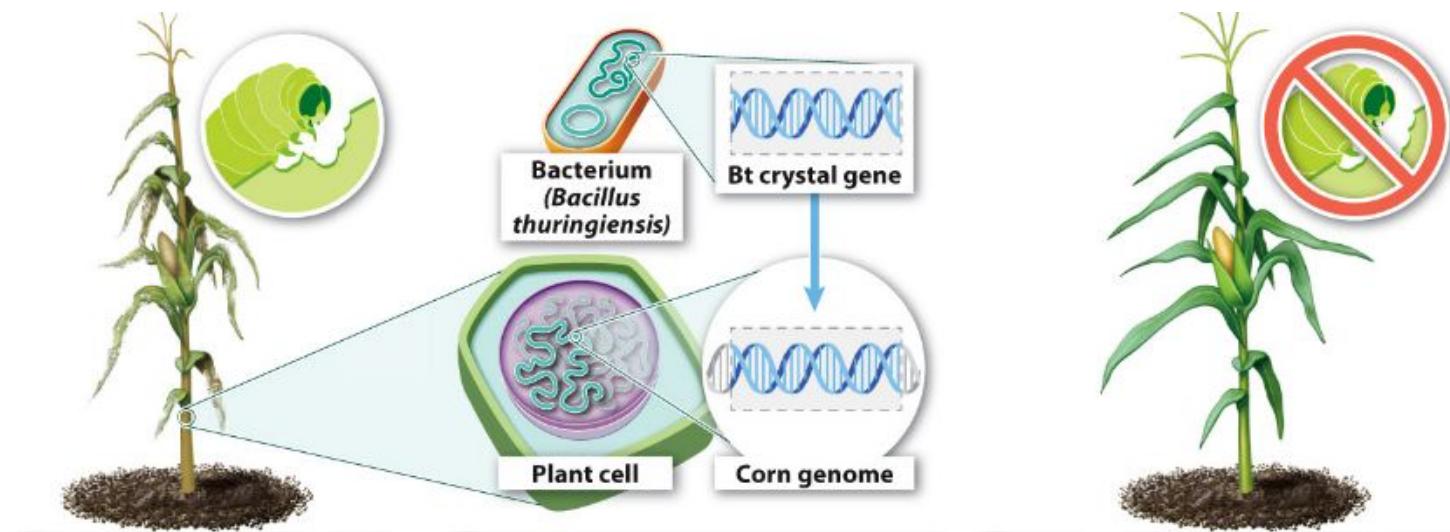


Unfortunately, domestication tends to decrease the genetic diversity

Background

Gene transformation

- Genome manipulation: Endogenous or foreign DNA



- Randomly insertion: Activation or block the expression

Background

Genome editing

- Genome manipulation: Edition of specific gene/s target/s
- Ethical issues – Eugenics!!!

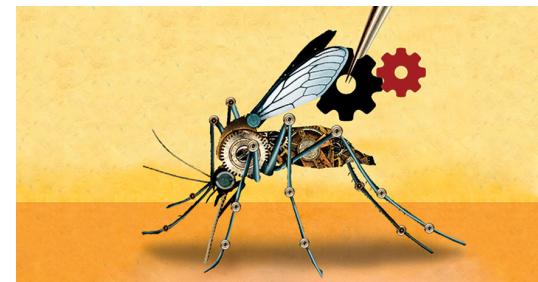


www.technologyreview.com

Myostatin gene



doi:10.1038/nature.2015.18448



www.sciencenews.org

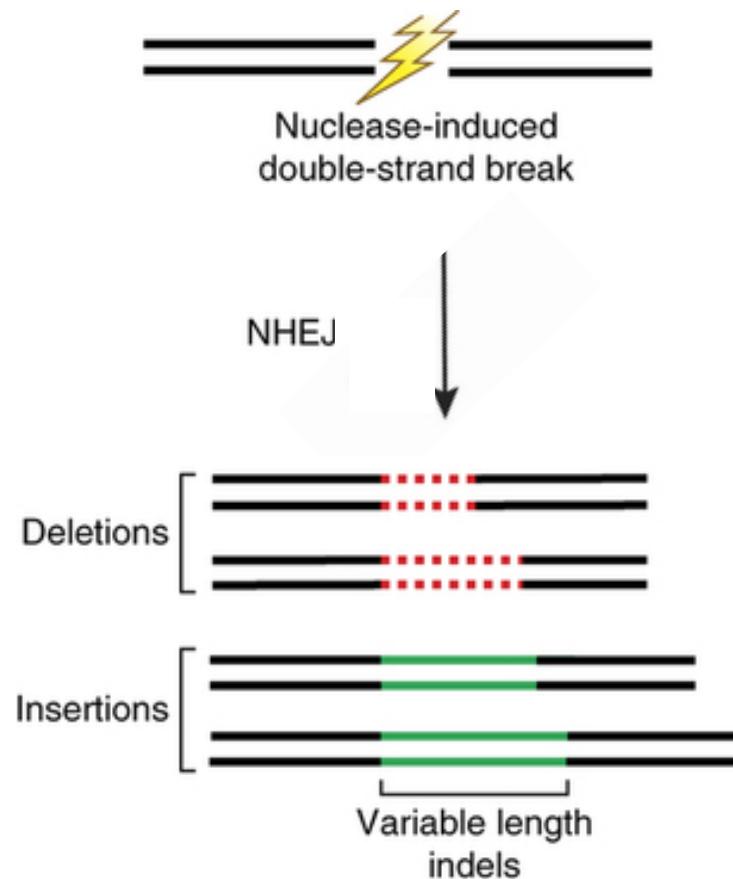
Growth hormone receptor

Gene drivers



Background

Gene editing foundation: DNA repair mechanism



Gene editing foundation: DNA repair mechanism



InDels

TGGATGTGTA ----- TTTCGGCAGGAAGCATACTGCCCTG -5
TGGATGTGTA~~GTC~~ttt **TCGG**CAGGAAGCATACTGCCCTG +1 (-2, +3)
TGGATGTGTA~~GTC~~TT**CGG**CAGGAAGCATACTGCCCTG -4
TGGATGTGTA~~GTC~~ttt **TCGG**CAGGAAGCATACTGCCCTG +1 (-1, +2)
TGGATGTGTA~~GTC~~att **TCGG**CAGGAAGCATACTGCCCTG +1 (-2, +3)
TGGATGTGTA~~GTC~~----- AAGCATACTGCCCTG -13
TGGATGTGTA~~GTC~~ gatggatgttagtc AATT**CGG**CAGGAA +15
TGGATGTGTA~~GTC~~TT**CGG**CAGGAAGCATACTGCCCTG -1
TGGATGTGTA~~GTC~~tcggcatgtg ATT**CGG**CAGGAAGCATAC +9 (-1, +10)
TGGATGTGTA~~GTC~~----- **TCGG**CAGGAAGCATACTGCCCTG -4
TGGATGTGTA~~GTC~~----- TT**CGG**CAGGAAGCATACTGCCCTG -5

TGGATGTGTA~~GTC~~ATT**CGG**CAGGAAGCATACTGCCCTG Wild type

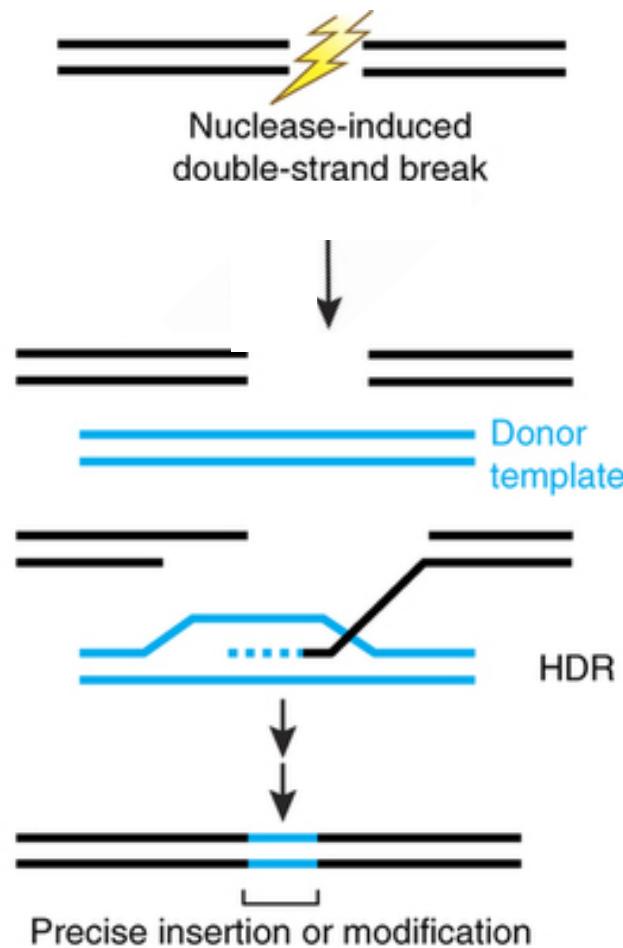


“Un-functional gene”



Background

Gene editing foundation: DNA repair mechanism

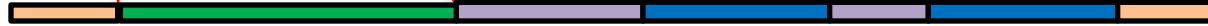


Genome editing

Original gene



Edited gene



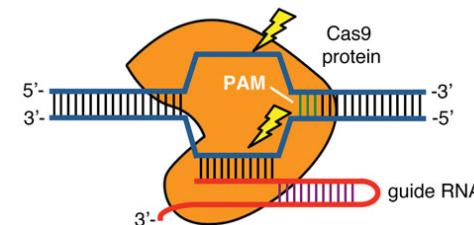
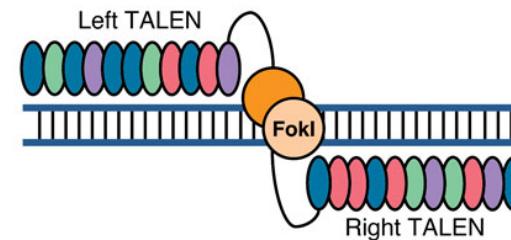
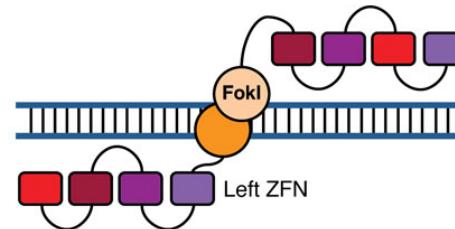
New allele



Background

Genome editing technologies

- Zinc-finger nucleases: ZFNs
- Transcription activator-like effector nucleases: TALENs
- Clustered, regularly interspaced, short palindromic repeat: CRISPR-Cas9



Objectives

2. Modification of the late blight resistance gene (RB) in transgenic plants using CRISPR/Cas9

Activities:

- 2.1** Block the expression of a recognition site of the RB transgene
- 2.2** Modify a recognition site of the RB transgene



Introduction

Late blight (*Phytophthora infestans*)



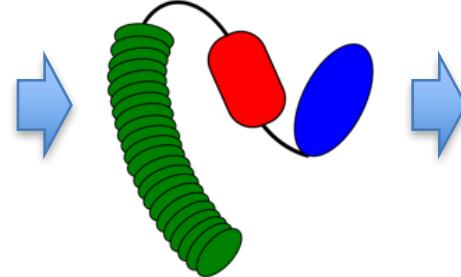
MSU potato breeding program.

- Irish potato famine in the 18th century
- Losses until 16% of the total global production
- Fungicides: Can be ineffective or indirectly select resistant strains

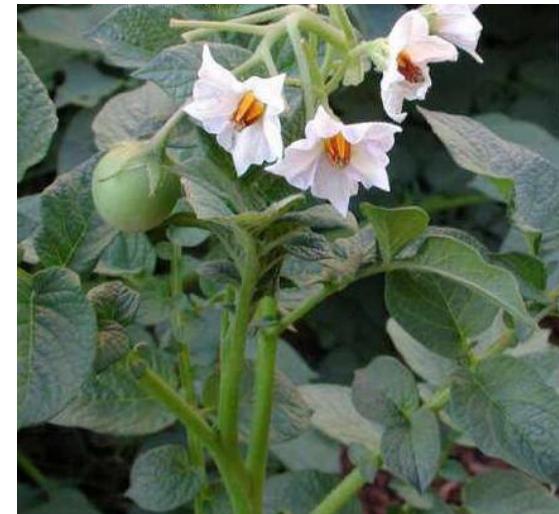




Solanum bulbocastanum



RB gene
(CC-NB-LRR)

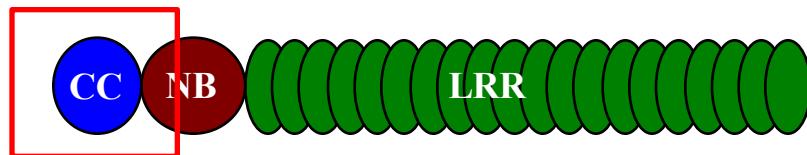


Solanum tuberosum

Katahdin + RB



Coiled-Coil domain in plants



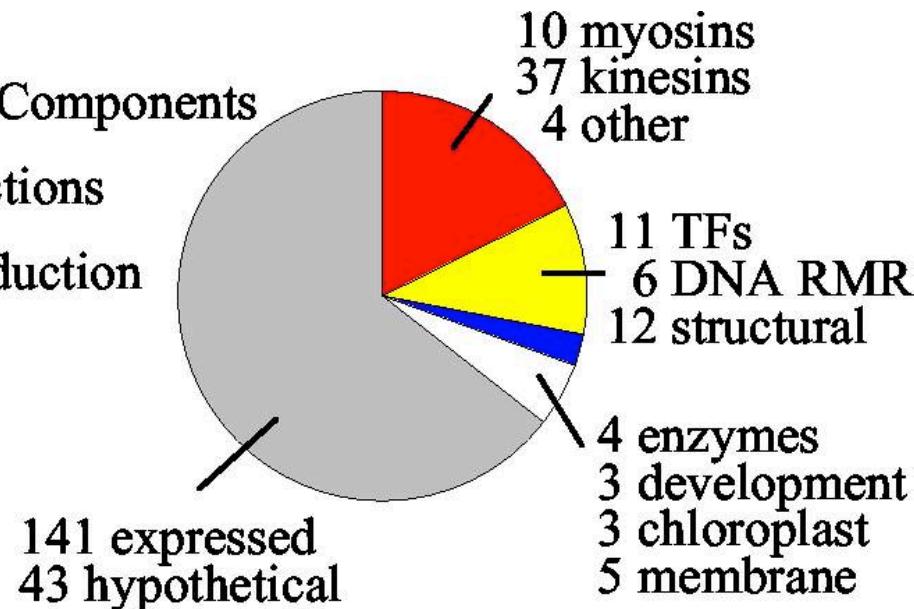
Genome name	Number of standard cc proteins	Number of short cc proteins	Number of fuzzy cc proteins	Total number of cc	% of proteins with cc
<i>A. thaliana</i>	256	134	895	1285	3.63
<i>Z. mays</i>	333	165	943	1441	2.68
<i>S. lycopersicum</i>	172	148	646	966	2.79
<i>S. tuberosum</i>	232	151	924	1307	2.32

Rackham *et al.* 2010.
PMID: 20813113



Gene ontology: CC in *Arabidopsis thaliana*

- 51 ■ Cytoskeletal Components
- 29 □ Nuclear Functions
- 7 ■ Signal Transduction
- 15 □ Other
- 84 ■ Unknown

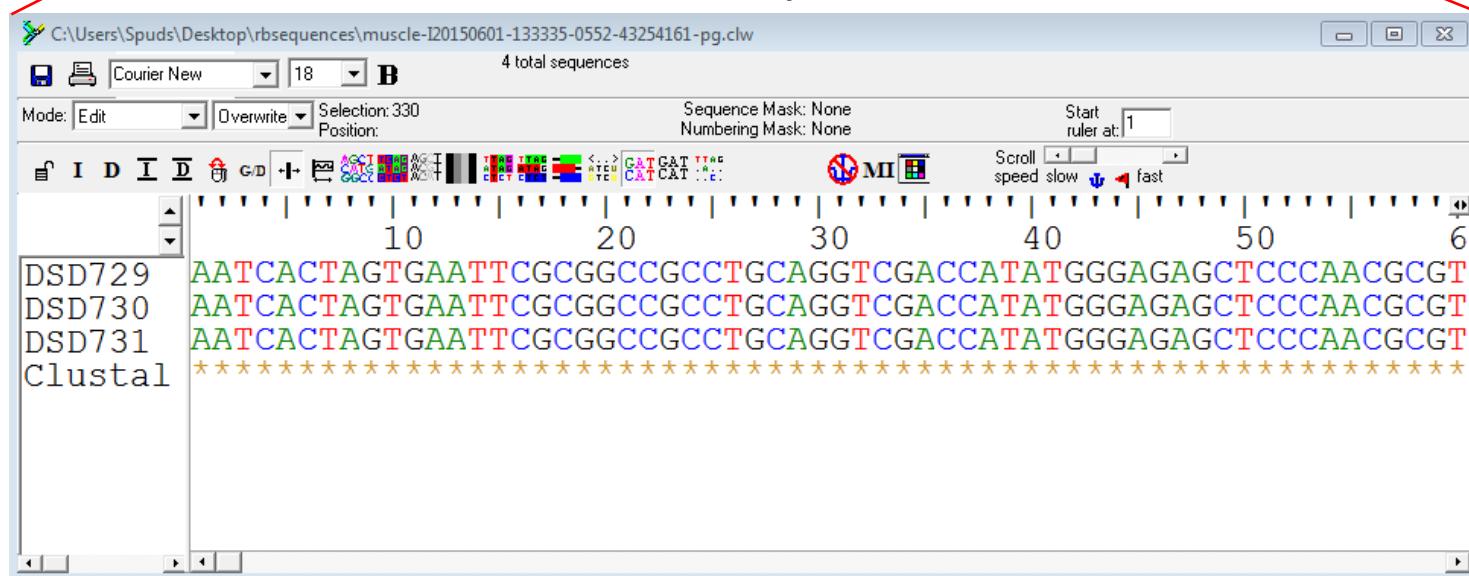


Rose *et al.*, 2004
PMID: 15020757

Amplification, cloning and sequencing of the RB transgene

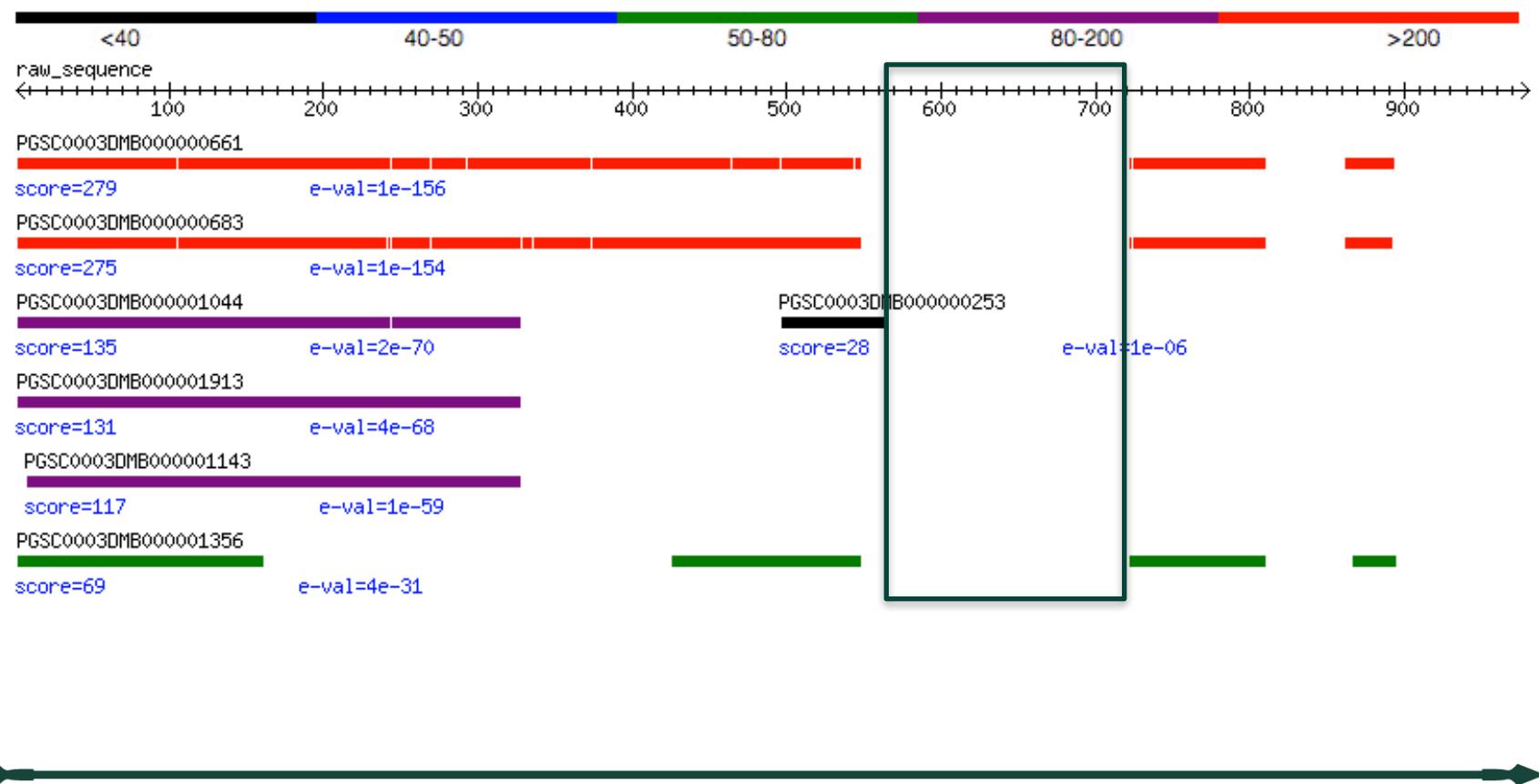


384 bp



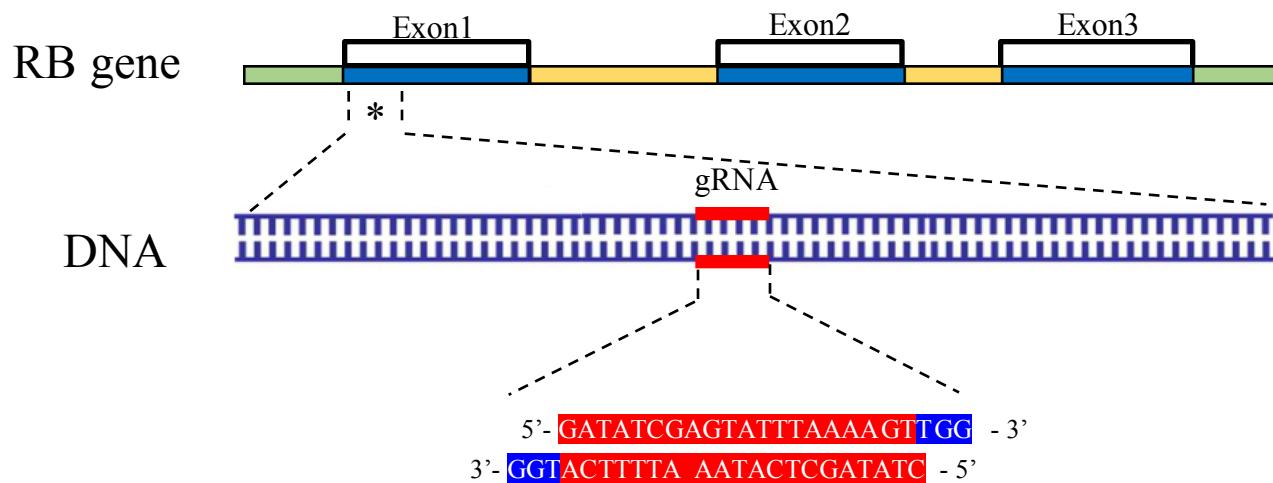
Comparison of CC among *S. tuberosum* genomic sequences

Spud db:



Preliminary results

gRNA design



gRNA insertion

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

col1	TTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGAAGCTGAGT
col3	TTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGAAGCTGAGT
col7	TTAAGTTGAAAACAATCTTCAAAAGTCCCACATCGCTTAGATAAGAAAACGAAGCTGAGT

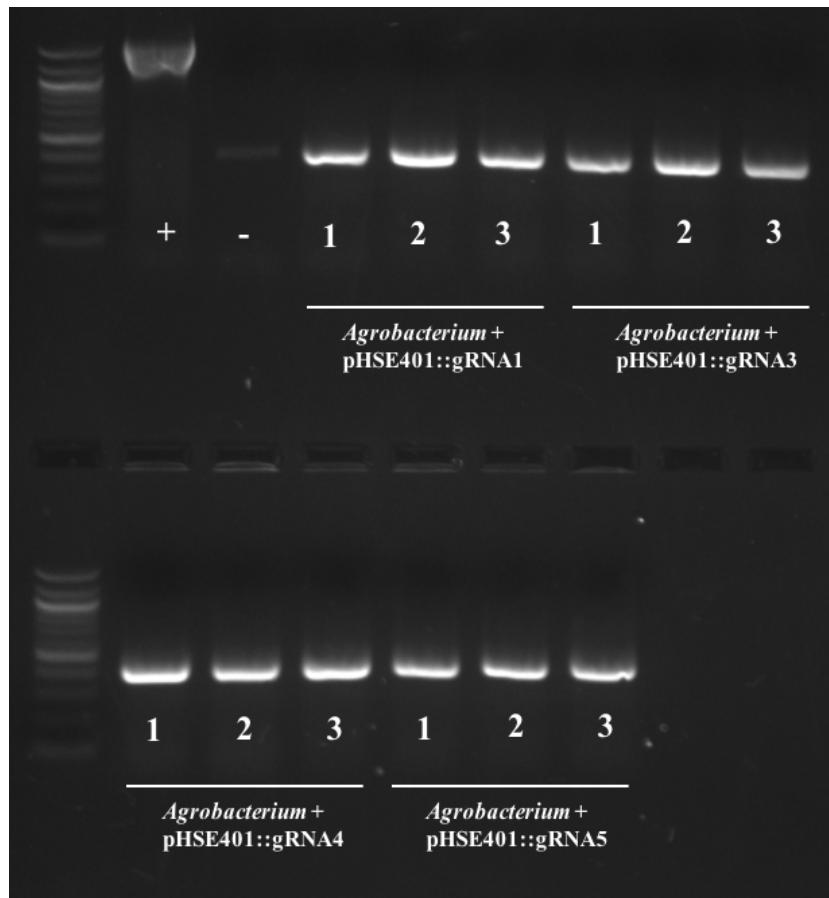
col1	TTATATACAGCTAGAGTCGAAGTAGTGATTG
col3	GATATCGAGTATTTAAAAGTGT
col7	TTTA GAG
col1	TTATATACAGCTAGAGTCGAAGTAGTGATTG
col3	GATATCGAGTATTTAAAAGTGT
col7	TTTA GAG

col1	CTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAG
col3	CTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAG
col7	CTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACC GAG

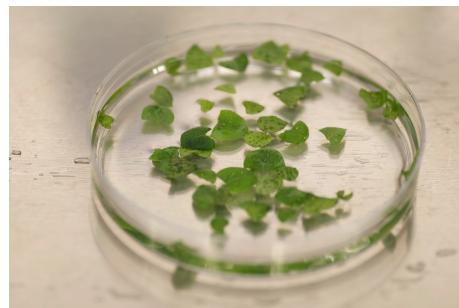
gRNA1 CTAGAGTCGAA~~GTAGTGATTG~~CTGGGAGAAC~~TCTGTGGCCT~~GTTTGAGCTAGAAATAGCAAGTTAAA
gRNA3 CTAGAGTCGAA~~GTAGTGATTG~~CAGATCGAAAGTACAACGAGGTTTGAGGCTAGAAATAGCAAGTTAAA
gRNA5 CTAGAGTCGAA~~GTAGTGATTG~~TTCCTTGTCAATTGATTAGGC~~GTTTGAGG~~GAGCTAGAAATAGCAAGTTAAA



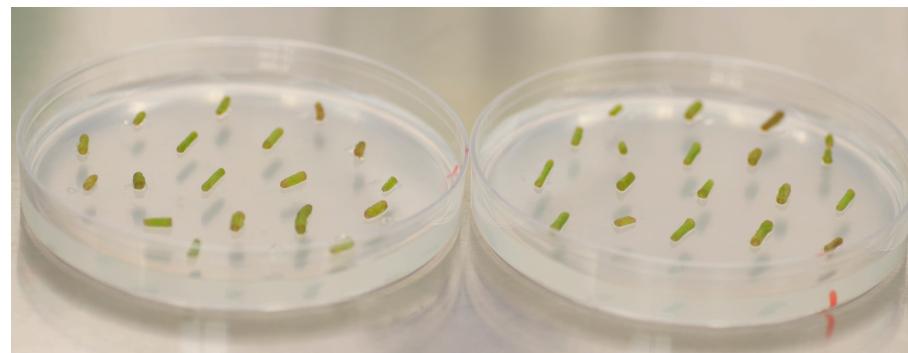
Agrobacterium transformation



Transformation assays



Infection with *A. tumefaciens*



Further Steps

- Perform leaf detachment assays
- Perform guideSeq analysis
- Start activity 2.

