



NRC-CMRC

*Plant
Biotechnology
Institute*

NRC-PBI Brassica genomics resources and DOTM/BOP TILLING Resource

Faouzi Bekkaoui

**Canadian Brassica Genomics Network
11 Dec 2008, Saskatoon**



National Research
Council Canada

Conseil national
de recherches Canada

Canada

Crop Genomics Technologies

Structural Genomics



Functional Genomics

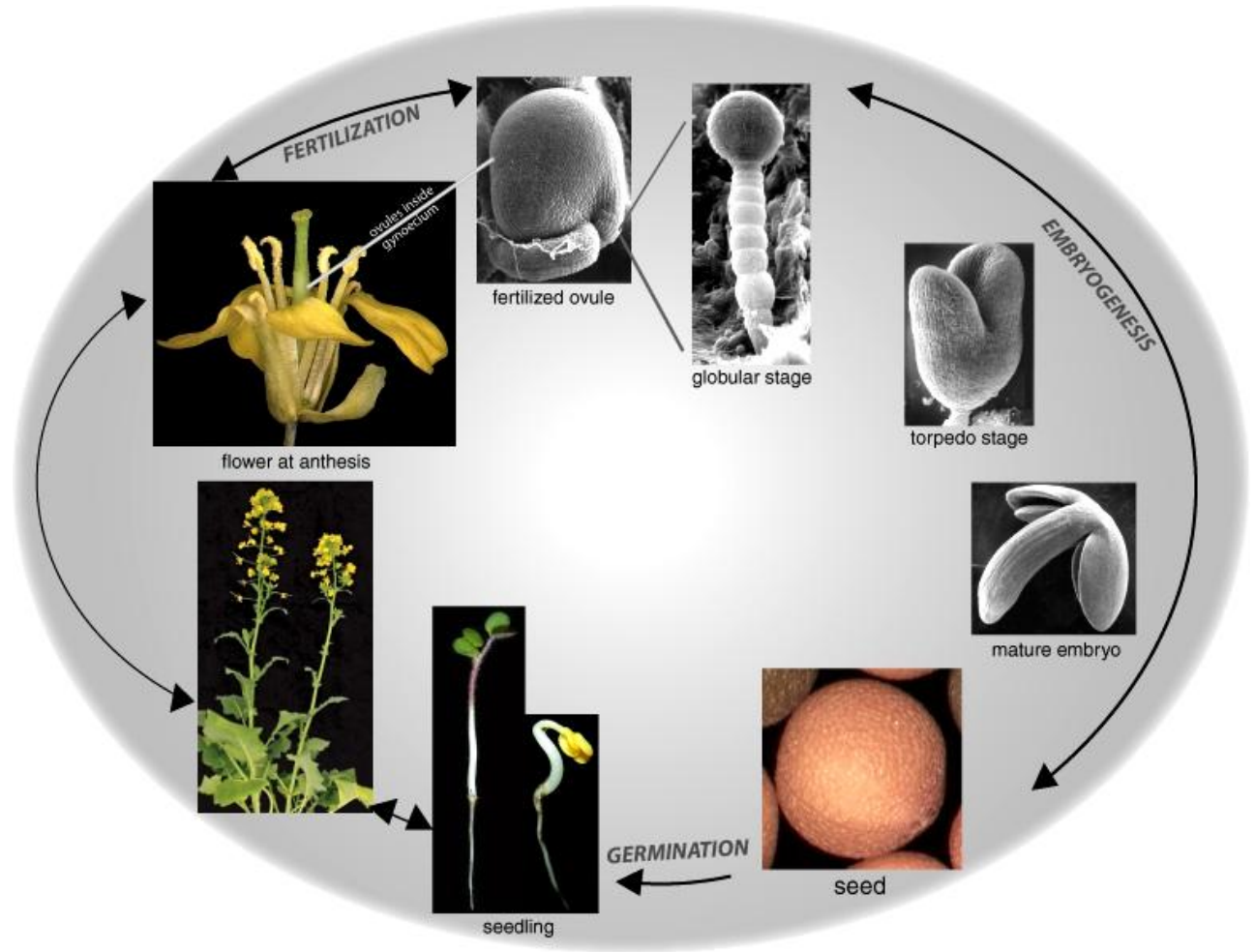


Improved knowledge
& Applications



- Brassica Expressed Sequence Tags (ESTs)
- DNA Genomic sequencing
- Bioinformatics
- Proteomics /Metabolomics
- DNA microarrays
- Plant hormone profiling
- Transgenics
- TILLING
- Field trials

Brassica Seed Development

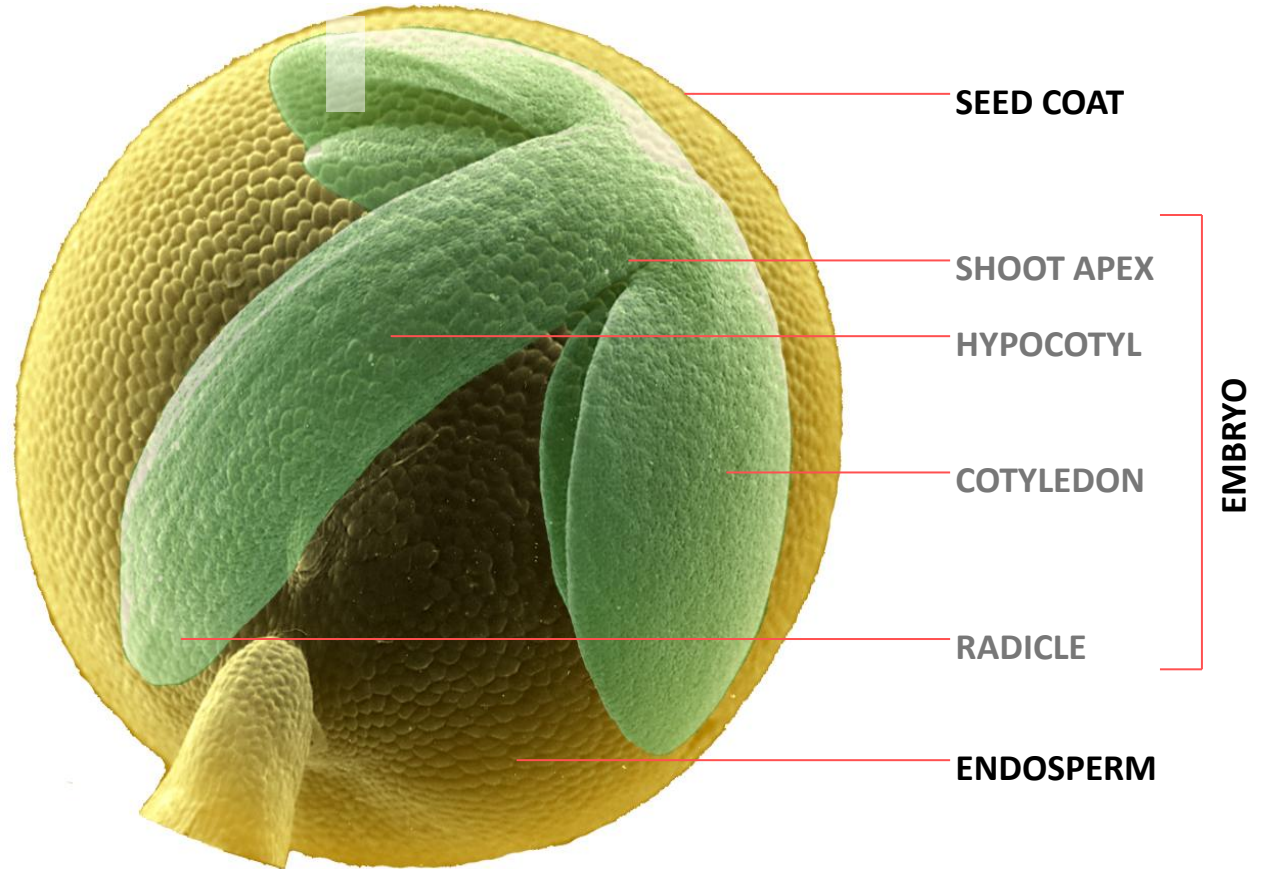


Example of targets

- Large seed size
- Uniform seed germination
- Reduced coat thickness

Brassica Seed

Components	%
Oils	45
Proteins	22
Carbohydrates	20
Water	9
others	4



Example of targets:

- Enhanced oil content
- Modified proteins
- Modified oils
- Reduced anti-nutritional factors

Germplasm

Brassica napus

- DH 12075
- Yellow seeded YN01-429

Dr. Gerhard Rakow
AAFC- Saskatoon



DNA sequencing

- Capillary DNA sequencing
 - Three 3730 XL ABI systems
- Next Generation Sequencing
 - Roche 454 Titanium FLX series
 - > 1M reads of 400 bases /10-h run



- EST data curation and analysis
- Development of software, e.g. FIESTA
- Data integration / Genbank submission
- DNA microarrays support



Brassica Seed ESTs

➤ ~ 436,000 seed ESTs

- Various stages of embryo development ~145,000
- Early seed stages under stress conditions ~34,300
- Microspore embryogenesis ~29,000
- Germination ~48,900
- Dormancy ~33,200
- Seed Coat ~121,800
- Endosperm ~23,500

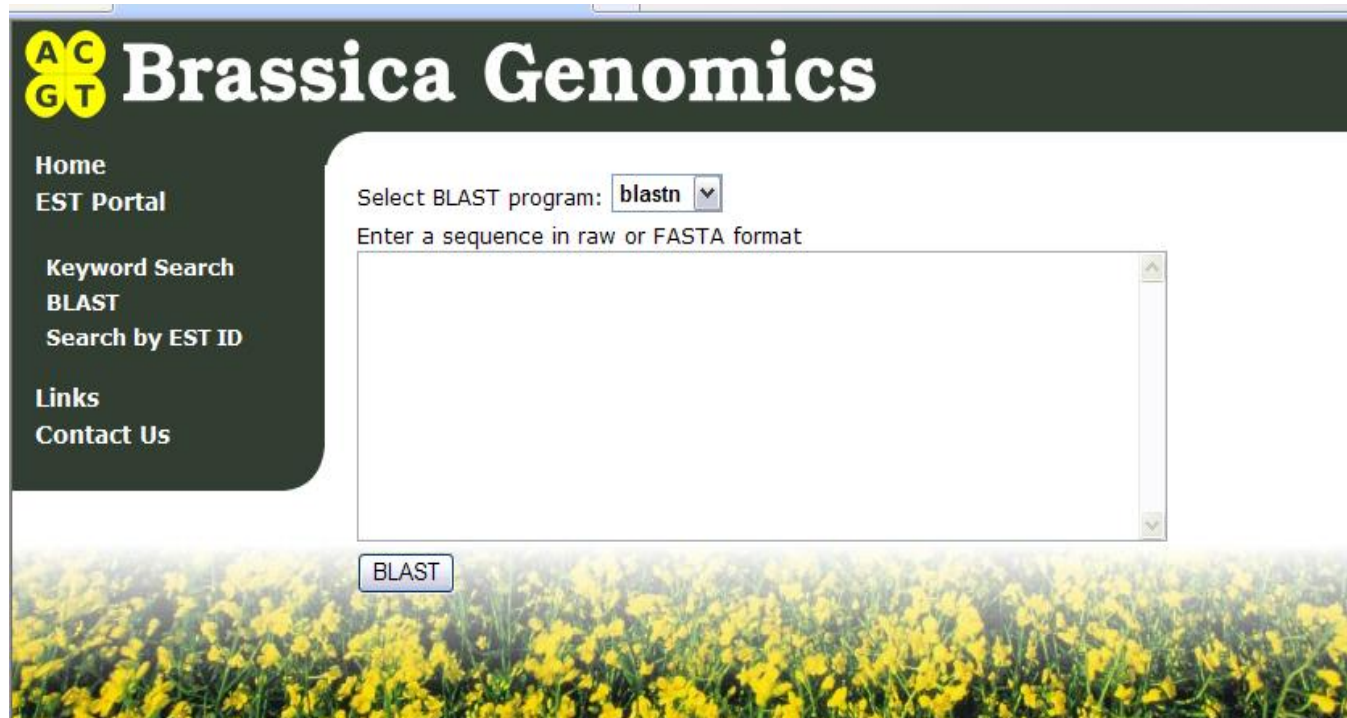
ESTs deposited in GenBank

Team:

R. Datla, F Georges, J Krochko, A Cutler, E Tsang, G Selvaraj and J Zou

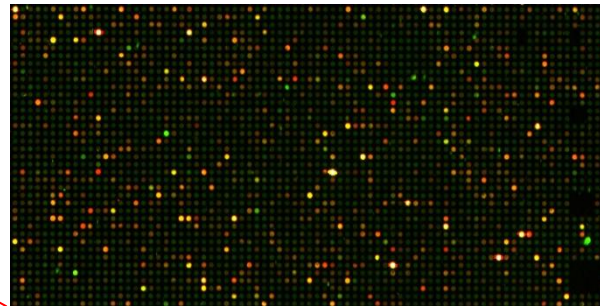
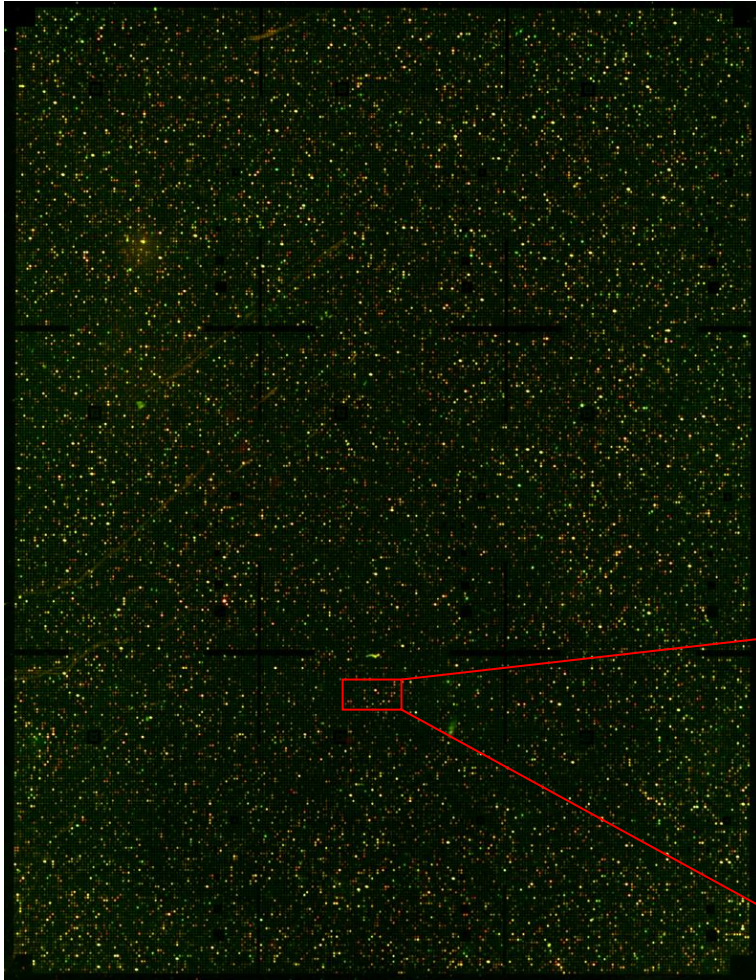
Access to ESTs

- www.brassicagenomics.ca/ests/blast.html



The screenshot displays the Brassica Genomics website interface. At the top left, there is a logo consisting of a yellow four-leaf clover with the letters 'A', 'C', 'G', and 'T' in each leaf, followed by the text 'Brassica Genomics' in a large, white, serif font. Below the logo, a dark green sidebar contains a list of navigation links: 'Home', 'EST Portal', 'Keyword Search', 'BLAST', 'Search by EST ID', 'Links', and 'Contact Us'. The main content area features a search form. At the top of the form, it says 'Select BLAST program:' followed by a dropdown menu currently set to 'blastn'. Below this, it prompts the user to 'Enter a sequence in raw or FASTA format' and provides a large, empty text input field. At the bottom of the form, there is a blue button labeled 'BLAST'. The background of the website is a photograph of a field of yellow rapeseed flowers.

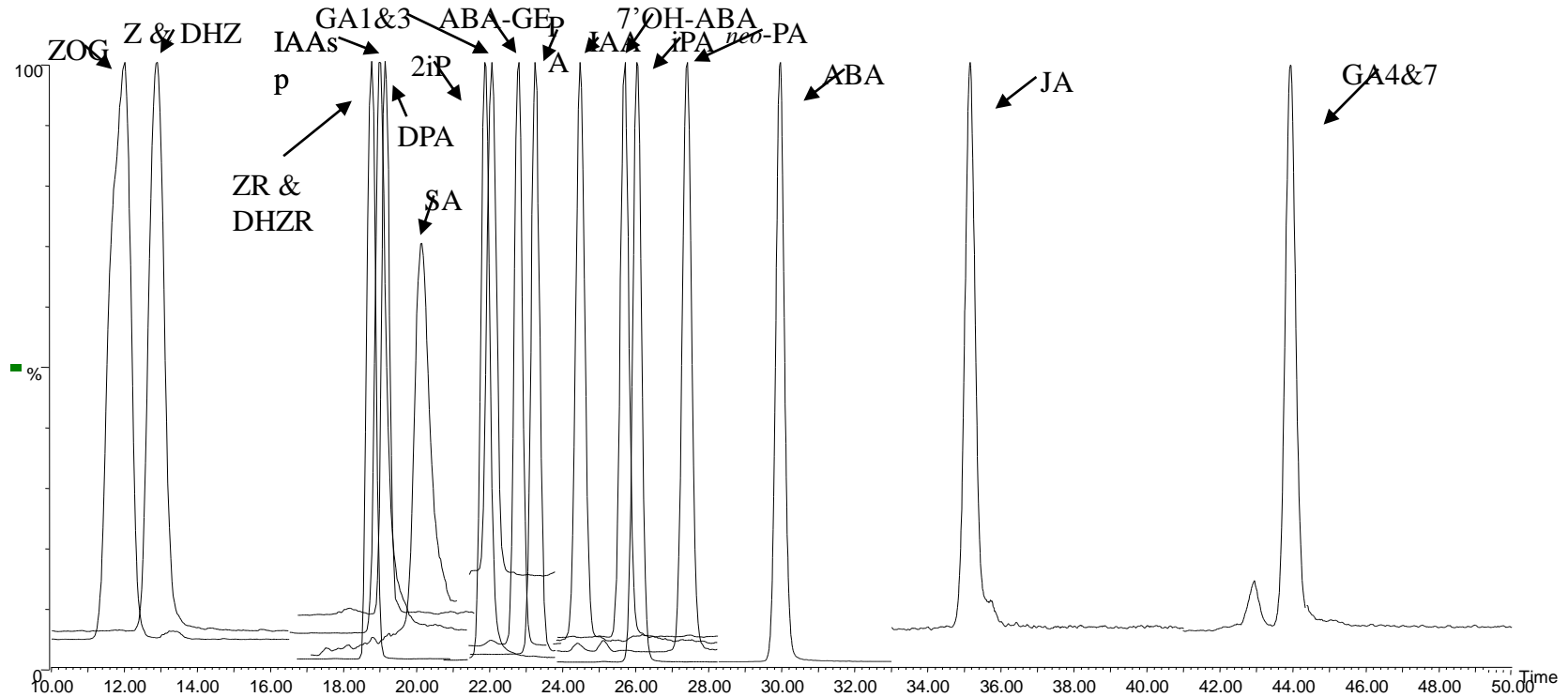
High density Brassica CombiMatrix 90K DNA array (developed with AAFC)



- Started with ~ 800,000 ESTs
- 90,000 selected for primer design & array preparation
- Estimated *Brassica napus* genes coverage: 68%

D Xiang, Y Cao, D Schwab, N Sharma, J Nowak, M Links, A Sharpe, I Parkin, A Cutler, G Selvaraj, P Fobert, W Keller, F Bekkaoui, R Datla

Targeted Metabolomics Plant Hormone Profilings



Contact : Irina Zaharia & Sue Abram

Transgenics

For each construct:

- Inoculate ~1200 hypocotyl explants
- Aim for ~20 transgenics
- Regenerants are screened first by phenotype on selection (colour, vigor, rooting) then by PCR for 35S promoter.
- Deliverables are rooted shoots in jars

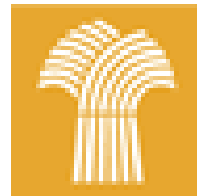


www.brassicagenomics.ca

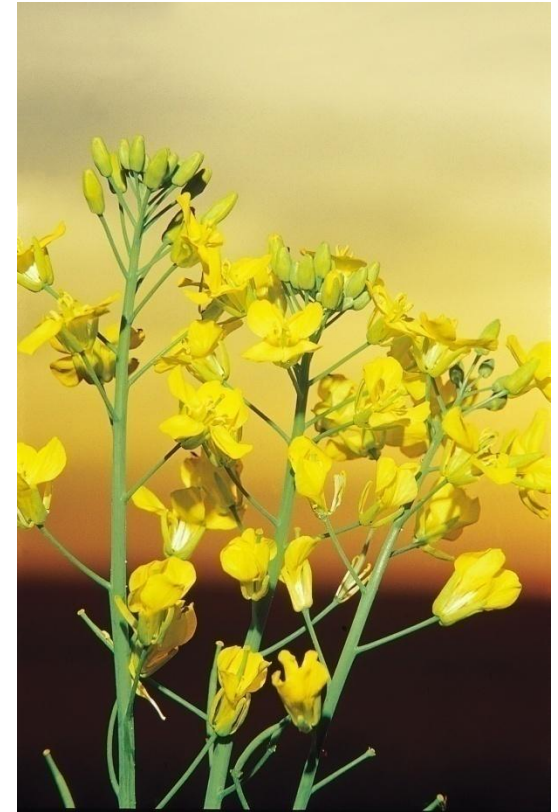
- **Objective**
 - Provide information on resources developed by PBI and collaborators
 - Link to other key Brassica genomic resources
- **Genomic Resources**
 - Brassica EST
 - DNA arrays
- **Projects / Programs**
 - Genome Canada projects / Federal initiatives
- **Workshops**
 - Applying genomics to Canola Improvement Workshop
 - Canola Industry Meeting
 - Cruciferae diversity: novel crops and traits workshop
- **Other Links**

Acknowledgements

- PBI Brassica genomics team
& Collaborators



Government of
Saskatchewan



GenomeAlberta



GenomePrairie



GenomeCanada

DOTM/BOP Brassica napus TILLING project

George Haughn (haughn@interchange.ubc.ca)

Erin Gilchrist (ering@interchange.ubc.ca)



- Designing
- Oilseeds for
- Tomorrow's
- Markets

**Bioactive
Oils
Program**

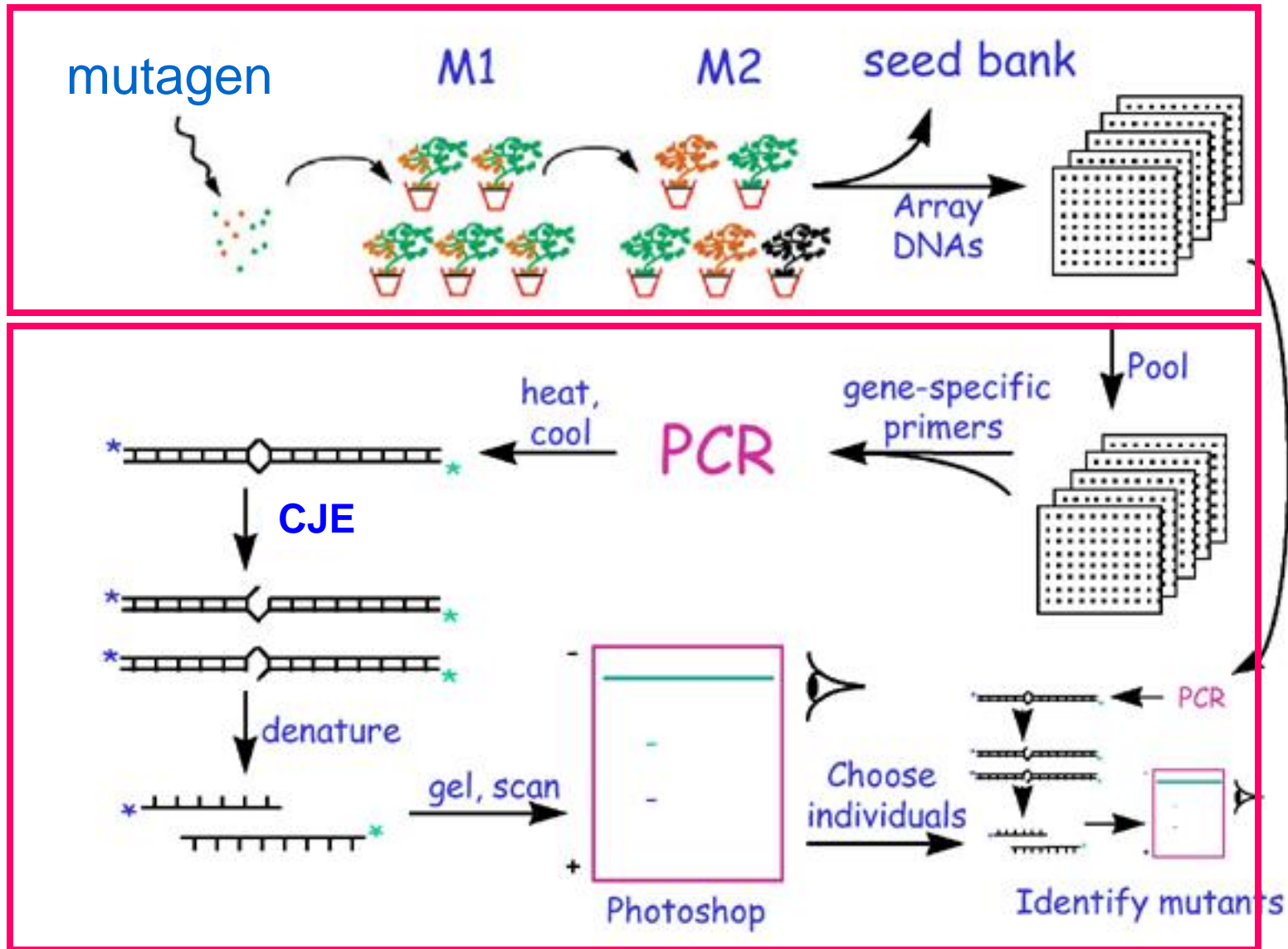
DOTM/BOP TILLING project

Objective:

Construct mutagenised population of 4000 – 6000 M₂
Brassica napus lines (from DH12075 seeds)

Request-driven TILLING on DNA from these lines to identify
genes important in seed coat development or oil seed
properties

TILLING



Population Status



Population Status

M1 grown/growing	4300
M2 grown/growing	2700
DNA extracted	1200



Summary

- *B. napus* TILLING
 - Number of M₁ lines grown/growing = 4300
 - Number of M₂ lines grown/growing = 2700
 - Number of lines screened using TILLING = 768
 - Number of genes examined = 5
 - DNA screened = 5 Mb
 - Number of unique mutations identified = 56
 - 38 sequenced (15 missense, 22 silent, 1 null)
 - Mutation rate > 1 in 90 kb

Approximately one in every 70 plants carries a mutation in any gene.

Availability to the Public of *Brassica napus* TILLING

A TILLING website interface for public requests is being designed in collaboration with the DOTM bioinformatics group.

Steps to setup up an open TILLING facility at UBC have been taken.

Public access to TILLING may be available as early as next spring.

George Haughn (haughn@interchange.ubc.ca)

Erin Gilchrist (ering@interchange.ubc.ca)



- Designing
- Oilseeds for
- Tomorrow's
- Markets

**Bioactive
Oils
Program**