

REDUCING THE LEVEL OF ANTI-NUTRITIONAL FACTORS IN CANOLA MEAL

**Randall Weselake
University of Alberta**

**Jeff Parker
Genome Alberta**

Canola Meal Research Meeting

September 28, 2007



GenomeCanada



GenomeAlberta

DESIGNING OILSEEDS FOR TOMORROW'S MARKETS



Presentation Outline

- The DOTM Team
- Project objectives and overview
- Gene expression and functional genomics
- Expressed sequence tags (ESTs)
- DNA microarray
- Tissue specific protein expression
- RNAi – transgenic expression interference
- Validation of gene involvement
- Prototype plants
- TILLING – mutation identification
- DOTM progress

The DOTM Team

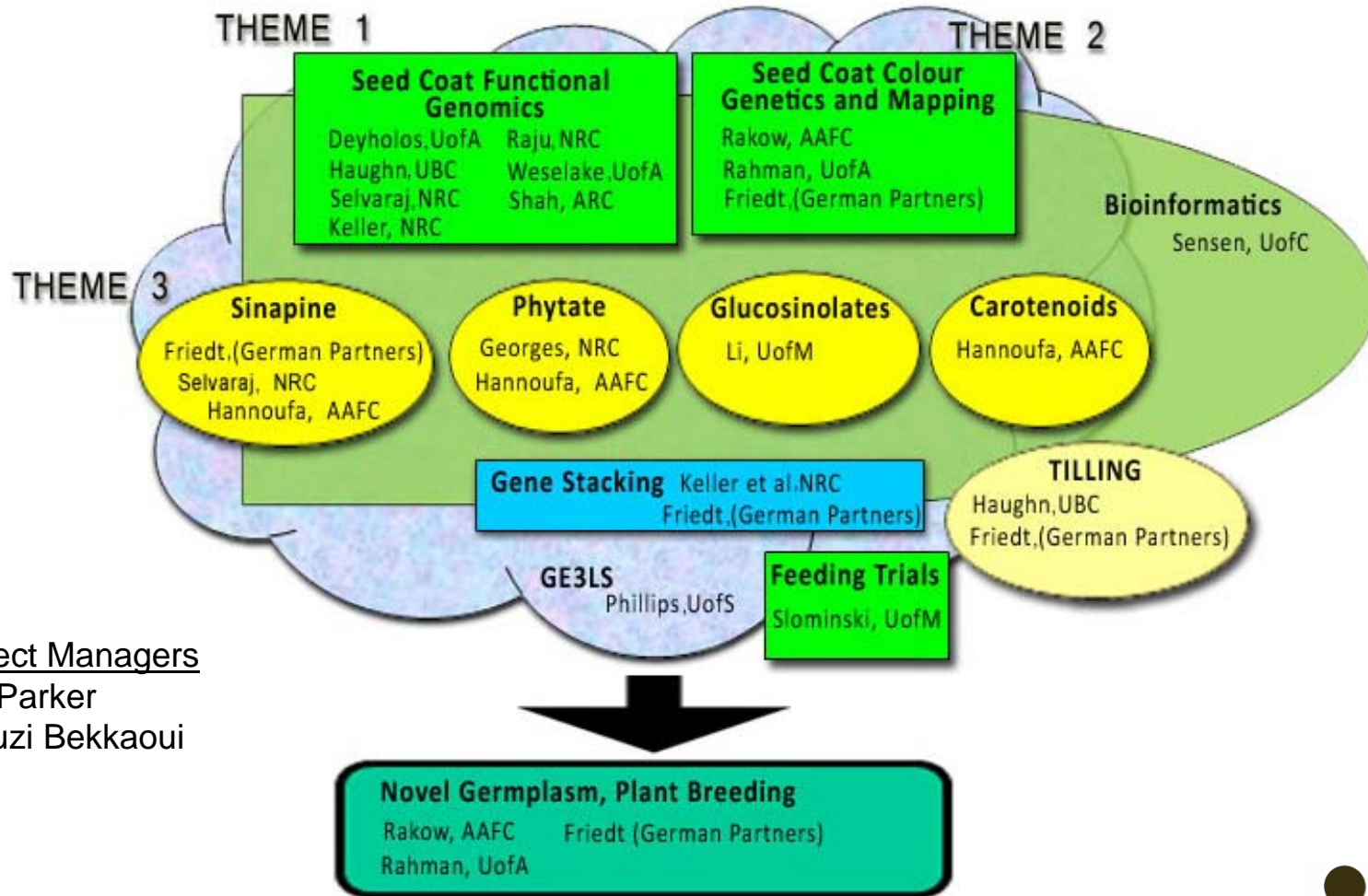


University of Alberta	NRC, Plant Biotechnology Institute
Randall Weselake *	Wilf Keller *
Mike Deyholos *	Gopalan Selvaraj *
Habibur Rahman	Raju Datla
University of Saskatchewan	Fawzy Georges
Peter Phillips	Alberta Research Council
University of Manitoba	Saleh Shah
Genyi Li	Agriculture & Agri-Food Canada
Bogdan Slominski	Gerhard Rakow
University of Calgary	Ali Hannoufa
Christoph Sensen *	University of British Columbia
German Consortium	George Haughn
Wolfgang Friedt	* - Steering Committee Members

Project Key Objectives

- To explore the functional genomics of the seed coat, yellow vs. black seed, fibre content and seed coat development.
- To develop base populations from different sources of the yellow seed coat trait and develop molecular markers for the various genes involved.
- To reduce specific anti-nutritional factors (ANF) in the seed meal and enhance specific beneficial nutritional factors.
- To develop prototype germplasm for breeding programs.
- To train Highly Qualified Personnel.

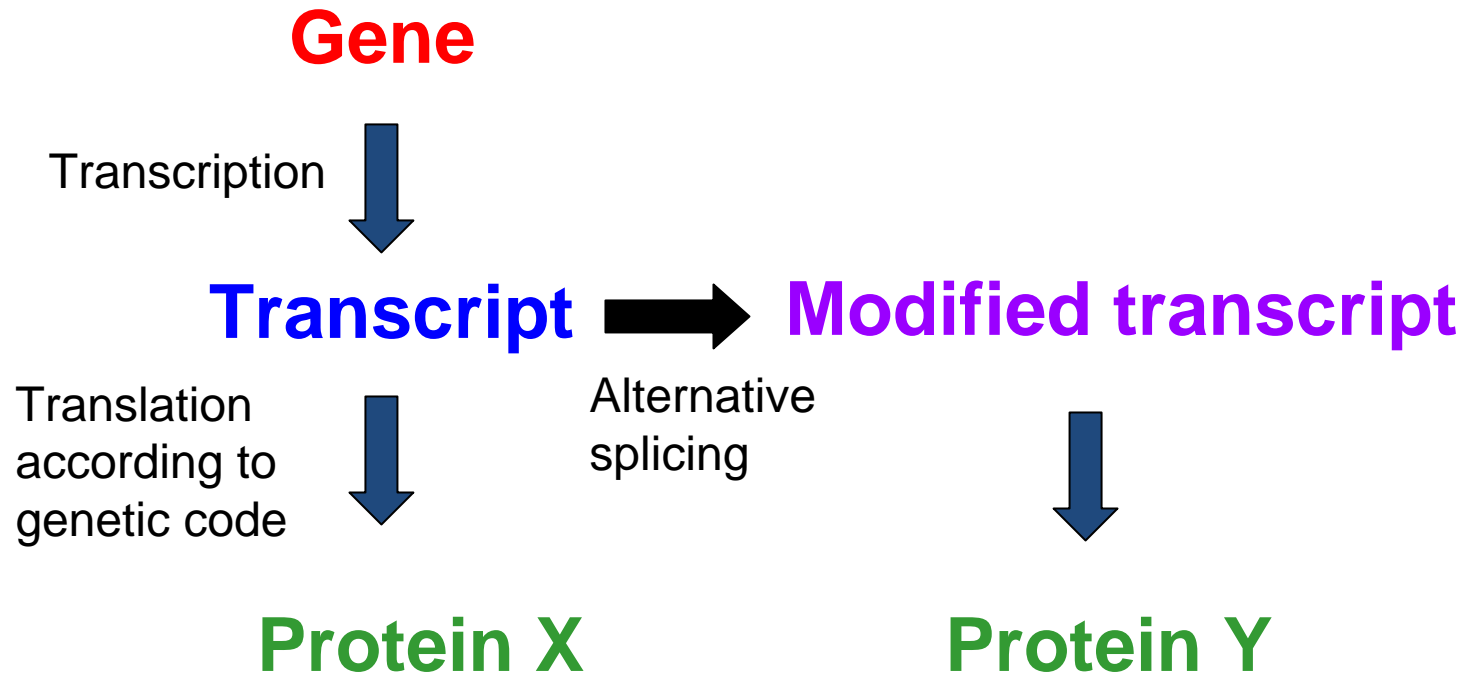
Project Overview



Project Managers
 Jeff Parker
 Faouzi Bekkaoui

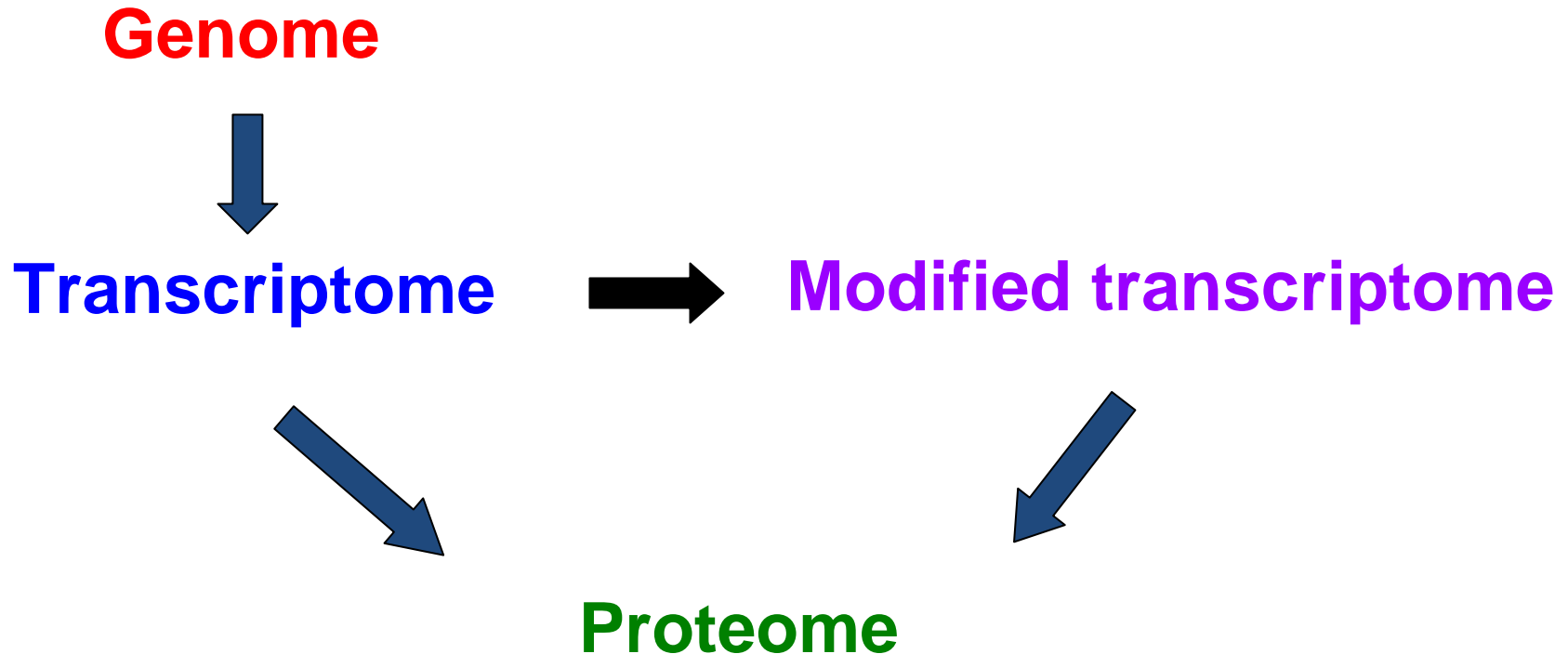
- Designing
- Oilseeds for Tomorrow's
- Markets

From Gene to Protein (Gene Expression)



- Gene** - a segment of DNA having a characteristic nucleotide sequence
- Transcript** - messengerRNA; a “mobile” form of the gene
- Protein** - has a characteristic amino acid sequence
- Many proteins are enzymes**
- Enzymes** drive biochemical reactions (e.g. those involved in the production of anti-nutritional factors)

Global Gene Expression



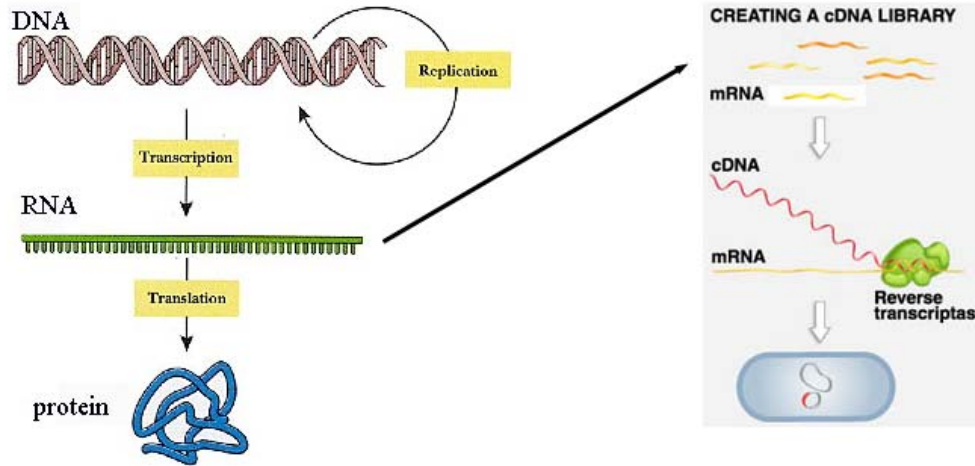
- ❑ Genome – “blueprint” containing all genes
- ❑ Transcriptome – a “snapshot” of the status of all of the transcripts
- ❑ Proteome – a “snapshot” of the status of all of the proteins
- ❑ The transcriptome and proteome can change with time and growth conditions

- ❑ In most cases we are only able to assess part of the transcriptome or proteome
- ❑ A multitude of enzymes are represented within the proteome
- ❑ Interactome: a “snapshot” of all protein-protein interactions within the proteome
- ❑ Metabolome: a “snapshot” of all metabolites in the cell; affected by enzyme action
- ❑ Technologies have been developed, and are under further development, to analyze the genome, transcriptome, proteome, interactome and metabolome.
- ❑ Sub-disciplines: genomics, proteomics & metabolomics
- ❑ Generate massive amounts of data, which require computational methods to analyze. This has resulted in the field of bioinformatics.

Technologies Applied in DOTM

- ❑ ESTs – Expressed sequence tags
- ❑ DNA microarray
- ❑ Tissue specific protein expression
- ❑ RNAi – Transgenic expression interference
- ❑ TILLING – Mutation Identification

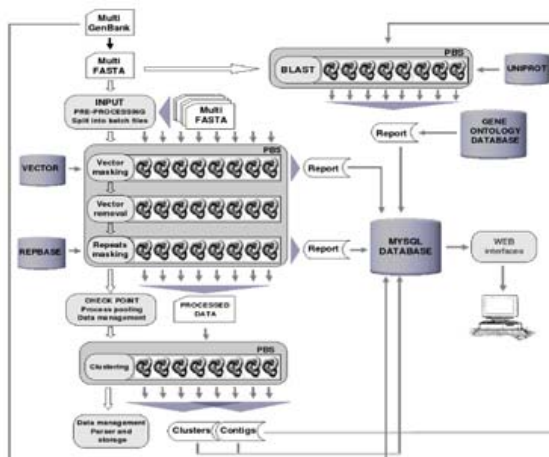
Expressed Sequence Tags (ESTs)



Bioinformatics

ESTs

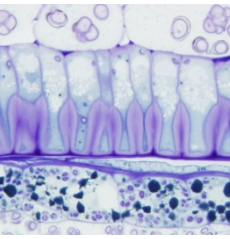
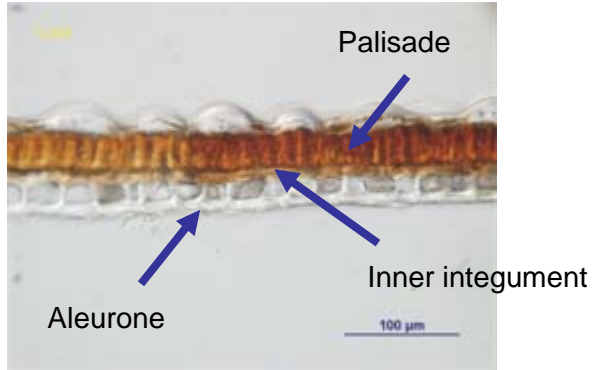
Sequencing



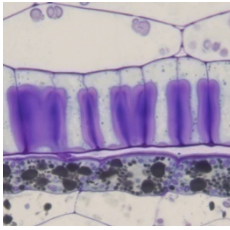
EST Libraries

- ❑ DOTM (Genome Canada Competition III)
-100,000 ESTs
- ❑ Enhancing Canola Through Genomics
(Genome Canada Competition II)
– 330,000 ESTs
- ❑ AAFC – 160,000 ESTs

DNA Microarray

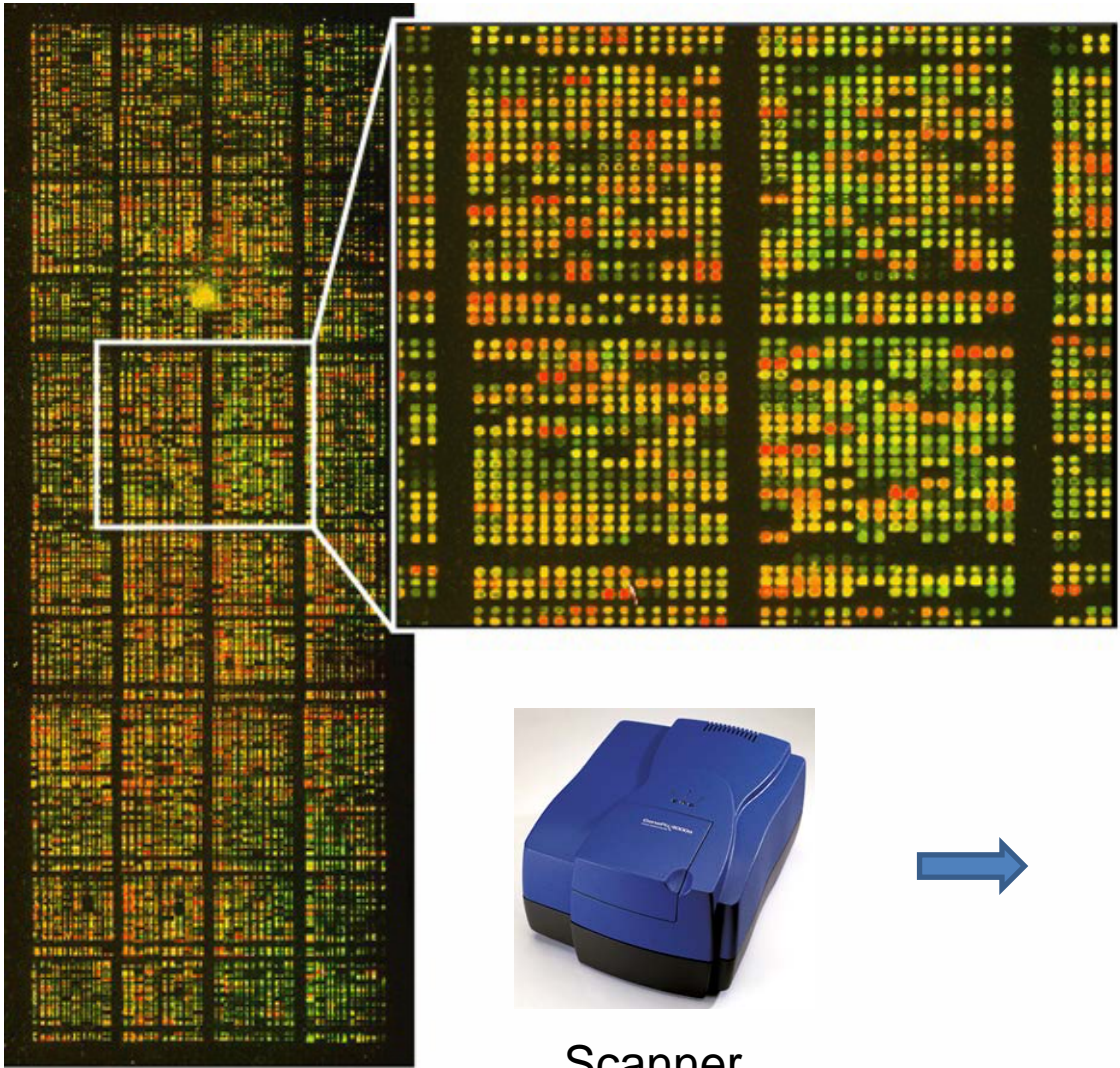


RNA



RNA

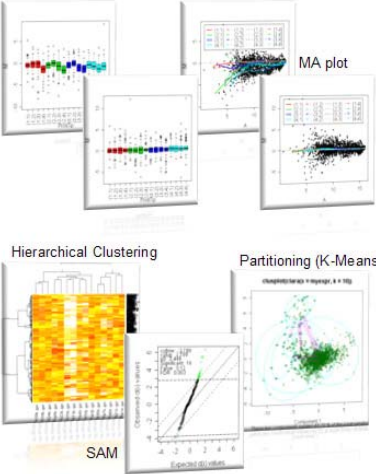




Scanner

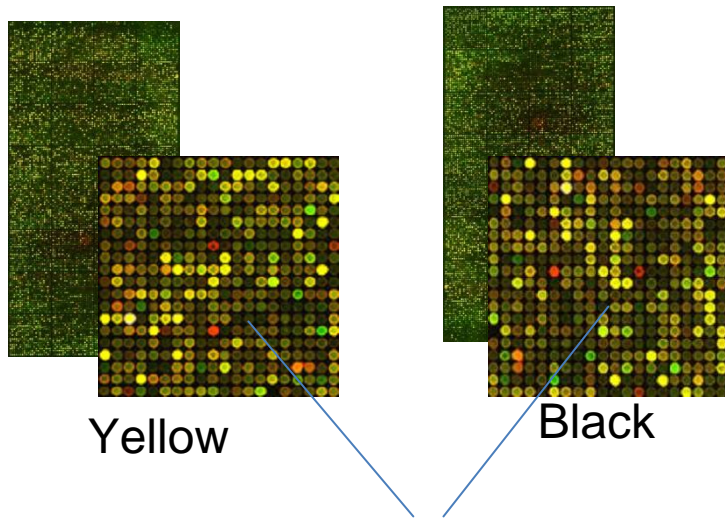


Bioinformatics

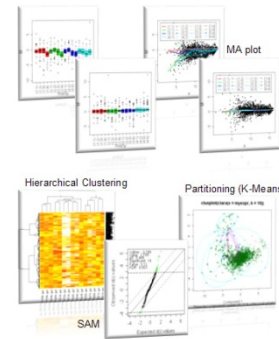


Super Computer

Identify the Differences

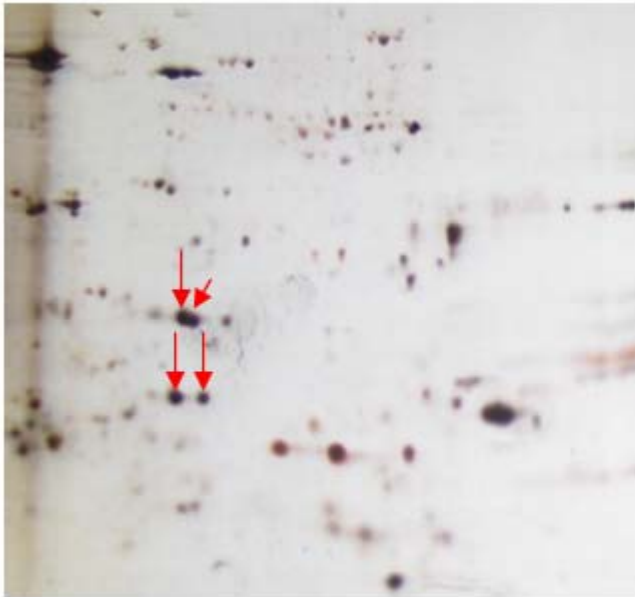


Bioinformatics



- Which genes are expressed in only the yellow seeded canola and not the black seeded canola?
- Identify the gene sequences and potential gene products (enzymes)

Protein Screening

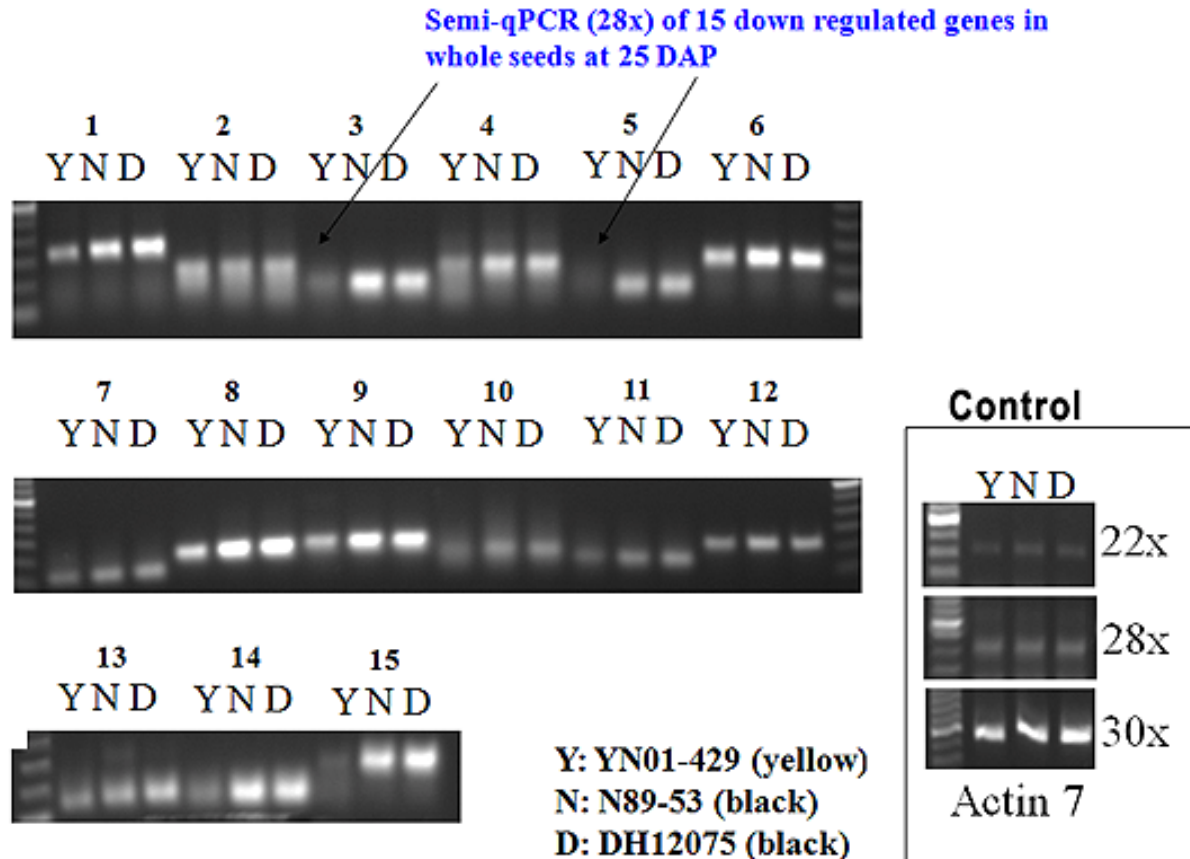


Brassica napus seed coat proteins pH 3-10 *Brassica napus* embryo proteins pH 3-10

Protein Screening

- ❑ Identify differentially expressed proteins
- ❑ Sequence the target proteins
- ❑ Infer gene sequences and screen gene targets from databases
- ❑ Screen ESTs for similar sequences

Validation of Gene Involvement



Prototype Plants

- Create RNAi constructs and produce transgenic prototypes
- Check for desired phenotype (were the changes sought after attained?)
- Increase seed in the field for feeding trials
- Transfer germplasm to plant breeders for cultivar development

TILLING – a non-GE approach

- ❑ TILLING: Targeting Induced Local Lesions IN Genomes
- ❑ Defined as a high-throughput method to identify specific gene knockouts in mutant populations
- ❑ Screen TILLING population for mutations in the desired gene
- ❑ Check mutation for phenotypic expression
- ❑ Transfer germplasm to plant breeders for cultivar development

DOTM Progress

- ❑ Microarray gene expression analyses conducted in *Arabidopsis* and *Brassica*
- ❑ Twenty candidate genes have been identified for further characterization
- ❑ > 100,000 ESTs sequenced and submitted to GenBank
- ❑ Genomic libraries and gene expression studies are nearing completion for the identification of genes and other genetic elements involved in seed coat cell wall formation.
- ❑ TILLING population development is ahead of schedule
- ❑ Functional characterization of 40 selected genes is being conducted through genetic transformation of *B. napus*
- ❑ 90,000 *Brassica* array is being developed and will be available to the broader research community

Acknowledgements

- Genome Canada
- Genome Alberta/Prairie
- Alberta, Saskatchewan and Manitoba - provincial
- AAFC and NRC – federal
- German partners

THANK YOU

