New DNA Marker Technologies for Tomato Breeding

Tomato Breeders’ Roundtable
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Charles Pick, DNA LandMarks
BASF – The Chemical Company

- The world’s leading chemical company
- Broad portfolio: Chemicals, Plastic & Fibers, Colorants & Finishing Products
- Agricultural Products & Nutrition
- Oil & Gas
- Sales 2008: €63.2 billion
- 97,000 employees worldwide

DNA LandMarks – a BASF Plant Science Company
BASF Plant Science

Global R&D network

- “Verbund“ of innovative technologies
- 8 sites in 5 countries
- founded in 1998
- > 700 employees in R&D
- several cooperations worldwide
- Budget of €400 million (2006 - 2008)
About DNA LandMarks

- Founded in 1995
- ~45 full-time employees
- BPS Centre of Excellence for sequencing and genotyping
- A leader in agricultural genomics
  - Project lead of the largest SNP development project in canola
  - Led the largest SNP validation project in tomato
  - Running the largest private genomics project in poultry (SNP validation followed by association mapping)
A time of tremendous change in molecular genetics

- Next generation sequencing technologies have dramatically increased throughput and lowered cost
A time of tremendous change in molecular genetics

- Genotyping costs have also dropped dramatically

### Historic Cost of Genotyping

<table>
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<tr>
<th>Year</th>
<th>Price per Datapoint</th>
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<tr>
<td>2002</td>
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<tr>
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*DNA LandMarks – a BASF Plant Science Company*
Highly abundant markers – allow very fine mapping of traits

SNPs can occur within gene sequences and may even be the source of mutation behind the phenotypic variation

Co-dominant – allows determination of zygosity, therefore more informative when screening for traits

Various technologies exist for SNP genotyping – can assay SNPs from simplex reactions (ABI Taqman) to >100,000 SNPs simultaneously (Affymetrix, Illumina)

SNP data is portable

SNPs likely to be a standard technology for the foreseeable future
SNP validation & mapping

- Mining EST databases for SNP markers
- Wet lab validation on commercial germplasm
- Mapping on public mapping populations

Conversion of existing trait-linked markers to SNPs

- Evaluation of existing public and licensed trait-linked tomato markers
- Multi-approach strategy for marker conversion
SNP Validation Project

- Consortium project involving 4 tomato breeding companies

- To develop 800-1000 validated SNP markers from 1428 *in silico* SNPs (eSNPs)

- To genotype validated SNPs on 24 tomato lines including 16 from consortium members, four public lines and the four parents of the two mapping populations

- Mapping of 500-600 validated SNPs on either of the two mapping populations

- To anchor the validated SNPs onto the tomato physical map when the tomato genome sequence is available.
Outline of the project

- **In silico SNP discovery (1428 eSNPs)**
- **Design and validation assays in two parental lines**
- **Genotyping population(s)**
- **Genotyping 22 additional lines**
- **Genetic mapping & locate SNPs on public map**
- **Validate SNP assays**
- **Calculate allele frequencies**
- **Anchor SNPs on Tomato genome sequence**
- **Project database**
- **SNP information**

**DNA LandMarks – a BASF Plant Science Company**
Sequenom MassARRAY® Platform

- iPLEX assay multiplexes up to 36 SNPs in a single-well reaction
- High flexibility: allow re-configuration of assays and samples by user
- High sensitivity and specificity: allow high accuracy and call rates
- Use of unlabeled primers cuts down the cost of the SNP assays
Basis of iPLEX Assay

**PCR Amplification**

Forward PCR primer

Reverse PCR primer

Genomic DNA

**PCR Product**

**iPLEX Reaction**

Primer extension with A terminator

Mass extend primer

Primer extension with G terminator

Desalting, spot on SpectroCHIP and MALDI-TOF MS

24-plex spectrum
Measures to Increase Efficacy

- Design iplex assays on batches 1 & 3 failed SNPs
  - 322 failed SNPs from batches 1 & 3 for iplex assay design
  - 316 SNPs in 12 iplexes tested (Batch 4)

- Mining of tomato sequences from a pilot allele-resequencing of IMPs
  - 140 SNPs selected for iplex assay design
  - 128 SNPs in 5 iplexes tested (Batch 5)
Summary of Achievements

- From 1,568 eSNPs, 1,156 SNP assays were validated and tested on a germplasm panel of 24 lines.
- 662 SNPs among the 1,156 SNPs are polymorphic among the germplasm panel of 24 lines.
- 462 SNPs are polymorphic among the 22 cultivated tomato lines.
- PIC values of all SNPs calculated based on genotypes of the 22 cultivated tomato lines.
- 309 SNPs mapped on the map of primary mapping population from the cross *S. lycopersicum* ‘LA3475’ x *S. pennellii* ‘LA0716’.
- 224 SNPs mapped on the map of the second mapping population from the cross *S. lycopersicum* ‘LA4024’ x *S. pimpinellifolium* ‘LA1589’.
- A total of 446 SNPs have map locations on the tomato maps.
Focused on existing trait-linked markers in tomato that are in the public domain or have been licensed by DNA LandMarks.

Existing assays were all CAPS (Cleaved Amplified Polymorphic Sequences) based.

CAPS are cumbersome and inefficient to run.

Total of 9 markers have been converted to date into SNPs – Pto, Mi, Tm2a, I2, I3, Sw-5, Ve, Ty-1 & Ty-3.

Combining these SNPs into a single Sequenom iplex has dramatically decreased assay costs (~$0.51/trait).
Acknowledgements

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