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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Ninth Session
Washington, D.C., June 21 to 23, 2005

THE POTENTIAL OF SNP MARKERS IN EXPRESSED GENES FOR IDENTIFICATION OF POTATO VARIETIES AND DETERMINATION OF DISTINCTNESS

Document prepared by experts from the Netherlands

1. The BMT agreed that, where agreed by the relevant experts, the presentations made at the meeting should be made available in the BMT document section of the UPOV website, as addenda to the relevant documents. This document contains a copy of the presentation given by Mr. Robert Cooke (United Kingdom), based on document BMT/9/13, and prepared by Mr. Ben Vosman (Netherlands).

The potential of SNP markers in expressed genes for identification of potato varieties and determination of distinctness

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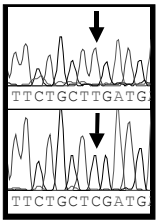
SNPs (Single Nucleotide Polymorphisms)

- SNPs are point mutations in the DNA

  AGGCTAGCGAGCTAATACGGG Genotype 1
  AGGCTAGCGAACTAATACGGG Genotype 2

- SNPs are generated randomly
- High number of possible markers
- Bi-allelic, co-dominant
- Easy databasing



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SNPs in this project

- SNPs extracted from EST databases (TIGR) and from GABI PoMaMo database

- > ESTs are expressed sequences
- Analyzed with Pyrosequencing™ technique
  - quantitative

Biotage

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SNP polymorphism in potato

- Normal (3-state) scoring: two homozygote and one heterozygote state
- In a tetraploid the situation is more complex: each SNP can be in five possible states: 0:4, 1:3, 2:2, 3:1 and 4:0
- Pyrosequencing is quantitative and allows scoring of all five allelic states of tetraploid potato

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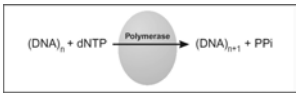
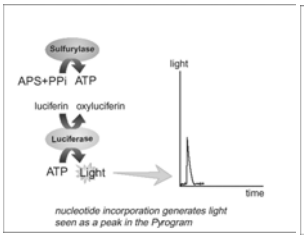
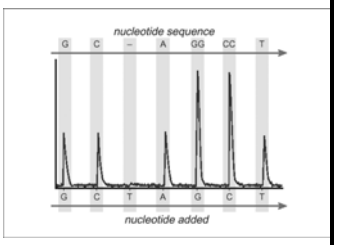
Pyrosequencing: principle







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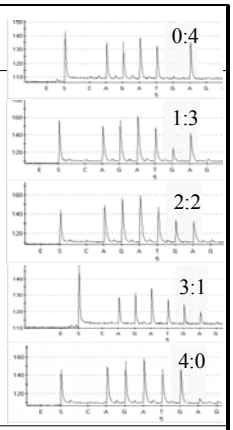
SNP analysis in potato

Tetraploid varieties

- 5 possible allelic states



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Analysis of 305 potato varieties using 38 SNPs

- All varieties can be uniquely identified, except mutants
- Two pairs of mutants identified
  - Profiles of mutants differed on 1 marker; reliability??
- 37 of 38 markers had PIC values between 0.4 and 0.7

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5-state versus 3-state scoring of 305 varieties

- All 38 SNPs
  - 5-state and 3-state scoring methods both uniquely distinguish all varieties except 2 pairs of mutants
- 12 most informative SNPs
  - 5-state scoring identifies all varieties uniquely, except for 3 pairs of varieties (including 2 mutant pairs)
  - 3-state scoring cannot distinguish 23 pairs of varieties

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DUS characteristics vs SNP markers

- DUS characteristics 1-50 were scored according to the UPOV guideline
- Most scores are on scale 1-9
- Euclidean similarities calculated for DUS and SNPs

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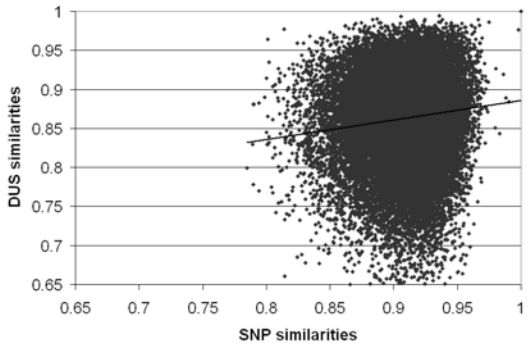
Genetic versus overall morphological s



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DUS characteristics vs SNP markers

- No clear relationship
- No possibilities for an option 2 approach

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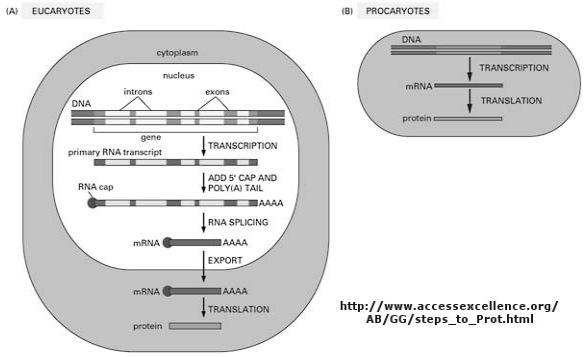
Expression of DNA



http://www.accessexcellence.org/AB/GG/steps_to_Prot.html

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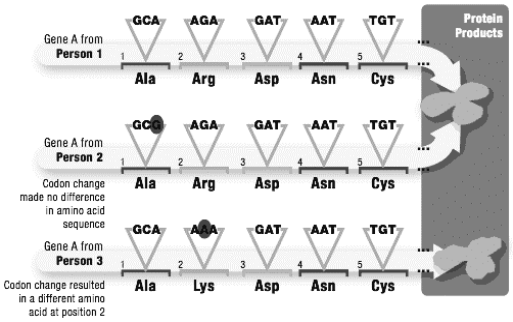
Different SNPs



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Type of SNPs

- Synonymous SNP
  - Alters the mRNA but not the protein sequence
- Non-synonymous SNP
  - 'Conserved' change of protein by replacing AA with AA with similar properties; function not necessarily affected
  - 'Non-conserved' alteration of protein by replacing AA with AA with different properties (charged vs neutral); likely to change function of protein
- Introduction of STOP codon
  - Disrupts protein; usually no functionality remaining

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SNPs in expressed DNA sequences

- The majority of the SNPs we used are in expressed DNA, since derived from ESTs
  - SNP polymorphisms are therefore expressed in the RNA
- Part of the SNP polymorphisms is also expressed in the protein (conserved and non-conserved mutations) (cf isozymes, seed storage proteins)
- The non-conserved mutations might change the phenotype (functional level)

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Another option for UPOV?

- SNPs (but also microsatellites) derived from EST sequences are expressed characters and should be acceptable for determining DUS under UPOV 1991

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Conclusions:

- SNPs are very effective markers for identification in potato
- Scoring of 5 states results in an increase in discriminative power of the markers
- An option 2 approach is not likely to work for potato
- SNPs derived from ESTs should be regarded as expressed characters under UPOV 1991

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