Generation of a genetic linkage map of *Mycosphaerella fijiensis* using SSR, VNTR and DArT markers

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Why generate a genetic linkage map of a pathogen?

- Transmission genetics
- Map based cloning/QTL analysis
- Assist in Genome assembly
- Develop independent markers for population studies

What markers did we use?

- DArT http://www.diversityarrays.com
  - STS
  - Cost efficient
  - Robust
  - Automatic scoring
  - Initial set-up of array
- SSR/VNTR markers
  - STS
  - Multiple alleles per locus (>50)
  - High variability/ high information value (10^2–10^6 mutations per locus per generation)
  - Length variation allows flexible instrumentation
  - Integration of datasets/construction of joined datasets
  - Cost efficient
  - Robust
  - Automatic scoring
  - Variation in the primer-site result in null-alleles
  - Transfer across species is difficult

SSR/VNTR markers

SSR or Microsatellites: repeat unit di, tri or tetra nucleotides in tandem repeats

VNTR or Minisatellites: repeat unit ranging from ~ 6 – 50+ tandem repeats

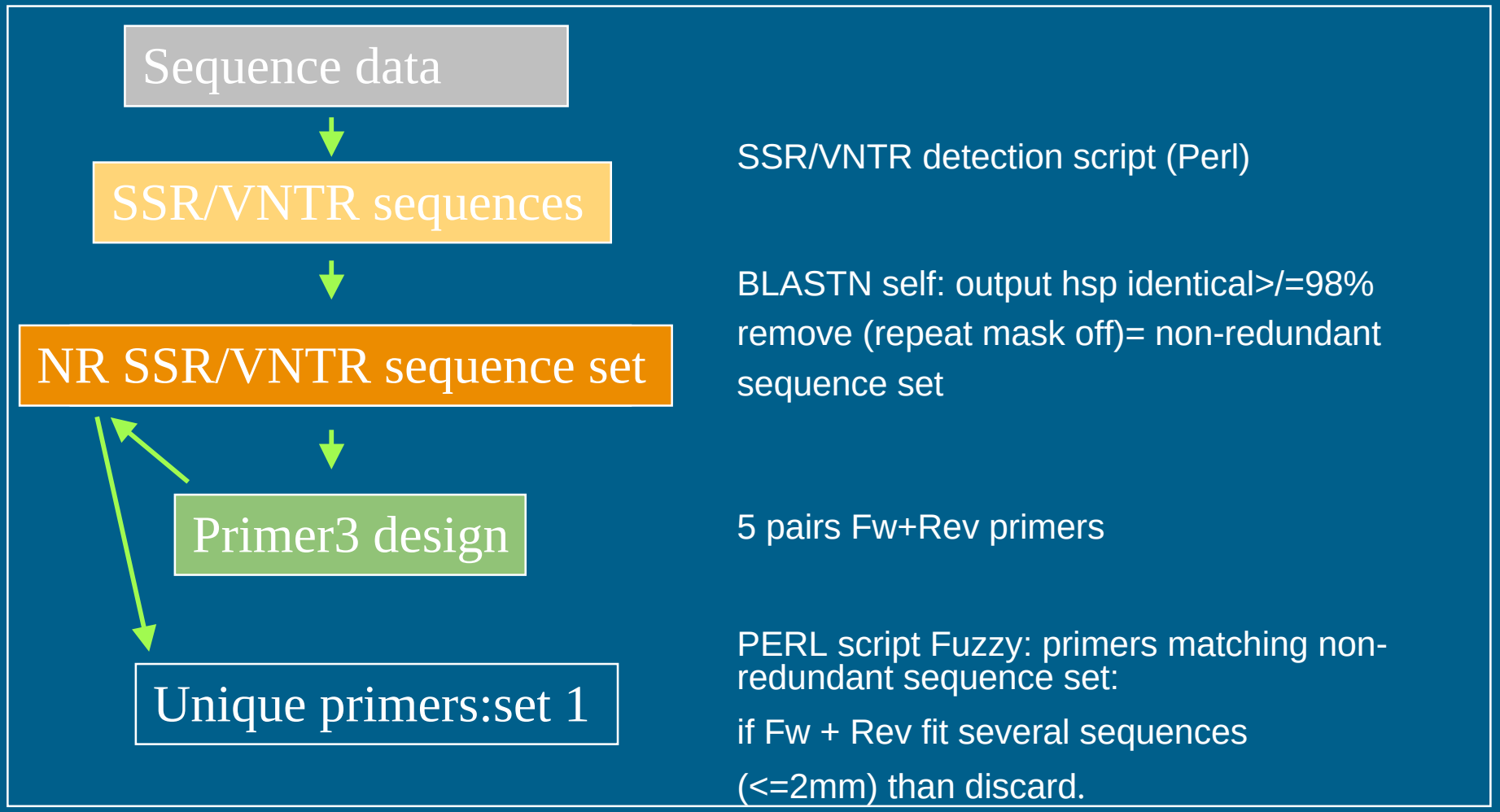
GAAGCGTTGTCGGAGGACGGGAGTCAACGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAAT
GAAGCGTTGTCGGAGGACGGGAGTCAAC

GGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAAT

GGGAGTCAATGGGAGTCAATGGGAGTCAATGGGAGTCAGATCAGGGTGGGTAGTAGATAG
GTCAATGGGAGTCAGATCAGGGTGGGTAGTAGATAG

- Polymorphism due to variation in the number of repeat units
- All alleles at a specific locus can be scored
- Variability is generated by slippage during replication or unequal crossing over

Overview SSR/VNTR detection/primer development module



http://www.jgi.doe.gov/index.html

VNTR Primer Design Results

1934_72597	60	188	Show sequence
0267_62598	60	344	Show sequence
3153_62600	60	348	Show sequence
3153_72608	60		Show sequence
4583_102610	60		Show sequence
4416_72611	60	226	Show sequence
0536_102615	60	278	Show sequence
0252_102618	60	136	Show sequence
3153_92621	60	331	Show sequence
0536_122624	60	323	Show sequence
0877_62625	60	253	Show sequence
1125_92626	60	273	Show sequence

Selected VNTR

Forward: CTCCTAGAGGCTACCCTGCC Reverse: GTATACTTCCGACCTCGGGC

>0252_102618 seq 4526 10 CTAGCTAATA CTAGCTAATA CTAGCTAATA CTAGCTAATA

AATAGGTATAGCTAAAAGTTATATATAGATATATATAGATTAAGGTATATATACTTATATTTAGATTATTAGATT
ATAAAACTAACCTAAGATATCCCTCTAATAAGTCTTCTATTACTAAAATTTAACCTTATCTTCCTAATAGTTAGT
TAAAGTATTATAAGCTCTATATATAAGATTTTAGTATATATTAAAAGACTAGTAGGCTAGATAGAGTCCTATATT
ATTATTATATACTTATTTGTTTATATCTCGTAAGTAGGCGTTATATATTTATTTAAACATCTTAGTAAATACTAA
GCTAATATTAGTAGAAACTATAACGTTATTACTAGACAACTTAGCCCTATATAAAATTGTTATATAATCTAGATA
TATTCGAAAATTAAAGAAATATATACGTTTACTTAGAGACCGTCGAGATATCGAAGTCGAGCTCCTAGAGGCTAC
CCTGCCGTCCGAGCTAATACTAGCTAATACTAGCTAATACTAGCTAATACTAGCTAATACTAGCTAATACTAGCT
AATACTAGCTAATACTAGCTAATACTAGCCCGAGGTCGGAAGTATACTTTCTATAATATCTTAGTAAGTATAAGA
AAATAGGTCTATAGTAACCGTCGTATAGAATACCCTCTACTAATGTAGTAGTAGATAGTAGAACTAAACCGTGC
CTCTCTAAGAATAGGATAGAGTATTTCCTATTAACTAAGCTCGATAGCCCTATACGGCTAGTATACCCGCCGAGC
CGGCTACCGAGTATCTCTATATAAGCGACCGCCGACCGAGTATCTCCGCGTATACGCTAAGCAGGCTTTACGCTA
GCGGATTAGTACTATAGTATACGTAATAACGTTATATAACTATTATATAGGATTTACTAAGATAGAATAGGAGAC
TTTACTTACTTAGGTTCTTATAGTAAGTACTAAGCCGATTCTAATAAAAACTATACTATTAAAACCGCGCAATCC
GGTTCTATAAGATCCGAAATTTTAGGAACAGTCGGTATACCTACTACCTACCTAGAAGAGGGGG

Repeat

primers

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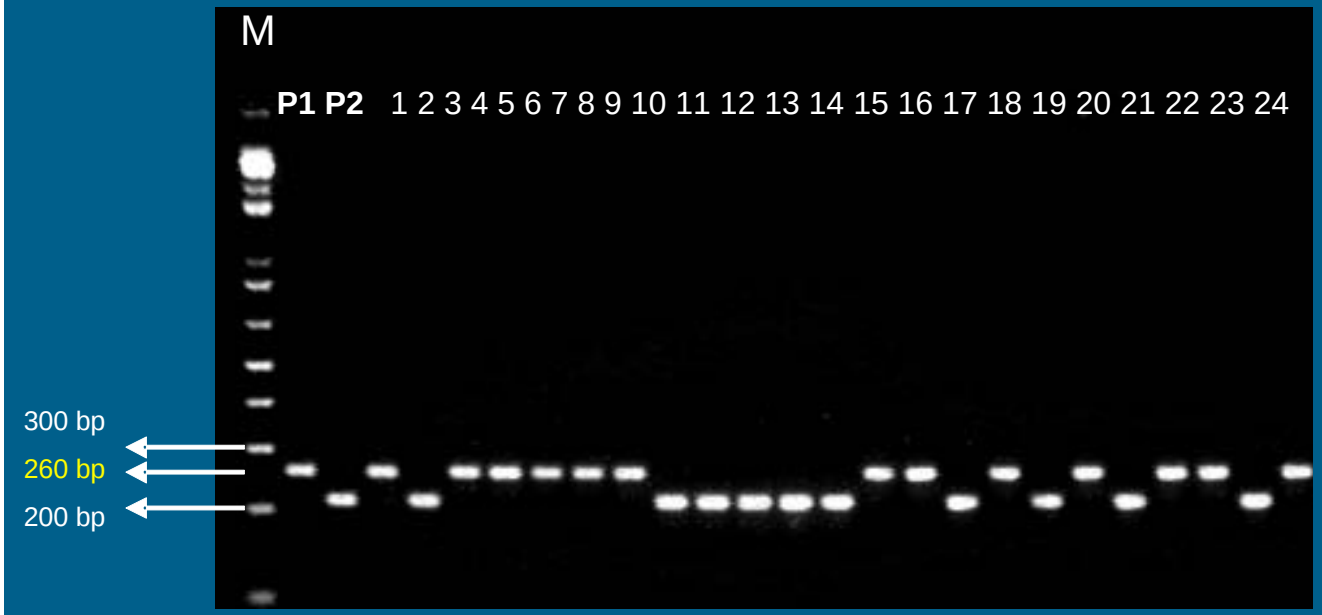
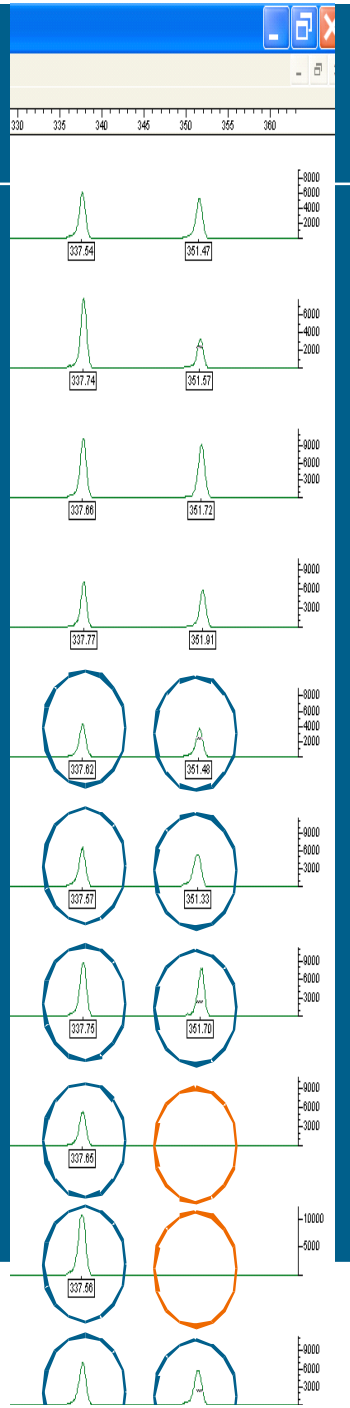
Mapping of VNTRs (Cirad 86 x Cirad 139a)

VNTR - 3786





3% agarose gel / 100 v/ 4h

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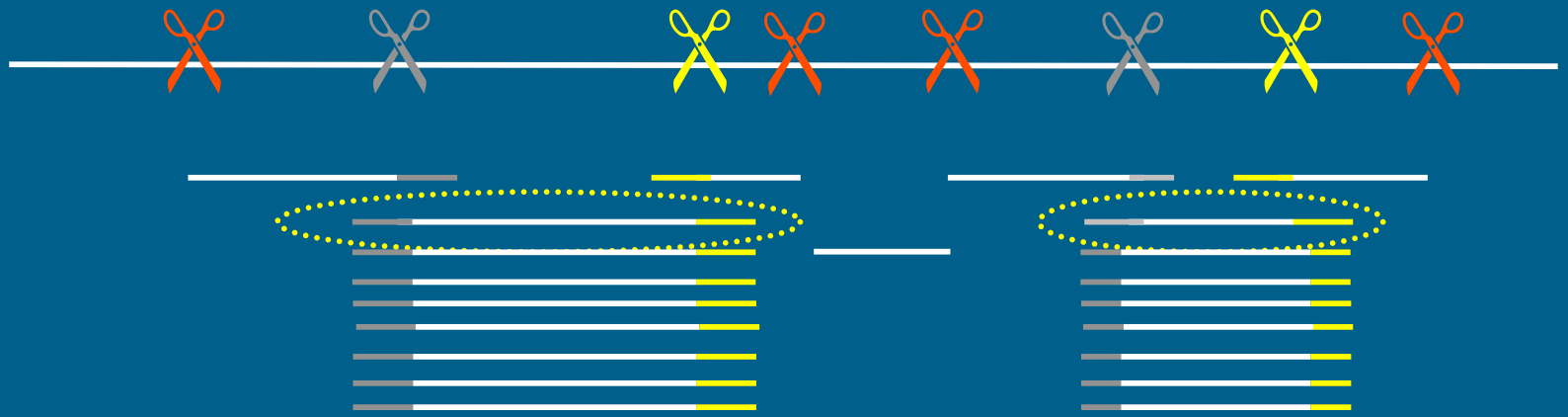
How to make a DArT micro-array?

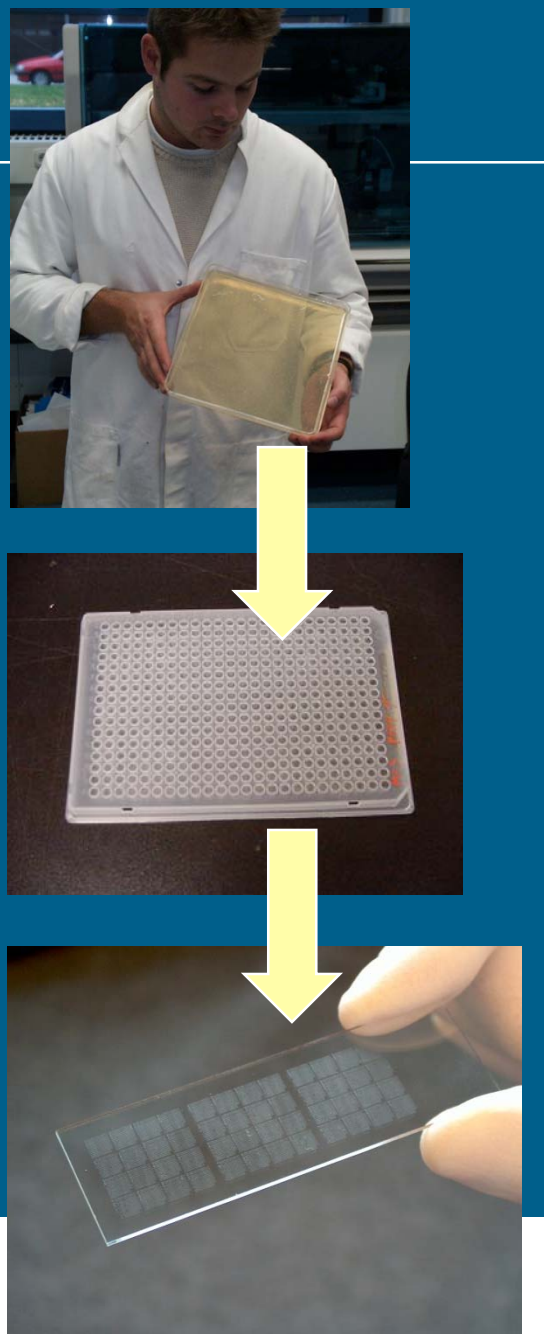
- Cut the reference DNA with 3 enzymes
  - *Hind* III (✂)
  - *Mse* I (✂)
  - *Rsa* I (✂)



- Ligate adapters to the *Hind* III and *Mse* I ends
- Amplify *Hind* III/*Mse* I fragments

Make micro-array (continued)

- *Hind* III/*Mse* I fragments from reference
- Individualize fragments
  - Cloning *Hind* III/*Mse* I fragments, using a pGemT-Easy vector.
  - Transfer vector with *Hind* III/*Mse* I fragments into E. coli.
  - Plate of colonies. Colony picking → Individualized fragments
  - Colony PCR
- Print in triplo on glass slide → micro-array



Use micro-array

DNA from genotype 1

↓

digest with enzymes↓

Amplify *Hind* III/*Mse* I fragments

↓

Label with Cy3

DNA from genotype 2

↓

digest with enzymes↓

Amplify *Hind* III/*Mse* I fragments

↓

Label with Cy5

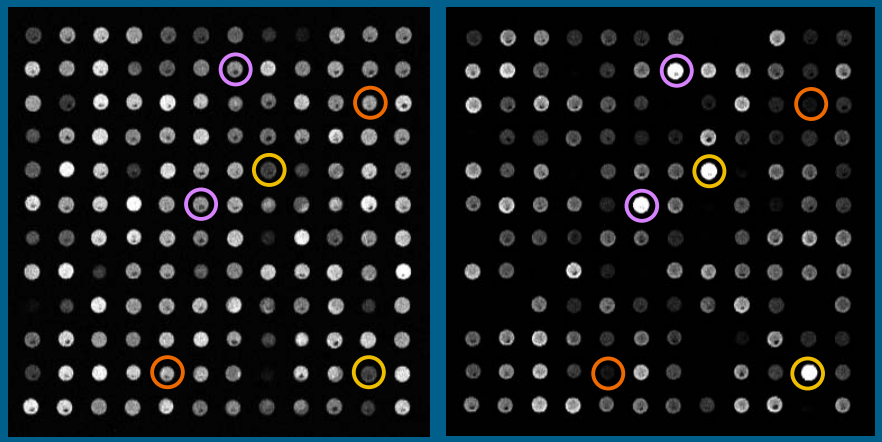
↓

Hybridize

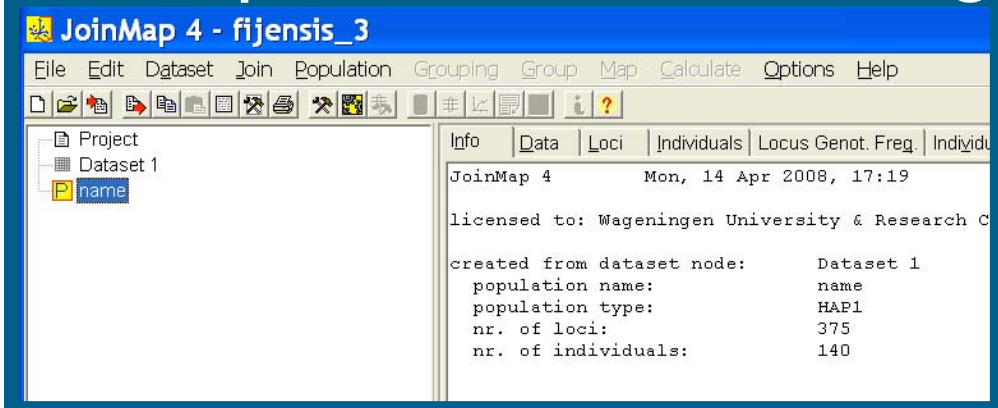
Molecular basis of polymorphism

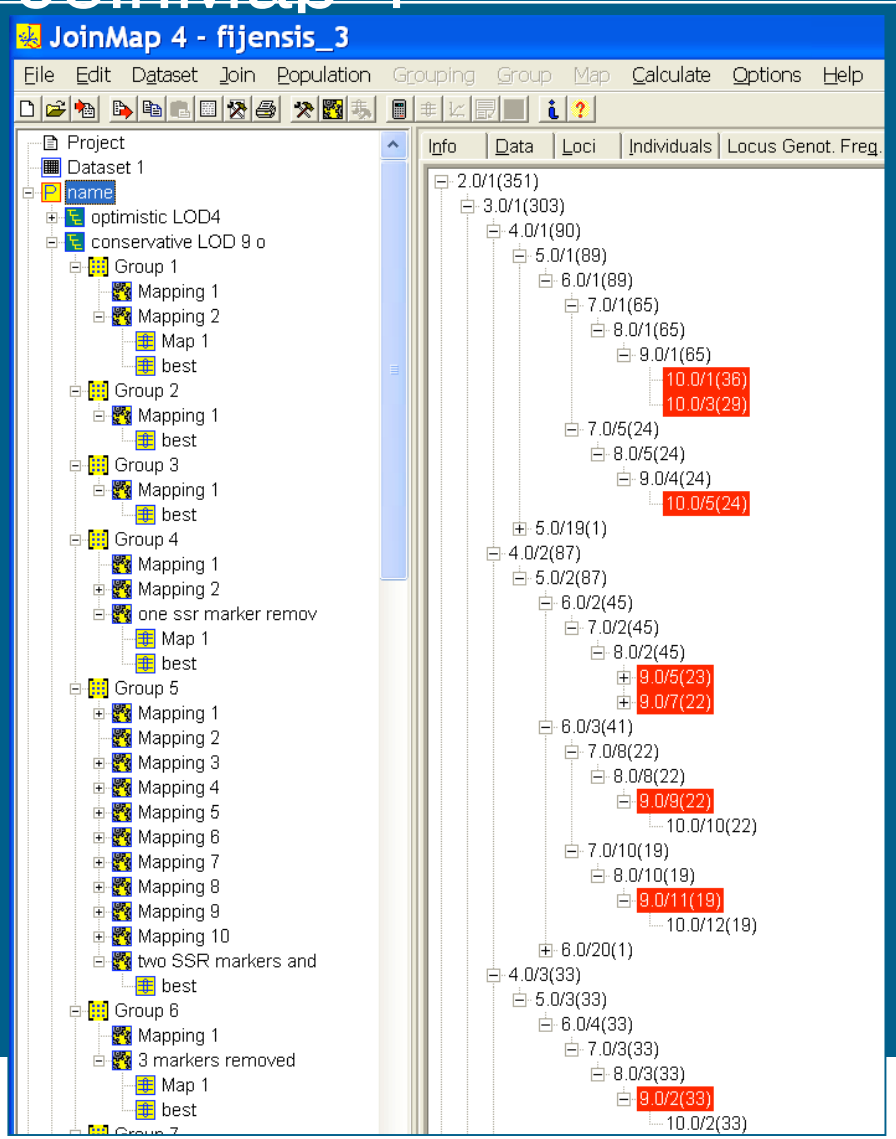
genotype 1

genotype 2



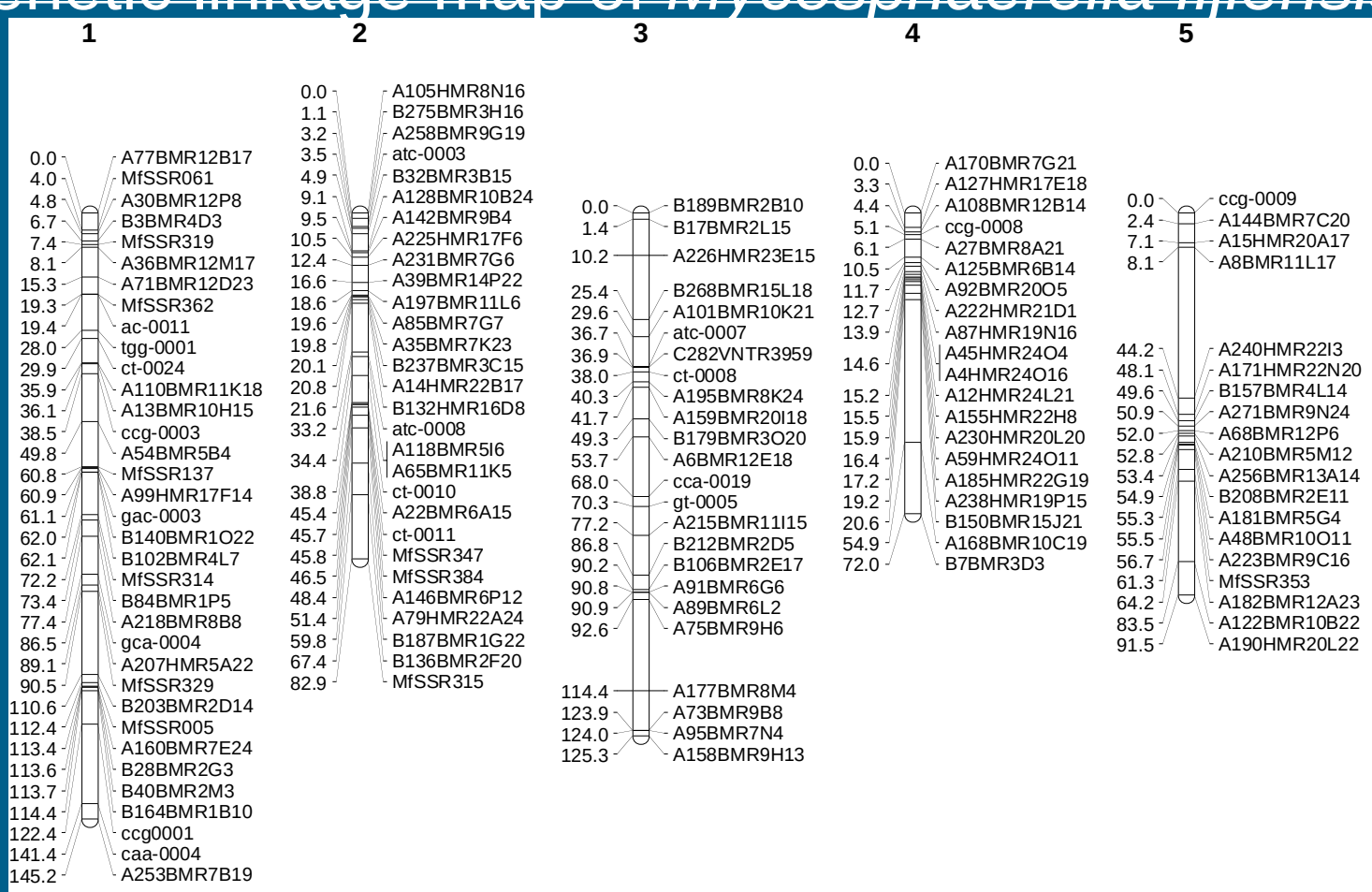
Map construction using JoinMap 4





DArT markers with a 90% genotype call rate and a 98.8% reliability score.

Genetic linkage map of *Mycosphaerella fijiensis*



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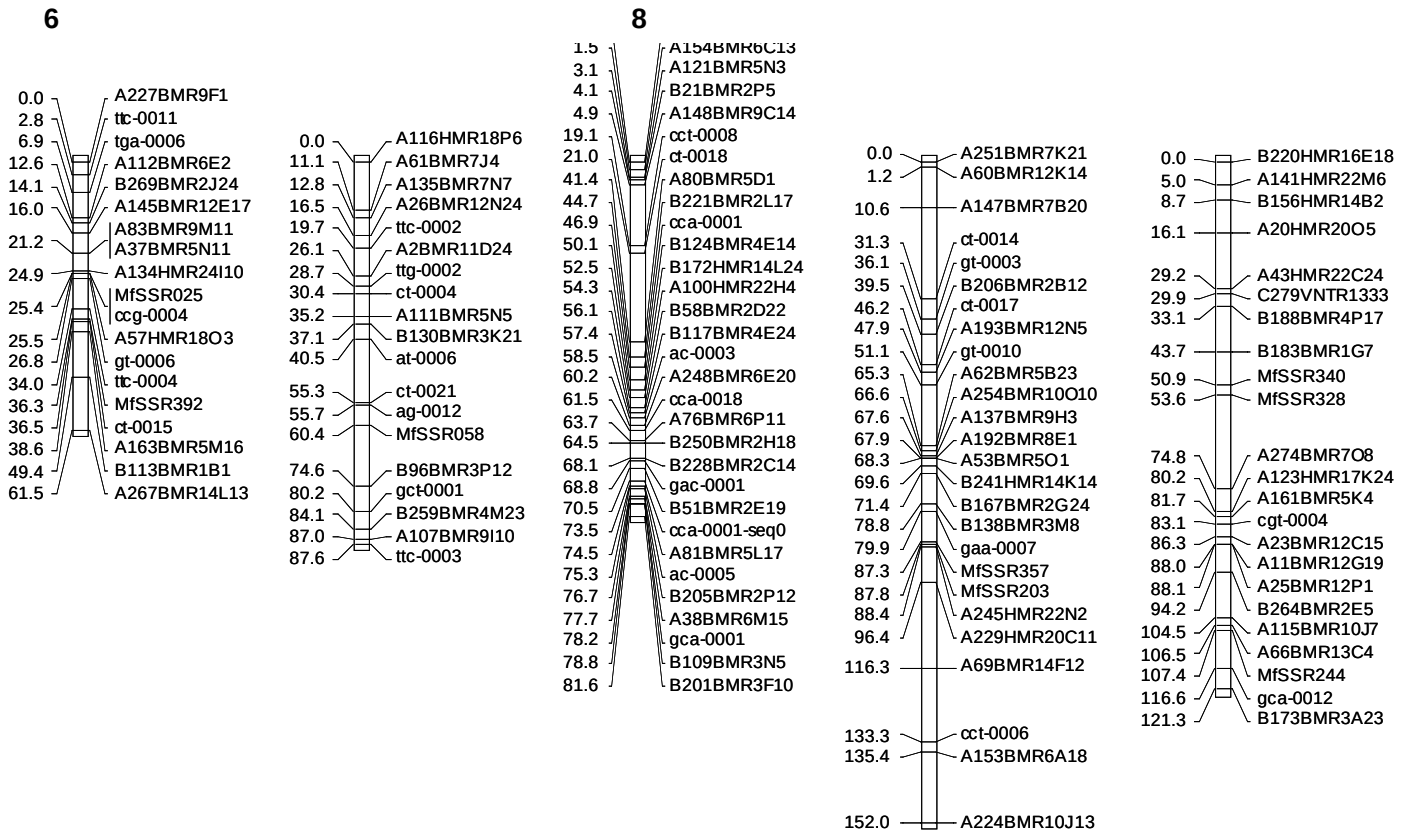
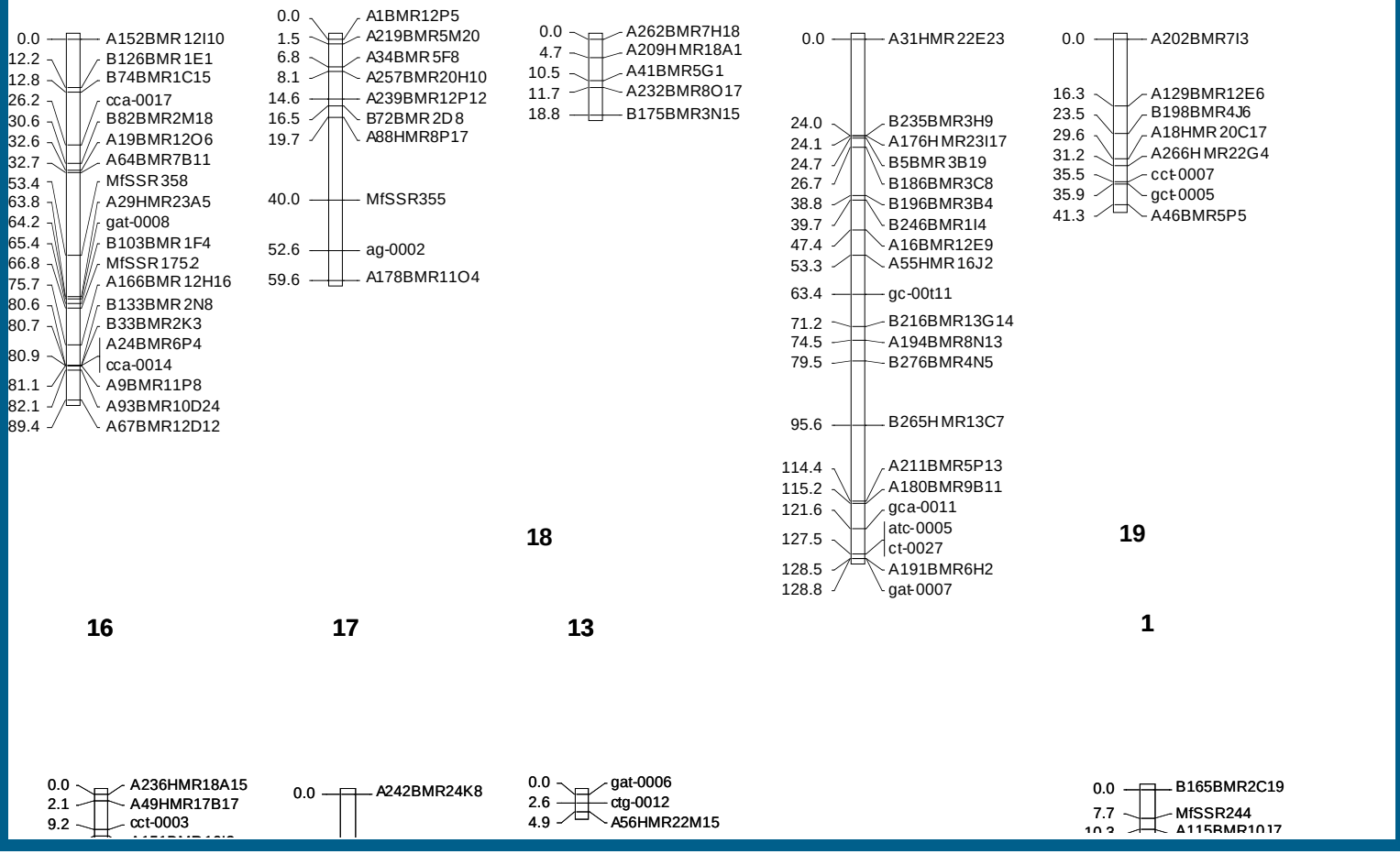


Genetic linkage map of *Mycosphaerella fijiensis*



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Mapping statistics

		positioned	total	percentage
All		325	375	87
DART		235	277	85
SSR		87	93	94
VNTR		3	5	60
removed	**distorted**	17	375	5
unlinked		26	375	7
removed	**friction**	15	375	4
replaced	**distorted**	4	375	1

- 19 linkage groups
- 1417 cM
- Possible additional linkages: 4-5, 6-7, 12-13, 15-16

Construction of a genetic linkage map of the fungal pathogen of banana *Mycosphaerella fijiensis*, causal agent of black leaf streak disease

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Abstract A genetic linkage map of the fungal plant pathogen *Mycosphaerella fijiensis*, causal agent of black leaf streak disease of banana was developed. A cross between the isolates CIRAD86 (from Cameroon) and CIRAD139A (from Colombia) was analyzed using molecular markers and the *MAT* locus. The genetic linkage map consists of 298 AFLP and 16 SSR markers with 23 linkage groups, containing five or more markers, covering 1,879 cM. Markers are separated on average by around 5.9 cM. The *MAT* locus was shown to segregate in a 1:1 ratio but could not be successfully mapped. An estimate of the relation between calculated using the genetic mapping data at 4,298.2 cM. This is the first genetic linkage map reported for this important foliar pathogen of banana. The great utility of the map will be for anchoring contigs in the genome sequence, evolutionary studies in comparison with other fungi, to identify quantitative trait loci (QTLs) associated with aggressiveness or oxidative stress resistance and with the recently available genome sequence, for positional cloning.

Keywords Ascomycete · *Mycosphaerella fijiensis* · Black leaf streak disease of banana · Genetic mapping ·

comparison

- More markers
- More progeny
- Higher LOD values
- More stable ordering
- More reliable
- More coverage
- STS markers

Future research

- Transmission genetics
- Map based cloning/QTL analysis
- Assist in Genome assembly
- Develop independent markers for population studies

A joined effort of …

- Plant Research International B.V., Wageningen University and Research Centre,
- Embrapa Cassava and Tropical Fruits, Brazil
- Biologie et Génétique des Interactions Plantes-Parasites, CIRAD-INRA-AGRO.M, Montpellier, France
- USDA-ARS, Crop Production and Pest Control Research Unit, Purdue University, IN, USA
- Embrapa-LABEX Europe - Wageningen University and Research Centre, Wageningen, The Netherlands

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- One-hundred and thirty-six F1 individuals from the CIRAD086 (Cameroon, *Mat*1-1) x CIRAD 139A (Colombia, *Mat*1-2) cross were evaluated. Eighty-seven (93) SSR (Simple-Sequence Repeat) markers, 3 (5) VNTR (Variable Number of Tandem Repeat) markers, the mating type (*Mat*) locus and 235 (277) DArT (Diversity Arrays Technology) markers were positioned in 19 linkage groups covering 1417 cM of the genome. The arrays containing individual fragments of the genomic representation of *M. fijiensis* generated DArT markers with a 90% genotype call rate and 98.8% reliability score. In total, 87% of the markers could be positioned reliably with high LOD scores (LOD <10). Due to its excellent genome coverage and high quality we decided to sequence the DArT markers to align this genetic map with the genome sequence of CIRAD086, which will considerably assist the genome assembly of this important fungus.