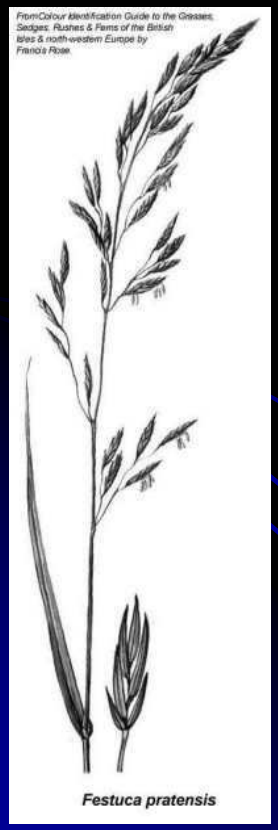
Genome mapping within the *Festuca-Lolium* complex



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Outline

DArTFest

-Development of the array

-Diversity study

-Genetic mapping

-Physical mapping

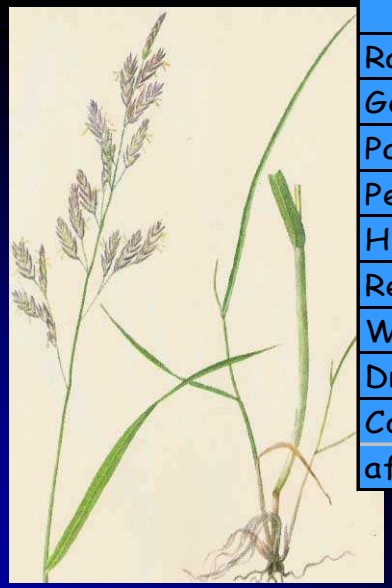
-Integration of the maps

-Characterization of Festulolium hybrids

-Conclusions and future work

FESTUCA - LOLIUM COMPLEX

- Ones of the most important forage and turf species
- Cross of the species of both genera results in fertile hybrids
- Hybrids display agronomically important attributes of both genera
- Chromosomes of both genera frequently pair and recombined with each other
- Chromosomes of both genera can be distinguished by GISH

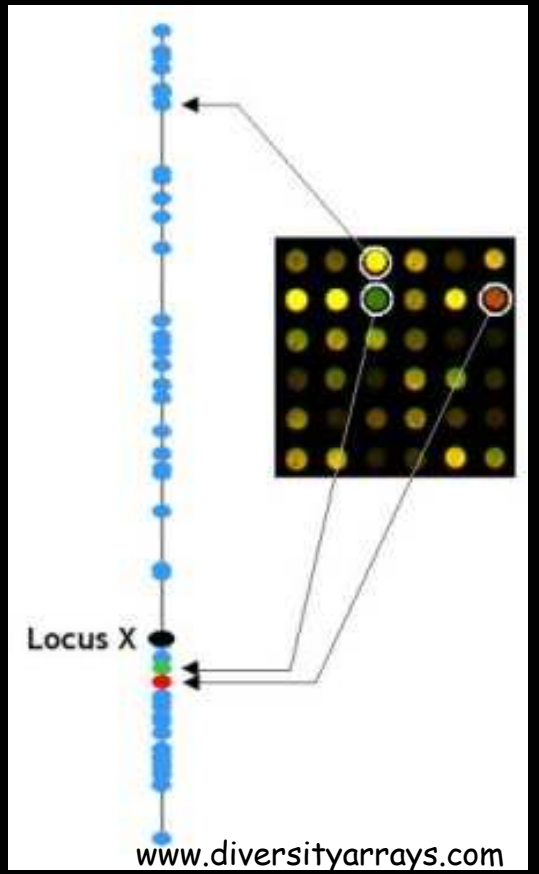


	Lm	Lp	Fp	Fa
Rapid establishment from seed	+	+/-	-	-
Good production in seeding year	+	+/-	-	-
Palatability	+	+/-	+	-
Persistency	-	+	+	+
High tillering density	-	+	-	-
Resistance to treading	-	+	-	-
Winter hardiness	-	+/-	++	+
Drought tolerance	-	-	+	++
Continued high production after second harvest year	-	-	+	+

DArT technology

Diversity Array Technology

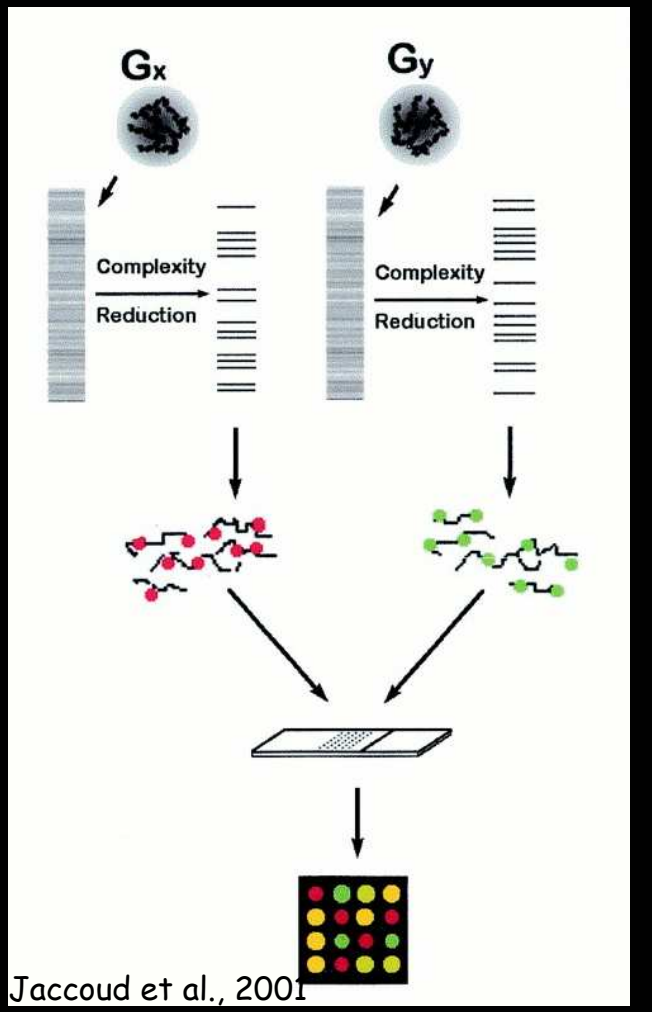
- Developed by Dr. Andrzej Kilian (Canberra, Australia) (Jaccoud et al., 2001)
- microarray technology
- dominant markers
- positives: high number of markers, anonymous markers (can be sequenced)
- negatives: low polymorphism in some species



DArT technology

DArT technology consists of several steps:

- Complexity reduction of DNA
- Library creation
- Microarray from libraries
- Hybridization of fluoro-labelled DNA
- Scanning of slides for hybridization signal (confocal laser)
- Data extraction and analysis (DArT software)



Jaccoud et al., 2001

DArT technology

Main use:

- identification of QTL
- simultaneous marker-assisted selection for several traits
- evaluation of genetic diversity
- varietal identification of crops
- monitoring the composition of complex DNA samples (microbial, populations, etc.) - also in hybrids
- methylation profiling
- Genetic and physical mapping

DArT*Fest*

A first DArT array for the *Festuca-Lolium* complex

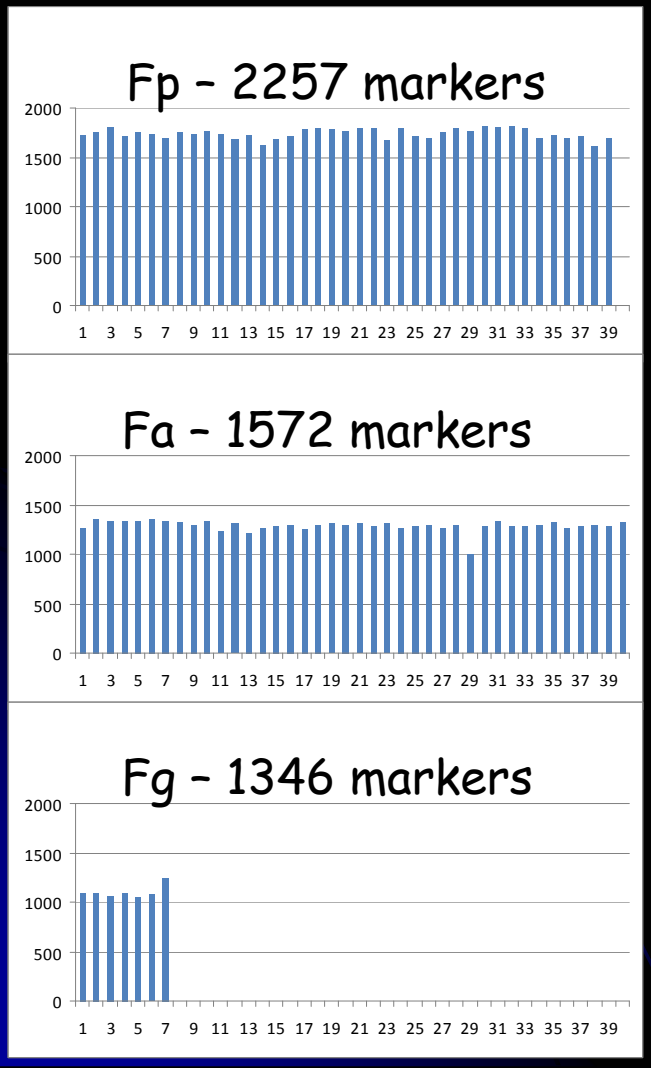
Development of array:

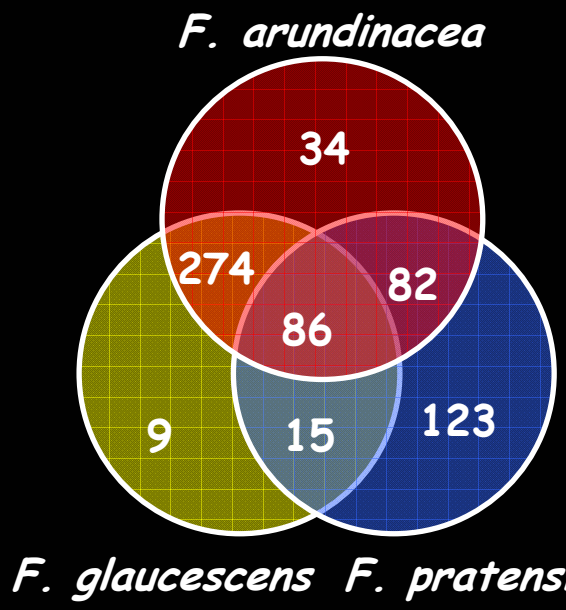
Material: 5 species
- 40 accessions of *Lolium perenne (2x, 4x)*
- 40 accessions of *L. multiflorum (2x, 4x)*
- 40 accessions of *Festuca pratensis (2x, 4x)*
- 40 accessions of *F. arundinacea (6x)*
- 7 accessions of *F. glaucescens (4x)*

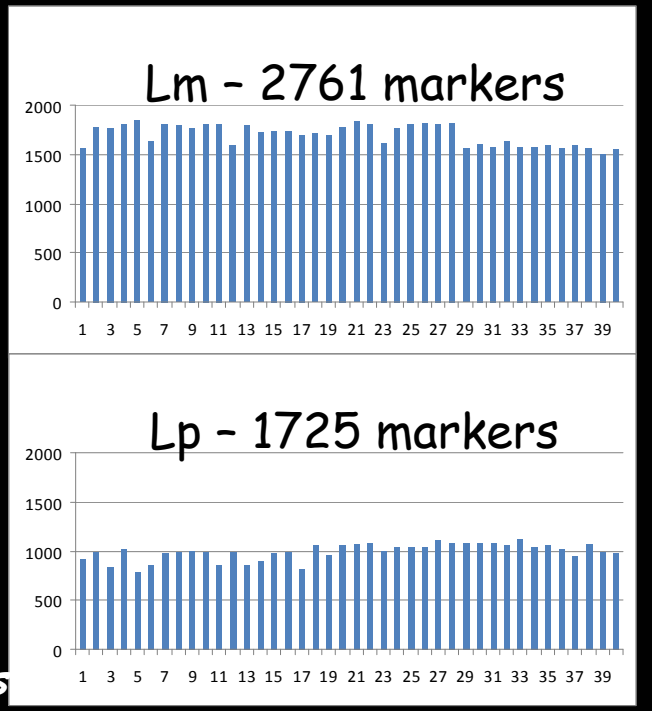
Array: 7680 probes

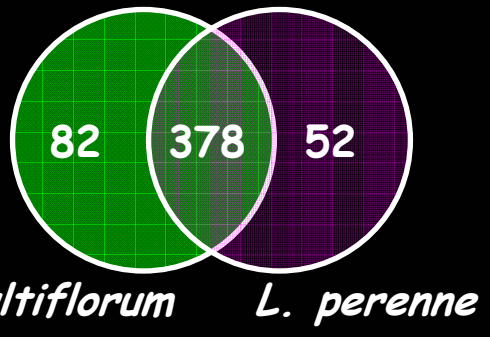
Number of polymorphic markers: 3884

DArT*Fest*: Genus- and species-specific markers



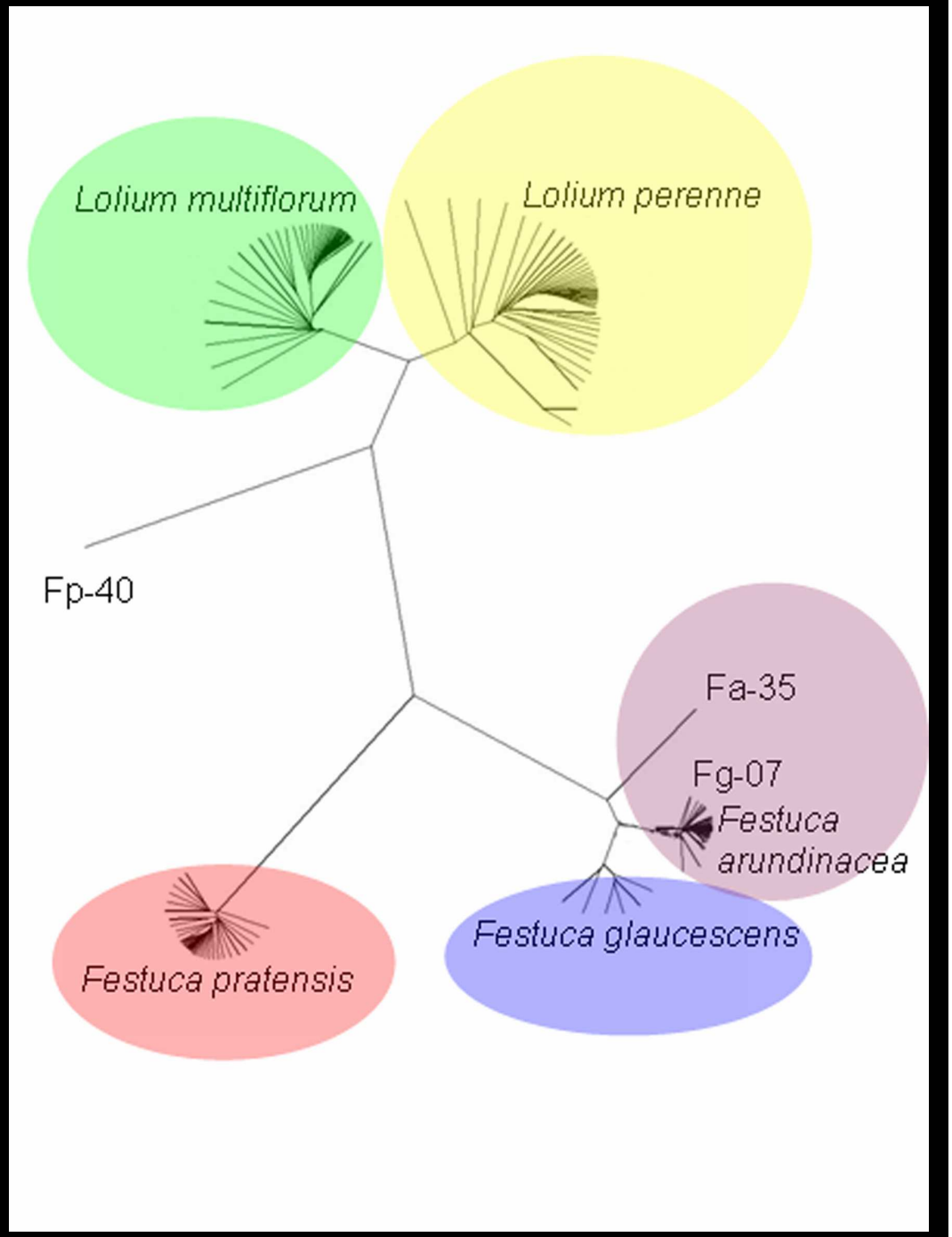




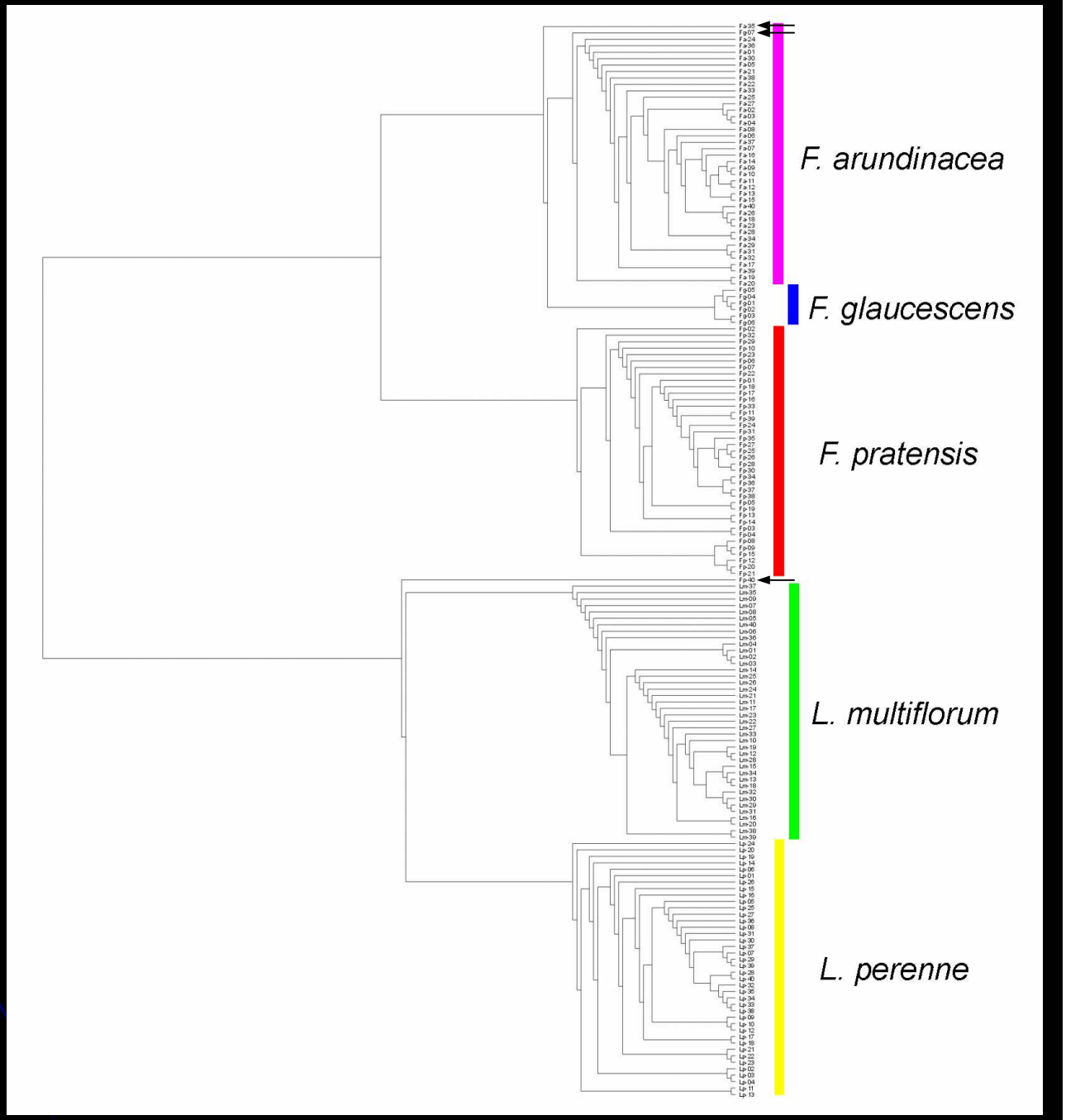


DArT*Fest*: Genetic diversity

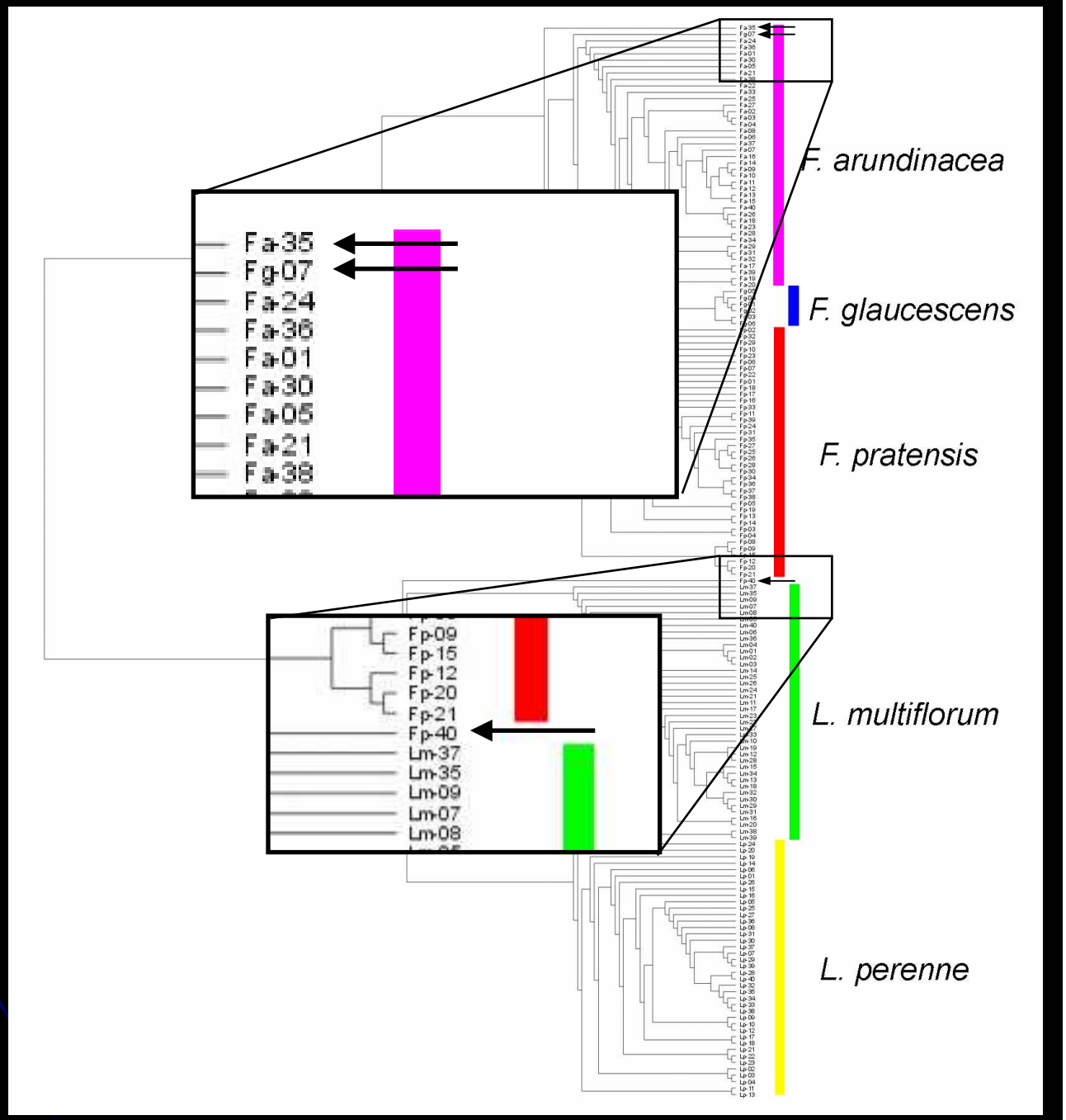
Based on the analysis of 2637 DArT markers



DArT*Fest*: Genetic diversity

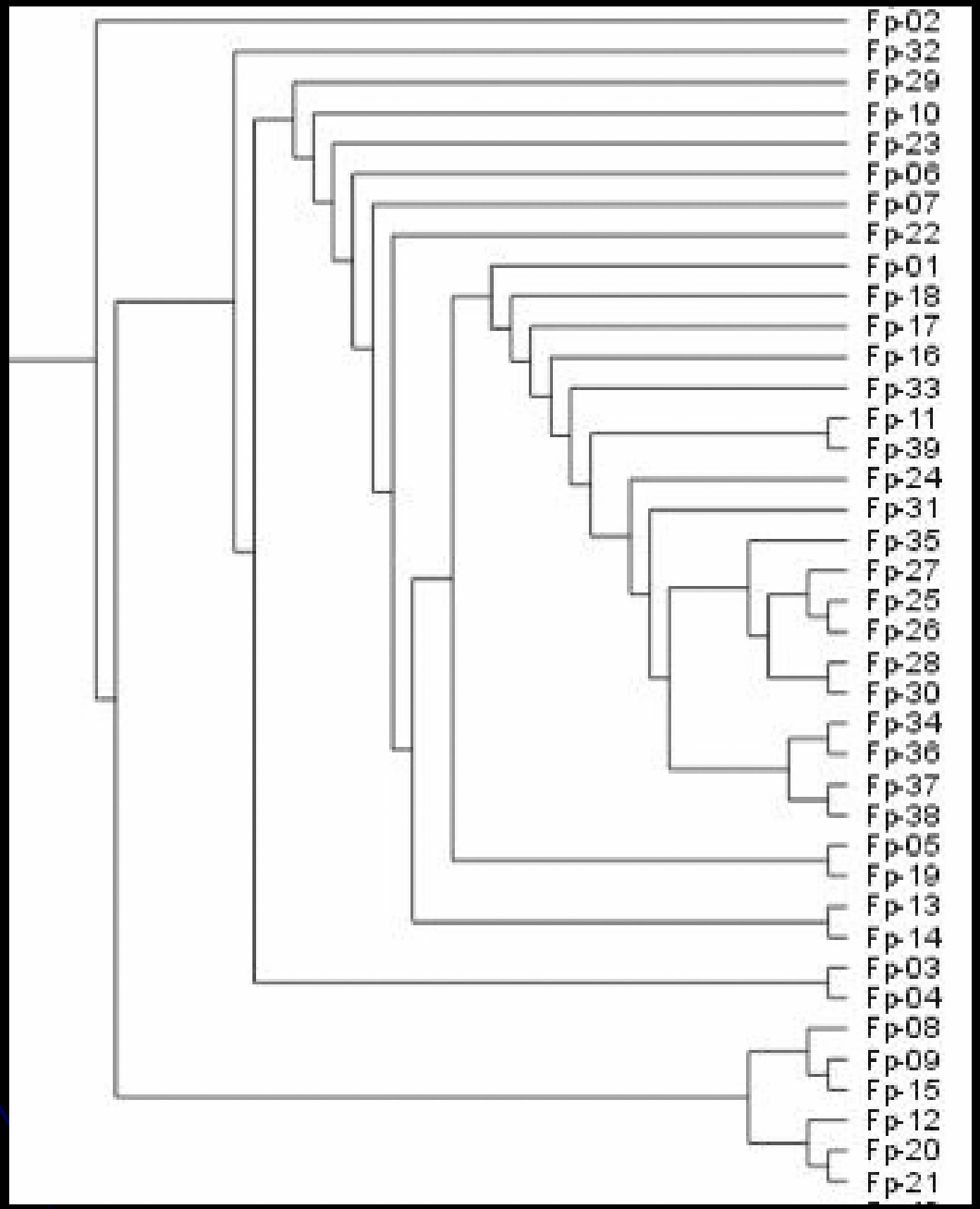


DArT*Fest*: Genetic diversity



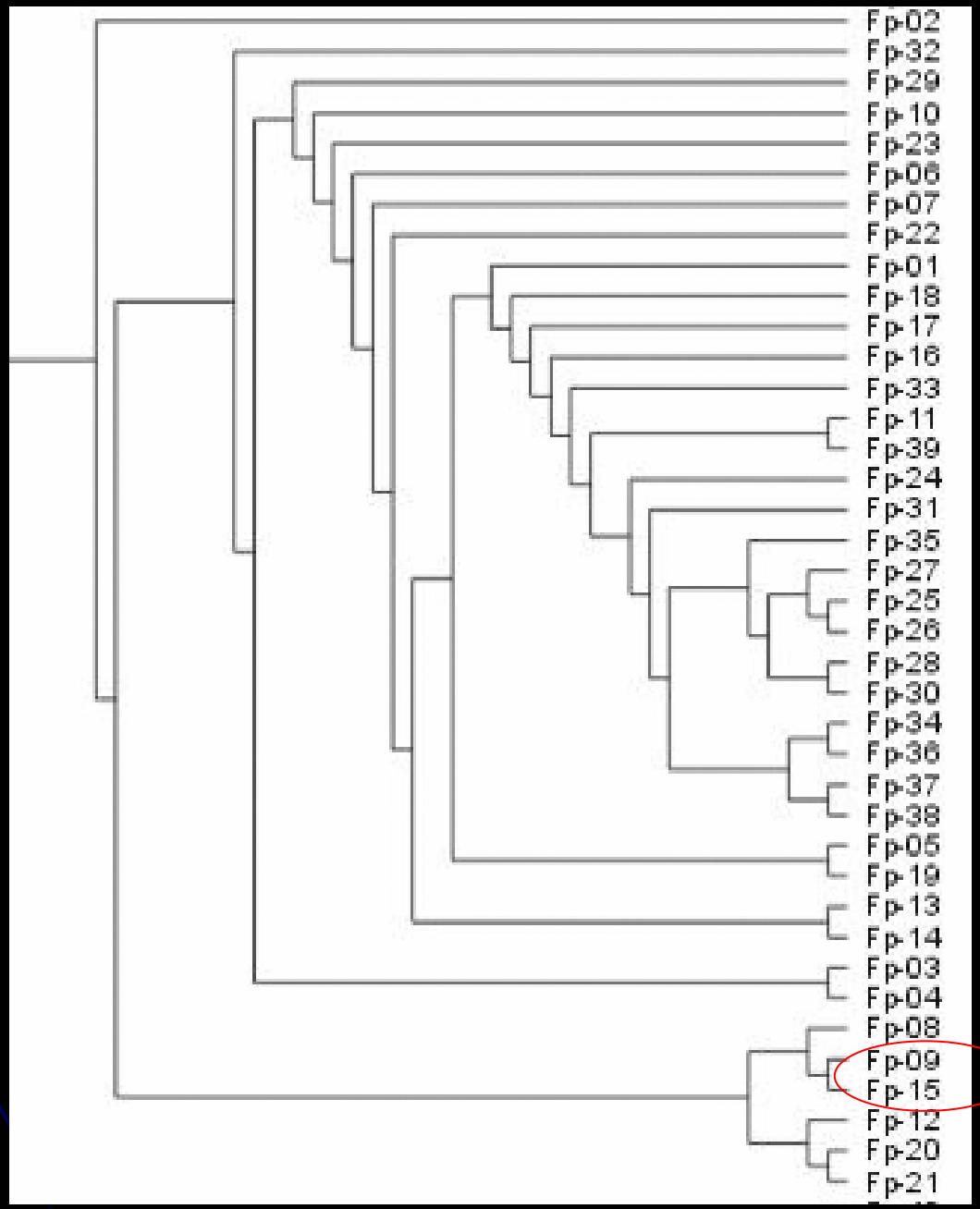
DArT*Fest*: Genetic diversity

(*F. pratensis*)



DArT*Fest*: Genetic diversity

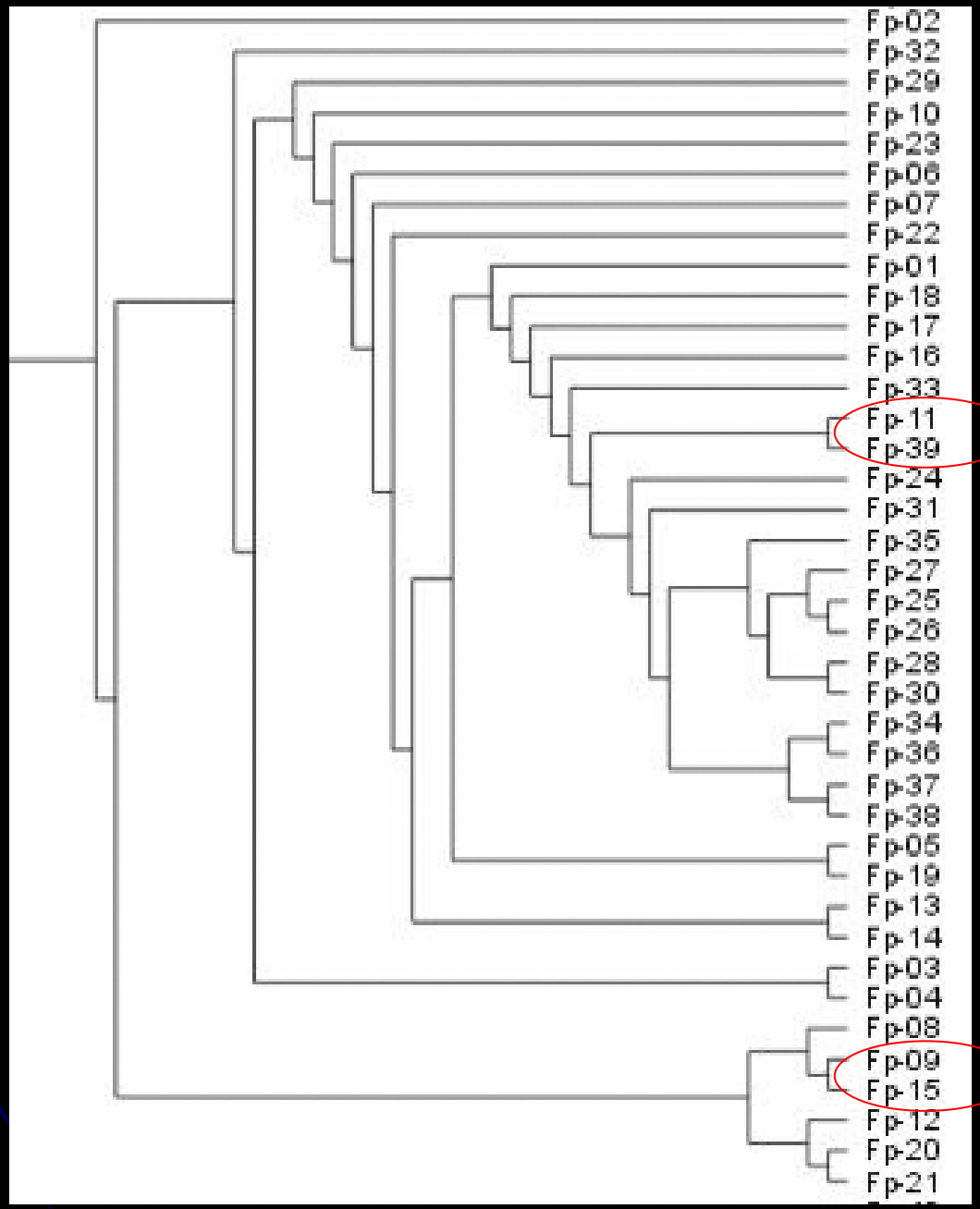
(*F. pratensis*)



Ecotypes from Lithunia (Acc. 3982 and 3985)

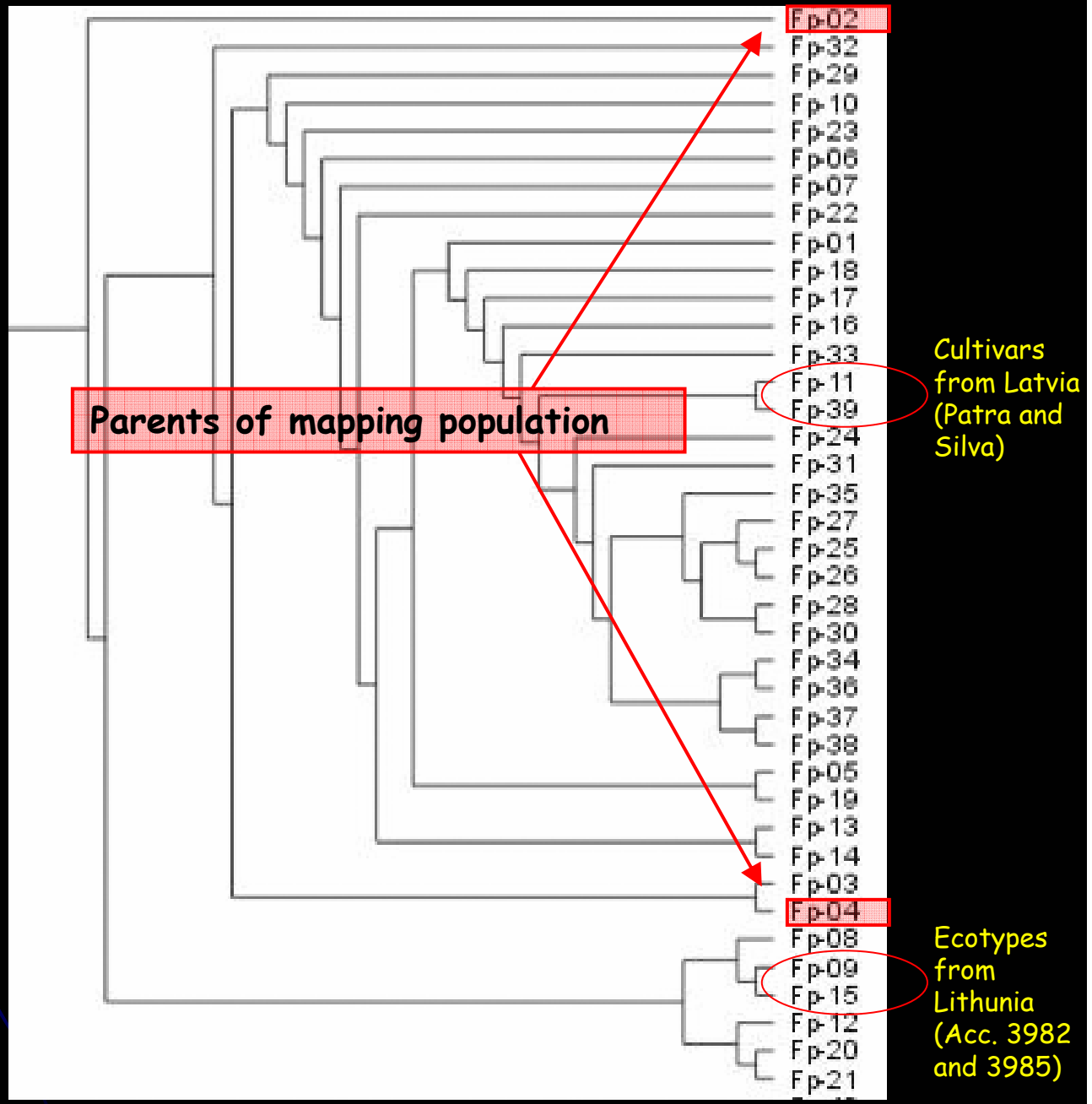
DArT*Fest*: Genetic diversity

(*F. pratensis*)



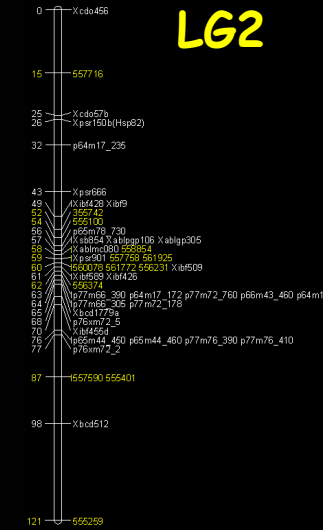
DArT*Fest*: Genetic diversity

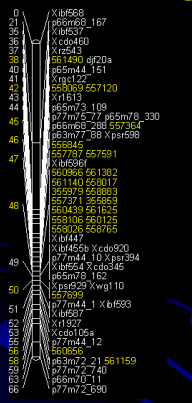
(*F. pratensis*)

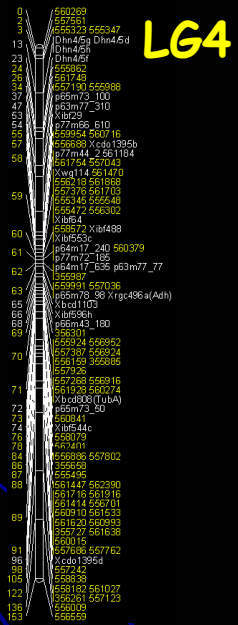


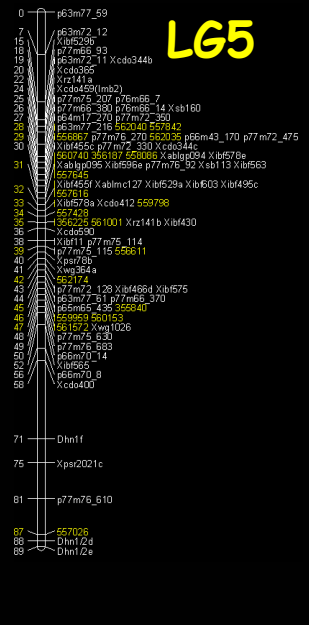
DArT*Fest*: Genetic mapping

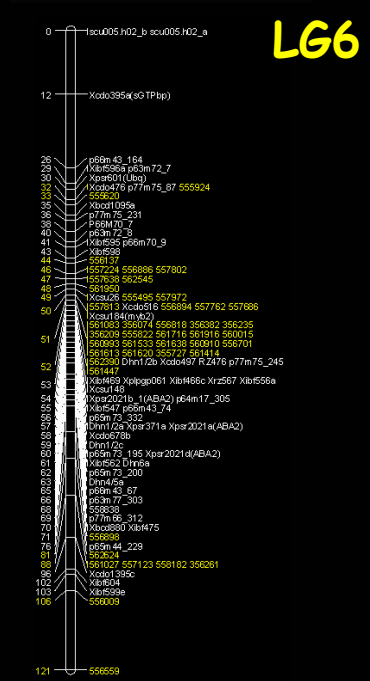
(*Festuca pratensis* - Odd Arne Rognli & Simen Rød Sandve)

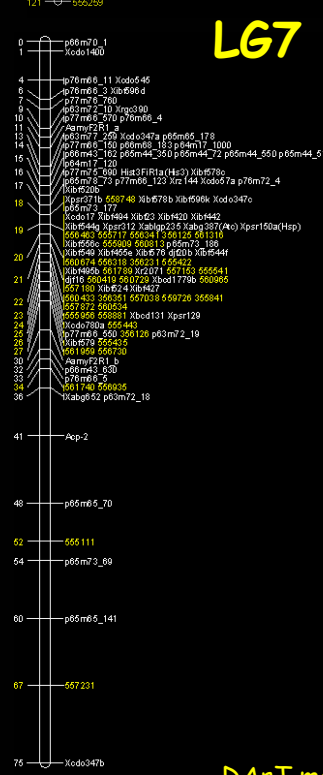












- 204 DArT markers (plus 466 others)
- The total length of genetic map 775cM (DArT genetic map by itself - 507cM; the original map - 605cM)

DArT markers
other markers

DArT*Fest*: Genetic mapping

(*Lolium multiflorum* – Roland Kölliker)

In progress

DArT*Fest* : physical mapping

Development of materials for the mapping

Fp (2n=2x=14) x Lm (2n=4x=28)

↓

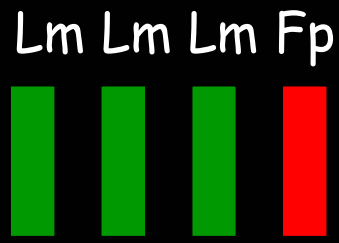
F1: (2n=3x=21, FpLmLm) x Lm (2n=4x=28)

↓

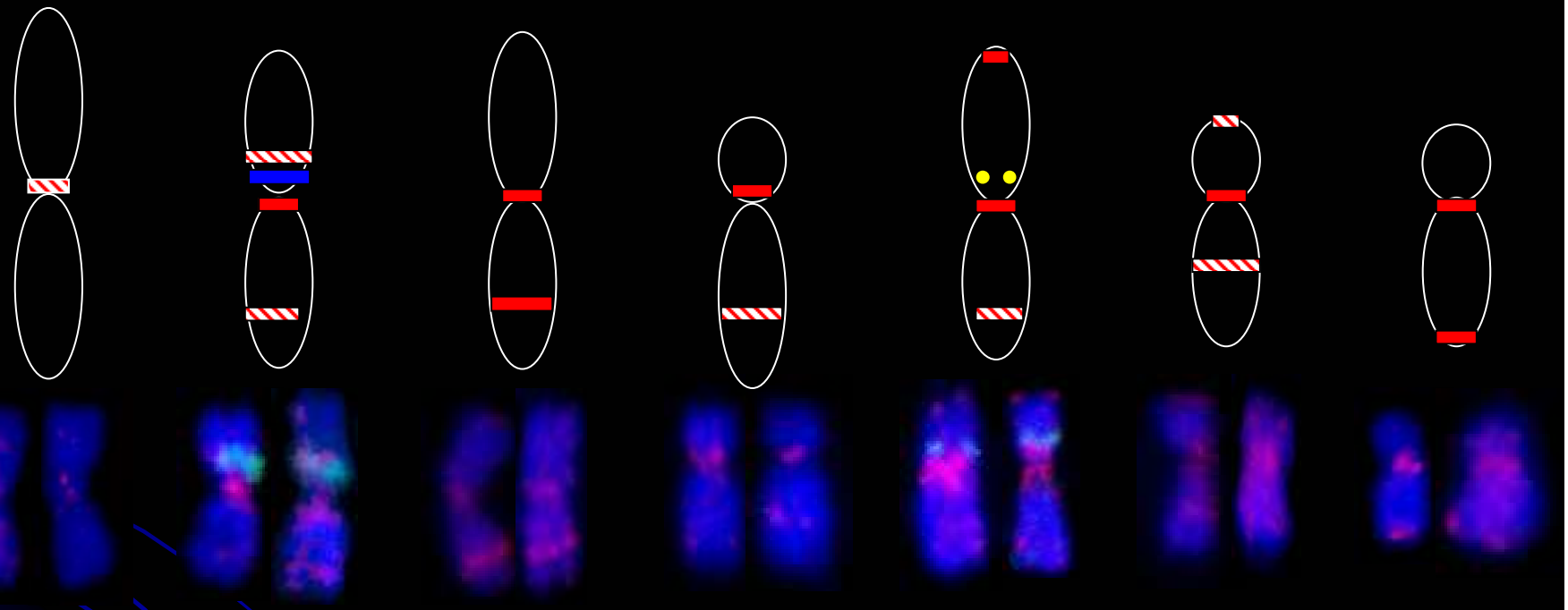
F2: (2n=4x=28, FpLmLmLm) x Lm (2n=4x=28)

↓

F3 etc. (2n=4x=28; 27Lm+1Fp)



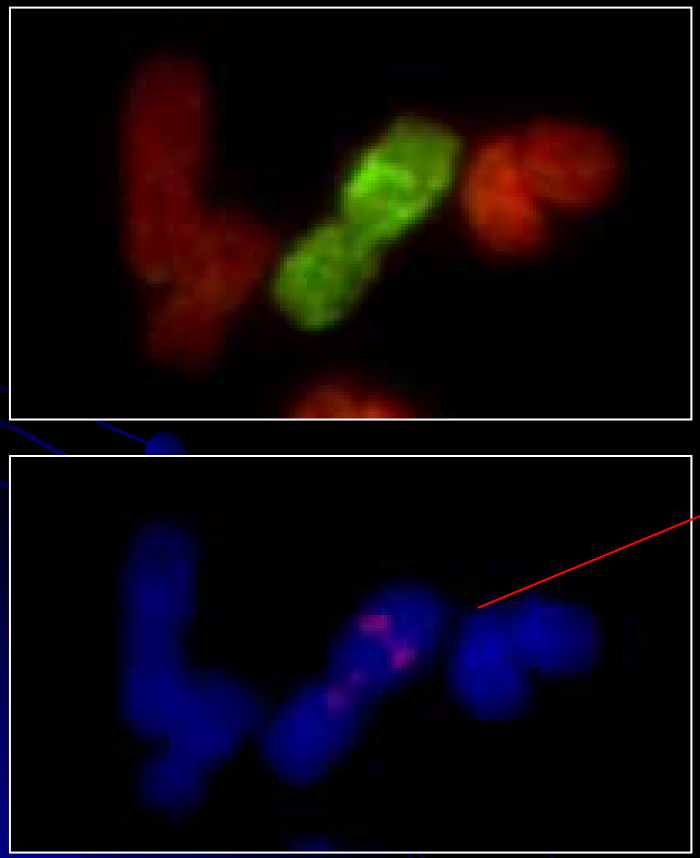
Cytogenetic mapping in *Festuca pratensis*



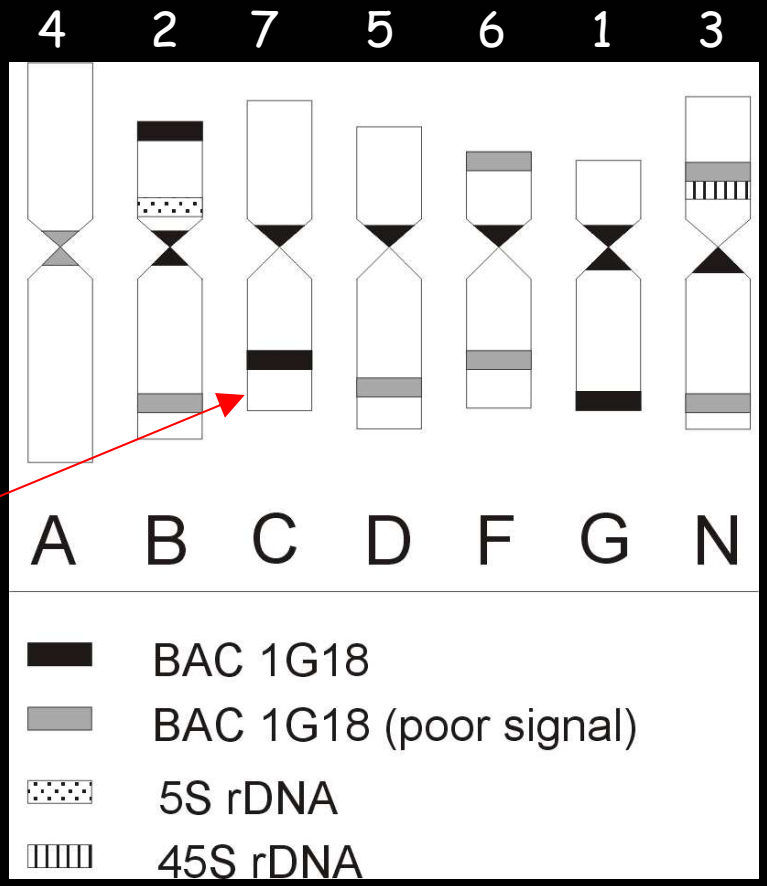
Chromosome 4	Chromosome 3	Chromosome 7	Chromosome 5	Chromosome 2	Chromosome 6	Chromosome 1
Chromosome A	Chromosome N	Chromosome C	Chromosome D	Chromosome B	Chromosome F	Chromosome G

BAC clone 1G18 — BAC clone 1G18 (weak signal) — 45S rDNA — 5S rDNA

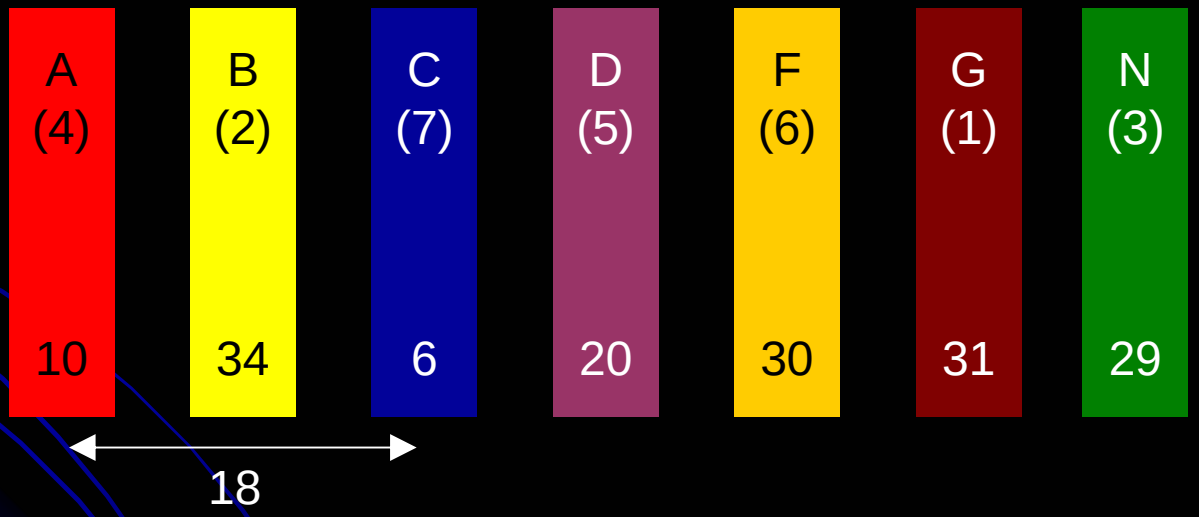
Cytogenetic mapping in *Festuca pratensis*



Chromosome C



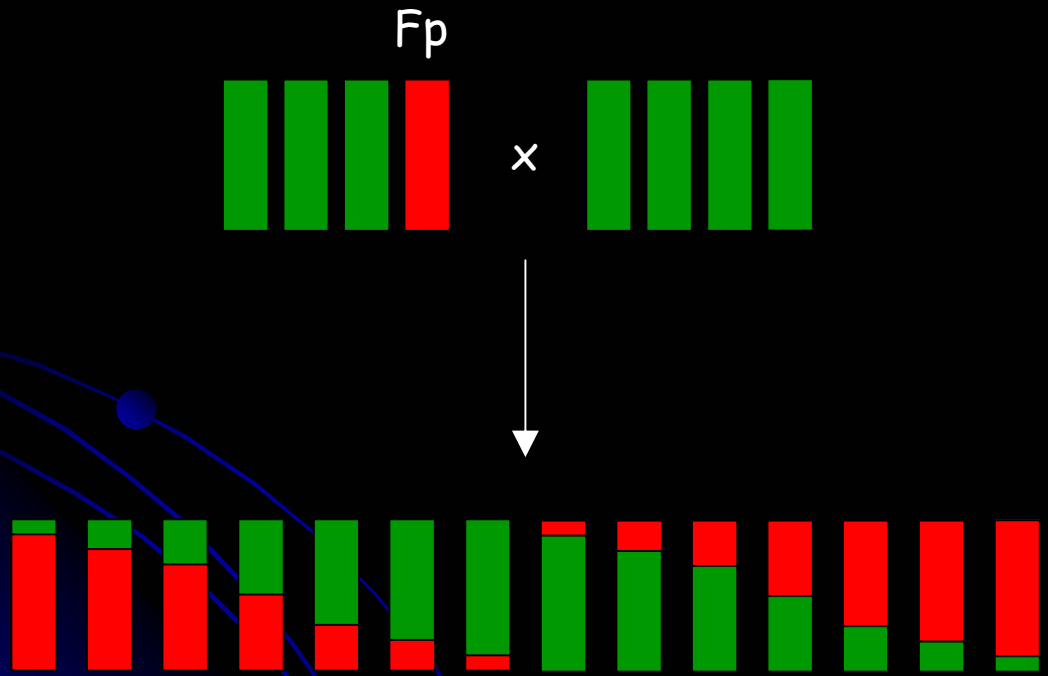
DArT*Fest*: Anchoring DArT markers to individual Fp chromosomes



=160 markers (out of 288 *F. pratensis* positive and *Lolium* negative)

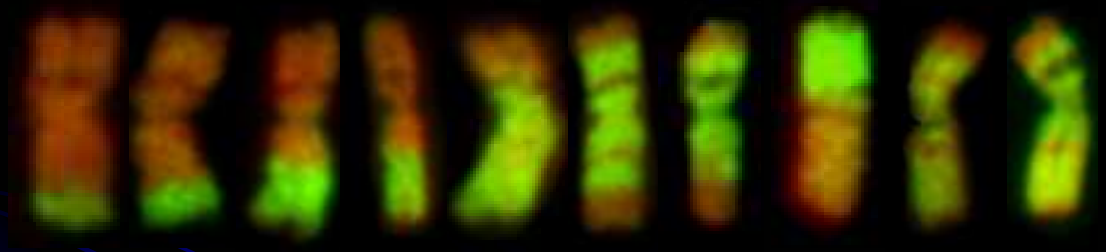
DArT*Fest*: physical mapping

- Mapping to subchromosomal regions (bins)
- Development of recombination lines



- More than 30 lines have been developed for each Fp chromosome

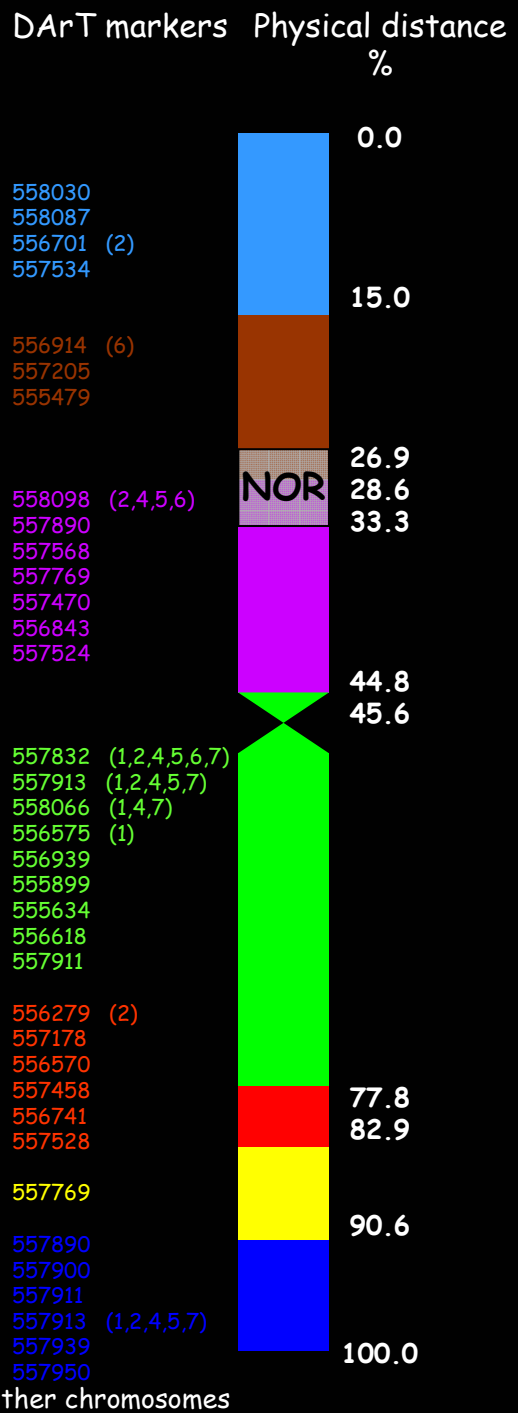
DArT*Fest* : Anchoring DArT markers to Fp chromosome bins



Chromosome 3 of *F. pratensis*

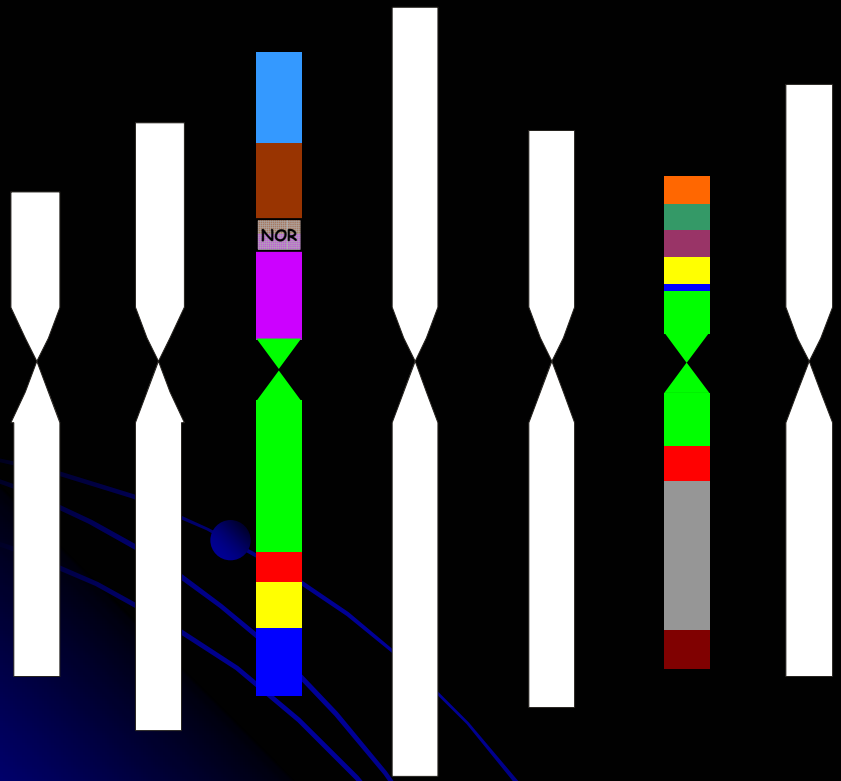
L. multiflorum

F. pratensis



*) detected in other chromosomes

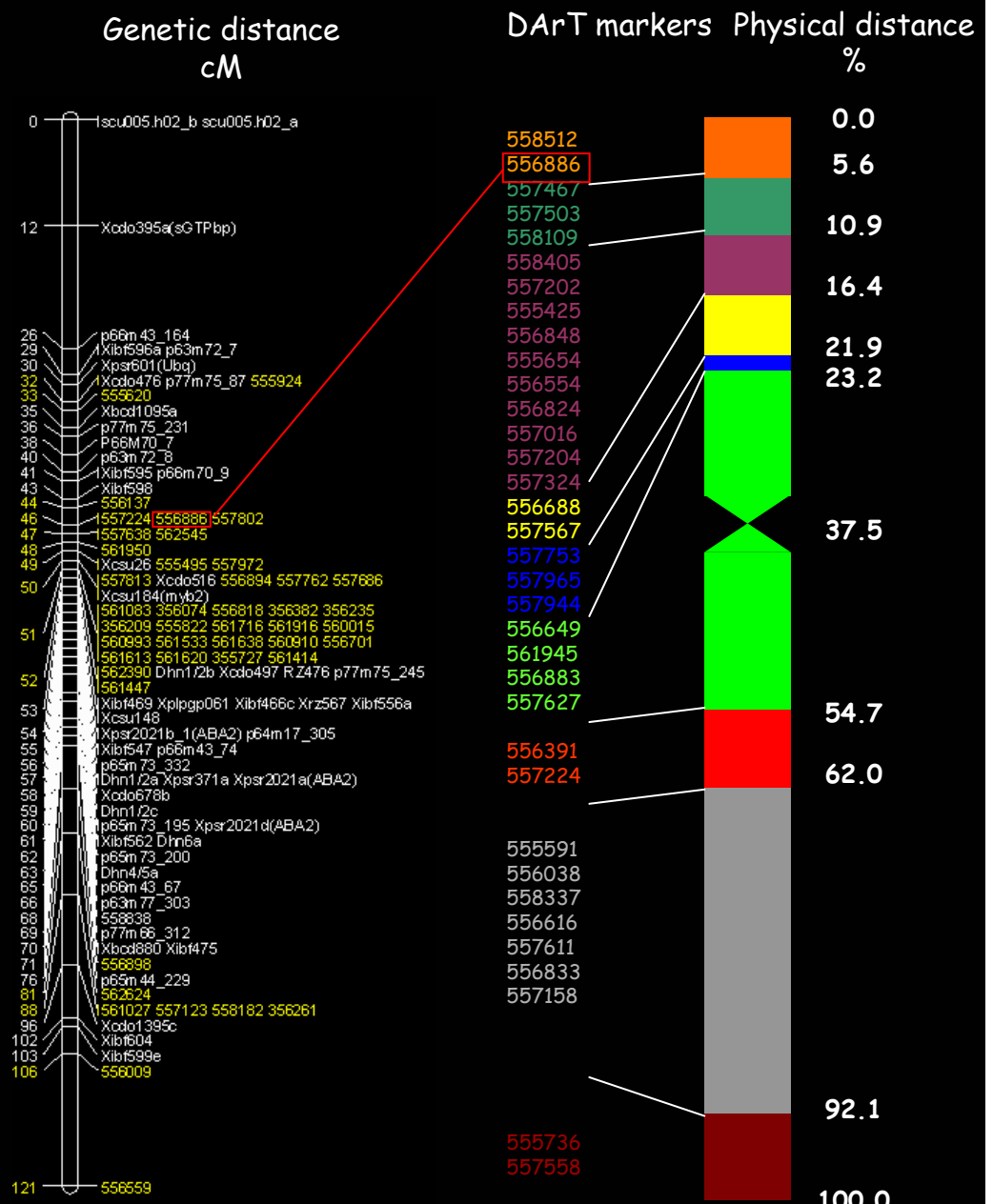
DArT*Fest*: Anchoring DArT markers to Fp chromosome bins



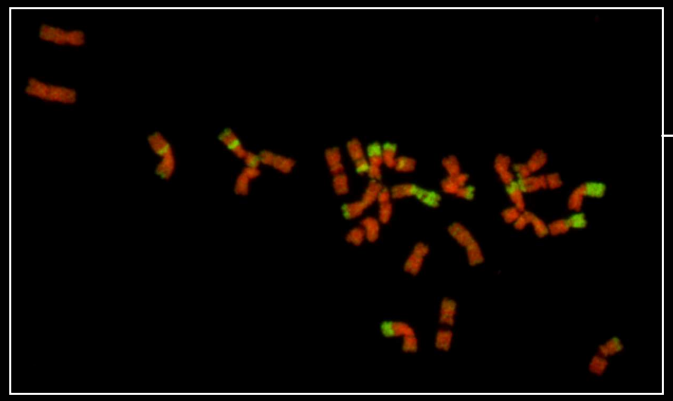
We expect to have about 10 bins per chromosome = dissect *F. pratensis* genome to about 70 bins

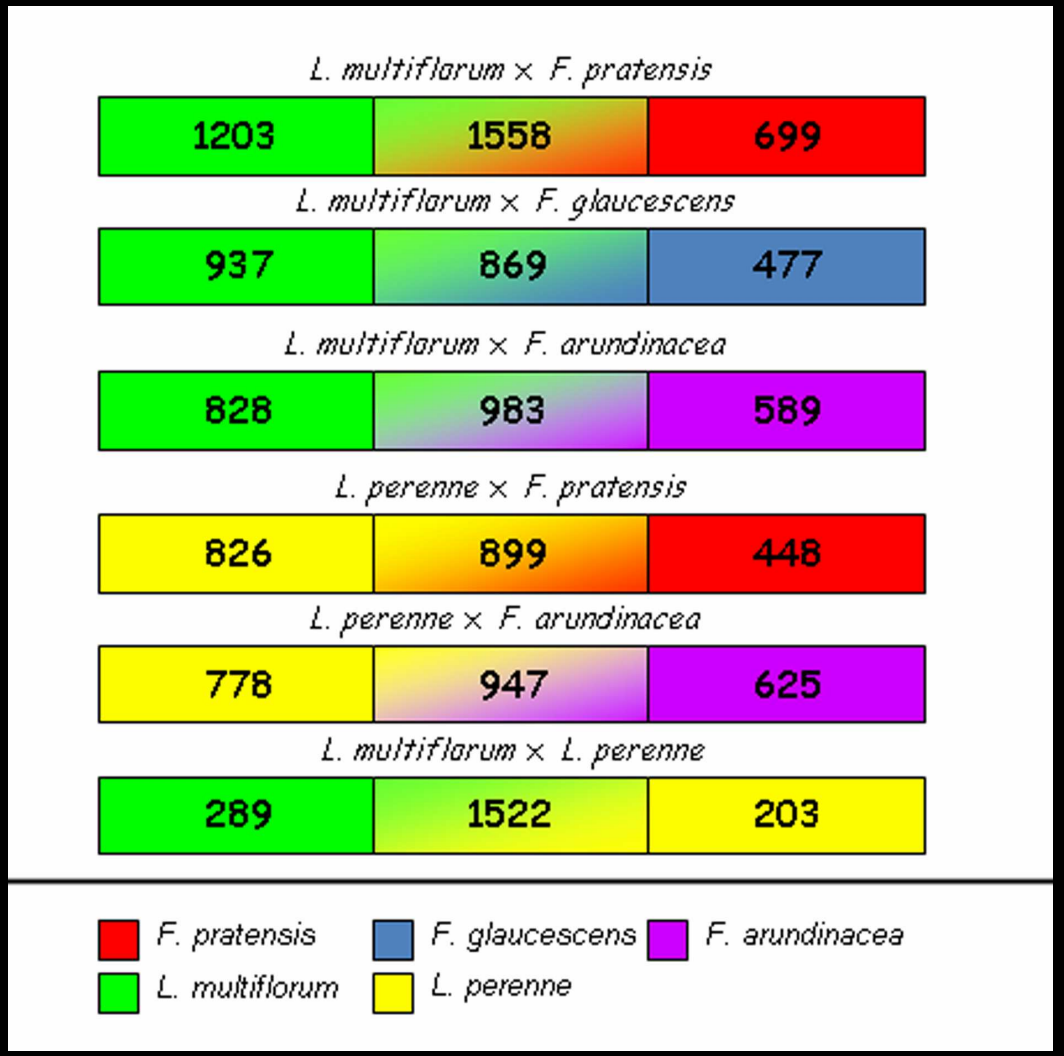
DArT*Fest*: Integration of genetic and bin maps

Festuca pratensis
chromosome 6

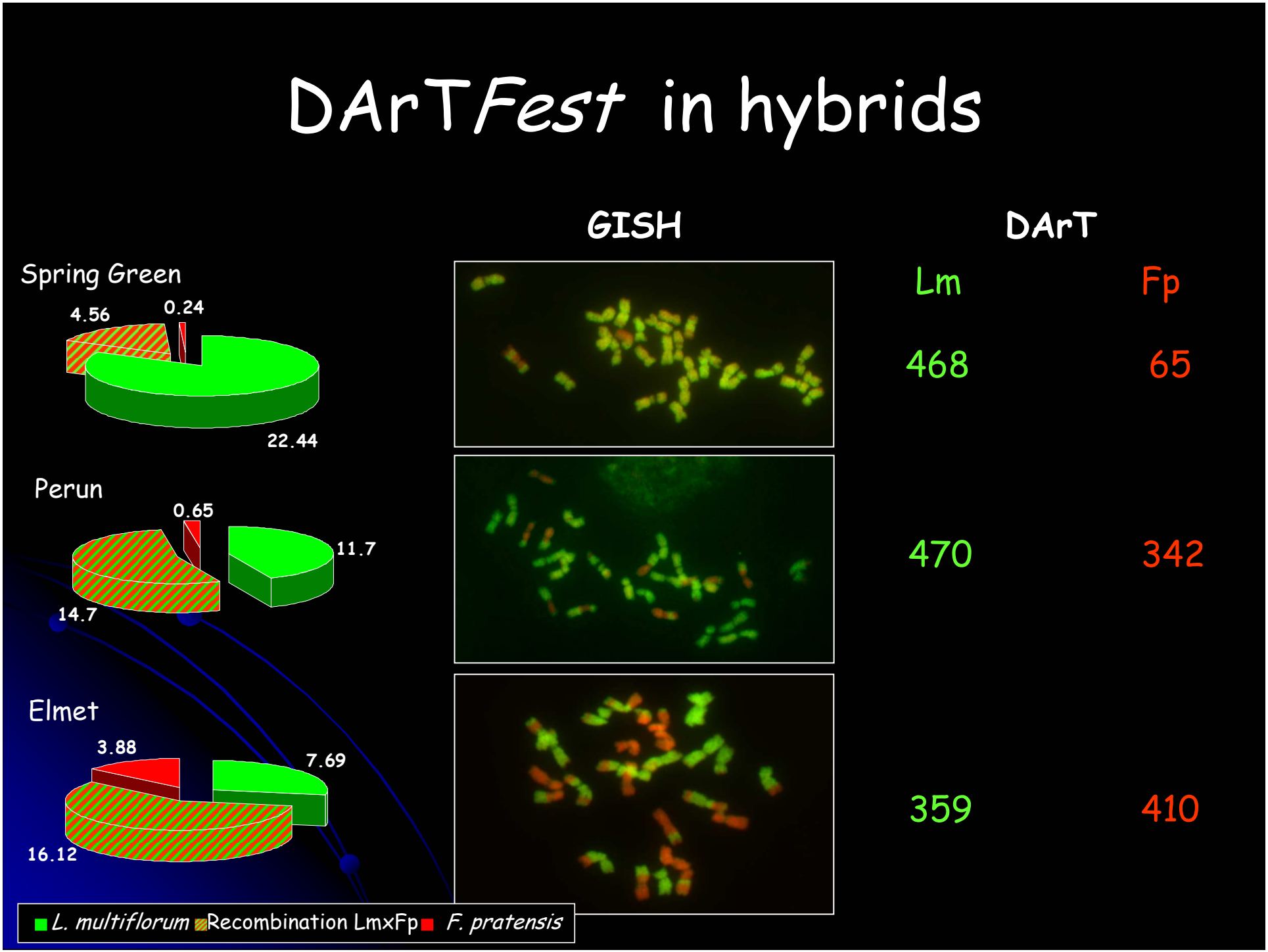


DArT*Fest* in hybrids



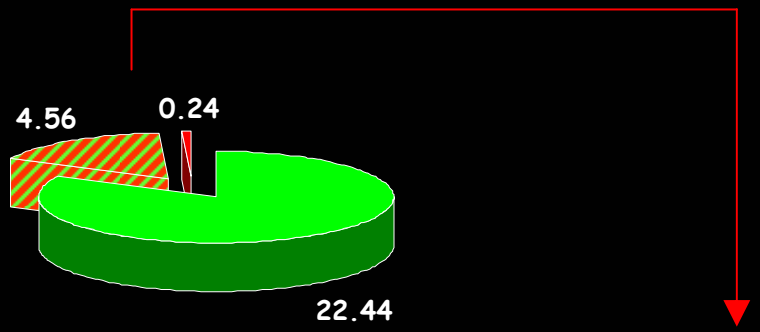


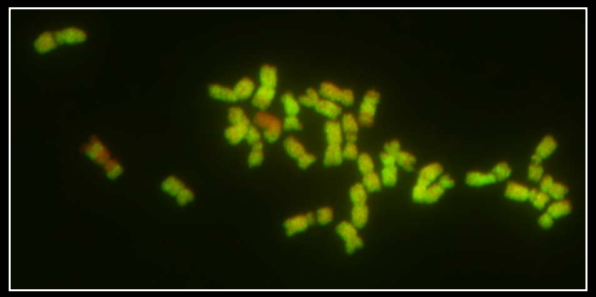
DArT*Fest* in hybrids

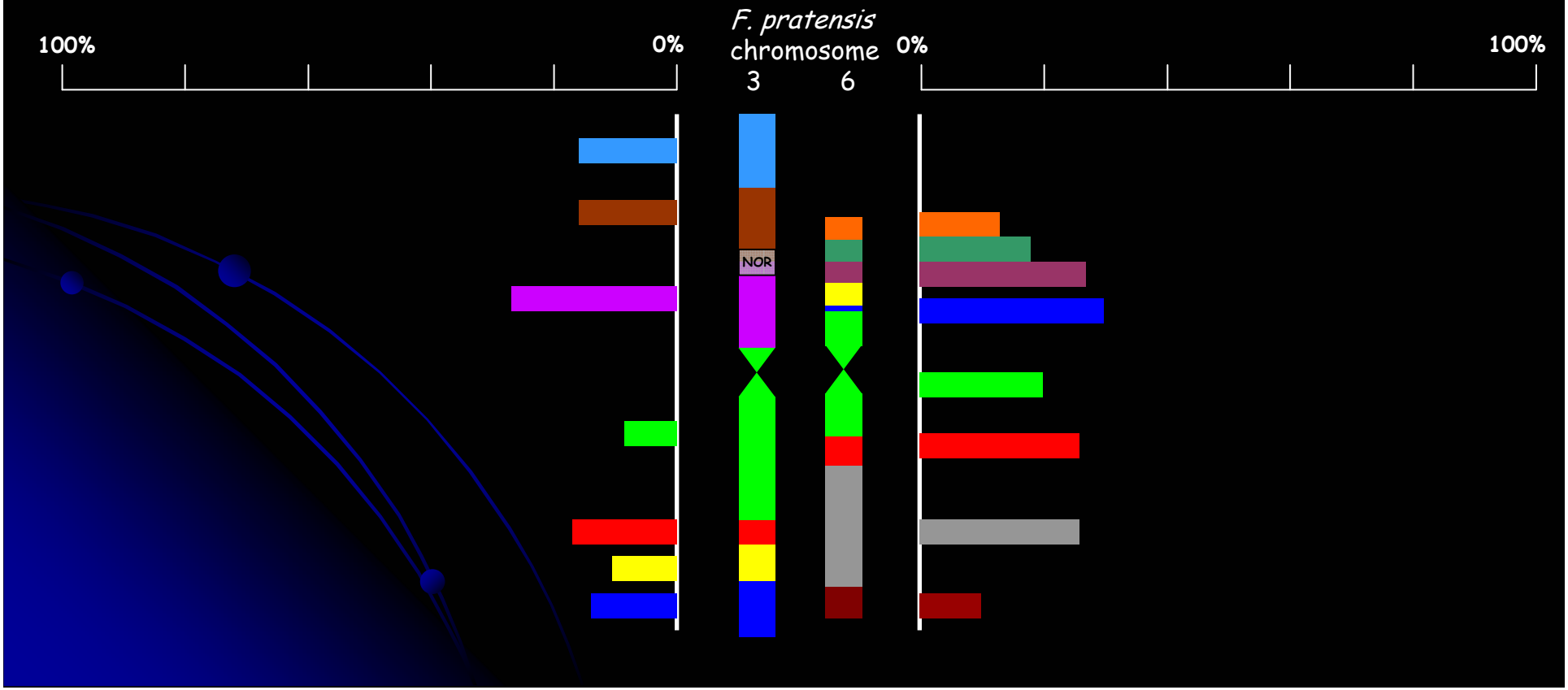


DArT*Fest* in hybrids

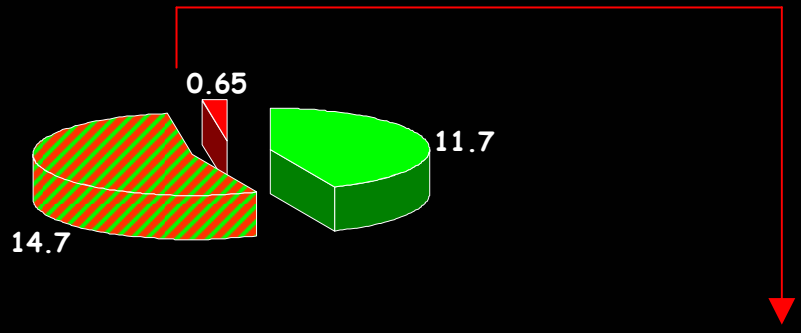
cv. Spring Green



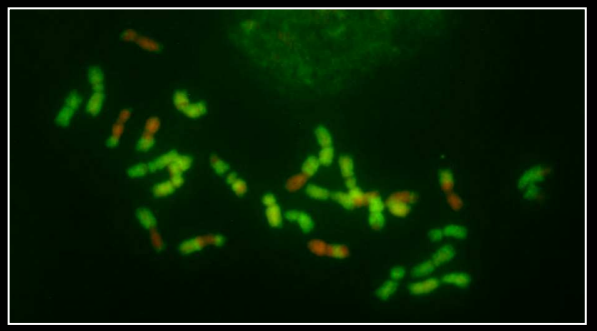


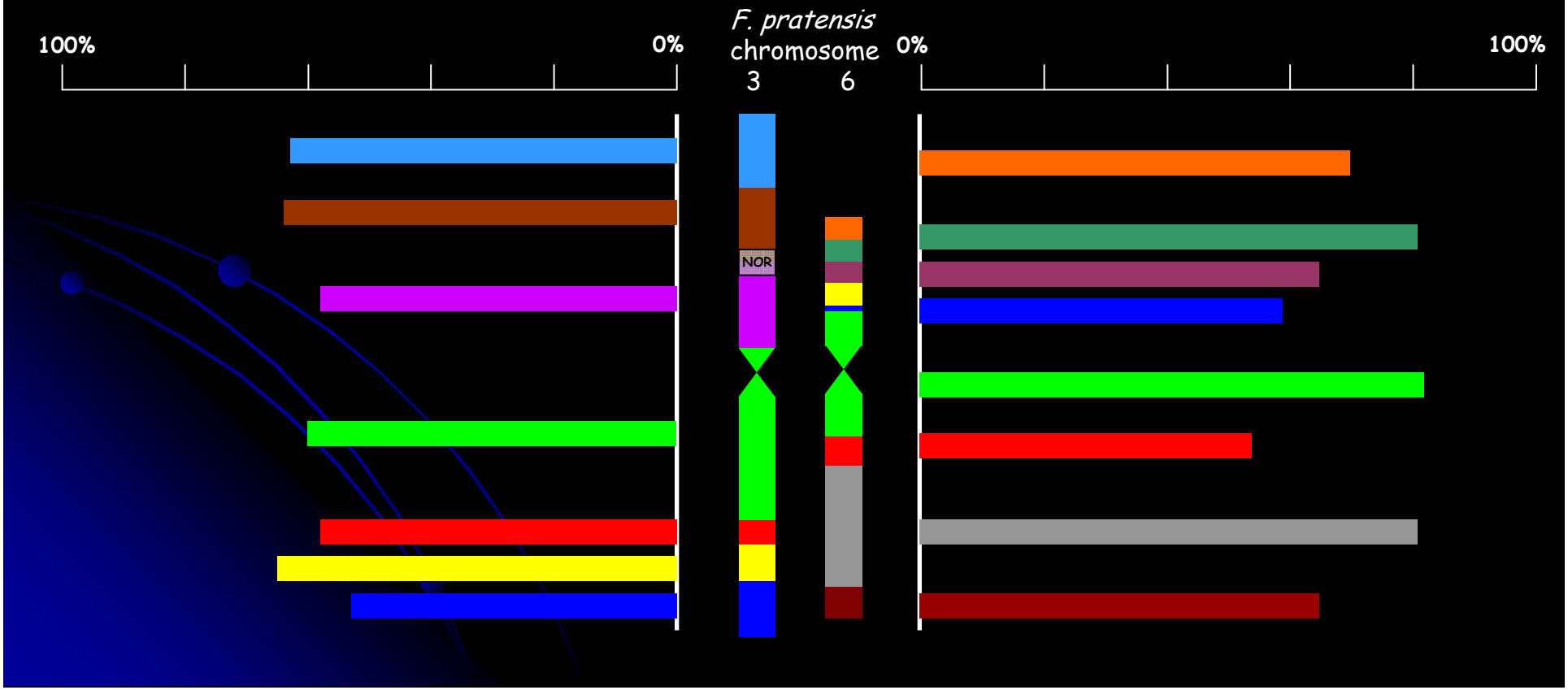


DArT*Fest* in hybrids

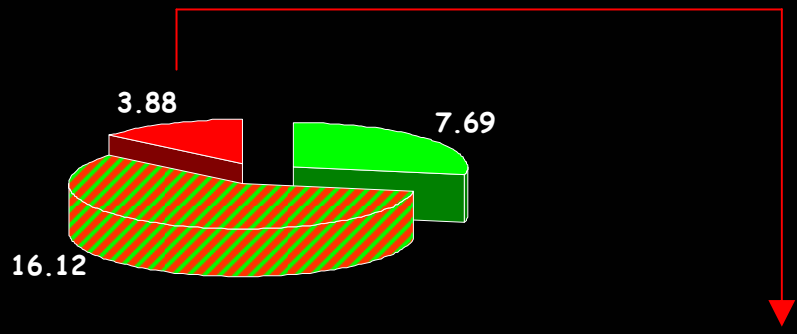


cv. Perun

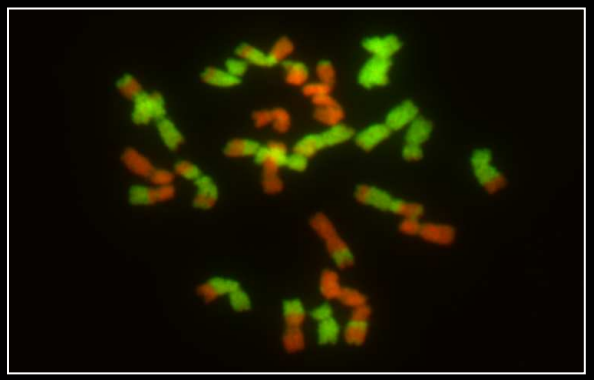


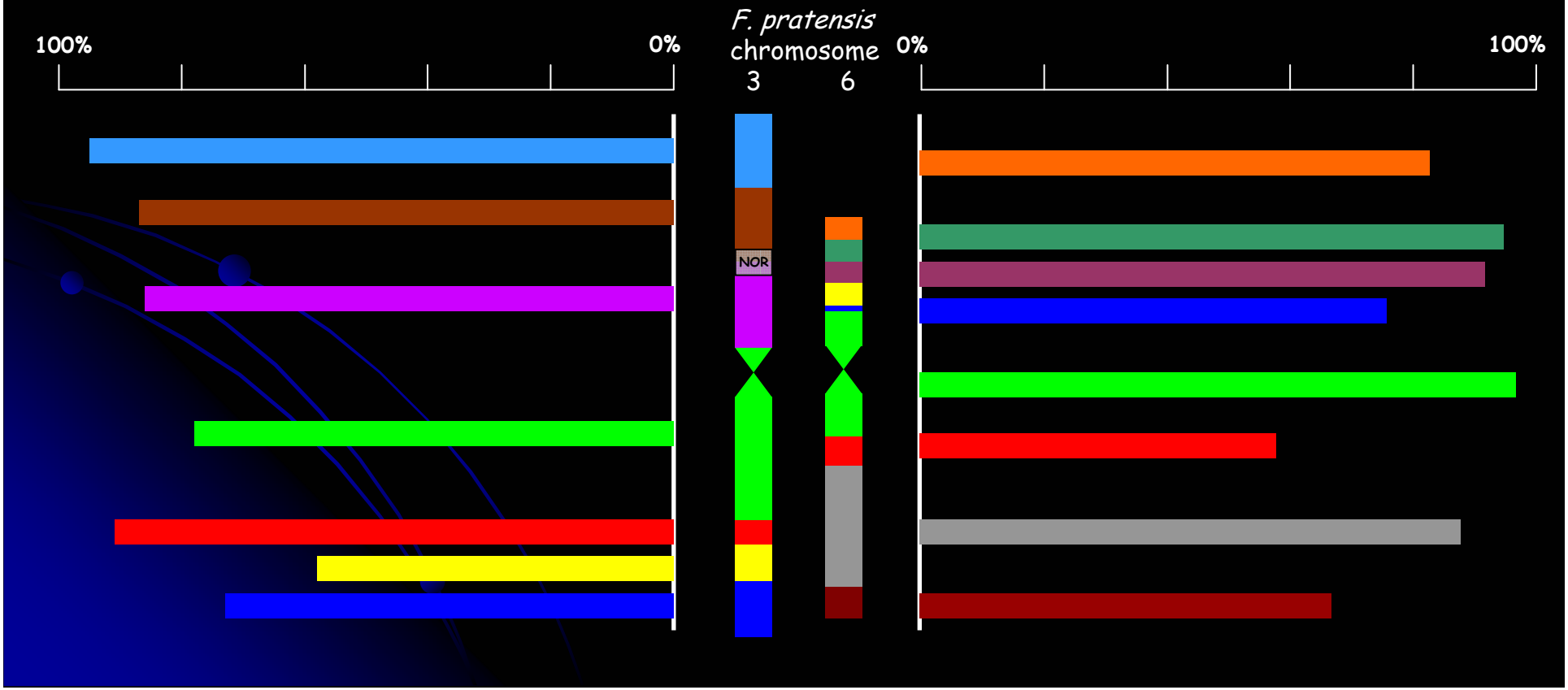


DArT*Fest* in hybrids



cv. Elmet





Conclusions

We have developed the first DArT array (DArT*Fest*) for *Festuca-Lolium* complex

The array contains 3884 markers and can be used for:

- Analysis of genetic diversity

- Genotyping

- Analysis of genomic constitution of hybrids

- Genetic and physical mapping

Future work

(1) Establishment of genetic maps with DArT markers

(2) Integration of genetic and bin maps

(3) Comparative analysis of species within the *Festuca-Lolium* complex

(4) Association of markers with agronomically important traits

(5) Sequencing of already mapped DArT markers and conversion to PCR markers

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Zurich, Switzerland
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IBERS, Aberystwyth University, Wales
Prof. Ian P. King, Dr. Leif Skot
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