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GENEVA

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MAIZE FINGERPRINTING:

IMPROVEMENT OF THE RFLP PROTOCOL AND SELECTION FOR PROBE QUALITY

Document prepared by experts from France

UPOV Working Group on Biochemical
and Molecular Techniques
VERSAILLES March 21-23, 1994

MAIZE FINGERPRINTING :
IMPROVEMENT OF THE RFLP PROTOCOL AND SELECTION FOR PROBE QUALITY

The use of molecular markers in the calculation of genetic distances between maize lines has been evaluated in a program initiated by the Comité Technique Permanent de la Sélection des Plantes Cultivées (CTPS) and three partners : GEVES, SEPROMA and INRA.

The main goals of this project were :

* to develop some molecular marker technologies (Restriction Fragment Length Polymorphism : RFLP) allowing the description of the genetic variability on a large number of genome sites.
* to compare the descriminant power of several criteria for the D. U. S. (Distinctness, Uniformity, Stability).

The molecular evaluation of the lines with RFLP markers has been performed at BIOCEM, the biotechnology lab of the Limagrain group in Clermont Ferrand. This experiment aimed to investigate several parameters playing a major role in the quality and the quantity of the information produced.

DESCRIPTION OF THE EXPERIMENT

DNA from one hundred and fifty maize lines, representative of the genetic variability used in Europe, were analysed with one hundred RFLP probes and three restriction enzymes. The choice of the probes was made according to previous knowledge of the probe quality and their position on the maize genome.

The whole experiment was done following a procedure which allowed the production of data as informative and reproducible as possible. To reach such a goal, the two the main parameters to be under control are the use of a well-defined protocole and a selected set of probes.

THE PROTOCOLE

In fingerprinting experiments, a very good quality of autoradiogram is required to allow an unambiguous detection and calculation of the molecular weight of all the bands. The main parameters are :

The distinctness of the bands

The presence or the absence of a band has to be readable without any doubt. This implies good quality and control of all the molecular biology procedures, from the plant material to the digested DNA ready to load (extraction and purification of heigh molecular weight DNA, enzymatic digestion, quantification of the DNA to avoid the risk of false negative in lane loaded with less DNA). The hybridization with the labelled probe has to be homogeneous on all the samples within and across experiments. No heavy nonpecific background is tolerated since it may mask the presence of some bands.

The use of an image analysis system, which allows the processing of a large amount of data (detection of the bands and evaluation of their molecular weight), requires even more stringent conditions.

The difference between the band levels

In this document the term "**band level**" is used to designate, for a probe x enzyme combination, the peak to which all the bands characterised by the same or a similar molecular weight, are assigned. This is to avoid ambiguous term such as "allele".

The accuracy of the assignment of the bands to the band levels is dependent on the quality of the electrophoresis. Distortions in the migration may induce some missassignation. In practice, an accurate assignation of the bands is obtained by

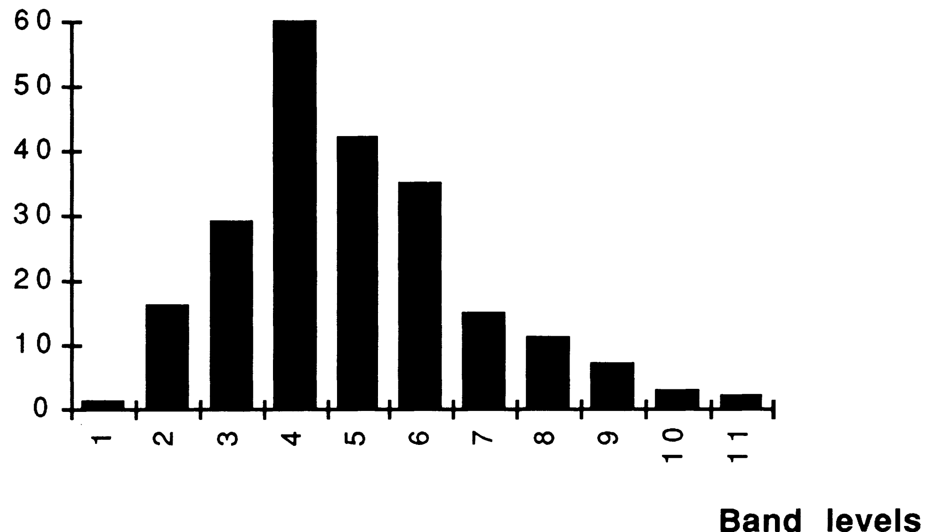
* conditions of electrophoresis under strict control
* the use of a reproducible DNA molecular marker (see Figures : lane ENZO). It displays 10 bands representing a good range of the molecular weights usually scored in RFLP experiments.
* the use of a protocole that allows lighting up the marker and the maize bands with the same intensity.
* an automated calculation of the molecular weights after capture of the autoradiogram with an image analysis system.

PROBE QUALITY

The use of a very standardized protocole is the first step to reach for making the RFLP a powerfull and reproducible technology. The second important parameter which will determine the effiency of the experiment is the quality of the probes. This quality is dependent on the ability of the probe to produce a strong hybridization signal. Beside the quality of the signal by itself the main feature is the information content, determined by the number and the frequency of the band levels.

The graph below shows the distribution of the probe x enzyme combinations (222) according to the number of the band levels observed in the 150 lines.

Number of probe x enzyme combinations



The average is close to five, but a wide range is observed, from non informative probe x enzyme combination up to very polymorphic ones (11 different band levels). The number of band levels is influenced by the polymorphism at the locus, but in maize, as a consequence of duplication in the genome, several probes hybridize at more than one locus (usually two). In such case the number of band levels is generally higher and results from the polymorphism present at two loci.

Using the traditional procedure (migration of bromophenol blue of 10 cm in agarose gels) a conflict appears between the ideal conditions in term of quantity of information (probes showing a maximum of band levels) and the possibility to distinguish the different band levels. The Figure 1 (BNL 16.06 HindIII) shows that nine different band levels have been determined for the 150 lines. Despite this high number it was possible to determine them with a good confidence (in some cases two close bands have been pooled in the same band level : band level number three from the top : 6479). In other cases the band levels are to close together to be scored without a high risk of missassignation (Figure 2 : UMC89 Hind III).

THE INFLUENCE OF THE ENZYME

The Figure 2 (UMC89 Hind III) and Figure 3 (UMC89 Eco RI) illustrate that even for probes giving a nice hybridization signal, the choice of the enzyme to be used for each probe, is critical. In this case it is not possible to discriminate between the different band levels with the enzyme Hind III, while Eco RV show the same number of them, easily distinguishable.

GENOME COVERAGE

An other important parameter is the coverage of the genome with polymorphic probes. The ideal description of the variability of germplasm would

require the use of markers evenly distributed on the chromosomes. One marker every 30 cM would yield a very accurate description of the variability. In practice it is never possible to reach this condition. Due to linkage the testing of two probes closely linked yield less information (some of it is redundant) than two unlinked ones. To overcome the problem of uneven distribution, some studies try to investigate the possibility of introducing the recombination fraction value between the probes in the calculation of the genetic distance (A. Bar-Hen). In this approach the probes hybridizing to a single locus are prefered since their position is known unambiguously.

CONCLUSION

The definitive choice of the probes has therefore to take into account the following parameters :

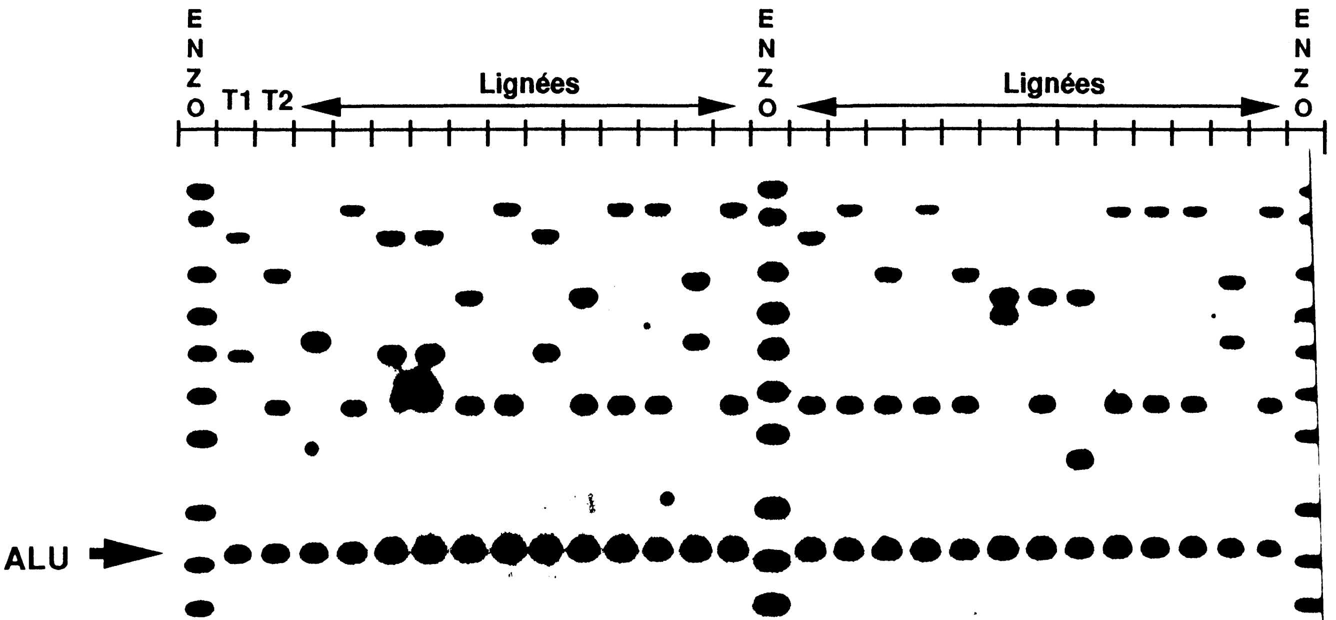
- **no hybridization back-ground**
- **good band intensity**
- **high polymorphism**
- **easily detectable difference between the band levels**
- **distribution on the genome**

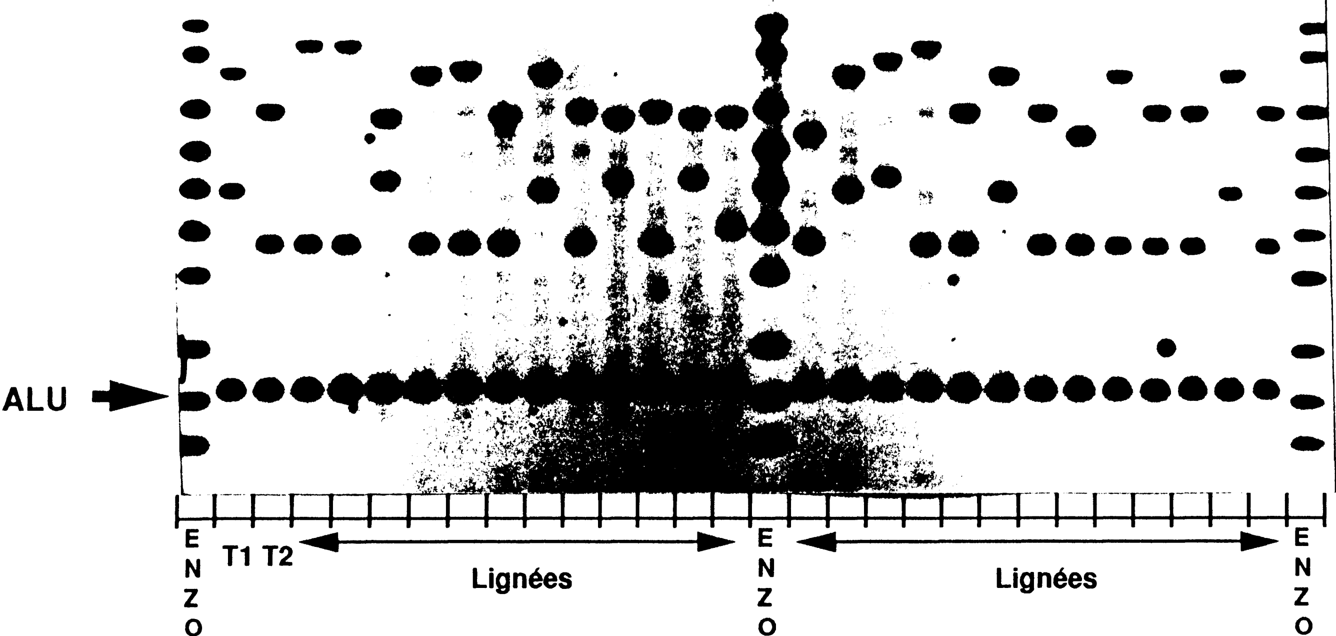
Few probes or probe x enzyme combinations fit all these parameters. An intensive screening of many probes on many lines has to be done. The use of many lines is necessary to be sure that all the band levels have been observed. This work is only possible if a large number of probes which saturate the genome are available. It is the case for maize but not for many other species for which a highly saturated map does not exist.

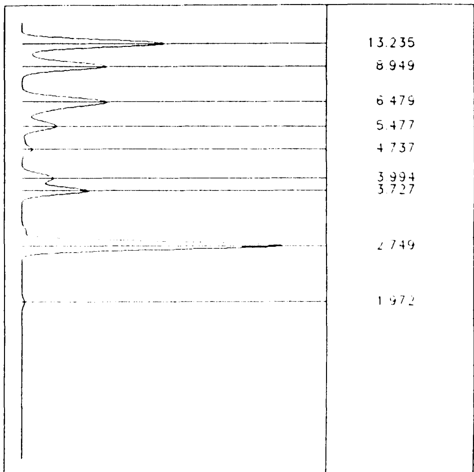
The screening of the good probes allowing a much faster interpretation of the data is an important parameter for the reduction of the cost of the analysis. **But the main advantage of having a set of good probes is that it increases the reproducibility of the technology which is the critical point for its widescale use.** The consistency of results obtained in different experiments and/or in different laboratories is certainly highly influenced by the quality of the probe used.

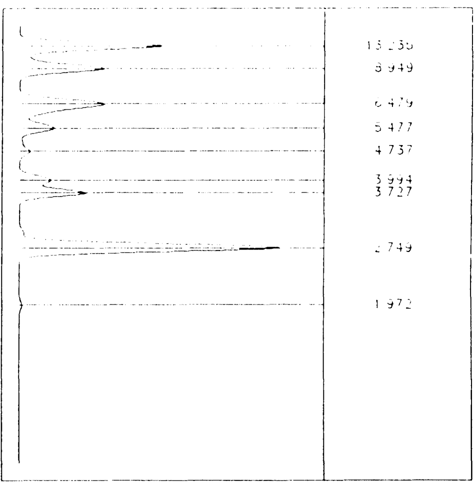
Laboratoire BIOCEM
Alain Murigneux
3/3/94

BNL 16.06 Hind III









UMC 89 Hind III

12.214
9.186
6.912

12.214
9.186
6.912

Lignées

Lignées

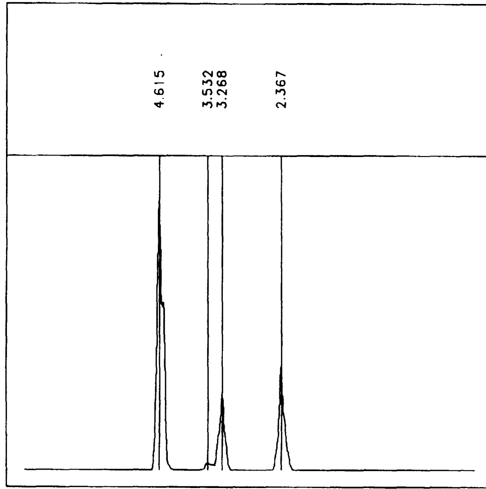
T1 T2

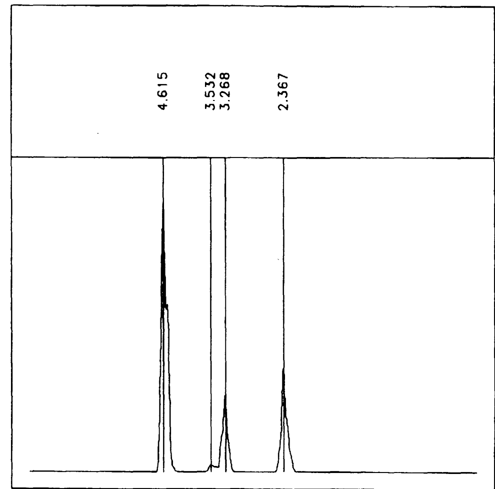
E N Z O

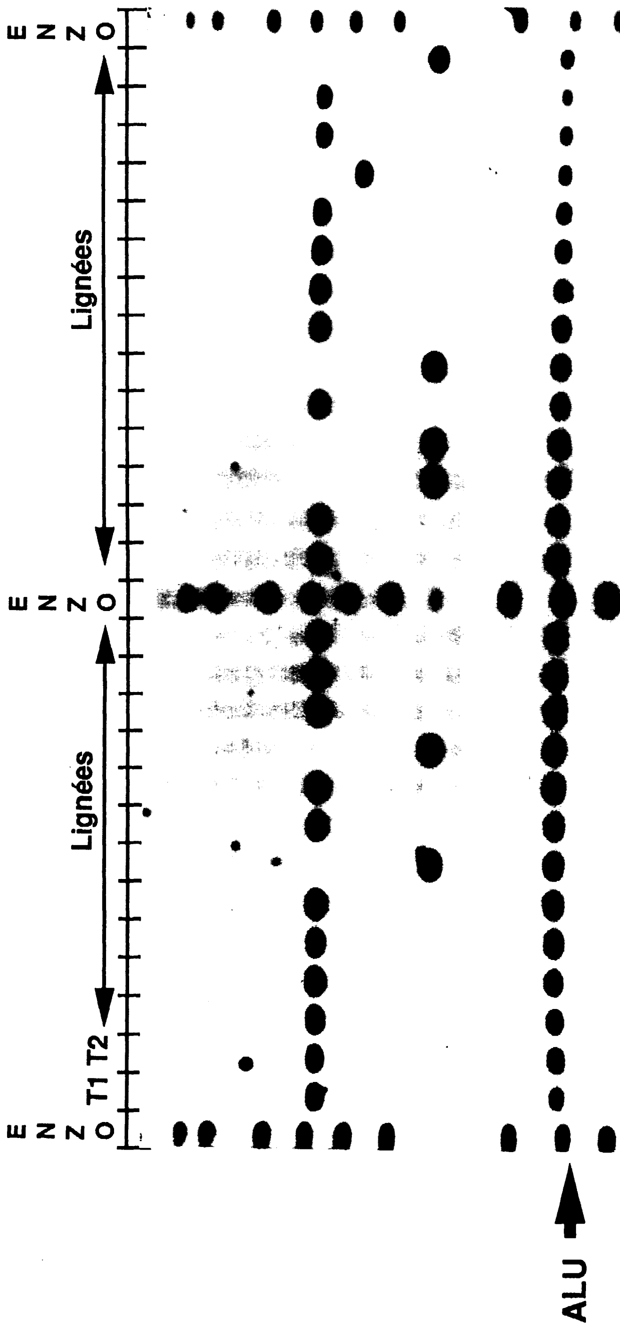
ALU

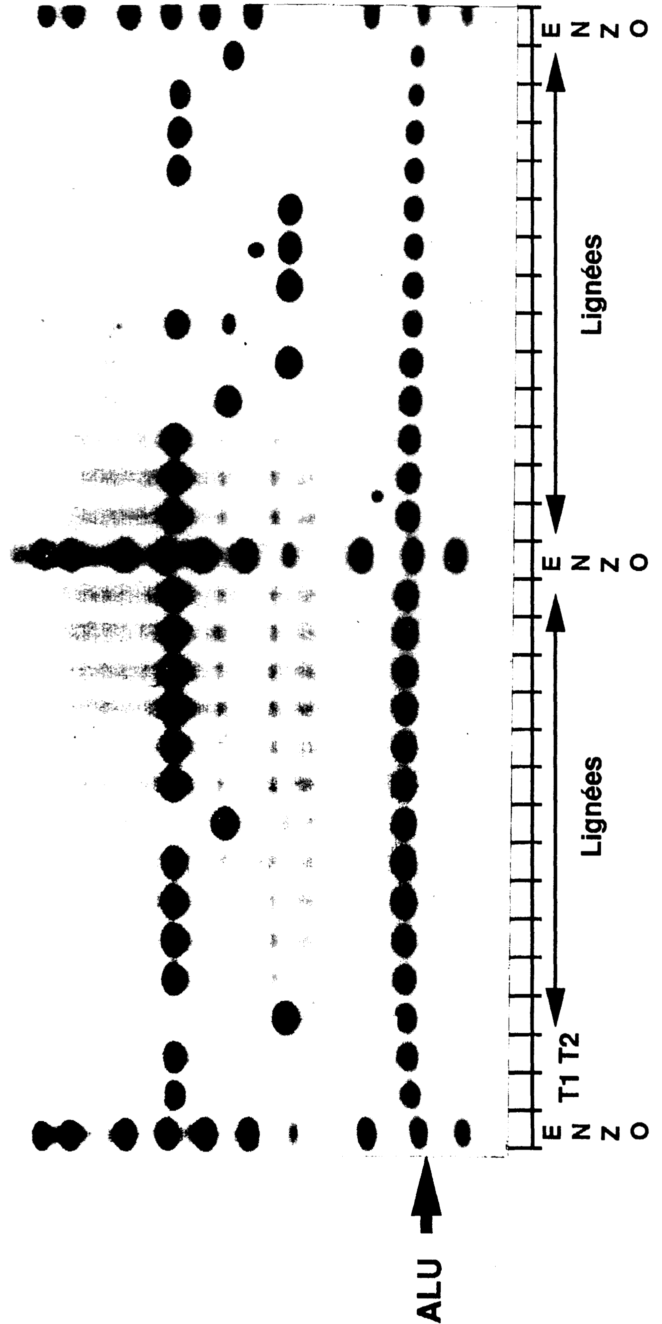
ALU

UMC 89 EcoR V









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