IDENTIFICATION OF GRAPE VARIETIES BY DNA FINGERPRINTING

Wine Grape Inspection Advisory Committee
California Department of Food and Agriculture
Final Report
June 30, 1991

Principal Investigator: Carole P. Meredith, Associate Professor
Department of Viticulture and Enology
University of California
Davis, California 95616
916-752-7535

Cooperating Personnel: Elizabeth B. Bandman, Staff Research Associate
John Bowers, Graduate Student
Bibiana Guerra, Graduate Student

Objective

To develop a method for the **unambiguous** identification of grape varieties based on their DNA sequence.

Background

The identification of individual humans by DNA fingerprinting is now well established and is accepted as legal evidence of identity in courts of law. Similar methodology can be applied to plants and there is no doubt that DNA fingerprinting can be used to identify grape varieties. Such conclusive identification would be useful in detecting the misrepresentation of wine varieties, in resolving international nomenclature differences, in protecting patented varieties, and in investigating the origins of the classic wine varieties.

Grape varieties have traditionally been identified by the appearance of their leaves, shoots and fruit, but this approach relies on human judgement and is subject to error. A biochemical method, isozyme analysis, is more objective but does not distinguish individual varieties and can affected by environment, season, or source of tissue. DNA fingerprinting, on the other hand, can be expected to distinguish individual varieties while being unaffected by the ambiguities introduced by environment and human judgement.

Methodology

The procedure employed is a variation of a technique known as Restriction Fragment Length Polymorphism (RFLP) analysis. DNA is extracted from a candidate plant and treated with an enzyme that cuts the DNA at specific sites, thus generating DNA fragments. The cut DNA is then placed at the end of a gel slab that is exposed to an electric field in which the DNA fragments migrate according to their size. The DNA fragments are then transferred from the gel to a membrane by blotting, such that their relative locations are maintained. The membrane is treated with a radioactively labeled probe (itself a small specific piece of DNA) that binds to those fragments with which it shares sufficient sequence homology. This membrane is then placed against a piece of x-ray film for sufficient time that the film is exposed at the locations at which the radioactive probe is bound. When the film is developed, a pattern of bands (resembling a bar code) is revealed that represents the size distribution of DNA fragments that are homologous to the probe.

The choice of probe is crucial. A probe representing the sequence of a single gene will produce only a single band (or two, if the form of the gene on one chromosome differs from that on its homologous chromosome). Probes employed for DNA fingerprinting usually do not represent specific genes, but instead are usually homologous to a type of DNA that can be found at a number of distinct sites throughout the genome. A probe of this type can produce complex patterns of many bands that vary from individual to individual. Such probes are now used for human fingerprinting and a single probe can distinguish individual human beings. Only identical twins share the same pattern. Individual grape varieties are also expected to exhibit distinct patterns. The vines within a variety are expected to have identical patterns because they were propagated vegetatively from a common source.

Research Accomplishments (July 1, 1990 through June 30, 1991)

The work has continued to focus on improving the methodology. Although it is tempting to begin to apply DNA fingerprinting to problems of interest, we have refrained from doing so until we have full confidence in the reliability of our results. Our main objectives have been to **optimize the resolution** and **insure the reproducibility** of the DNA fingerprint patterns that we obtain. Good resolution means well-defined bands and low background. Diffuse bands or high background reduce the confidence with which fingerprint patterns can be discerned. Reproducibility is essential--the same variety must yield the same fingerprint pattern time after time, regardless of when the vine is sampled or what tissue is used or what environmental conditions prevail.

DNA quality. Of critical importance to both resolution and reproducibility is DNA purity. The DNA extracted from a vine must be free of contaminating substances, particularly phenolic compounds and polysaccharides. Insufficiently pure DNA will not migrate normally during electrophoresis, so fragments will not be separated. DNA that is not clean will also not be cut by the restriction enzyme at every correct site, resulting in an array of incompletely cut DNA fragments in addition to, or instead of, the ones that are characteristic

of the variety being analyzed. Such incomplete enzymatic digestion would obviously produce a false fingerprint pattern. Grape DNA is particularly difficult to purify and some varieties are more difficult than others. We have devoted a great deal of attention to DNA extraction and have modified standard methods to the point where we now usually obtain high yields of high quality DNA.

Digestion conditions. The conditions under which the DNA is treated with the restriction enzyme is also crucial to complete digestion. Various parameters, including digestion volume, repeated digestion, ratio of enzyme to DNA, can influence the extent to which the DNA is completely digested. We have examined these parameters and demonstrated that different digestion treatments of the same DNA sample can produce false banding patterns resulting from incomplete digestion.

Restriction enzymes. There are many different restriction enzymes available. Each is specific for a different sequence of DNA subunits; it cuts the DNA at every site at which it recognizes this sequence. Each enzyme will thus produce a different array of characteristic fragments. The enzymes also vary in their effectiveness. We have evaluated about 10 different enzymes and eliminated two (*Bgl* and *Bam*). Best results to date (i.e., large number of distinct bands) have been obtained with *EcoRI*, *HinfI*, and *DraI*, but the other enzymes have not yet been eliminated.

DNA probes. We have investigated two kinds of DNA probes, those consisting of fragments of grape DNA and those from other species. Of the latter type, we have tested two widely used probes. One is a synthetic probe, pHSD42, based on a human DNA probe (33.6, Jeffreys et al., 1985) that has been widely used for DNA fingerprinting of humans as well as a number of other animal species and a few plants (Dallas, 1988). The other probe is M13, a bacterial virus widely used for molecular biology research. Hybridization with M13 produces individual-specific fingerprints in a wide range of organisms and has been used on a number of plant species (e.g., Nybom et al., 1990a,b). Our initial efforts with M13 were unsatisfactory, and we therefore concentrated our efforts on pHSD42. Our best results with this probe produce a large number of bands, but many are difficult to distinguish because of high background and crowded groups of bands. An example of fingerprints of some wine varieties produced with the enzyme *EcoRI* and the probe pHSD42 is shown in Figure 1.

We obtained a modified form of the M13 probe, pCZ1, in which a key section of the M13 genome has been removed from the rest of the virus and inserted into a bacterial plasmid (Zimmerman et al., 1989). Fingerprints produced with this probe are better resolved than those obtained with HSD42, with lower background, but there are fewer bands. Figure 2 illustrates the same wine varieties as shown in Figure 1 cut with the same enzyme and probed with pCZ1. Figure 3 is a schematic diagram of the patterns illustrated in Figure 2 for 8 of the varieties.

While both pHSD42 and pCZ1 produce variety-specific fingerprints, the bands are not well-resolved and therefore subject to variable interpretation. An ideal fingerprint probe would produce well-resolved bands of sufficient number and variability so as to insure variety

specificity. Probes obtained from grape DNA are likely to produce sharply resolved bands with minimal background because they share greater sequence homology with the DNA being tested than do probes from unrelated species. We are in the process of screening a large number of grape DNA probes in order to identify one or more that will produce complex and variable patterns of bands.

We produced a grape DNA library consisting of several hundred potential probes. Of approximately 20 probes screened to date, none has produced pattern complexity approaching that of pHSD42 or pCZ1. Results obtained with one of these probes, 4G3, is shown in Figures 4 and 5. Variety-specific patterns were not observed. From 33 grape varieties, only 24 banding patterns were obtained, insufficient to separate individual varieties. Furthermore, we now know that some of the bands produced by this probe are not real, but are artifacts resulting from contaminated DNA samples. We are continuing to screen additional grape DNA probes in search of those that will produce complex and variable banding patterns. We are considering the possibility that several probes of relatively low complexity might be combined to yield useful results.

Reproducibility. To determine whether our methods are sufficiently advanced to repeatedly produce the same fingerprint from the same variety, we compared 9 samples of Cabernet Sauvignon DNA that had been obtained from vineyard shoots, vineyard shoot tips, shoots forced from dormant wood in the laboratory, and cambium scrapings from dormant wood. Some samples were obtained from fresh tissue, some from frozen tissue. Plant material was collected on several dates throughout the year and not always from the same vine. DNA was digested with the enzymes *HinfI* and *EcoRI* and probed with HSD42 and pCZ1. Several of the DNA samples were poorly digested with *HinfI*, resulting in aberrant banding patterns. Results obtained with *EcoRI*, however, were much more consistent (Figure 6). One DNA sample was incompletely digested (lane 2 in Figure 6). All other DNA samples produced identical fingerprint patterns. (Because of insufficient DNA, lane 9 produced only faint bands that are not visible in the figure. Examination of the original autoradiogram, however, reveals the same pattern as the other lanes.) This evidence of reproducibility is most encouraging. We are not yet confident, however, that we can obtain such results consistently.

At the present time, we can obtain variety-specific fingerprints (from the limited number of varieties we have examined) with two non-grape DNA probes, but the poorly-resolved patterns are not sufficiently clear to be used with confidence. With grape DNA probes, we can obtain patterns that are much more clear, but we have not yet identified a probe that produces a sufficiently complex pattern.

These results were reported at the Annual Meeting of the American Society for Enology and Viticulture (Meredith et al., 1991).

References Cited

Dallas, J. F. 1988. Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite probe. Proceedings of the National Academy of Science USA 85:6831-6835.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Individual-specific "fingerprints" of human DNA. Nature 316:76-79.

Meredith, C. P., E. B. Bandman, B. Guerra, and J. E. Bowers. 1991. Identification of grape varieties by DNA fingerprinting. American Society for Enology and Viticulture, 42nd Annual Meeting, 20-22 June 1991, Seattle. Technical Abstracts, p. 16.

Nybom, H., S. H. Rogstad and B. A. Schaal. 1990a. Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus, Prunus and Rubus* (Rosaceae). Theoretical and Applied Genetics 79:153-156.

Nybom, H., B. A. Schaal and S. H. Rogstad. 1990b. DNA "fingerprints" can distinguish cultivars of blackberries and raspberries. Acta Horticulturae 262:305-310.

Zimmerman, P. A., N. Lang-Unnasch and C. A. Cullis. 1989. Polymorphic regions in plant genomes detected by an M13 probe. Genome 32:824-828.

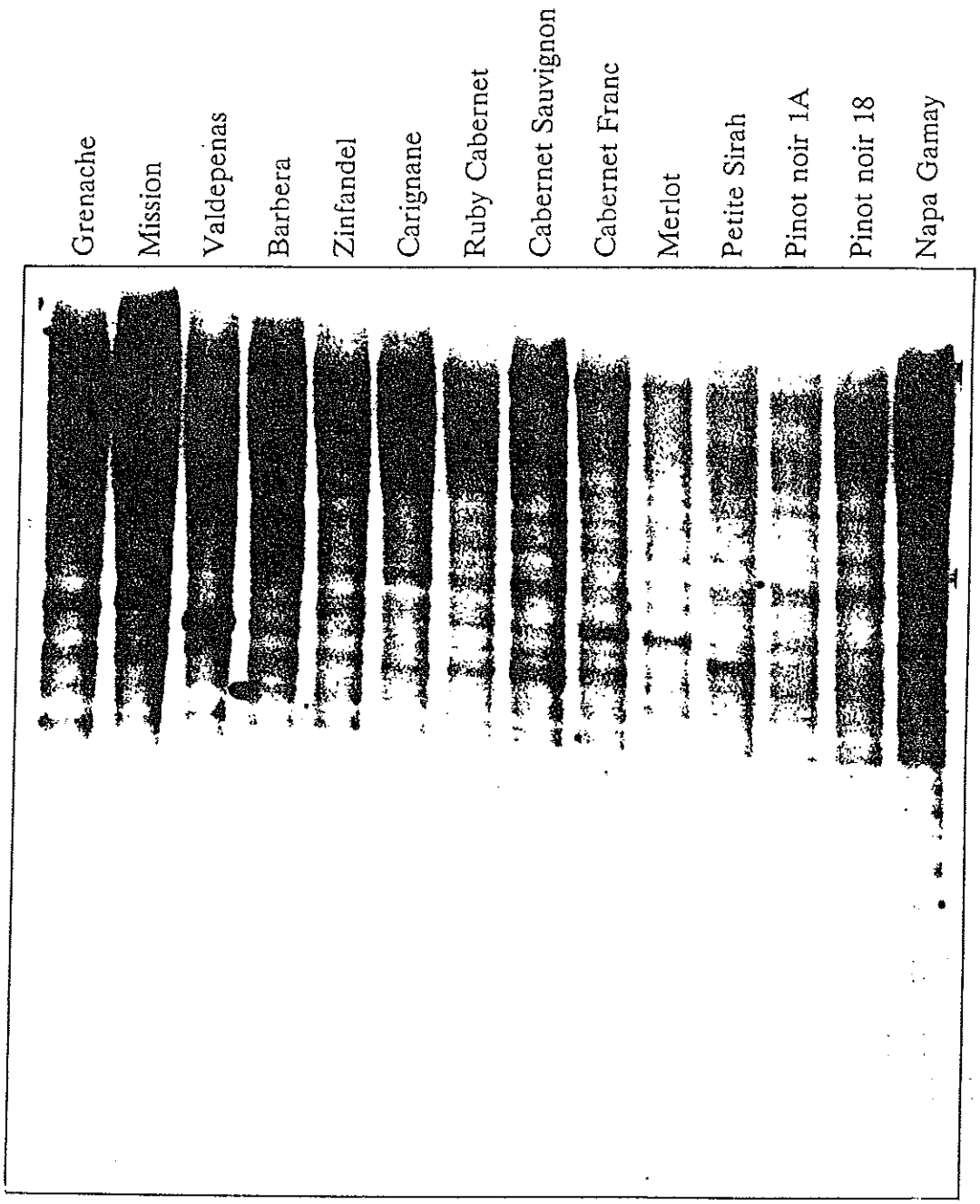

Figure 1. DNA fingerprints of 13 red wine varieties produced with the enzyme *EcoRI* and the probe pHSD42.

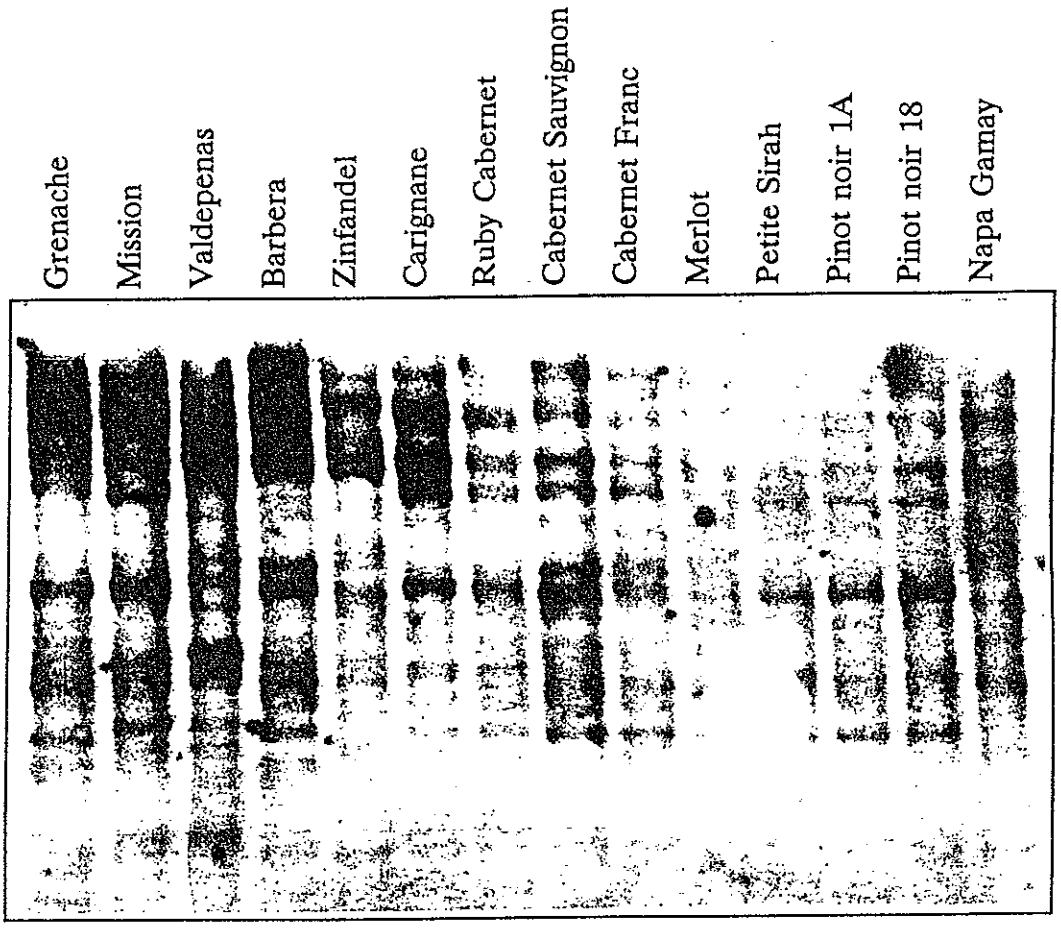

Figure 2. DNA fingerprints of 13 red wine varieties produced with the enzyme *EcoRI* and the probe pCZ1.

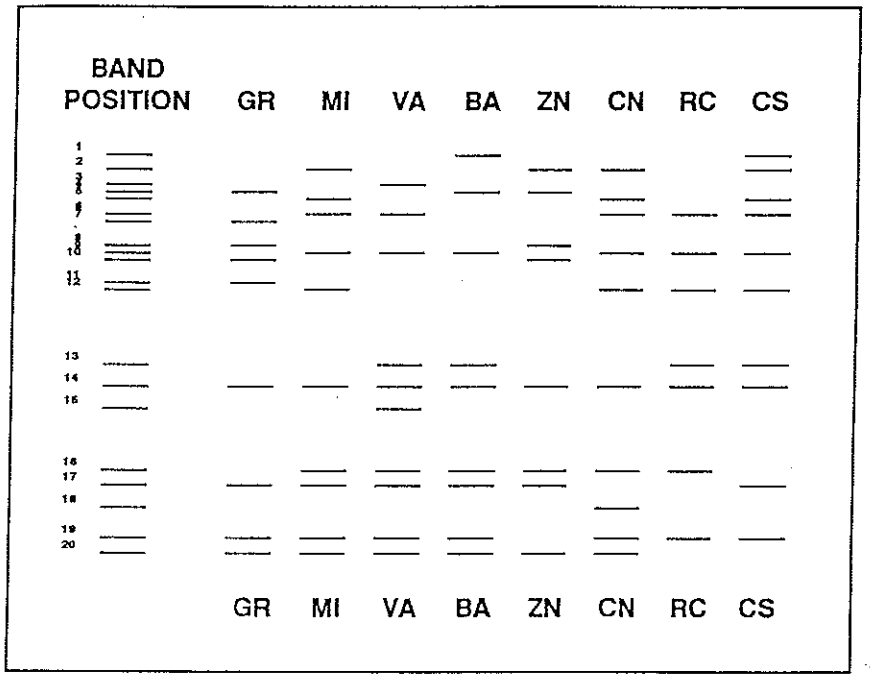

Figure 3. Schematic diagram of the banding patterns illustrated in Figure 2 for 8 varieties.

GR	Grenache	ZN	Zinfandel
MI	Mission	CN	Carignane
VA	Valdepenas	RC	Ruby Cabernet
BA	Barbera	CS	Cabernet Sauvignon

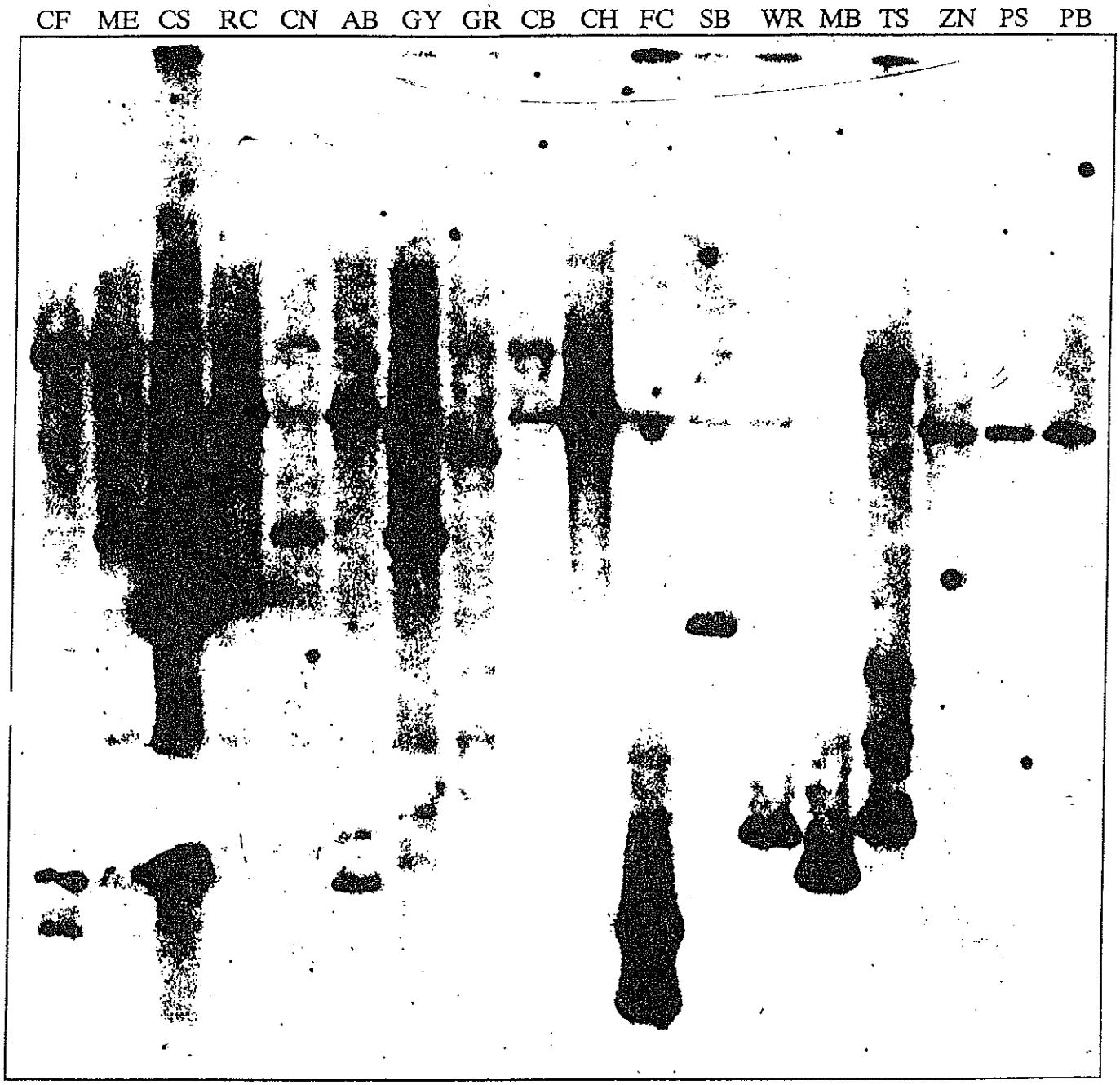

Figure 4. DNA restriction fragment patterns of 18 wine varieties produced with the enzyme *EcoV* and the probe 4G3.

CF	Cabernet Franc	GY	Napa Gamay	WR	White Riesling
ME	Merlot	GR	Grenache	MB	Muscat blanc
CS	Cabernet Sauvignon	CB	Chenin blanc	TS	Thompson Seedless
RC	Ruby Cabernet	CH	Chardonnay	ZN	Zinfandel
CN	Carignane	FC	French Colombard	PS	Petite Sirah
AB	Alicante Bouschet	SB	Sauvignon blanc	PB	Pinot blanc

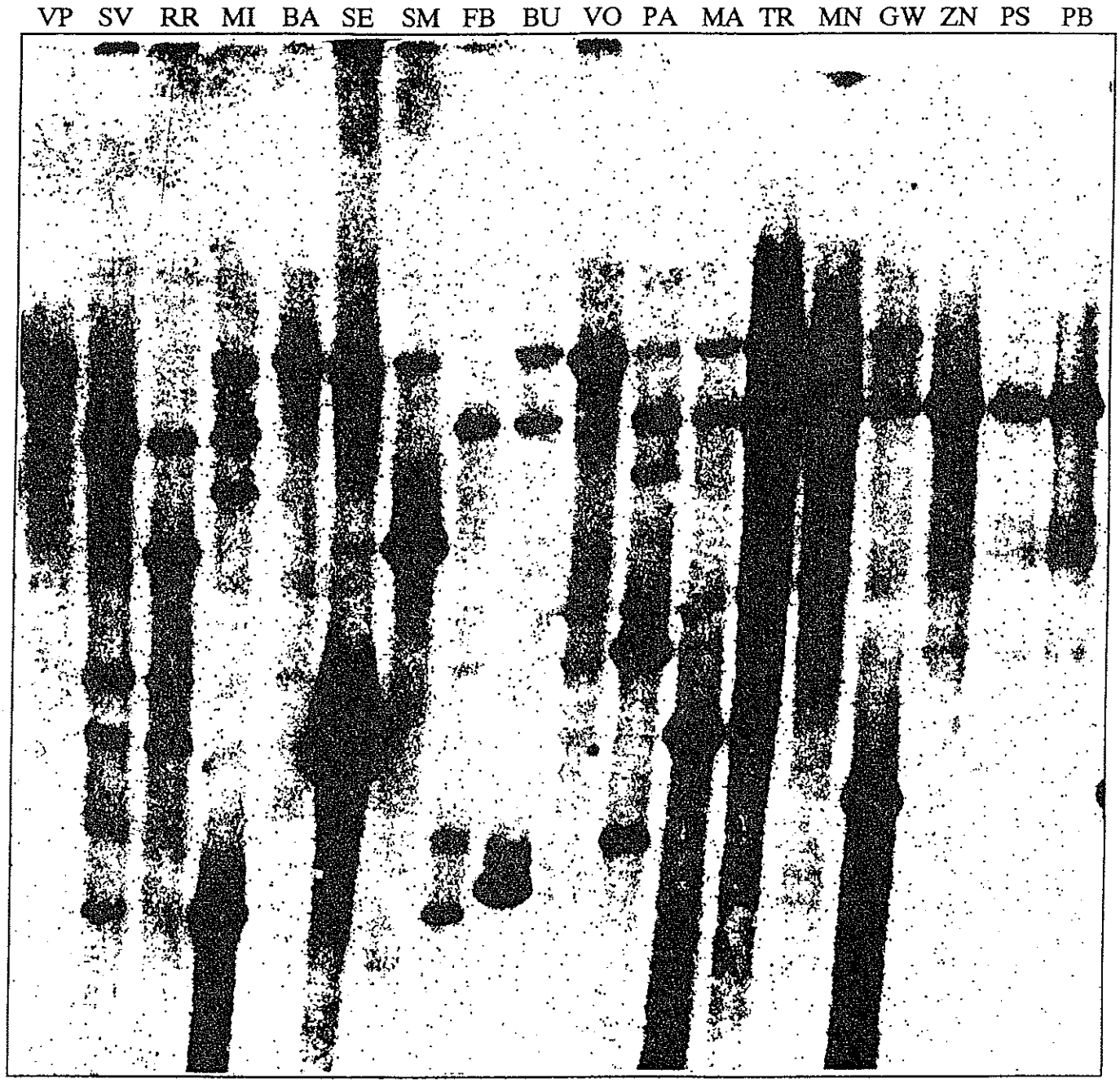

Figure 5. DNA restriction fragment patterns of 18 wine varieties produced with the enzyme *EcoV* and the probe 4G3.

VP	Valdepenas	SM	Semillon	TR	Trousseau
SV	Salvador	FB	Folle Blanche	MN	Meunier
RR	Rubired	BU	Burger	GW	Gewürztraminer
MI	Mission	VO	Voignier	ZN	Zinfandel
BA	Barbera	PA	Palomino	PS	Petite Sirah
SE	St. Emilion	MA	Muscat Alexandria	PB	Pinot blanc

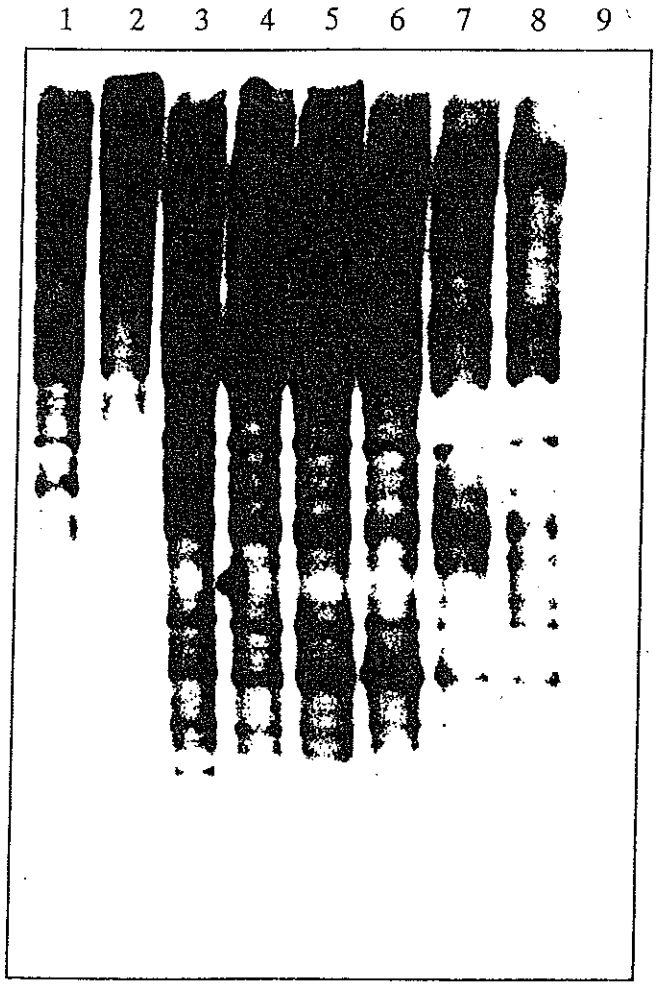

Figure 6. DNA fingerprints of 9 DNA samples of Cabernet Sauvignon 8 produced with the enzyme *EcoRI* and the probe pCZ1.

Lane	Vine	Tissue	Date harvested	Condition	Date DNA extracted	Extraction method
1	A	shoot tips	4/90	frozen 8 mo.	12/90	CTAB, pH 6.6
2	A	shoot tips	4/90	frozen 8 mo.	12/90	CTAB, pH 8.3
3	A	shoot tips	4/90	frozen 4 mo.	8/90	CTAB
4	A	shoots	8/90	fresh	8/90	CTAB
5	B	shoots	8/90	dormant wood	12/898/90	CTAB
6	B	shoots	1/91	"	1/91	CTAB, supernatant
7	B	shoots,lvs	1/91	"	1/91	CTAB, pellicle
8	B	shoots	12/90	"	12/90	Dellaporta, supernatant
9	B	shoots	12/90	"	12/90	Dellaporta, pellicle