ISSN 1009 – 3095 *Journal of Zhejiang University*(*SCIENCE*) V.2, No.4, P.431 – 435, Oct. – Dec., 2001
http://www.periodicals.com.cn; http://www.zju.edu.cn;
http://lib.zju.edu.cn/English; http://www.zjupress.com

COMPARISON OF DIFFERENT ENZYMES AND PROBES AND THEIR COMBINATIONS IN DNA FINGERPRINTING

FU Yan (傅　衍)[1], NIU Dong (牛　冬)[1], RUAN Hui (阮　晖)[2],
CHEN Haiyan (陈海燕)[1], Ponsuksili S.[3], Horst P.[3].

([1]*College of Animal Science, Zhejiang University*; [2]*College of Agricultural Engineering & Food Science, Zhejiang University, Hangzhou, 310029, China*)
([3]*Institute of Animal Science, Humboldt University of Berlin, Berlin, 14195, Germany*)

Received Dec.6, 2000; revision accepted Apr.18, 2001

Abstract: In the present study, eight combinations of restriction enzymes and oligonucleotide probes were tested for detecting VNTR polymorphism. More than a hundred loci were detected by all enzyme-probe combinations. The influences of breed, enzyme and probe as well as their interactions were analysed, and the mean value of DNA fingerprint data was calculated for the enzymes and probes. The results will provide some valuable information for studying the genetic relationship of individuals or populations using DNA fingerprinting.

Key words: DNA fingerprinting, restriction enzyme, oligonucleotide probe, chicken
Document code: A　　**CLC number:** Q341$^+$.1

INTRODUCTION

DNA fingerprinting is a method for producing the DNA fragment band pattern, specific for the genome of a certain individual. The production of the individual specific band pattern is based on the simultaneous detection of several highly polymorphic, repetitive genomic regions, which were hybridized with DNA probes consisting of repeats of core-sequences of different minisatellite-loci, or with oligonucleotide probes complementary to simple tandem repetitive sequences (Jeffreys et al., 1985; Ali et al., 1986). Because DNA fingerprints (DFPs) are individual-specific they were mainly used in forensic science. It has become obvious that DNA fingerprints can be useful in animal breeding (Jeffreys et al., 1987a) and in evolutionary studies (Jin and Chakraborty, 1994). Investigations of farm animal populations showed that DNA fingerprints also provided bands typical of a certain population (Jeffreys et al., 1987b). On the basis of such line specific bands Haberfeld et al. (1992) determined the average parental genome proportions in animal groups of different breeding steps. For genetic studies using DNA fingerprinting, different results will be produced by using different probes, enzymes and their combinations. Therefore knowledge of the influences of the probes, enzymes and their combinations on average percent bandsharing, the number of scorable bands, polymorphic bands and the number of loci estimated is prequisite for achieving correct results of DNA fingerprinting.

MATERIALS AND METHODS

1. Experiment animals

Three groups of chicken of different origin were used, one inbred line, eight exotic lines and three commercial lines. These lines came from different sources, as presented in Table 1.

2. Chemicals and enzymes

Chemicals were received from the companies of Merck, Darmstadt, Carl Roth, Karlsruhe, Sigma, Diesenhofen and Gibco BRL, Eggenstein.

Special chemicals bought from certain companies were: Amersham: Nylon membrane, Hybond N+, restriction endonuclease AluI; Biometra: TE-equilibrated Phenol, Proteinase K; Boehringer: Anti-digoxigenin, Fab-fragments, blocking reagent; Gibco-BRL: Restriction endonucleases AluI and HinfI.

Table 1 Source of stocks used in the present study

Groups of chicken	Character of source	Origin	Strain	Number of animal
Commercial line	Breeding company	Germany	Broiler, male line	14
Commercial line	Breeding company	Germany	Rhode Island Red	14
Commercial line	Breeding company	Germany	White Leghorn	12
Exotic line	Laboratory line	Germany	Bankiva	14
Exotic line	Laboratory line	Egypt	Fayoumi	13
Exotic line	Laboratory line	Egypt	Dandarawi	15
Exotic line	Laboratory line	India	Kadakanath	15
Exotic line	Laboratory line	Indonesian	Nunukan	14
Exotic line	Breeding strain	Thailand	Taiwan White	7
Exotic line	Breeding strain	Thailand	Taiwan Brown	7
Exotic line	Fancy breeder	Germany	Silkie	15
Inbred line	Laboratory line	Switzerland	White Leghorn inbred line	13

3. Restriction enzymes and oligonucleotide probes

In this work, the restriction enzymes AluI and HinfI were used to digest genomic DNA of the described populations. To produce the DNA fingerprint profiles, the following oligonucleotides were used: $(CA)_8$, $(CAC)_5$, $(GGAT)_4$ and $(GACA)_4$.

4. DNA fingerprint production

After extraction from blood samples genomic DNA was dissolved in TE buffer and then stored at 4 ℃. Forty μl mixture containing 5 μg DNA and 50 units of the restriction endonucleases AluI or HinfI was incubated overnight at 37 ℃. DNA fragments were electrophoresed in 0.7% agarose gels (1V/cm for 40 h) and transferred to nylon membranes. The oligonucleotide probes were labelled with digoxigenin. The DNA fingerprint patterns were produced by immunological detection of the digoxigenated probes.

5. DNA fingerprint analysis

%(BS(% bandsharing) is basis index used to analyse DNA fingerprint data, and is defined as: $\%BS = [2n_{xy}/(n_x + n_y)] \times 100$, where n_{xy} is the number of common fragments between the individual x and y, n_x and n_y are the number of bands in individual x and y.

RESULTS AND DISCUSSION

1. Analysis of variance

After hybridization with genomic fragments digested by AluI and HinfI using the oligonucleotide probes mentioned above, the average % bandsharing, the number of scorable bands and the number of polymorphic bands in each individual were detected and used as dependent variables in analysis of variance. Results of variance analysis of these DNA fingerprint data are presented in Table 2.

Table 2 Analysis of variance of average percent bandsharing, number of scorable bands and polymorphic bands

Source of variation	*d.f.*	Average percent bandsharing		No. of scorable bands		No. of polymorphic bands	
		MS	*Pr* > *F*	*MS*	*Pr* > *F*	*MS*	*Pr* > *F*
Breed	11	25697.3	0.000	223.4	0.000	5157.7	0.000
Enzyme	1	7.7	0.706	2523.7	0.000	404.8	0.000
Probe	3	252.2	0.003	9567.9	0.000	1900.6	0.000
Breed * Enzyme	11	50.0	0.525	207.8	0.000	51.1	0.000
Breed * Probe	33	265.0	0.000	289.9	0.000	88.6	0.000
Enzyme * Probe	3	63.0	0.326	838.5	0.000	244.8	0.000
Error	107	54.6		8.7		13.0	
	3						
R^2		0.83		0.85		0.83	
C.V.		13.37		6.77		18.37	

R^2: coefficient of determination; *C.V.*: coefficient of variation; *MS*: mean squares; *Pr* < *F*: significance probability value associated with the *F*-value; *d.f.*: degrees of freedom

The effect of breed was significant for all dependent variables tested. The effect of enzyme was significant for the number of scorable bands and for the number of polymorphic bands, whereas it was not for average % bandsharing. The effect of probe and the interaction of breed and probe were significant for all dependent variables tested. The effect of interaction of breed × enzyme and enzyme × probe was not significant for average % bandsharing but highly significant for the number of scorable and polymorphic bands. The coefficient of determination was 0.83 for the average % bandsharing and the number of polymorphic bands and 0.85 for the number of scorable bands.

The breeds used in this experiment had extreme difference regarding their genetic make-up, ranging from inbreeding line to outbreeding line. Different oligonucleotide probes detected different loci. Different restriction enzymes produced different numbers of scorable bands and polymorphic bands with statistical significance. The number of scorable bands and polymorphic bands were absolute values, whereas the average bandsharing was a relative value and not significantly affected by the choice of enzyme.

2. Mean values of DNA fingerprint data calculated for the enzymes and the probes

The restriction enzyme HinfI provided DFPs with higher levels of average % bandsharing than the restriction enzyme AluI (Table. 3), but the difference was not significant. Furthermore the enzyme HinfI provided significantly more scorable bands and more polymorphic bands than AluI. Therefore the probability P of identical DFPs between two unrelated individuals obtained with restriction enzyme HinfI was lower than that with AluI.

Table 3 Mean values and standard deviations of DNA fingerprint data calculated for the restriction enzymes

Restriction enzyme	% Bandsharing	No. of scorable bands	No. of polymorphic bands	Probability (P)
AluI	55.2 ± 17.3^{a}	42.2 ± 7.4^{a}	19.0 ± 8.3^{a}	2.4 ± 10^{-23}
HinfI	45.1 ± 17.9^{a}	45.1 ± 6.4^{b}	20.3 ± 8.6^{b}	7.0 ± 10^{-25}

Means with different superscripts within trait differ significantly at $P < 0.05$;

The average % bandsharing values obtained with the oligonucleotide probe $(CA)_8$ were the highest (Table. 4), followed by the values provided by using $(GACA)_4$. The difference of these values of both probes was not significant. The average % bandsharing detected by the probe $(CAC)_5$ was significantly lower than the values obtained with the probes $(GGAT)_4$ and $(CA)_8$. The oligonucleotide probe $(GACA)_4$ and $(GGAT)_4$ showed the same level of average % bandsharing.

Table 4 Mean values and standard deviations of DNA fingerprint data calculated for the oligonucleotide probes

Oligonucleotide probe	% Bandsharing	No. of scorable bands	No. of polymorphic bands	Probability (P)
$(CAC)_5$	53.8 ± 17.4^{c}	47.3 ± 5.8^{a}	21.9 ± 8.1^{a}	5.8 ± 10^{-27}
$(GGAT)_4$	55.0 ± 17.6^{b}	47.2 ± 5.5^{a}	21.2 ± 8.8^{b}	3.7 ± 10^{-26}
$(GACA)_4$	55.9 ± 18.4^{ab}	35.0 ± 4.9^{c}	15.9 ± 7.6^{d}	3.2 ± 10^{-19}
$(CA)_8$	56.4 ± 17.0^{a}	45.1 ± 3.7^{b}	19.7 ± 8.0^{c}	3.1 ± 10^{-24}

Means with different superscripts within trait differ significantly at $P < 0.05$;

More scorable bands were detected by the oligonucleotide probe $(CAC)_5$ than by the oligonucleotide probe $(GGAT)_4$, but the difference was not significant. The lowest average number of scorable bands detected by the oligonucleotide probe $(GACA)_4$ was 35.0, and significantly lower than the values observed with the other probes $(CAC)_5$, $(GGAT)_4$ and $(CA)_8$, were 47.3, 47.2 and 45.1 respectively.

Significantly different numbers of polymorphic bands were obtained by using different probes, was observed similarly in the case of the number of scorable bands. The oligonucleotide $(CAC)_5$ yielded the highest number of bands,

followed by probe $(GGAT)_4$. It was found that the more number scorable, the higher was the chance to find polymorphic bands. The probability of identical DNA fingerprints of unrelated individuals was the lowest with the oligonucleotide probe $(CAC)_5$ and the highest with the probe $(GACA)_4$. The choice of restriction enzyme influenced the average number of scorable and polymorphic bands but not the average % bandsharing because bandsharing is the ratio of the number of bands. The values of average % bandsharing, estimated with both restriction enzymes, had correlation of 0.86.

3. The number of loci estimated

The number of loci detected by the enzyme-probe combinations were estimated by two methods. The results are shown in Table 5. The first method was based on the frequency of bands, (Yuhki and O'Brien, 1990). The second method to estimate the number of loci detected was based on bandsharing (Lynch, 1990).

Table 5 Effect of the two restriction enzymes and four oligonucleotide probes used on the number of loci estimated by two methods

Restriction enzyme	Oligonucleotide probe	Average number of loci estimated with method 1 (a)			Average number of loci estimated with method 2 (b)		
		Mean	*SD*	*SE*	*Mean*	*SD*	*SE*
	$(CA)_8$	27.0	2.83	0.58	28.0	7.04	1.44
	$(CAC)_5$	28.1	3.65	0.75	24.7	7.26	1.48
	$(GACA)_4$	20.9	2.13	0.43	17.4	4.33	0.88
	$(GGAT)_4$	28.3	4.05	0.83	28.4	8.39	1.71
AluI		25.2	4.46	0.64	23.3	7.28	1.05
HinfI	$(CA)_8$	27.0	4.20	0.61	26.0	8.77	1.26
AluI	$(CAC)_5$	25.5	2.64	0.76	26.2	6.59	1.90
AluI	$(GACA)_4$	28.9	3.54	1.02	24.4	6.88	1.99
AluI	$(GGAT)_4$	19.8	1.86	0.54	16.7	4.42	1.28
AluI		26.5	3.58	1.03	25.8	7.16	2.07
HinfI	$(CA)_8$	28.5	2.2	0.64	29.9	7.27	2.10
HinfI	$(CAC)_5$	27.3	3.74	1.08	25.1	7.91	2.28
HinfI	$(GACA)_4$	22.1	1.72	0.50	18.1	4.32	1.25
HinfI	$(GGAT)_4$	30.0	3.88	1.12	31.0	9.01	2.60

(a) the method to estimate the average number of loci based on the frequency of bands (Yuhki and O'Brien, 1990)
(b) the method to estimate the average number of loci based on bandsharing (Lynch, 1990)
SD: standard deviation; *SE*: standard error of the mean

In estimating the number of loci detectable by the enzyme-probe combinations, it was assumed that the alleles were in Hardy Weinberg equilibrium; and that bands of the same molecular weight and intensity represent the same allele of a particular locus.

For both methods the oligonucleotide $(GGAT)_4$ detected the highest number of loci and $(GACA)_4$ detected the lowest number of loci. It can be presumed that the sequence GGAT is more frequent than other sequences in the genome of chickens. Considering restriction enzymes, HinfI provided a higher number of loci than AluI. It can also be presumed that the genomic DNA was cut more frequently in the neighbourhood of simple repeat sequences by restriction enzyme HinfI than by AluI, thus providing more detectable fragments in DFPs.

The enzyme-probe combination of HinfI $(GGAT)_5$ detected the highest number of loci, whereas the combination AluI (GACA)4 provided the lowest number of loci. Other enzyme probe combinations detected respectively 22 to 28 loci and 18 to 30 loci, when method 1 and 2 were used. Both estimation methods, however yielded only approximate values, either overestimating or underestimating the number of detectable loci. It could not be determined which method was better.

The need to examine a large number of loci is evident from the observations that the reliability of statistical estimates, such as heterozygosity and genetic distance, depends more on the number of loci than on the number of individuals investigated (Nei, 1978; Nei and Chesser, 1983; Mathur and Ponsuksili, 1994). Through the use

of two or three oligonucleotide probes detecting independent sets of polymorphic loci, it should not be difficult to detect 30 – 40 loci by DFPs (Lynch, 1990). This provides statistical reliability.

The present study showed that each combination of enzyme-probe provided about 20 – 30 loci, estimated on the basis of bandsharing (Lynch, 1990) and on the basis of band frequency. If we assume that each probe detected independent loci, then about 100 loci can be detected with four different probes. Such a large number of loci is substantially powerful for statistical tests. In contrast, the use of the minisatellite probe 33.6 provided 8 to 10 loci in individual human cell lines (Gilbert et al., 1990), 13 and 4 loci in female and male of Red grouse, 25 and 16 loci in female and male Ring-necked pheasant and 1 and 9 loci in both sexes of Indian peafowl (Hanotte et al., 1991).

References

Ali, S., Müller, C.R., Epplen, J.T., 1986. DNA fingerprinting by oligonucleotide probes specific for simple repeats. *Hum. Genet.*, **74**: 239 – 243.

Armour, J.A.L., Povey, S., Jeremiah, S. et al., 1990. Systematic cloning of human minisatellites from ordered array charomid libraries. *Genomics*, **8**: 501 – 512.

Gilbert, D.A., Lehman, N., O'Brien, S.J., et al., 1990. Genetic fingerprinting reflects population differentiation in the California Channel Island fox. *Nature*, **344**: 764 – 767.

Haberfeld, A., Dunnington, E.A., Siegel, P.B., 1992. Genetic distances estimated from DNA fingerprints in crosses of white Plymouth Rock chickens. *Animal Genetics*, **23**: 167 – 173.

Hanotte, O., Burke, T., Armour, J.A.L. et al., 1991. Cloning, characterization and evolution of Indian peafowl Pavo christatus minisatellite loci. *In*: DNA fingerprinting. Birkhauser Verlag, Basel, p.193 – 216.

Jeffreys, A.J., Wilson, V., Thein, S.L., 1985. Hypervariable "minisatellite" regions in human DNA. *Nature*, **314**: 67 – 73.

Jeffreys, A.J., Wilson, V., Thein, S.L. et al., 1986. DNA "fingerprints" and segregation analysis of multiple markers in human pedigrees. *American J. Human Genetics*, **39**: 11 – 24.

Jeffreys, A.J., Hillel, J., Hartley, N., 1987a. The implications of hypervariable DNA-regions for animal identification. *Animal Genetics*, **18** (*Suppl.*): 141 – 142.

Jeffreys, A.J., Wilson, V., Kelly, R., et al., 1987b. Mouse DNA "fingerprints": analysis of chromosome localization and germ-line stability of hypervariable loci in recombinant inbred strains. *Nucl. Acids Res.*, **15**: 2832 – 2836.

Jin, L. and Chakraborty, R., 1994. Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. *Mol. Biol. Evol.* **11**: 120 – 127.

Lynch, M., 1990. The similarity index and DNA fingerprinting. *Mol. Biol. Evol.*, **7**: 478 – 484.

Mathur, P.K., Ponsuksili, S., Groen, A.F. et al., 1994. Estimation of genetic variability within and beween populations using DNA fingerprints. Proceeding of the 5th world congress on genetics applied to livestock production, Guelph, **21**: 528 – 531.

Nakamura, Y., Julier, C., Wolff, R. et al., 1987. Characterization of a human "minisatellite" sequence. *Nucleic Acids Res.*, **15**: 2537 – 2547.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583 – 590.

Nei, M. and Chesser, R.K., 1983. Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.*, **47**: 253 – 259.

Yuhki, N. and O'Brien, S.J., 1990. DNA variation of the mammalian major histocompatibility complex reflects genomic diversityand population history. *Proc. Natl. Acad. Sci., USA*, **87**: 836 – 840.

Zischler, H., Nanda, I., Schafer, R.et al., 1989. Digoxigenated oligonucleotide probes specific for simple repeats in DNA fingerprinting and hybridization in situ. *Hum. Genet.*, **82**: 227 – 232.