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R E V I E W:

Genetic Diversity: Detection of Gene Variation at the DNA Level and Utilization of Gene Markers on Locating QTLs

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ABSTRACT

Advanced techniques of molecular biology have provided the opportunity to study genetic diversity within and among breeds at the single gene level. Many DNA markers, either of genomic DNA or cytoplasmic DNA, have been generated recently by utilizing molecular techniques, such as RFLP, microsatellites, PCR-RFLP, RAPD, sequencing etc. PCR-based techniques have recently progressed rapidly for the detection of both known- and unknown-mutation detections that may be applied in locating gene marker for economically important traits. There are basically two different approaches of locating quantitative trait loci (QTLs), candidate gene and random approaches. The first approach is based on prior supporting knowledge of physiological and biochemical evidence, showing that the gene is involved in the trait(s) of interest, while the random marker approach attempts to locate gene markers by measuring genotypes at a large number of loci with unknown phenotypic effects, in the hope that the loci are linked to a QTL influencing the trait of interest.

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Key words: gene variation, QTLs, gene marker.

DETECTION OF GENETIC DIVERSITY

Modern molecular technologies enable genetic variation to be identified directly at the DNA level. By utilizing genetic markers such as RFLP, minisatellite, microsatellite, PCR-RFLP and RAPD (Cushwa and Medrano, 1996), it may be possible to directly monitor the DNA variation responsible for phenotypic differences between lines of individuals. The ability to visualize polymorphisms at the DNA sequence level, combined with the development of powerful experimental designs, makes possible the ultimate objective of breeding for the improvement of production traits in livestock by DNA marker-based approaches.

The most important new technique that is now incorporated into almost all mutation-detection methods is the polymerase chain reaction (PCR), a reaction that exponentially amplifies defined regions of the genome (Saiki *et al*., 1988). PCR-based techniques have recently progressed rapidly for the detection of both known- and unknown-mutation detections. For known mutations, the detection techniques are PCR-RFLP, ligase chain reaction (LCR), allele specific amplification (ASA), amplification refractory mutation system-PCR (ARMS-PCR) and short tandem (microsatellite) repeat-PCR (STR-PCR), while for unknown mutations, the techniques are heteroduplex polymorphism assay (HPA), single strand conformation polymorphism (SSCP), constant denaturant capillary electrophoresis (CDCE), quantitative reverse-transcriptase PCR (QRT-PCR) and dideoxy fingerprinting (ddF) a method that combines SSCP and Sanger dideoxy sequencing. Many DNA markers, either of genomic DNA or cytoplasmic DNA, have been generated recently by utilizing molecular techniques, such as restriction fragment length polymorphism (RFLP), Microsatellites, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), random amplified polymorphic DNA (RAPD), sequencing, etc. Each of these methods has its own suitability, and advantages or disadvantages.

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphisms are genetic markers in which polymorphisms are detected and visualized in two steps; restriction endonuclease digestion and Southern blot hybridization (Winkelmann and Hodgetts, 1992). Restriction endonucleases recognize specific sequences, usually 4 to 8 bp long and catalyze endonucleolytic cleavages, resulting in fragments of defined length. These fragments can be separated by gel electrophoresis, transferred to a solid support such as nitrocellulose filter (Southern, 1975) and detected by hybridizing with radioactively labelled probes (Hallerman *et al*., 1987; Jeffreys, Wilson and Thein, 1985), consisting of cloned DNA sequences homologous to a particular DNA fragment or some portion of it (Beckmann and Soller, 1983).

RFLPs have been widely used in both plant and animal agriculture as a means of identifying parentage and investigating polymorphic genetic loci affecting traits for marker assisted selection (Beckmann and Soller, 1987; Rafalski and Tingey, 1993). A major drawback of RFLPs, however, is the limited degree of variability of such loci. For example, a study of the Holstein-Fresian dairy cattle breed indicated that most RFLPs involved two allelic variants with the frequency of rare allele less than 0.10 (Hallerman *et al*., 1988). In addition, RFLPs will be uninformative in pedigree analysis whenever critical individuals are homozygous (Jeffreys *et al*., 1985). Kashi *et al*. (1986) also suggested that RFLPs have relatively little value for practical marker-based breeding applications; this is especially so since RFLP analysis cannot be automated for large scale applications (Rafalski and Tingey, 1993).

Microsatellites

Microsatellite, a new class of genetic marker, is a technique based on length variation within tandem arrays of di-, tri-, or tetra nucleotide motifs. Variable numbers of these nucleotide repeats may be amplified by PCR, and then detected as length variants by gel electrophoresis (Weber and May, 1989). This technique has low technical difficulties compared to RFLP analysis (Cushwa and Medrano, 1996), although sequence information is required to generate appropriate primers for PCR. In eukaryotic genomes, dinucleotides repeats, such as (AC)n, (AG)n and (AT)n have been shown recently to be abundant and highly polymorphic (Weber and May, 1989). Microsatellites have been used for genotyping and constructing genetic maps in livestock species (MacHugh *et al*., 1994; Yeh *et al*., 1996).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

PCR-RFLP (Prosser, 1993; Unanian *et al*., 1994) is a widely applied technique for the detection of DNA fragments of up to several kilobases (Mitchelson, Cheng and Kricka, 1997). This relatively new technique detects the availability of restriction endonuclease cleavage sites at a locus in amplified DNA fragments from target template. Base changes, insertion or deletion at the restriction site will result in different lengths of DNA fragments, which can be analyzed by UV detection when the digested fragments are run on an ethidium bromide-stained agarose gel.

This relatively new technique of identifying gene variants is based on PCR amplification and restriction endonuclease digestion of single loci (Lucy *et al*., 1993; Unanian *et al*., 1994). Base changes, insertions and deletions at the restriction site will result in different lengths of DNA fragments, which can be visualized using agarose gel electrophoresis.

This technique generates the same type of polymorphism as with traditional RFLP, but without the need for Southern blotting (Cushwa and Medrano, 1996). It is therefore more sensitive and rapid compared with standard blotting and hybridization (Weber and May, 1989). In addition, this technique requires very little DNA, less than 100ng, compared to 2-10 g of DNA in traditional RFLP (Rafalski and Tingey, 1993). It has been extensively used for detecting gene polymorphisms in the growth hormone gene in cattle (Lucy *et al*., 1993; Mitra *et al*., 1995; Unanian *et al*., 1994, Sutarno, 1997), kappa-casein, beta-casein and beta lactoglobulin in cattle (Dellama and Zago, 1996; Sabour *et al*., 1996), MHC genes (Teutsch *et al*., 1996), ABO blood group genes (Fukumori *et al*., 1995; Sasaki and Shiono, 1996), and alcohol dehydrogenese (ADH) aldehyde dehydrogenese (ALDH) genes (Suzuki *et al*., 1994), and bovine mitochondrial DNA (Sutarno, 2002).

Random amplified polymorphic DNA (RAPD)

This method is based on the PCR amplification of genomic regions using short arbitrary oligonucleotide primers (Williams *et al*., 1990). Amplification with the arbitrary primers results in several discrete products, since each short oligonucleotide primer is capable of amplifying a number of fragments from different loci in the same PCR reaction (Waugh and Powell, 1992). These can be separated by agarose gels and visualized by staining with ethidium bromide.

The sensitivity of RAPD in detecting polymorphisms due to single base mismatch between primer and template and mutations that affect the primer binding site or prevent amplification, has been demonstrated (Williams *et al*., 1993), and applied extensively for genetic analysis in mice (Cheah *et al*., 1994), and in many livestock animals, such as cattle (Kemp and Teale, 1994), goats (Cargill *et al*.,, 1995), chickens (Plotsky *et al.,* 1995) and horses (Bailey and Lear, 1994).

Cushwa and Medrano (1996) suggested that RAPD markers are very useful for identifying breed or species-specific markers and the estimation of genetic divergence between breeds. The technique offers a quick and efficient assay for screening DNA polymorphism at a very large number of loci. The difficulty of reproducibility of this technique (Rafalski and Tingey, 1993), may be overcome by eliminating variation in DNA concentration, and maintaining consistent reaction conditions and thermal profile during amplification.

Utilization of gene markers

Because most traits of economic importance in livestock are quantitative in nature and controlled by many quantitative trait loci (QTLs) as well as environmental factors, it is generally impossible to genotype a certain animal with respect to an economic trait based on the examination of the phenotypic characteristics only. Traditionally, the genetic basis of polygenic traits has been approached by biometrical-statistical analysis, dealing with all the

numerous loci with individual small contributions to the total of the quantitative trait variation. A gene marker for a quantitative trait is defined as a variable DNA sequence that co-occurs with a variable quantitative trait, either because it directly influences the trait or because it is linked to another DNA sequence that influences the trait (Lymbery, 1996). Gene markers will be very useful additions to the traditional biometric approach to accurately selecting superior animals with the trait of interest (Soller, 1994; Schlee *et al*., 1994; Whittaker *et al*., 1995). There are basically 2 major approaches to locating genes that influence quantitative phenotype traits: the candidate marker and random marker approach (Cheverud and Routman, 1993).

Quantitative traits

Quantitative genetics is concerned with the inheritance of quantitative differences between individuals (Falconer, 1976). Quantitative traits caused by many genes, each having a small effect on the total phenotype, and by the environment in which those genes are expressed. Genes influencing quantitative traits are called quantitative trait loci (QTLs). In other word, a quantitative trait locus (QTL) is the location of a gene that affects a trait that is measured on a quantitative scale.

APPROACHES TO LOCATING GENE MARKERS

Candidate gene approach

The approach of locating candidate gene markers is based on prior supporting knowledge of physiological and biochemical evidence, showing that the gene is involved in the trait(s) of interest. An example of this approach is in choosing growth hormone and insulin-like growth factor 2 (IGF-2) loci for studying genes affecting murine growth (Winkelmann and Hodgetts, 1992), since the products of the genes, growth hormone and insulin-like growth factor-2, are known to be important in somatic growth.

The advantages of the approach are basically drawn from the involvement of the gene in determining the phenotypic trait. This approach is relatively straight forward, only focusing on relevant genes for the phenotypic characters of interest, can measure directly to the genotypic value, and is suitable for analyses which determine the contribution of the candidate locus to the total phenotypic variance of the trait. Moreover, the results are interpretable in term of trait physiology and the gene of interest can be located from known protein products by standard techniques.

The first step in finding candidate markers is to identify a locus or loci of interest, based on prior evidence of biochemical and physiological correlation with the phenotype, and then identify molecular variants at or near the loci. Molecular variants are usually identified by RFLP, PCR-RFLP, microsatellite or any other molecular methods. After molecular variants are identified and grouped into genotype classes, differences in phenotypic value between the classes can be statistically tested using suitable analyses. The disadvantage of the candidate gene approach is that it is limited to certain traits with known biochemical and physiological causation. In addition, molecular variation at the candidate gene locus must exist and be both segregating and measurable in the population under study (Cheverud and Routman, 1993).

Random marker approach

In contrast to the candidate gene approach, the random marker approach attempts to locate gene markers by measuring genotypes at a large number of loci with unknown phenotypic effects, in the hope that the loci are linked to a QTL influencing the trait of interest. Generally, for the detection of linkage, the markers and QTL need to be located within at least 20cM to as close as 1cM (Soller *et al*., 1976; Terwilliger, 1995), so variable markers are required throughout the genome.

The approach has been used recently for detecting linkages to economically important traits of livestock species, such as cattle (Barendse *et al*., 1994; Bishop *et al*., 1994; Bishop, 1995), sheep (Crawford *et al*., 1995), pigs (Archibald *et al*., 1995), and chickens (Burt *et al*., 1995). It has also detected many QTLs in agricultural plants (Paterson *et al*., 1990; Edwards *et al*., 1992).

The advantages of this approach are that it is not limited to the traits that have already known biochemical and physiological causation and it searches variable markers on the entire genome, so it provides more possibilities for finding multiple QTLs even in locations that have not been previously been known to affect the trait of interest. This approach is suitable for locating QTLs of crosses between genetically and phenotypically divergent populations, since they will provide more variable markers.

The disadvantages of the approach are that the measures of the genetic effect are hard to interpret in term of specific biochemical and physiological relationship to the traits, since the loci detected are of unknown function. Moreover, the approach may be more suitable for experimental crosses between divergent populations than for the study of QTLs in natural populations, where specific crosses cannot be made. In addition, the approach is less valuable than the candidate marker approach, and eventual cloning of the QTL requires a linkage map containing a candidate sequence or a high-resolution physical map, which are not available for all species.

CONCLUSION

New technologies in molecular genetics potentially enable direct analysis of genetic diversity and traits at

the level of the gene. Coupled with innovations in quantitative genetics, advances in molecular genetics enable us to identify, map and measure the effects of quantitative trait loci (QTL) affecting production traits. The isolation and analysis of single genes are now standard molecular genetic techniques, as is the ability to transfer the isolated gene from one animal (plant) to another animal (plant), to produce a transgenic. It is therefore possible that these new techniques of molecular genetics will have a significant impact on animal/ plant breeding in the near future.

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