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Short Cuts for Gene Tagging

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Abstract: The construction of linkage maps and QTL analysis takes a considerable amount of time and effort and may be very expensive. QTL mapping experiments are notorious for their unwieldy size. Most QTL experiments have involved >200 individuals and in some cases as many as 2000 or more. Therefore, alternative methods that can save time and money would be extremely useful, especially if resources are limited. Several approaches have been suggested to extract more information from analysis of smaller populations. Three 'short-cut' methods used to identify markers that tag QTLs are Bulked Segregant Analysis (BSA), selective genotyping and comparative QTL mapping.

Key words: Bulked segregant analysis, selective genotyping, comparative QTL mapping, gene populations

INTRODUCTION

There are two general approaches to understanding the function of a gene: forward genetics and reverse genetics (Alberts *et al*., 2007). Reverse genetics is a particular approach in discovering the function of a gene that usually goes in the opposite direction of what is called forward genetic screens associated with classical genetics. Forward genetics refers to a process where studies are initiated to determine the genetic underpinnings of observable phenotypic variation. It begins with a well-characterized phenotype and then works toward identifying the gene(s) responsible for the phenotype. In many cases the observable variation has been induced using a DNA damaging agent (mutagen) such as T-DNA tagging, transposon tagging and gene or enhancer traps which require inserting foreign DNA into a host genome. Genetic mapping approaches such as Quantitative Trait Loci (QTL) mapping and association mapping are also forward genetic approaches and are often used because gene transfer is not required (Tierney and Lamour, 2005; White *et al*., 2007).

The objectives of QTL mapping analysis comprise: To provide knowledge towards a fundamental understanding of individual gene and interactions, to enable positional clonin and to improve breeding value estimate and selection response through marker assisted selection in plants and animals (Collard *et al*., 2005; Holland, 2004).

The effective population size for QTL analysis is a very important consideration that has a direct impact on the resolution of the map and the accuracy of the QTL location. Population size also affects the genetic gains breeders achieve using Marker-Assisted Selection (MAS). If the population is not large enough in a QTL analysis, certain putative QTL will not be detected and therefore gains these candidate QTLs in MAS will be reduced. Large population sizes are not always feasible due to the space and time constraints on the researcher; therefore, some strategies have been implemented to maximize information from smaller populations, including selective genotyping, bulk segregant analysis and comparative QTL mapping (Tuzun and Bent, 2006).

MATERIALS AND METHODS

Selective genotyping: An excellent approach for efficiently mapping QTLs which influence a single phenotype (only) is Selective Genotyping (also known as distribution extreme analysis or trait-based marker analysis) (Paterson, 1998). Selective genotyping may be a useful alternative to decrease the number of marker genotypings. The principle underlying selective genotyping was first described by Lebowitz *et al*. (1987). For crosses between inbred lines, Darvasi and Soller (1992) derived approximate formulae for the marker contrast and variance after selective genotyping has been applied. Selective genotyping in an outbred population should be applied within families.

Bovenhuis and Spelman (2000) derived formulae for marker contrast after selective genotyping in outbred populations (Bovenhuis and Spelman, 2000; Weller, 2001; Darvasi and Soller, 1992).

These individuals harbor more "information" than phenotypically average individuals, since they are more likely to contain a high proportion of the + or - alleles, respectively, at the QTLs affecting the targer trait. By phenotyping a large population and then selecting only the most extreme individuals for genotyping , one can obtain equal or greater information about QTLs than from exhaustive mapping of randomly chosen individuals (Paterson, 1998).

Lander and Botstein (1989) point out that most of the evidence on existence of QTLs for a trait comes from the highest and lowest performing individuals. Therefore, if the cost of genotyping is limiting, it make sense to genotype only the extreme individuals (Dear, 1997).

Another approach, Reverse QTL Mapping, relies on the selective phenotyping and genotyping with a limited number of individuals/markers in a two step procedure. The first step is a classical QTL analysis on a fraction of a segregating population (e.g., 200 individuals) to identify the possible major QTLs for the trait of interest. The second step is to use markers flanking the major QTL and screen the entire population (e.g., 2000 individuals) to identify QTL isogenic recombinants (QNIRs): individuals that carry a recombination at one QTL region and bear identical homozygous genotypes at the other QTL. These QNIRs are then genotyped with sufficient markers at the recombinant QTL region to precisely map the recombination events. By increasing progeny or clones of the QNIRs, highly accurate phenotyping data can be obtained and precise localization of the QTL gene could be reached within a sub-centimorgan interval (Wye *et al*., 2000).

A limitation of selective genotyping is the fact that it is suitable for analysis of only one phenotype at a time. This limitation often proves serious in applied experiments such as plant and animal breeding that require evaluation for many independent characteristics (Paterson, 1998).

Another drawback with this method is that the estimated QTL effect is severely biased upwards if only genotyped individuals are used to estimate the effect. This is a major problem if the QTLs are to be used in MAS, as the overestimation of the QTL variance will erode the advantage of using the marker information. Methods have been derived to correct for this bias (Darvasi and Soller, 1992; Ruvinsky and Marshall-Graves, 2005). Furthermore, single point analysis cannot be used for QTL detection because the phenotypic effects would be grossly overestimated; interval mapping methods must be used (Lander and Botstein, 1989).

RESULTS AND DISCUSSION

Bulk segregant analysis: Bulk segregant analysis was developed by Michelmore and his coworkers to rapidly identify marker linked to any specific gene or trait (Tabata, 2003). The high costs of screening large population for marker allele frequencies can be markedly reduced by use of selective DNA pooling (Darvasi and Soller, 1994; Lipkin *et al*., 1998). The marker contrast is determined by differences in allele frequency in pooled DNA samples of individuals taken from extreme high and low tails of the phenotypic distribution of the population. In practice, this is similar to a selective genotyping but treats the two extremes in a phenotypic distribution as a single DNA sample. The entire population is then genotyped with these polymorphic markers and a localized linkage map may be generated. This enables QTL analysis to be performed and the position of a QTL to be determined (Muir and Aggrey, 2003).

While this approach is an effective way to map genes which account for 100% variance in a trait, both theoretical and empirical results suggests that it has limited applicability to QTL mapping. Among the phenotypically extreme individuals for a polygenic trait, rare QTLs with unusually large effects may be fixed and therefore may be detected as a chromosome segment which is polymorphic between the pools. However, the majority of QTLs, with much smaller phenotypic effects, will remain heterogeneous in the pools and therefore will escape detection (Paterson, 1998; Muir and Aggrey, 2003).

Combination of BSA approach and candidate genes can be a very effective approach in discovering genes having major effects on quantitative traits. It allows candidate genes potentially associated with phenotypic variation to be tested for differences in allele frequency between populations derived from a given genetic background and selectively improved for that trait of interest. BSA has been successfully used in the development of markers tightly linked to disease-resistance genes (Varshney *et al*., 2004; Chen *et al*., 2006).

Comparative QTL mapping: An evolutionary approach to QTL mapping is becoming an increasingly powerful means to expedite QTL analysis.

Three QTLs that affect seed mass (size) correspond closely in sorghum, rice and maize and at least five additional QTLs correspond between two of these genera. Among seven QTLs that account for 52% of Phenotypic Variance Explained (PVE) in sorghum seed mass, five (on linkage groups A, C, E, F and I) correspond to five of eight QTLs that account for 78% of PVE in rice (Smith and Frederiksen, 2000; Meksem and Kahl, 2005).

CONCLUSION

The finding that diverse taxa within common taxonomic families often share similar gene order over large chromosomal segments has been basis for comparative mapping, alignment of the chromosomes of different taxa based on common reference loci. The structural similarity of chromosomes in different taxa is often accompanied by functional similarity in the locations of genes influencing common phenotypes (Paterson, 1998).

REFERENCES

Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, 2007. Molecular Biology of the Cell. 5th Edn., Garland Science, New York.

Bovenhuis, H. and R.J. Spelman, 2000. Selective genotyping to detect quantitative trait loci for multiple traits in outbred populations. J. Dairy Sci., 83: 173-180.

Chen, T.H.H., M. Uemura and S. Fujikawa, 2006. Cold hardiness in Plants: Molecular Genetics, Cell Biology and Physiology. CABI Publishing, Wallingford, UK., pp: 288.

Collard, B.C.Y., M.Z.Z. Jahufer, J.B. Brouwer and E.C.K. Pang, 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142: 169-196.

Darvasi, A. and M. Soller, 1992. Selective genotyping for determination of linkage between a marker locus and quantitative trait locus. Theoret. Applied Genet., 85: 353-359.

Darvasi, A. and M. Soller, 1994. Selective DNA pooling for determination of linkage between a molecular marker and a quantitative trait locus. Genetics, 138: 1365-1373.

Dear, P.H., 1997. Genome Mapping: A Practical Approach. Oxford University Press, Oxford, UK.

Holland, J.B., 2004. Implementation of Molecular Markers for Quantitative Traits in Breeding Programs-challenges and Opportunities. In: New Directions for a Diverse Planet, Fischer, T., N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell and D. Lloyd (Eds.). Regional Institute Ltd., Gosford, NSW, Australia.

Lander, E.S. and D. Botstein, 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics, 121: 185-199.

Lebowitz, R.J., M. Soller and J.S. Beckmann, 1987. Trait-based analysese for the detection of linkage between marker loci and quantitative trait loci in crosses between inbred lines. Theoret. Applied Genet., 73: 556-562.

Lipkin, E., M.O. Mosig, A. Darvasi, E. Ezra, A. Shalom, A. Friedmann and M. Soller, 1998. Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling using dinucleotide microsatellite markers: Analysis of milk protein percentage. Genetics, 149: 1557-1567.

Meksem, K and G. Kahl, 2005. The Handbook of Plant Genome Mapping: Genetic and Physical Mapping. Wiley-VCH, New York, USA.

Muir, W.M. and S.E. Aggrey, 2003. Poultry Genetics, Breeding and Biotechnology. CABI Publishing, Wallingford, UK.

Paterson, A.H., 1998. Molecular Dissection of Complex Traits. CRC Press, Boca Raton, FL., USA.

Ruvinsky, A. and J.A. Marshall-Graves, 2005. Mammalian Genomics. CABI Publishing, Wallingford, UK.

Smith, C.W. and R.A. Frederiksen, 2000. Sorghum: Origin, History, Technology and Production. John Wiley and Sons Inc., New York.

Tabata, S., 2003. Brassica and Legume: From Genome Structure to Breeding. Springer Verlage, Berlin, Heidelberg.

Tierney, M.B. and K.H. Lamour, 2005. An introduction to reverse genetic tools for investigating gene function. The Plant Health Instructor.

Tuzun, S. and E. Bent, 2006. Multigenic and Induced Systemic Resistance in Plants. Springer, New York.

Varshney, A., T. Mohaptra and R.P. Sharma, 2004. Development and validation of CAPs and AFLP markers for white rust resistance gene of *Brassica juncea*. Theoret. Applied Genet., 109: 153-159.

Weller, J.I., 2001. Quantitative Trait Loci in Animals. CABI Publishing, Wallingford, UK.

White, T.L., W.T. Adams and D.B. Neale, 2007. Forest Genetics. CABI Publishing, Wallingford, UK.

Wye, C., J. Rouppe van der Voort and J. Peleman, 2000. Reverse QTL mapping (RQM), an efficient approach for high resolution mapping of QTL. Poster No. Proceedings of the Plant and Animal Genome Conference VII, San Diego.