AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 03, Issue: 07 (July, 2026)
Available online at http://www.agrimagazine.in
©Agri Magazine, ISSN: 3048-8656

QTL-seq: An Efficient Next-Generation Sequencing Approach for Rapid QTL Discovery

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Quantitative trait loci sequencing (QTL-seq) is a powerful next-generation sequencing (NGS)-based approach that combines bulked segregant analysis (BSA) with whole-genome resequencing to enable rapid identification of genomic regions associated with important agronomic traits. Conventional QTL mapping requires more number of markers development, genotyping and linkage analysis, making the process laborious, time-consuming, and costly. QTL-seq overcomes these limitations by sequencing DNA pools from individuals exhibiting extreme phenotypes and identifying trait-associated genomic regions using SNP-index and Δ(SNP-index) analyses. This approach has efficiently accelerated the discovery of major-effect QTLs controlling disease resistance, abiotic stress tolerance, yield, quality, flowering time and other economically important traits across diverse crop species. QTL-seq can be applied to different mapping populations including F_2, recombinant inbred lines, doubled haploids, backcross populations, and near-isogenic lines with F_2 populations being the most widely used due to their rapid development and high genetic variability. In addition to its speed, cost-effectiveness and genome-wide SNP discovery capability, QTL-seq facilitates marker-assisted selection through the development of tightly linked molecular markers.

Keywords: Bulked segregant analysis (BSA), Next-generation sequencing (NGS), Quantitative trait loci sequencing (QTL-seq) , SNP-index, Marker-assisted selection (MAS), Gene discovery.

Introduction

Traditionally, QTLs have been identified by linkage analysis of progeny derived from a cross between parents showing contrasting phenotypes for a trait of interest. To perform linkage analysis, DNA markers capable of discriminating between parental genomes are required. Due to this requirement, parents for crosses are selected from genetically distantly related cultivars. This entails that parents may be different in many QTLs controlling a given phenotype, complicating the isolation of individual loci. On the other hand, whenever closely related parents are used, identification of sufficient DNA markers for linkage analysis becomes a limiting step. Bulked segregant analysis (BSA) is an elegant method to identify DNA markers tightly linked to the causal gene for a given phenotype. Following a cross between parental lines showing contrasting phenotypes, the resulting F 2 progeny are scored for segregation of the phenotype. Two bulked DNA samples are generated from the progeny showing contrasting phenotypes, and DNA markers exhibiting differences between the two bulks are screened. In the original reports, DNAs bulked from F2 progeny were screened with

restriction fragment polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD) markers to identify the markers linked to the traits of interest. Later, BSA was applied to identify QTL which is sometimes called 'selective DNA pooling'. However, in these analyses, the availability of DNA markers was the main factor limiting the effectiveness of the methods. Furthermore, genotyping of each marker for the two bulked DNAs is still time-consuming and costly.

Recent development of whole genome sequencing has accelerated the analysis of QTLs. Plant QTL identification using whole-genome resequencing of two DNA bulks of progeny (each with 20–50 individuals) showing extreme phenotypic values by next-generation sequencing (NGS) technology. Since this approach has a wide applicability in QTL identification in plant species, including crops, we propose to name the method QTL-seq as specifically applied to plant species. Because it does not require DNA marker development and the most time-consuming and costly procedure needed for conventional QTL analysis, QTL-seq allows the rapid identification of QTLs. The wider applicability and added advantages of QTL-seq over other traditional QTL mapping strategies available in diverse plant species have been truly realized. Henceforth, its usage is in rapid genome-wide scanning and fine mapping of major genes underlying robust QTLs.

Principle

QTL-seq which is a modern version of the classical BSA combined with WGR. In this approach, a mapping population derived from a cross between two contrasted parents is used. The progenies are phenotyped and the tails of the distribution are divided into two extreme bulks of 10–20 individuals, which are sequenced at above 6x coverage. For each genomic position, the proportion of short reads harboring SNPs with the sequence of one of the parents chosen as the reference (the so-called SNP index) is estimated and the difference between the SNP index of the low-trait bulk and that of the high-trait bulk, called the D(SNP index), is calculated. A large D(SNP index) characterizes the genomic fraction that has an association with the phenotypic value. The principle of QTL-seq is shown in Figure 1 and is explained by taking rice as an example. QTL-seq combines bulked-segregant analysis and whole-genome resequencing for rapid identification of the genomic regions that differ between the two parents used in a genetic cross and also contribute to the higher and lower values of the traits of interest among the resulting progeny. For QTL mapping using QTL-seq first generate a mapping population by crossing two cultivars showing contrasting phenotypes for the traits of interest. In Figure 1, The trait of interest is plant height and cultivars A and B have low and high stature, respectively. Different kinds of mapping populations can be used for QTL-seq depending on the traits to be studied. Recombinant inbred lines (RILs) and doubled haploids (DH) show a high degree of homozygosity and individuals in each line can be regarded as proxy clones that allow replicated measurements of the phenotype, and thus are suitable for detecting QTLs of minor effects. The advantage of using an F2 population is the short time required for its generation. However, no replicated measurements are possible for each genotype. As a result, the approach is not suitable for

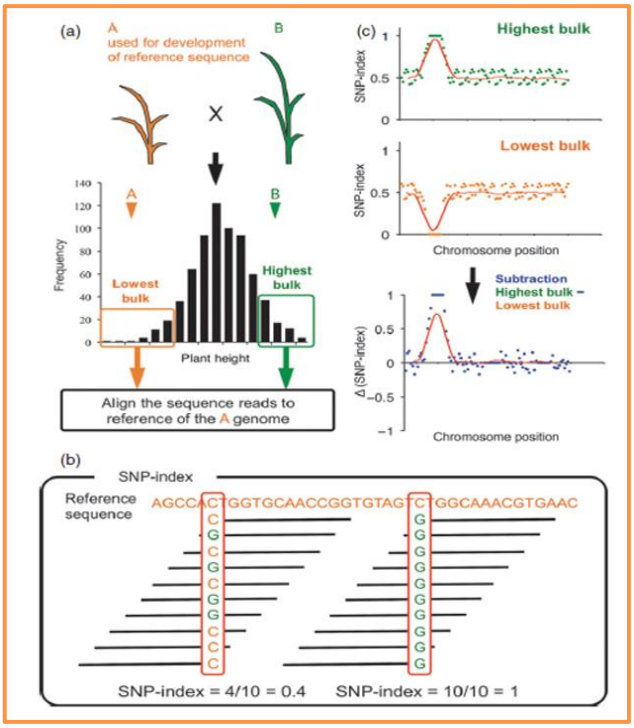

Figure 1. A simplified scheme of QTL-seq as applied to rice.

detecting minor effect QTLs. After the progeny of a mapping population are measured for the focused trait then score segregation of the phenotype. If the number of QTLs involved in the trait variation is multiple, the frequency distribution of measured values will be close to the normal (Gaussian) distribution (Figure 1a). Here, results focused on the multiple progenies showing extreme phenotypes, i.e. those exhibiting the highest and the lowest extreme values. Sampled DNA from 10 to 20 individuals from each extremity and bulked them to generate 'Highest' bulk and 'Lowest' bulk. Each of the bulked DNAs was applied to whole genome resequencing with a > 6x genome coverage. Expected results of the bulked DNA to contain genomes from both parents in a 1:1 ratio for the majority of genomic regions. However, final results detect unequal representation of the genomes from the two parents in the genomic regions harboring QTL for the phenotypic difference between 'Highest' and 'Lowest' bulks.

To examine the relative amount of the genomes derived from the two parents, an evaluation requires for the proportion of short reads corresponding to each of the two parental genomes that can be discriminated by single nucleotide polymorphisms (SNPs) available between the two. After aligning the sequence data to the reference sequence of either of the two parents, counts the number (k) of short reads harboring SNPs that are different from the reference sequence. It defined the proportion of k in the total short reads (n) covering a particular genomic position (=k n^{-1}) as the SNP-index (Figure 1b). The SNP-index is 0 if the entire short reads contains genomic fragments from the parent that was used as a reference sequence. The SNP-index is 1 if all the short reads represent the genome from the other parent. A SNP-index of 0.5 means an equal contribution of both parents' genomes to the bulked progeny. Accordingly, the SNP-index is calculated for all the SNPs detected between the two parents, and the relationships between SNP-index and SNP position in the genome is graphically represented (Figure 1c).

Types & size of Populations Suitable for QTL-seq

QTL-seq can be applied to several types of segregating populations, including F_2, $F_{2:3}$, backcross (BC), recombinant inbred lines (RILs), doubled haploids (DHs), and near-isogenic lines (NILs)**.** The choice of population depends on the crop, breeding objectives, trait inheritance and available resources. Among these the F_2 population is considered the most desirable and widely used because it combines high genetic variation which enables efficient detection of QTLs. In F_2 population individuals exhibiting extreme phenotypes can be readily selected to construct contrasting DNA bulks for sequencing making the approach both rapid and cost-effective. Additionally, F_2 populations require only a single generation after crossing, allowing researchers to identify major-effect QTLs much faster than populations that require several generations of selfing such as RILs or DHs. Although RILs provide greater genetic stability and higher mapping resolution due to accumulated recombination events but their development is time-consuming and labor intensive. Similarly, backcross populations are useful for validating or fine-mapping QTLs inherited from a specific parent, whereas NILs are primarily employed for QTL confirmation rather than initial discovery. Due to its simplicity, rapid development, high heterozygosity, and effectiveness in detecting major QTLs, the F_2 population is preferred for QTL-seq studies. An F_2 population of 150–300 individuals is generally sufficient, while 200–500 individuals provides optimal mapping resolution. Larger populations improve recombination and QTL localization, whereas 500–1,000 or more individuals are recommended for detecting minor-effect QTLs.

Comparative analysis of QTL-seq and related gene/QTL mapping methods

Method	Population	Sequencing	Detects	Best Application
MutMap	EMS mutant F_2	DNA	Causal mutation	Gene cloning
BSA-seq	F_2	DNA	Major loci	Rapid mapping
BSR-seq	F_2	RNA	Expression + SNPs	Candidate gene discovery
QTL-seq	F_2/RIL/DH	DNA	Major QTLs	Marker-assisted breeding
QTG-seq	Advanced recombinant population	DNA	Quantitative trait genes	Gene identification
GWAS	Natural population	DNA	Marker-trait association	Diversity panels

Method	Population	Sequencing	Detects	Best Application
Conventional linkage mapping	Biparental population	Marker genotyping	Major & minor QTLs	Comprehensive genetic analysis

Advantages

- For overlapping traits, multiple QTL detection is possible if accurate genotyping is done after phenotyping
- QTL-seq provides an affordable alternative to traditional QTL mapping with reduced genotyping requirements
- This approach overcomes the need for prior development of dense molecular markers through direct genome-wide SNP discovery
- QTL-seq is a fast method for genome-wide SNP detection using next generation sequencing technologies
- This strategy provides high resolution mapping that allows rapid identification of QTLs of major effect
- It can generate markers like KASP-Kompetitive Allele Specific PCR for MAS
- QTL identification for traits like Disease Resistance, Yield Traits**,** Quality Traits, Abiotic Stress and Flowering

Limitation

- This technique is less effective for minor-effect QTLs detection and novel QTL validation
- It requires a reference genome which is not easily available for all crops
- In QTL-seq, efficient NGS tools with high sequencing depth needed
- QTL-seq is influenced by phenotyping accuracy
- In polyploid species, duplicated nature of allo and auto polyploids genomes can cause problems with false-positive SNP calls masking significant
- QTLs. Population structure and it's parameters directly affects results and it may give false positive results in this technique.
- Advanced Bioinformatics tools like Bowtie, SAM tools, Picard, GATK, BCF tools, QTLseqr, QTL-seq pipeline, IGV etc., and experts are required to operate all tools

Applications

Crop	Trait	Application of QTL-seq	Outcome	Reference
Rice (*Oryza sativa*)	Blast resistance	Rapid mapping of genomic regions associated with blast resistance using resistant and susceptible bulks	Identified major QTLs for blast resistance, facilitating marker-assisted breeding	Takagi *et al.* (2013)
Rice (*Oryza sativa*)	Seedling vigor	Whole-genome resequencing of extreme bulks to identify QTLs controlling early seedling growth	Rapid detection of major QTLs for seedling vigor	Takagi *et al.* (2013)
Rice (*Oryza sativa*)	Bacterial blight resistance	QTL-seq combined with BSA to identify novel resistance loci	Novel QTL qBBchr8 identified and KASP markers developed for marker-assisted selection	Wongsa *et al.* (2025)
Potato (*Solanum tuberosum*)	Potato cyst nematode resistance (H1 gene)	Modified polyploid QTL-seq for tetraploid genome analysis	Developed tightly linked DNA markers for efficient breeding	Yamakawa *et al.* (2021)
Sweet potato (*Ipomoea batatas*)	Storage root anthocyanin content	Polyploid QTL-seq for mapping quality trait in hexaploid genome	Identified SNP clusters and tightly linked markers for anthocyanin content	Yamakawa *et al.* (2021)
Chickpea (*Cicer arietinum*)	Drought tolerance	QTL-seq to detect genomic regions controlling drought-related traits	Candidate QTLs identified for drought tolerance breeding	Chaturvedi *et al.* (2026)
Soybean (*Glycine max*)	Seed protein content	Whole-genome resequencing of contrasting bulks	Major QTLs identified for seed quality improvement	Zhang *et al.*(2026)
Groundnut (*Arachis hypogaea*)	Late leaf spot resistance	QTL-seq for polyploid-specific identifying LLS associated loci	Discovery of significant QTLs for LLS resistance.	Clevenger *et al.* (2018)

Conclusion

QTL-seq has been invented as a rapid and cost-effective strategy for identifying major quantitative trait loci controlling agriculturally important traits. By integrating bulked segregant analysis with whole-genome resequencing this technique eliminates the need for extensive marker development and significantly reduces the time and effort required for conventional QTL mapping. To identify novel QTLs associated with disease resistance, abiotic stress tolerance, yield, quality, flowering, and other complex polygenic traits in numerous crop species this method has been successfully applied. Among the available mapping population F_2 population remains the preferred choice due to its rapid development, high genetic variation, and suitability for detecting major-effect QTLs. Furthermore, QTL-seq enables the rapid development of closely linked molecular markers that accelerate marker-assisted breeding programmes. Although challenges such as accurate phenotyping, high sequencing depth, reference genome availability, polyploid genome complexity and limited detection of minor-effect QTLs remain continuous advancements in sequencing technologies. Bioinformatics tools and genome assemblies are expected to further enhance its efficiency and applicability. Overall, QTL-seq has become an indispensable genomic tool for rapid QTL discovery and molecular breeding, contributing significantly to the development of improved crop varieties with enhanced productivity, resilience and quality.

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