UNIGENOME
Leading Genomics Innovations

GENOTYPING
BY SEQUENCING

A Lifesciences Division of
Unipath
SPECIALTY LABORATORY Ltd.

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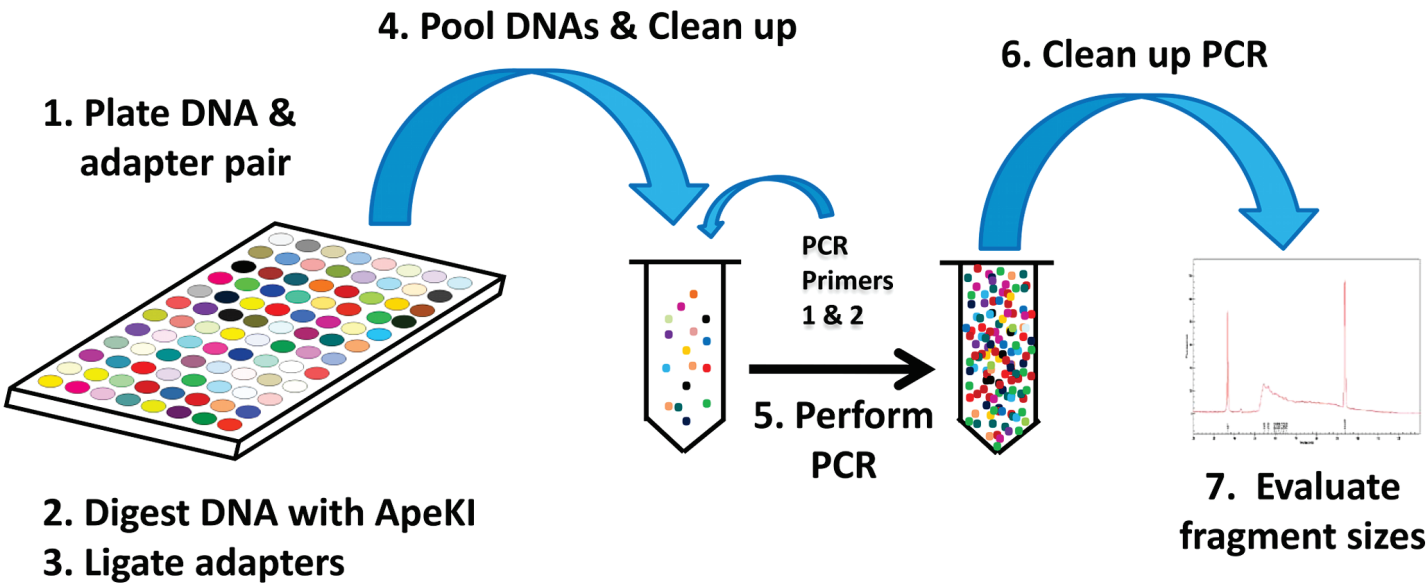
Genotyping by Sequencing

Plant breeding can be accomplished through two major strategies, classical breeding and molecular breeding. It needs a long period and several generations to select and evaluate useful genotypes, the use of molecular markers like single nucleotide polymorphism (SNP), have been identified and effectively used in plant breeding to assist phenotypic selections in crop improvement. Genotyping-by-sequencing (GBS) is a novel application of NGS protocols for discovering and genotyping SNPs in crop genomes and populations. GBS provides a rapid and low-cost tool to genotype breeding populations, allowing plant breeders to implement GWAS, genomic diversity study, genetic linkage analysis, molecular marker discovery, and genomic selection (GS) under a large scale of plant breeding programs. In contrast to GWAS studies, which require 1000s to millions of markers to generate sufficient information and coverage [1] GBS does not require genome sequence to identify the SNPs.

GBS Workflow



GBS includes digestion of genomic DNA with restriction enzymes followed by the ligation of barcode adapter, PCR amplification and sequencing of the amplified DNA pool on a single lane of flow cells [2]. Bioinformatic pipelines are required to analyze and interpret GBS datasets. As an ultimate MAS tool and a cost-effective technique, GBS has been successfully used in implementing genome-wide association study (GWAS), genomic diversity study, genetic linkage analysis, molecular marker discovery and genomic selection under a large scale of plant breeding programs.

GBS Applications

GBS enables "deep sequencing" of RAD-tags at 30-60 x coverage to discover SNPs, which can be linked to a trait or population. Optimized enzymatic digestion reduces the genome complexity. PstI, SbfI, SgrAI, ApeKI etc are the most commonly used enzymes in plants and SbI, PstI, NotI, Eag1, BamHI, BbvC1 etc. are used in animals. Combination of enzymes set can be used to prepare libraries for parental as well as progeny lines and sequencing on HiSeq or Novaseq platform using paired-end or single-end library in combination of 48, 96 or 192 samples or as per request.

Highly skilled, qualified and experienced team of scientists and researchers at Unigenome has executed many projects on SNP genotyping by GBS. Unigenome has developed expertise in the field of GBS: SNP Discovery, association analysis, LD analysis and haplotyping of closely related markers using various tools & software. GBS based on the Illumina platform has proven to be a fast and cost-effective means of SNP discovery.

Sample requirement for GBS/RAD:

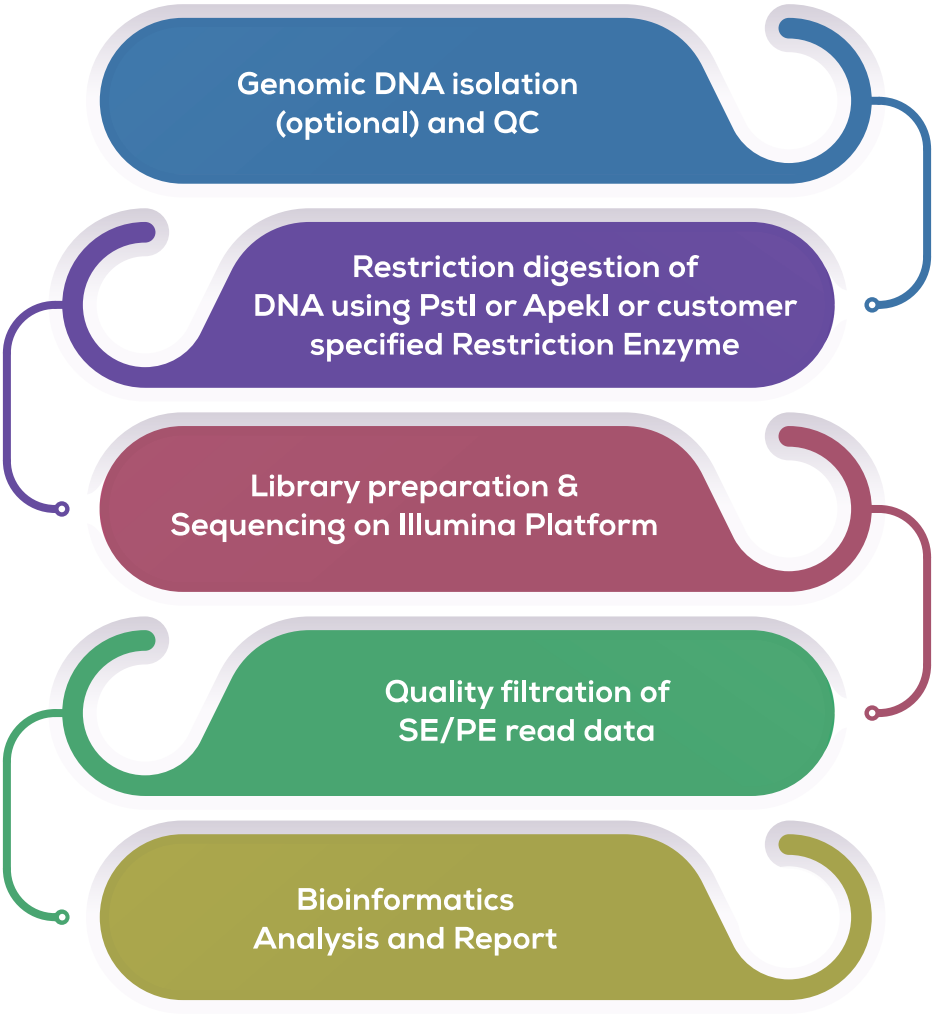
- Genomics DNA sample: 8-10µg of high molecular weight intact double stranded genomic DNA, free from RNA contamination should be provided.
- gDNA should have an absorbance ratio (A260/280) of ~1.8 to 2.0 with minimum 300-500ng/µL concentration.

Quality control of genomic DNA samples:

- Genomic DNA samples must be qualified for both qualification and quantification by 1% agarose gel electrophoresis & Qubit/Nanodrop respectively.

Note: All types of samples should be transported in -20^{0}C to Unigenome, Ahmedabad, Gujarat, India.

Brief methodology for GBS/RAD:



GBS/RAD data analysis/deliverables:

- De-multiplexing of reads based on sample barcodes
- Clean reads after filtration of adapter and low quality bases
- Identification of tags from reads
- Mapping of tags to reference genome
- SNPs identification
- SNPs filtration based on quality, coverage and reads depth
- Genome-wise distribution of SNPs
- SNPs annotation
- Comprehensive compiled report and data deliverables

Advance Analysis deliverables:

It includes genome-wise association analysis using SNP generated using GBS and phenotypic data of trait of interest:

- Linkage disequilibrium analysis
- Kinship analysis
- Association analysis for identification of SNP associated with trait
- Manhattan plot for trait associated SNPs
- QQ-plot for trait associated SNPs

Note: Customized analysis as per client's need is provided with additional charges

References:

- Edwards, D., and Batley, J. (2010).Plant genome sequencing: applications for crop improvement. Plant Biotechnol. J. 8,2–9. doi: 10.1111/j.1467-7652.2009.00459.x
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S.,et al. (2011).A robust, simple genotyping -by-sequencing (GBS) approach for high diversity species. PLoS ONE 6:e19379. doi: 10.1371/journal.pone.0019379

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