Genotyping By Sequencing (GBS)/Restriction site Associated DNA Sequencing (RAD):

GBS/RAD-seq is a method that uses Illumina HiSeq2000/2500 next-generation sequencing platform to discover and score tens to hundreds of thousands of SNP markers in hundreds of individuals in model or non-model organisms. It is a “complexity-reduction” technique designed to reliably interrogate a segment of genome, the method involves cutting down a genome anywhere from 0.1 to 15% with at least one restriction enzyme and sequencing the ends of the resulting fragments for either genetic marker discovery or genotyping. It allows you to interrogate a scalable number of loci even in a highly heterozygous species that leads to study of QTL mapping and population genomics. GBS enables “deep sequencing” of RAD-tags at 30-60 x coverage to discover SNPs, giving researchers confidence that technology can identify genetic variation linked to a trait or population. The complexity of the genome is reduced by digesting the DNA with optimized enzymes. The most commonly used enzymes in plants are PstI, SbfI, SgrAl, ApeKI etc, and in animals are Sbi, PstI, Notl, Eagl, BamHI, BbvC1 etc. Combination of enzymes set can be used to prepare libraries for parental as well as progeny lines and sequencing on HiSeq 2000/2500 platform using paired-end or single-end library can be carried out by multiplexing for 12, 24, 48, 96, 192 samples or as per request. Xcelris Genomics has executed many projects on SNP genotyping by GBS. Xcelris Genomics has developed expertise in the field of GBS: SNP Discovery, association analysis, LD analysis and haplotyping of closely related markers using various tools & software’s (including Bowtie, Stacks, RAD tool, JoinMap, TASSEL etc). GBS based on the Illumina HiSeq2000 platform has proven to be a fast and cost-effective means of SNP discovery.

Sample requirement for GBS/RAD:
- gDNA 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 RNA samples:
- gDNA samples are subjected to both qualification and quantification by 1% agarose gel electrophoresis & Qubit/Nanodrop respectively.

Note: All types of samples should be transported in -20°C to Xcelris genomics, Ahmedabad, Gujarat, India.

Brief methodology for GBS/RAD:
- gDNA isolation (optional)
- Quality check of gDNA
- Restriction digestion of DNA using Pstl or Apekl or customer specified
- Library preparation
- Sequencing on Illumina HiSeq 2000/2500
- Data generation
- Quality filtration of SE/PE read data
- High quality raw data in (fastq)

GBS/RAD data analysis/deliverables:
- Mapping on the reference genome/draft genome
- The complete list of SNPs are provided in excel sheet
- Polymorphic tags in the parental strains are identified
• Polymorphic tags identified in the parental lines act as reference for F2 hybrid tags to study the segregation pattern.
• Following criteria to assess the quality of RAD markers for linkage map construction are provided:
  ▸ Total number of loci detected
  ▸ The percentage of polymorphic loci
  ▸ The number of missing allele calls for polymorphic loci
  ▸ Comparable genome coverage along with Linkage map length
  ▸ Identification of significant QTLs
• LD analysis and haplotyping of closely related markers
• Haplotype block analysis
• Comprehensive compiled report and data

Optional:
• Submission of data to NCBI (raw data)
• Further analysis, figures, tables, required by specific journals are provided as per customer’s requirement.

Timeline: It depends upon project size and scope, number of RE selected, technology used and amount of data required. The generalized time line is six to ten weeks.

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<tr>
<th>Data Generation</th>
<th>HiSeq 2000</th>
<th>HiSeq 2500</th>
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<tr>
<td>Number of Samples Multiplex</td>
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<td>1 x 100 bp</td>
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<tr>
<td>12 plex/lane</td>
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