**Choosing most suitable restriction enzyme (RE) and optimization**

To perform SNP genotyping on Bittergourd 93 samples including parental genotypes using Gentotyping By Sequencing method (GBS); sequencing for generating Linkage map using JOINMAP v4.1 & QTL analysis using QTL cartographer v2.5. Choosing most suitable restriction enzyme is most important for success of sequencing.

ApekI Enzyme will be used for library preparation. Preparing **one set of 94 Plex** library (93 lines+blank). Generating sequencing RAW data for the optimized GBS library using Illumina True Seq V4 Chemistry.

**Bioinformatics Analysis includes**:

* Filtering the high quality reads from the RAW data.
* Assembly of high quality filtered reads (Analysis using UNEAK pipeline from TASSEL as the reference genome is not available).
* Mining out polymorphic SNP markers from the assembled data.

**Sample Information**:

* 93 Accessions
* Negative Control (BLANK)
* **ApeKI Enzyme Library:** 93 SAMPLES +Blank (refer to the plate format given below)

|  |
| --- |
| **BITTERGOURD PLATE MAP**  |
|  | **1**  | **2**  | **3**  | **4**  | **5**  | **6**  | **7**  | **8**  | **9**  | **10**  | **11**  | **12**  |
| **A**  | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  | 11  | 12  |
| **B**  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  |
| **C**  | 25  | 26  | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  |
| **D**  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  | 46  | 47  | 48  |
| **E**  | 49  | 50  | 51  | 52  | 53  | 54  | 55  | 56  | 57  | 58  | 59  | 60  |
| **F**  | 61  | 62  | 63  | 64  | 65  | 66  | 67  | 68  | 69  | 70  | 71  | 72  |
| **G**  | 73  | 74  | 75  | 76  | 77  | 78  | 79  | 80  | 81  | 82  | 83  | 84  |
| **H**  | 85  | 86  | 87  | 88  | 89  | 90  | 91  | 92  | 93  |   |   |   |

**Restriction Enzyme Genome Optimization:**

ApeKI

EcoT22I

MspI/PstI

PstI

***Note: Best Library fragment distribution was identified using ApekI Enzyme. Hence ApekI was chosen for library preparations for all Bitter gourd Accessions.***

 **Optimized Library details:**

* **Single** End adapters were used for library preparation.
* For preparing each library we have used 94 different barcodes for tagging samples. These barcodes are of variable length from 5-10 nt in lengths.

**Adaptors sequences for *ApeKI***

**The ApeKI barcode adapters:**

5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTxxxx and

5′-CWGyyyyAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT, where “xxxx” and

“yyyy” denote the barcode and barcode complement, respectively.

 **The ApeKI common adapter**:

5′-CWGAGATCGGAAGAGCGGTTCAGCAGGAATGCCGAG and

5′-CTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

**Final Bitter gourd Library QC Results on Agilent’s Bioanlyzer 2100:**



 **Summarised Details for Final Libraries:**

* 94 well plate generated good ApeKI Enzyme Libraries which have passed the QC criteria.
* There are no adapter dimers and the library fragments are well within the sequencing size range of the Illumina system, for Sequencing using Illumina True Seq Version 4 Chemistry.