WHITE PAPER

Introducing GenoPlex-One

Efficient Multiplexing of Thousands of Samples and Hundreds of SNPs in a Single Reaction Within One Day for Genotyping

Overview

GenoPlex-One is an innovative genotyping solution designed to streamline the multiplexing of thousands of DNA samples while analyzing hundreds of SNPs in a single PCR step. This approach allows for pooling in one reaction for NGS sequencing. Focusing on efficiency, high-throughput capabilities, and accuracy, GenoPlex-One enables researchers to conduct large-scale genetic testing with ease. The data output from SNP scatter plots closely resembles results from single PCR end-point genotyping by tallying the read depth of the two alleles. By combining hundreds of reactions into one tube, GenoPlex-One significantly increases throughput—potentially hundreds of times—while completing the process within a single day.

Key Features

- **One-Step Multiplexing:** The GenoPlex-One enables the multiplexing of dozens to hundreds of SNPs in a single reaction through a streamlined one-step PCR workflow (Figure 1).
- **Flexible Target Design:** The GenoPlex-One system is a highly cost-effective solution that supports targeted genotyping designs of **20-500 markers.**
- **Cost-Effective Workflow:** No transfers between reaction stages—minimize the risk of sample cross-contamination while reducing consumable and maintenance costs.
- **Ultimate Throughput:** it supports a daily throughput of **6,000 to 10,000** samples, suitable for large-scale genotyping projects.
- **End-to-End Data Analysis:** Molbreeding offers MEES (MolBreeding's End-to-End Solution), delivering a comprehensive pipeline for genotyping data processing and analysis.

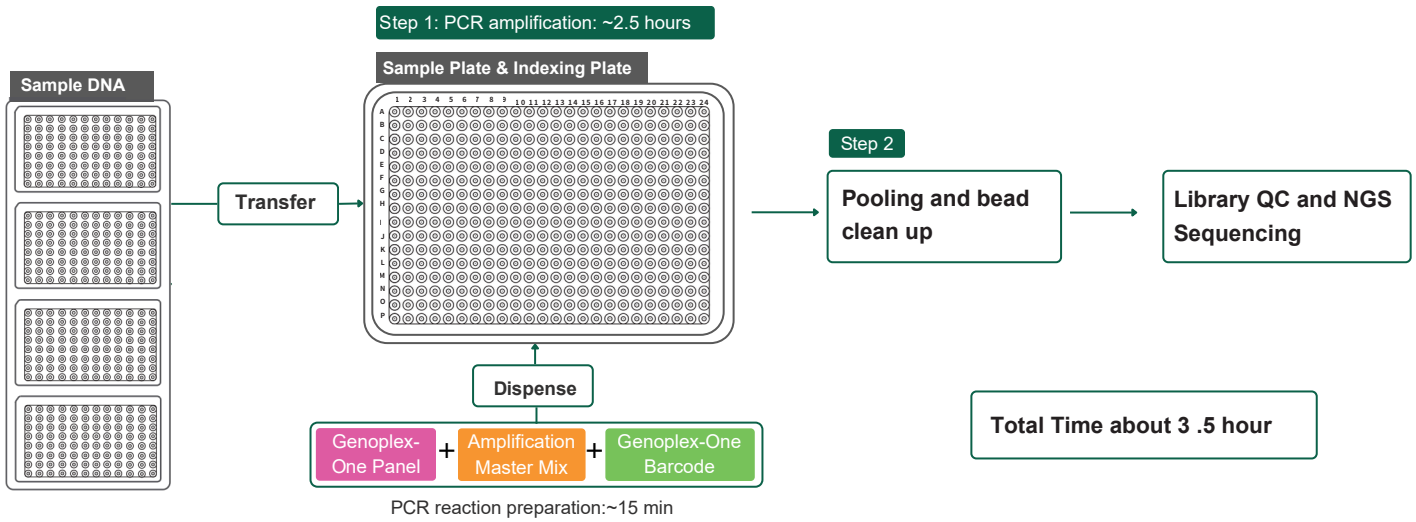

Figure 1. The Genopledx-One Workflow for 384-Format PCR Setup and SNP Genotyping

Following a one-step PCR amplification (~2.5 hours) and a quick pooling/purification step (~1 hour), this process allows for the genotyping of hundreds of SNPs in a single, efficient workflow.

Workflow Efficiency

GenoPlex-One allows for the rapid assembly of a set of hundreds of SNP markers, facilitating a single-step PCR to prepare all amplicons for next-generation sequencing (NGS). Each sequencing run can process between 6,000 to 10,000 samples, with total sequencing durations ranging from **8 to 24 hours**, depending on the specific sequencer settings and configurations.

We repeat the same panel and the same set of samples, the concordance rate of two replication is 99.8% (Figure 2, Figure 3).

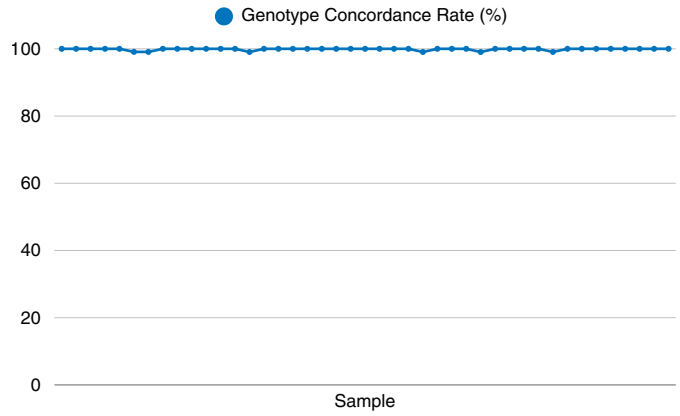

Figure 2.The concordance rate of 99.8% between the two replicates.

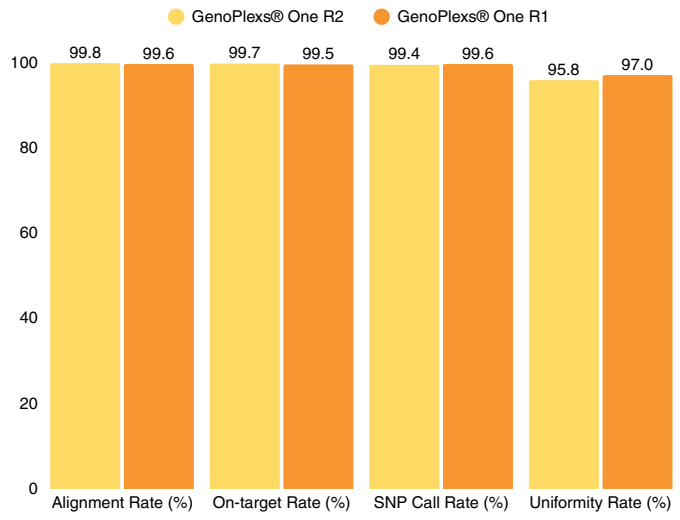

Figure 3. Dataset for two replicates: GenoPlex-One exhibits high stability with an average SNP call rate of 99.5%, and a uniformity rate greater than 95.8%.

Design and Performance

The GenoPlex-One system is a cost-effective solution that excels with designs featuring fewer than 500 SNPs, achieving a remarkable 90% success rate for randomly selected SNPs. Evaluations of three key panels—Maize, Pepper, and Tomato—demonstrated alignment rates exceeding 90%, with Pepper and Tomato surpassing 99%.

All panels exhibited an impressive call rate and uniformity of over 94% (see Table 1). Following replications, this consistency is reliably maintained. Each GenoPlex-One panel for Maize, Pepper, and Tomato achieved approximately 90% design success, with overall alignment exceeding 90% to the reference genome and equivalent call rates (see Figure 4). This robust performance highlights GenoPlex-One's effectiveness for advanced genotyping applications.

Parameter	Maize (96 SNPs)	Pepper (110 SNPs)	Tomato (134 SNPs)
Alignment Rate (%)	91.9	99.6	99.9
On-target Rate (%)	93.2	99.5	99.9
SNP Call Rate (%)	94.7	98.8	97.9
Uniformity Rate (%)	94.8	97	95.6
Genotype Concordance Rate (%)	99	99.9	99

Table1.Dataset of three Genoplex-One panels

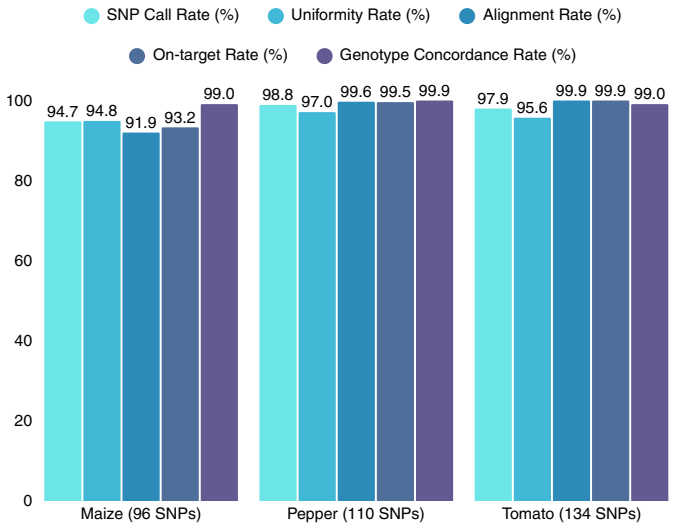

Figure 4.The Performance of three Genoplex-One panels

In our analysis of these panels, we specifically examined the signal characteristics from diploid samples with two alleles. For heterozygous genotypes (e.g., A/T), the read signals exhibited nearly equal strength from both alleles, resembling the fluorescence signals typical of end-point genotyping. The accompanying scatter plots visualize the genotype classifications based on allele depth, distinguishing between homozygous mutants, homozygous wild-types, and heterozygotes (Figure 5).

Sequence Input Requriments

The minimum input DNA amount is 50 ng, we recommend to use 50-150 ng per sample for library preparation.

Conclusion

Genoplex-One stands as a robust solution tailored for high-throughput genotyping, integrating advanced design features and workflows that enhance efficiency and accuracy. By leveraging this innovative technology, researchers can accelerate their genetic studies and make significant strides in their respective fields.

Call to Action

We invite researchers, breeders, and industry partners to collaborate with us in applying the Genoplex-One system. We can design customized Genoplex-One panels to meet your specific genotyping goals, or provide ready-to-use Genoplex-One kits for in-house testing, enabling efficient, flexible, and cost-effective genotyping solutions.

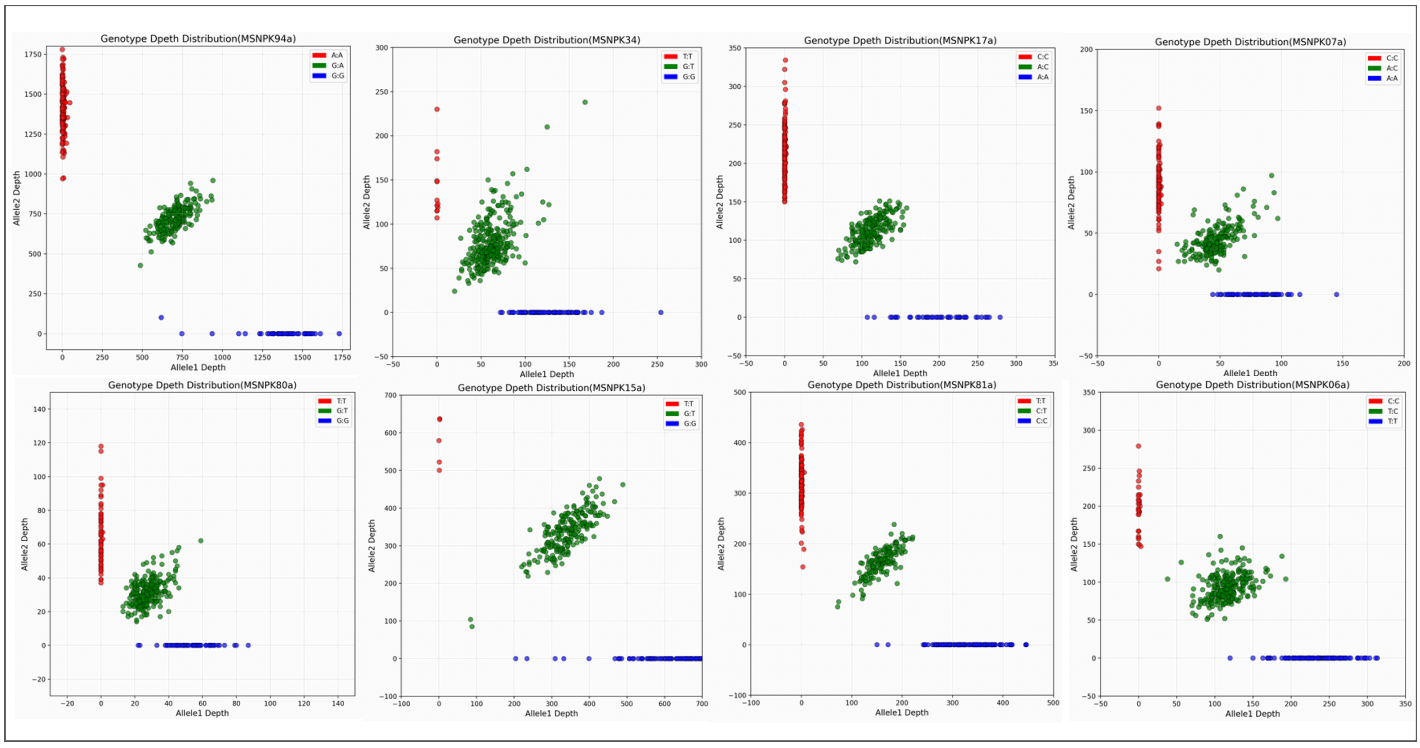

Figure 5.Scatter plot of genotype depth distribution.

MolBreeding Biotechnology | cs@molbreeding.com

Americas | 10 Pasteur, Suite 150,Irvine, CA 92618 United States | +1-515-508-9174
EMEA | Unit 10, West Point Business Park, West Road, Harlow, Essex, CM20 2BU United Kingdom | +44 (0) 1279 940 983
Asia | 538B, 5/F, Building 1W, 1 Science Park W Ave, Science Park, Pak Shek Kok, Hong Kong SAR, China | + (852) 5130-1856