

AgriType® 2.0 Streamlined and High-Throughput Targeted Genotyping by Sequencing Solution

Streamlined and high-throughput targeted genotyping by sequencing solution for molecular breeding

Highlights

- **High-Throughput Genotyping System**
96-well and 384-well plate compatible
- **Streamlined, Automation-Friendly Workflow**
Simple, three-step workflow with less than 25-min hands-on time that enables operators to walk away with easy automation integration
- **Scalable, Adaptable Genotyping System**
Target up to 7000 relevant markers per pool
- **Cost-Effective Sequencing on Major Platforms**
Process thousands of samples at a time on either Illumina®, or Ion Torrent™ NGS platforms
- **High-Quality, Reproducible Results**
Prepare high-quality NGS libraries with high call rates, uniformity and on-target rates for efficient use of sequencing reads

The AgriType® 2.0 Targeted Genotyping by Sequencing Solution is designed and developed to address large-scale genotyping projects in agrigenomics. The AgriType solution is powered by our advanced primer design algorithm and an efficient ultra-high multiplex PCR-based target enrichment chemistry. AgriType provides a cost-effective solution to construct NGS libraries to quickly and efficiently screen hundreds to thousands of markers of interest to accelerate genotyping studies in fields such as aquaculture, livestock farming, and seed breeding.

Relevant molecular breeding applications include but are not limited to Marker assisted selection (MAS), Genomic selection (GS), Quantitative trait locus (QTL) screening, Marker assisted back crossing (MABC), and Trait mapping.

AgriType® 2.0 Custom Targeted Genotyping by Sequencing Solution Specifications for Sequencing Platforms

Parameter	Specification
Enrichment Method	Multiplex PCR
Platform	Illumina®, Ion Torrent™
Number of Markers	50-7000 markers per pool with potential to discover novel SNPs
Amplicon Size	107 – 275 bp
Number of Primer Pools	1 or 2 pools
Input DNA Requirement	50 – 200 ng
Sample Types	Extracted DNA from seeds, leaves, tissue, degraded DNA
Total Assay Time	3-5 hours for Illumina (depending on panel size)
Hands-on time	20-25 minutes
Mean Genotyping Call Rate	≥ 95%
Coverage Uniformity (targets with > 0.2X mean coverage)	≥ 90%
On-Target Aligned Reads	≥ 90%
Suggested Sequencing Coverage	100X per amplicon

Streamlined Targeted Sequencing Workflow

The AgriType 2.0 genotyping solution was developed to streamline library preparation workflow, increase throughput, and facilitate automation of either 96-well or 384-well workflow. The multiplex PCR-based protocol can be completed in a range of 3-5 hours, with 25 minutes of hands-on time, via a three-step process with minimal tube-to-tube transfers. Libraries are pooled post-indexing PCR for a simplified bead purification step. This simple workflow allows easy integration onto all major automation platforms.



AgriType® 2.0 Target Enrichment and Library Preparation

3-5 hours of total assay time, 20-25 minutes of hands-on time

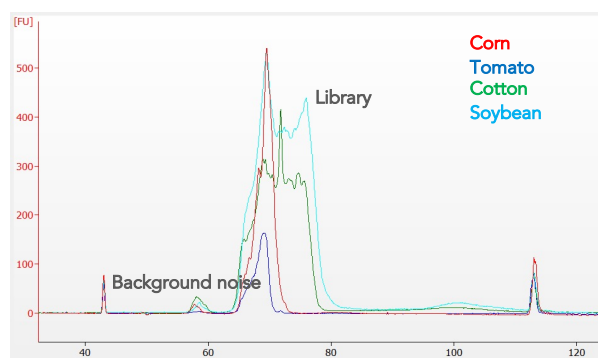
AgriType® 2.0 Targeted Genotyping by Sequencing Solution

High-Quality AgriType® NGS Panels for All Panel Sizes

AgriType panels at 100X average amplicon coverage show consistently high sequencing metrics across various crop types and panel sizes for cost effective sequencing. Using extracted DNA from leaf and seed samples from distinct crops, libraries were prepared and sequenced at 100X coverage on an Illumina MiSeq. The table below shows uniformity, on-target, and mean call rates for a few crops.

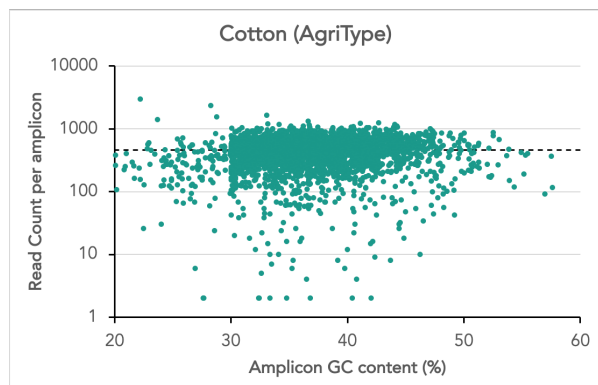
Organism	Panel Size	Uniformity (0.2x Mean Coverage)	% On-Target Rate	Mean Call Rate
Tomato	436	96%	99%	98%
Cotton	2550	95%	92%	97%
Soybean	6808	90%	92%	95%

As shown in the trace below, the negligible background noise across different sample types attributes to high-quality and clean sequencing performance.



High Coverage Uniformity Reduces Dropouts and Saves Costs

Paragon Designer combined with AgriType Chemistry offers uniform amplification of hundreds to thousands of targets with high consistency, low GC bias, and minimal dropouts.



Increased Sample Multiplexing for Higher Throughput

High uniformity, call rates, and on-target metrics allow efficient use of sequencing space. Below is a representative table of the maximum number of samples accommodated in an associated Illumina sequencer with 100X average amplicon coverage with 2x150bp length. Paragon Genomics offers 2688 Illumina and 384 Ion Torrent index combinations to support high-throughput workflows. Multi-lane, or multi-chip workflows can further increase throughput and reduce sequencing time.

Panel Size	Sequencer, Kit Type	Samples Per Run
1000	MiSeq (v2 micro v2)	40, 150
	MiniSeq (Mid, High-output)	80, 250
	NextSeq (Mid, High-output)	1300, 4000
	NovaSeq (SP, S1)	8000, 16000
2500	MiSeq (v2 micro, v2, v3)	16, 60, 100
	MiniSeq (Mid, High-output)	32, 100
	NextSeq (Mid, High-output)	520, 1600
	NovaSeq (SP, S1)	3200, 6400
5000	MiSeq (v2 micro, v2)	8, 30
	MiniSeq (Mid, High-output)	16, 50
	NextSeq (Mid, High-output)	260, 800
	NovaSeq (SP, S1)	1600, 3200

Scalable Panel Content that Can Evolve to Meet New Challenges

AgriType custom NGS panels can be designed to multiplex up to 7000+ amplicons per assay. New targets can be easily added without sacrificing performance, allowing your molecular breeding assays to evolve and stay current. Our superior primer design ensures that targets, including those in difficult regions, are successfully amplified to generate maximum coverage, minimizing assay failure due to dropouts of the desired targets.

Pricing

For pricing information regarding your high-throughput genotyping projects, please enquire for a free consultation with our specialists at sales@paragongenomics.com or 415-855-0957.

Learn More

To learn more about the AgriType® Targeted Genotyping by Sequencing Solution, visit <https://www.paragongenomics.com/applications/molecular-breeding-genotyping-by-sequencing/>

Paragon Genomics, Inc. | 3521 Investment Blvd Suite 1, Hayward CA 94545, USA | +1.650.822.7545
www.paragongenomics.com | techsupport@paragongenomics.com

© 2022 Paragon Genomics, Inc. All rights reserved. All trademarks are the property of Paragon Genomics, Inc. or their respective owners.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

2 | PS4001-01

