

AgriGenomics

AgriType® Auto

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 Assay**
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 7,000 relevant amplicons 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 accurate call rates, excellent uniformity and on-target rates for efficient use of sequencing reads

The AgriType® Auto Targeted Genotyping by Sequencing Solution is designed and developed to address large-scale agrigenomics projects. The AgriType solution is powered by our advanced primer design algorithm and an efficient ultra-high multiplex PCR-based target enrichment chemistry. AgriType products provide a cost-effective solution for constructing NGS libraries to quickly and efficiently screen hundreds to thousands of markers of interest, thereby accelerating 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 Auto Custom Targeted Genotyping by Sequencing Solution Specifications for Sequencing Platforms

| Parameter | Specification |
|---|--|
| Enrichment Method | Multiplex PCR |
| Platform | Illumina®, Ion Torrent™ |
| Number of Amplicons | 7 – 7,000 amplicons per pool with novel SNP discovery capabilities |
| Amplicon Size | Flexible, custom to application |
| Number of Primer Pools | Flexible (1 pool recommended) |
| Input DNA Requirement | 50 – 200 ng |
| Sample Types | Extracted DNA from seeds, leaves, tissue, degraded DNA |
| Total Library Preparation Time | 3 – 5 hours (depending on panel size) |
| Hands-on time | 20 – 25 minutes |
| Mean Genotyping Call Rate | ≥ 90% |
| Coverage Uniformity (targets with > 0.2X mean coverage) | ≥ 90% |
| On-Target Rate | ≥ 90% |
| Suggested Sequencing Coverage | 100X per amplicon |

Streamlined Targeted Sequencing Workflow

The AgriType Auto genotyping solution was developed to streamline the library preparation workflow, increase throughput, and facilitate automation of both 96-well and 384-well setups. The multiplex PCR-based protocol can be completed in a range of 3 to 5 hours, with just 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 into all major automation platforms.



AgriType Auto Targeted Enrichment and Library Preparation

3 – 5 hours of library prep time, 20 – 25 minutes of hands-on time

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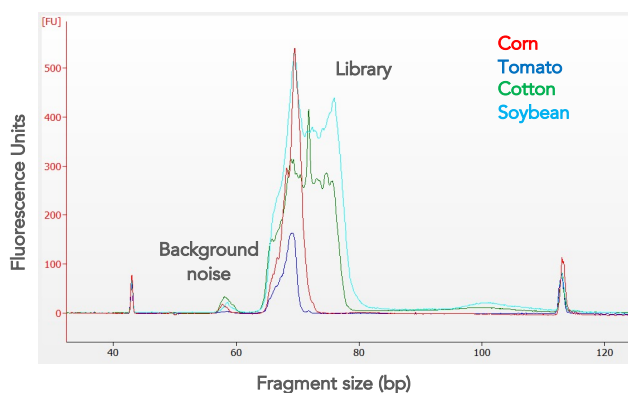
AgriType® Auto Targeted Genotyping by Sequencing Solution

High-Quality AgriType NGS Panels for All Panel Sizes

AgriType panels show consistently robust 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 selected crops.

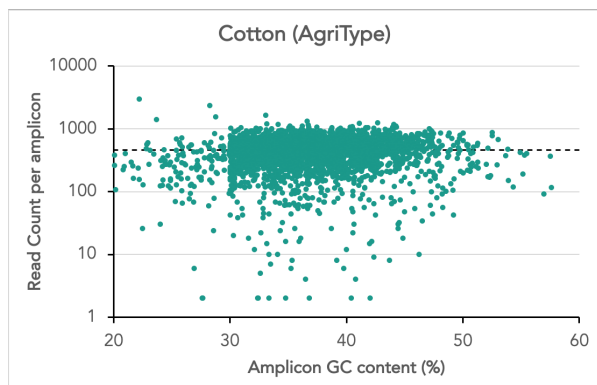
| Organism | Panel Size (Amplicon count) | Uniformity (0.2x Mean Coverage) | % On-Target Rate | Mean Call Rate |
|----------|-----------------------------|---------------------------------|------------------|----------------|
| Tomato | 400 | 98% | 100% | 99% |
| Cotton | 2500 | 96% | 91% | 98% |
| Soybean | 7000 | 87% | 94% | 93% |

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



High Coverage Uniformity Reduces Dropouts and Saves Costs

Paragon Designer combined with AgriType Auto 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 and 2x150bp read length. Paragon Genomics offers up to 2,688 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) | 1,300 / 4,000 |
| | NovaSeq (SP / S1) | 8,000 / 16,000 |
| 2500 | MiSeq (v2 micro / v2 / v3) | 16 / 60 / 100 |
| | MiniSeq (Mid / High-output) | 32 / 100 |
| | NextSeq (Mid / High-output) | 520 / 1,600 |
| 5000 | NovaSeq (SP / S1) | 3,200 / 6,400 |
| | MiSeq (v2 micro / v2) | 8 / 30 |
| | MiniSeq (Mid / High-output) | 16 / 50 |
| | NextSeq (Mid / High-output) | 260 / 800 |
| | NovaSeq (SP / S1) | 1,600 / 3,200 <small>*can be doubled with 2 flow cells</small> |

Scalable Panel Content that Can Evolve to Meet New Needs

AgriType Auto custom NGS panels can be designed to multiplex up to 7,000 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 expected 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 other products in the AgriType Portfolio, such as AgriType Prime for higher sensitivity, higher multiplexing, and crude lysate compatibility, visit <https://www.paragongenomics.com/applications/molecular-breeding-genotyping-by-sequencing/>

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