#### Agrigenomics

## AgriType<sup>®</sup> Targeted NGS Product Portfolio

#### Highlights

- Specialized Solutions for Unique Applications AgriType<sup>®</sup> Prime and Auto are tailored to specific parameters to meet the range of requirements from various agrigenomic applications.
- Fast Custom Turnaround Time Custom designs specific to your markers of interest ready in 1-2 weeks and delivered to your lab in 4-6 weeks.
- Streamlined, Automation-Friendly Workflow Generate sequence-ready libraries in 3 hours using a simple, three-step workflow. AgriType Auto is compatible with 384 well plates for ultra-high throughput needs.
- High-Quality, Reproducible Results Prepare high-quality NGS libraries with high and accurate genotyping call rates for cost-efficient sequencing.

#### AgriType Targeted Solutions: Prime & Auto Specifications

Parameter	Prime	Auto	
Enrichment Method	Multiplex PCR		
Target Multiplexing Sample	20,000+	7,000+	
DNA Input Requirement	> 1ng	>50ng	
DNA Input Quality	Low quality and crude lysate compatible	High quality purified DNA	
Total Assay Time	3-5 hours (depending on panel size)		
Automation compatibility	96 well low to medium throughput	96 well & 384 well high throughput	
Sequencing and Data	Uniform amplification, High call rates, Cost-efficient sequencing		
Sequencing Compatibility	Illumina <sup>®</sup> , Ion Torrent™		
Suggested Sequencing Coverage	100X per amplicon		
Workflow	*See next page for details		

# Scalable and Flexible Targeted Genotyping by Sequencing Solutions

Next-generation sequencing (NGS) technologies have accelerated research efforts in the fields of environmental, plant and animal agrigenomics. These applications include genotyping, genomic prediction, marker-assisted selection and breeding, species authentication, *de novo* sequencing and variant identification. The AgriType product portfolio was specifically designed to address these agricultural research needs. Powered by a proprietary primer design algorithm, an ultra-high multiplex PCR-based target enrichment chemistry, and a proprietary background cleaning technology, AgriType products offer cost-effective and highly scalable solutions to construct NGS libraries for quick and efficient screening of up to tens of thousands of markers and to deliver accurate and high call rates.

#### AgriType Prime and AgriType Auto Workflows

To overcome the drawbacks of traditional amplicon-based library preparation NGS methods, we developed a portfolio of high-performing amplicon-based library preparation solutions powered by Paragon Genomics' patented AgriType technology. The AgriType portfolio offers two products, the *AgriType Prime* and the *AgriType Auto*.

*AgriType Prime* technology utilizes a proprietary background cleaning chemistry that removes non-specific PCR products to produce the cleanest and highest quality NGS libraries for superior sequencing data. This product is tailored to research and discovery-based applications that require the highest multiplexing, use the lowest quality and quantity samples, and need highly sensitive detection. AgriType Prime offers a comprehensive solution to tackle the most difficult genomic questions in agricultural biology.

*AgriType Auto* has been adapted specifically for ultra-high throughput genotyping applications and developed to be compatible with standard liquid handling systems. Though its sample input and panel size range differ from AgriType Prime, AgriType Auto features 384-well compatible, further streamlined automation-friendly workflow that is perfect for traditional high-volume genotyping projects, like HTP molecular breeding. Combined with the ability to batch up to 6,144 samples with Paragon's combinatorial dual-indexes in a single sequencing lane, AgriType Auto offers an elegantly simple, streamlined solution for your high-throughput needs.



#### Streamlined Workflow of the AgriType Portfolio

The AgriType Auto workflow replaces Prime's background cleaning step (step two) with an in-plate dilution step and eliminates the in-plate magnetic bead purification steps for more streamlined automation.

AgriType Prime	AgriType Auto
1. Multiplex PCR	1. Multiplex PCR
Magnetic Bead Purification	
2. Background Cleaning	2. In-Plate Dilution
Target Amplicons + Non-specific Products	CONCORD CONCO
Magnetic Bead Purification	
3. Indexing PCR	3. Indexing PCR
Magnetic Bead Purification	Pooled Bead Purification
Sample Barcodes	Sample Barcodes

AgriType Auto's final pooled bead purification step after indexing PCR can be configured to the user's preference. 384 well plates after indexing PCR can be pooled equi-volume to 96well configurations, 8 strip tubes, or even pooled into a single tube. This enables minimal sample handling and reduced reagent usage.

Both the AgriType Prime and Auto workflows are either single tube or single plate workflows\*, which reduces consumable cost and loss of material. The minimal material transfer and reduced complexity of the workflow leads to improved reproducibility.

\*Three-step workflow is all contained in a single plate; exact number of plates for AgriType Auto is contingent upon configuration of final bead purification.

#### AgriType Prime: High-Quality Data with Low Quality Input

Consistent genotyping performance and high marker call rates are key to accurate assessment and selection for agricultural production. For applications requiring high sensitivity such as for discovery or *de novo* research, AgriType Prime offers a robust solution, compatible with all sample types, including crude lysates. The technology can interrogate up to 20,000+ amplicons in a single assay and is powered by Paragon's proprietary background cleaning technology that removes non-specific PCR products, resulting in best in-class target enrichment and efficient use of sequencing reads.

Corn, cotton, and soybean panels ranging from 250 to 6800 amplicons were used to generate AgriType Prime target-enriched NGS libraries with crude lysate input. Sequencing results indicate high uniformity, mapping, and on-target rates.

Organism	Corn (n=3)	Cotton (n=3)	Soybean (n= 16)
Panel Size	250	2500	6800
Uniformity (0.2X Mean Coverage)	98.8 ± 0.6%	92.8 ± 2%	90.7 ± 1.4%
% Mapped Reads	99.6 ± 0.3%	99.8 ± 0%	99.5± 0.5%
% On-Target Rate	98.6 ± 0.3%	85.3 ± 0.9%	87.9 ± 4.0%

#### High Concordance with Whole Genome Sequencing Data

A 2,000 amplicon panel was used to test the concordance of AgriType Prime solution. Eight samples that were previously genotyped using whole genome sequencing (WGS) were used to construct AgriType Prime target-enriched NGS libraries and the data compared. Of the 2,000 markers tested, 99.8% showed high concordance with WGS results, and perfect concordance was obtained at the sample level.

Threshold	% Markers	% Samples
>95% Concordant	99.8%	100%



#### AgriType Auto: High Genotyping Call Rates with Streamlined Workflow

AgriType Auto custom NGS panels can be designed to multiplex up to 7,000+ amplicons per tube. The Auto solution showcases a low volume, 384 well-compatible workflow with minimal hands-on time (approximately 25 minutes). Like Prime, the panel is based on our superior primer design algorithm. This 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.

Tomato, cotton, and melon panels were used to generate AgriType Auto Targeted NGS libraries with purified DNA. Sequencing results demonstrate the assay's robust metrics and accurate call rates.

Crop	Tomato	Cotton	Melon
Panel Size	440	2,500	500
Mean Uniformity (0.2X)	96.4%	94.7%	97.0%
Mean On-target rate	100%	92.2%	99.5%
Genotype Call Rate	98.5%	96.3%	N/A

\*Additional data on different crops can be found in the AgriType Auto specific product sheet.

Paragon Genomics offers up to 6,144 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 turnaround time.

### Flexible Design, Fast Turnaround Time, and Excellent Support

Use AgriType solutions to accelerate your large-scale molecular breeding programs. Custom designs are created to meet each project's specific needs. AgriType panels enable revision and updates to the design by simple spike-ins of new targets. As agricultural needs and our understanding of trait markers are ever-dynamic, new targets allow your molecular breeding assays to evolve and stay current. Our PhD-level expert scientists are here to provide speedy and comprehensive support throughout the design, ordering, and technology adoption process, including excellent after-sales technical support. Depending on ordering size and complexity, AgriType custom NGS panels are designed, manufactured, and delivered in just a few weeks. The process is simple. First, obtain a quote based on your volume and marker count. Second, define your targets. Third, review and approve the design. Once the design is approved and an order is placed, we will deliver your custom AgriType solution in 4 to 6 weeks.

#### Accessories Ordering Information

CleanPlex Indexed PCR Primers and CleanMag<sup>®</sup> Magnetic Beads are ordered separately to complete the workflow. For indexing options on other sequencers, and additional product configurations please visit www.paragongenomics.com/store/

Product	SKU
CleanPlex® Plated Dual-Indexed PCR Primers for Illumina® Set A, B,C, D 96-well (12 x 8 indexes, 96 reactions)	716033 716034 716035 716036
Plated Dual-Indexed PCR Primers for Illumina® Plates 1-64, indexes 1-6144 (6144 reaction)	717001
CleanMag Magnetic Beads (20 mL)	718005
CleanMag Magnetic Beads (60 mL)	718003
CleanMag Magnetic Beads (450 mL)	718004

#### Pricing

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

#### Learn More

To learn more about the AgriType Portfolio and gain access to additional literature, please visit:

https://www.paragongenomics.com/applications/molecular-breedi ng-genotyping-by-sequencing/

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