



# AgriType Auto NGS Kit

## User Guide

This user guide is for the following products for sequencing on Illumina® and Ion Torrent™ NGS platforms:

- AgriType Auto Custom Panels

Please see the product documents page for appropriate user guides for each version of the AgriType Portfolio.

Get the latest user guide at: [www.paragongenomics.com/product\\_documents/](http://www.paragongenomics.com/product_documents/)

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## Revision History

Document	Date	Description of Change
UG6001-01	February 2022	<ul style="list-style-type: none"><li>Initial version</li></ul>
UG6001-02	May 2023	<ul style="list-style-type: none"><li>Revised for new product branding and official release of AgriType Auto.</li></ul>

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# Overview

## Product Information

AgriType® Auto Panels are a fast, robust, and versatile agrigenomics Genotyping by Sequencing (GBS) solution with next-generation sequencing (NGS) on Illumina® and Ion Torrent™ sequencing platforms. This target enrichment and library preparation solution allows simultaneous interrogation of up to 7,000 markers at a time for high call rate while minimizing total sequencing requirements with its highly uniform coverage of your targets of interest.

AgriType Auto Panels generate highly accurate data while maintaining easy and streamlined workflow for high throughput processing. AgriType Auto panels are custom designed to target user-defined genomic regions of interest. To accommodate high throughput applications and laboratories, this library preparation kit utilizes a plate-based approach for easy sample handling that can be easily automated using typical liquid handling systems.

AgriType Auto Panels are powered by Paragon Genomics' proprietary technology, which combines an advanced primer design algorithm to generate best-in-class target enrichment performance and efficient use of sequencing reads. More than 7,000 amplicons can be multiplexed per primer pool with the sensitivity to discover new SNPs in these samples.

AgriType Auto Panels feature a fast and simple workflow that can be completed in 3-5 hours from input DNA to indexed and amplified NGS libraries. The workflow involves just 2 PCR steps with an intermediate dilution in between, followed by a pooled library purification using magnetic beads. Tube-to-tube transfer is minimized throughout the protocol to preserve DNA material and prevent handling errors and sample mix-up.

The first step of the AgriType Auto workflow is a multiplex PCR reaction that uses target-specific primers to amplify targets of interest. The second step is a dilution reaction optimized to reduce non-specific amplification. The last step is a PCR reaction that uses CleanPlex® Indexed PCR Primers to amplify and add sample-level indexes to the NGS libraries. CleanMag® Magnetic Beads are recommended for library purification. See the Workflow section for a detailed depiction of the AgriType Auto workflow.



### AgriType® Auto Target Enrichment and Library Preparation

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

## Applications

The AgriType Auto Panels can be used to detect genomic markers in a wide variety of agrigenomic applications like marker assisted selection, genomic selection, quantitative trait locus screening, trait mapping, genetic modification screening & QC, and more. AgriType Auto Custom NGS Panels can be built from 50 to 7,000 amplicons. Each amplicon may also include more than one marker. Amplicons can be designed for various lengths to accommodate different sample types and the needs of specific applications.

The table below shows some of the organisms, sample types, and applications that users have studied using AgriType Auto Panels for early access. This is not meant to be an exhaustive or restrictive list.

Organisms	Sample Types	Applications
<ul style="list-style-type: none"> <li>• Livestock such as Bovine, Porcine, Poultry, etc.</li> <li>• Plant/Crops such as Soybean, Corn, Cotton, Tomatoes, etc</li> </ul>	<ul style="list-style-type: none"> <li>• Genomic DNA from animal tissue, seeds, leaves, etc.</li> <li>• Degraded DNA</li> </ul>	<ul style="list-style-type: none"> <li>• Genotyping by Sequencing</li> <li>• Variant detection and discovery</li> <li>• Species identification</li> <li>• GM Marker screening</li> </ul>

## Other Applications

For other targeted sequencing applications, Paragon Genomics also offer a while variety of both ready to use panel and custom panels for infectious disease, oncology, drug discovery, and more.

Visit our applications page for additional information: <https://www.paragongenomics.com/applications/>

## Compatible Sequencing Instruments

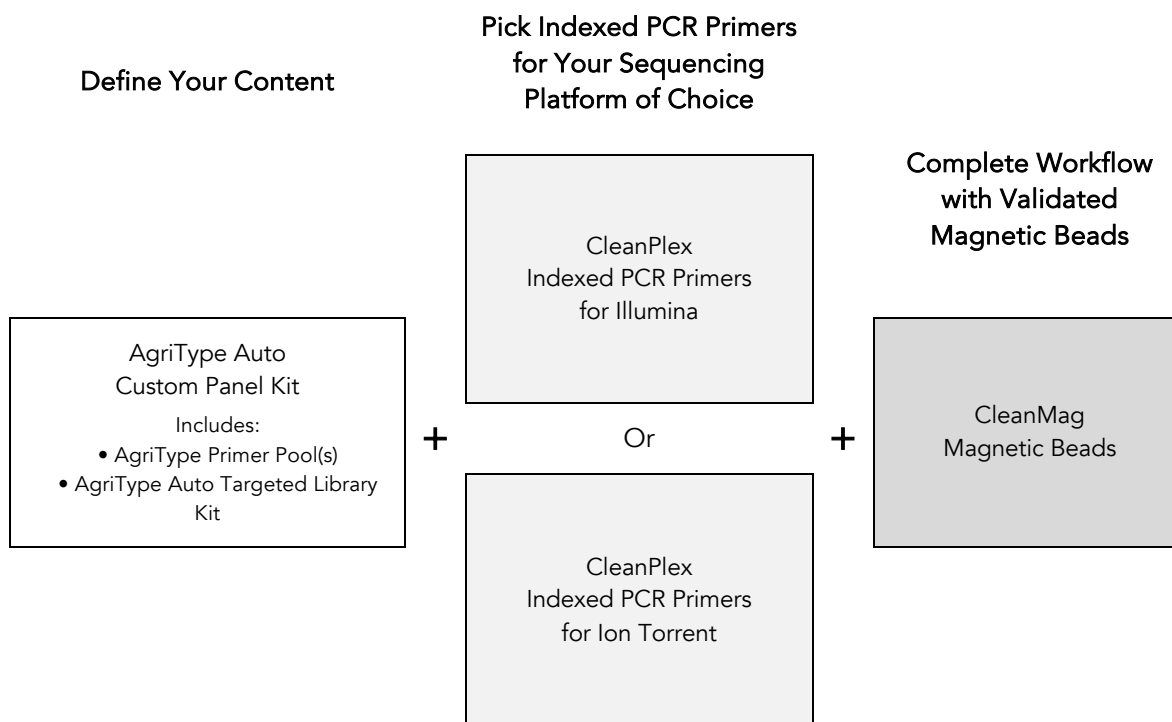
CleanPlex Indexed PCR Primers for Illumina are used to generate AgriType Auto target-enriched NGS libraries that are compatible with Illumina sequencing platforms, including NovaSeq® 5000/6000 Systems, HiSeq 3000/4000 Systems, HiSeq 2500 System, NextSeq® Series, MiSeq® System, MiniSeq® System, and iSeq® System.

CleanPlex Indexed PCR Primers for Ion Torrent are used to generate AgriType Auto target-enriched NGS libraries that are compatible with all Ion Torrent sequencing platforms, including Ion PGM System, Ion Proton System, and Ion GeneStudio S5 Series.

## Kit Contents

The protocol outlined in the AgriType Auto Panel User Guide requires the following components, which need to be ordered separately:

- AgriType Auto Custom Panel Kit
- CleanPlex Indexed PCR Primers
- CleanMag Magnetic Beads (or equivalent)



AgriType Auto Panel — Kit Contents, Store at -20°C

Panel	SKU	Size (Reactions)	Components	
			Primer Pool	AgriType Auto Targeted Library Kit
AgriType Auto Custom Panel Kits	937110	96	1	1-pool, 96 rxns
	937111	384	1	1-pool, 384 rxns
	937112	768	1	1-pool, 768 rxns
	937113	4608	1	1-pool, 4608 rxns
	937114	9216	1	1-pool, 9216 rxns

An AgriType Auto Targeted Library Kit is included in every AgriType Auto Custom Panel Kit. Please note that the AgriType Auto Targeted Library Kit is **not** sold separately.

AgriType Auto Custom Panel + Library Kit — Kit Contents, Store at –20°C (not sold separately)							
Configuration			1-Pool				
Size			96 Rxns	384 Rxns	768 Rxns	4608 Rxns	9216 Rxns
Component	Cap Color	SKU	937110	937111	937112	937113	937114
5X mPCR Mix	Green		192 µl	768 ul	1536 ul	9.3mL	18.5mL
5X 2nd PCR Mix	Blue		480 µl	1.92mL	3.84mL	23.04mL	46.08mL
TE buffer	Clear		3.4mL	20mL	40mL	200mL	300mL



## Required Materials and Equipment Not Included

- CleanPlex Indexed PCR Primers (visit [www.paragongenomics.com/store](http://www.paragongenomics.com/store) for more indexing options)

CleanPlex Dual-Indexed PCR Primers for Illumina — Store at –20°C			
	SKU	Size (Reactions)	Format
CleanPlex Dual-Indexed PCR Primers for Illumina Set T	716021	9	Random 3 i7 indexes + 3 i5 indexes (9 tubes)
CleanPlex Dual-Indexed PCR Primers for Illumina Set A	716006	96	12 i7 indexes +
	716017	384	8 i5 indexes (20 tubes)
CleanPlex Dual-Indexed PCR Primers for Illumina Set B	716018	96	12 i7 indexes +
	716019	384	8 i5 indexes (20 tubes)
CleanPlex Plated Dual-Indexed PCR Primers for Illumina Set A	716033	96	Prepooled i5 and i7 indexes (96-well plate)
	716045	384	
CleanPlex Plated Dual-Indexed PCR Primers for Illumina Set B	716034	96	Prepooled i5 and i7 indexes (96-well plate)
	716046	384	
CleanPlex Plated Dual-Indexed PCR Primers for Illumina Set C	716035	96	Prepooled i5 and i7 indexes (96-well plate)
	716047	384	
CleanPlex Plated Dual-Indexed PCR Primers for Illumina Set D	716036	96	Prepooled i5 and i7 indexes (96-well plate)
	716048	384	
CleanPlex Plated Dual-Indexed PCR Primers for Illumina Plate 1-64	717000	96	Prepooled i5 and i7 indexes (96-well plate)

CleanPlex Single-Indexed PCR Primers for Ion Torrent — Store at –20°C			
	SKU	Size (Reactions)	Format
CleanPlex® Single-Indexed PCR Primers v2 for Ion Torrent Set T	716016	8	8 tubes
CleanPlex® Plated Single-Indexed PCR Primers v2 for Ion Torrent Set C	716053	192	96 indexes, (96-well plate)
CleanPlex® Plated Single-Indexed PCR Primers v2 for Ion Torrent Set D	716054	192	96 indexes (96-well plate)
CleanPlex® Plated Single-Indexed PCR Primers v2 for Ion Torrent Set E	716055	192	96 indexes (96-well plate)
CleanPlex® Plated Single-Indexed PCR Primers v2 for Ion Torrent Set F	716056	192	96 indexes (96-well plate)

- CleanMag Magnetic Beads, or equivalent — eg. Agencourt™ AMPure™ XP Kit (Beckman Coulter, A63880, A63881, or A63882)

CleanMag Magnetic Beads — Store at 2–8°C			
	SKU	Size (Volume)	Reactions 1-pool panels
CleanMag Magnetic Beads, 1 ml	718001	1 ml	<3 3 rxns
CleanMag Magnetic Beads, 5 ml	718002	5 ml	<166 rxns
CleanMag Magnetic Beads, 20 ml	718005	20 ml	<666 rxns
CleanMag Magnetic Beads, 60 ml	718003	60 ml	< 2000 rxns
CleanMag Magnetic Beads, 450 ml	718004	450 ml	< 15000 rxns

- For PCR tubes or strips, CleanMag Magnetic Rack (SKU 719001) or equivalent magnetic racks designed for PCR strip workflows
- For 96-well PCR plates, CleanMag Magnetic Plate (SKU 719002) or equivalent magnetic plates designed for PCR plate workflows. We recommend twin.tec PCR Plate 96, semi-skirted, colorless (Catalog #: 951020303).

CleanMag Magnetic Rack & Plate		
	SKU	Compatibility
CleanMag Magnetic Rack	719001	2 rows of 12 tubes each for PCR strip tubes
CleanMag Magnetic Plate	719002	96 well PCR plates, full or semi- skirted compatible

\* For Magnetic Racks of 1.5mL or higher volume tubes for batch purification, please use industry standard magnetic racks such as the ThermoFisher MagJET Separation Rack (cat no: MR02)

- 70% ethanol (freshly prepared)
- TE Buffer
- Nuclease-free water
- Nuclease-free, low bind, or 96/384-well PCR plate with adhesive film
- Nuclease- free, low bind, 1.5 ml or larger tube for pooled magnetic bead purification based on total sample count
- Pipettors and low-retention pipette tips
- Thermal cycler
- Mini-centrifuge, 96/384-well plate centrifuge
- Qubit® Fluorometer and dsDNA HS (high sensitivity) Assay Kit, or equivalent
- Optional: Agilent® 2100 Bioanalyzer® Instrument and Agilent High Sensitivity DNA Kit, or equivalent

## Optional Materials and Equipment Not Included

- For 384-well PCR plates, designed for PCR plate workflows, we recommend any of the following:
  - VWR PCR Plates with Barcode for 384 Thermocyclers (Catalog #: 89218-288)
  - twin.tec PCR Plate 384 colorless (Catalog #: 951020702)
  - BIO-RAD Hard-Shell PCR Plates 384-well (Catalog #: HSP3801)
- PCR plate Seal, we recommend Axygen Aluminum Sealing Film (REF: PCR-AS-600)

## Storage, Handling, and Usage

AgriType Auto Kits and CleanPlex Indexed PCR Primers are shipped on blue ice (ice packs). Upon receipt, immediately store AgriType Auto Kits and CleanPlex Indexed PCR Primers at -20°C in a constant-temperature freezer. Do not store in a freezer with auto-defrost or frost-free features. Do not store at -80°C.

CleanMag Magnetic Bead solution is shipped at room temperature or on blue ice (ice packs). **Magnetic beads may be frozen during transit.** This single freeze thaw will not affect the performance of the beads. Upon receipt, immediately store CleanMag Magnetic Beads at 2°C to 8°C in a constant-temperature refrigerator. Do not freeze. Do not store at -20°C with other reagents. Always ensure that all frozen components are fully thawed and have been vortexed and spun down to bring all liquids to the bottom of the tubes prior to use.

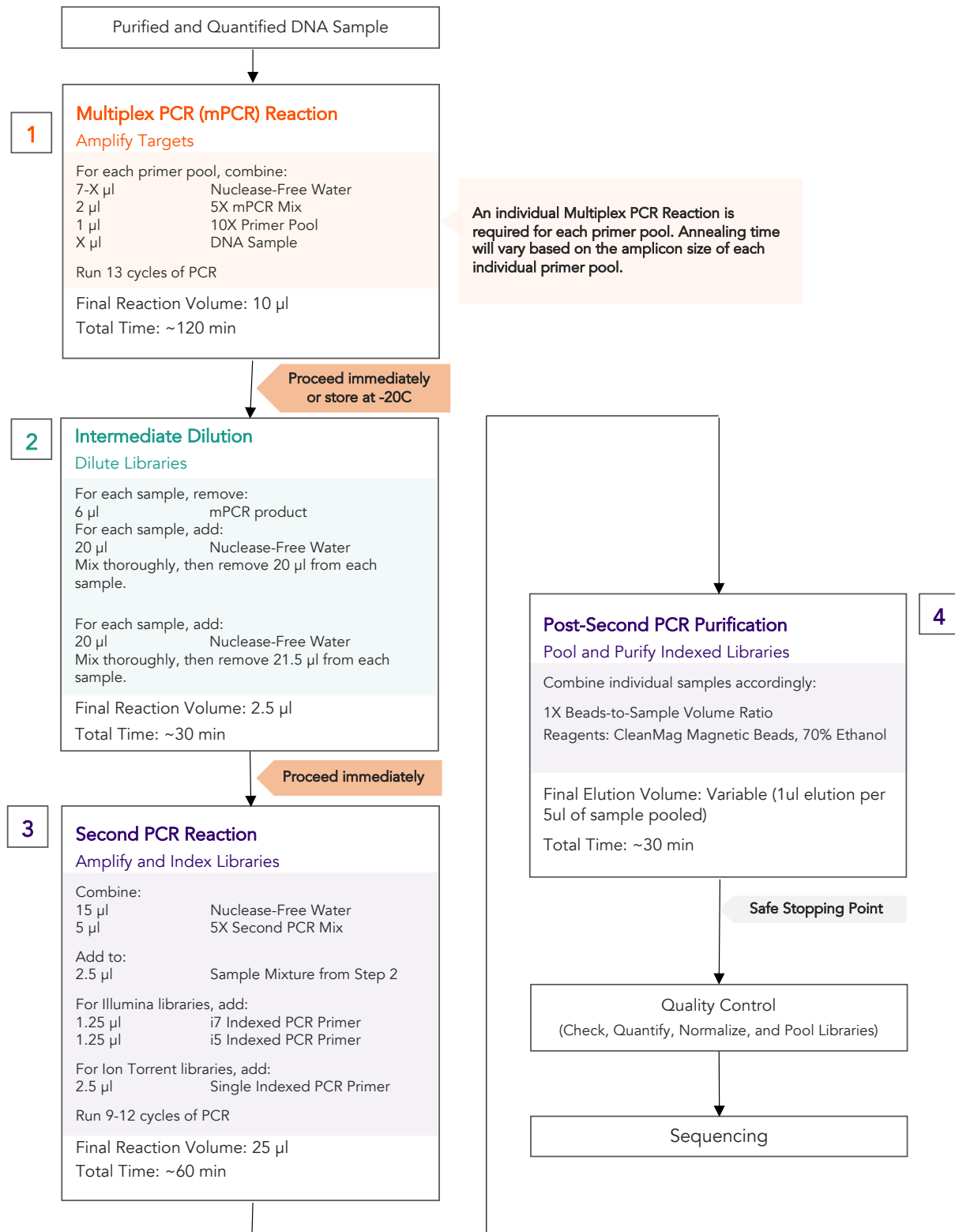
The components containing enzymes (5X mPCR Mix, and 5X 2nd PCR Mix) are specially formulated for storage at -20°C without freezing to avoid freeze-thaw cycles to retain their full activity. Please avoid storing these components below -23°C.

AgriType Auto Kits are developed, designed and sold exclusively for research use only. None of the products or their individual components have been tested for use in diagnostic procedures.

For hazard information, please refer to the Safety Data Sheet (SDS), which is available upon request.

## Workflow

The following diagram illustrates AgriType Auto Panel's targeted NGS library preparation workflow.



# Protocol

## Best Practices

- When using the kit after prolonged storage, briefly vortex and spin the tubes in the kit to bring the liquid to the bottom of the tubes. Store the tubes containing enzymes (5X mPCR Mix and 2nd PCR Mix) on ice during their respective procedures. All other components, including primer pools, may be thawed at room temperature, mixed thoroughly by vortexing and spun-down before use.
- Use good laboratory practices to minimize cross-contamination. If possible, perform PCR setup in an isolated area or room to minimize cross-contamination between samples, multiplex PCR (mPCR) primers, or indexed PCR primers. Always change pipette tips between samples and change gloves frequently. Clean all workstations and tools with 10% bleach followed by water, then alcohol at the end of each work day.
- Use a calibrated PCR thermal cycler as specified by the manufacturer's user guide. Validated thermal cyclers include Bio-Rad C1000, Eppendorf Mastercycler series, and Applied Biosystems GeneAmp PCR System 9700. Generally, a thermal cycler set with the highest ramp speed, such as 5°C/second and higher, is not recommended. For thermal cyclers with adjustable ramp speed, we recommend 3°C/second up and 2°C/second down speed, or use the default setting (no ramp adjustment).
- To ensure accurate assembly of reactions, withdraw viscous solution (such as 5X mPCR Mix, and 5X 2nd PCR Mix) slowly from containers and dispense it slowly into the reaction mixtures. A good practice is to remove excess from outside of tip and rinse the tip by pipetting up and down several times after dispensing viscous solutions into aqueous mixture. Thoroughly pipette or vortex mix each assembly to ensure solutions are homogeneous prior to PCR and incubations. Prepare master mixes and aliquot reaction mixtures into plated wells carefully. Remember to briefly spin the 96/ 384-well PCR plate after mixing.
- Always keep working solutions and PCR products on ice until needed.
- The protocol is designed to minimize the number of well-to-well transfers in order to avoid or reduce sample loss. For a single-pool AgriType Auto Panel, the entire protocol is performed following a single-tube workflow, with no tube-to-tube transfers.
- While working with 96/384-well PCR plates, take extra care to ensure thorough mixing of all samples and proper sealing to avoid cross contamination between samples.
- The final magnetic bead purification step should be performed carefully to minimize residual supernatant and ethanol washes, and to minimize bead loss. Using a strong magnetic rack or plate specifically designed for handling of tubes is critical for a successful bead purification.
- Assign sample indexes to specific samples before starting the protocol.
- Always prepare a master mix of reagents when working with multiplex reactions. Prepare appropriate volume of excess of each master mix to allow for pipetting losses.

## Input DNA / Panel Concentration Requirements

- DNA input typically ranges from 10-200ng per reaction. This input is dependent on both the genomic size of the organism and the targeted panel size. Smaller genomes have more template copies per ng of DNA, and therefore require less input material. Larger panels require more input material to minimize non-specific products. A titration is recommended to determine the best input for your genome and panel. Please note that this product is not compatible with crude lysate material. For crude lysate, please refer to AgriType Prime chemistry or contact our technical support team for alternative options at [support@paragongenomics.com](mailto:support@paragongenomics.com).

Working range is established by a variety of factors; please refer to the table below only as a general guide.

Genome size (bp)	Example Crop	Suggested approximate input (ng)
0.5 billion	Melon	~>20ng
1 billion	Soybean	~>50ng
2.5 billion	Corn, Cotton	~>75ng

- The maximum volume of DNA input per Multiplex PCR Reaction is 7  $\mu$ l. Inputs that are outside of the recommended input range may still work, but could produce a lower quality, but acceptable library.
- For the most uniform representation of samples in sequencing, normalize sample input for each sequencing batch. Fluorometric methods such as Qubit dsDNA HS Assay Kit (Thermo Fisher, Cat. No. Q32851 or Q32854) can be used. UV spectrophotometry methods (e.g. NanoDrop™ spectrophotometer) are not recommended because it can significantly overestimate the DNA concentration. However, when samples are within the working range of input, per sample DNA input normalization may not be necessary with sufficient sequencing depth.
- After DNA extraction, keep material at high concentration for prolonged storage, since DNA is less stable in solution at lower concentrations, and to ensure sufficient input per sample. As a reference, the data presented below were generated with DNA that had been extracted with a commercially available kit from Qiagen.
- Avoid freeze-thawing dilute DNA samples when possible, and measure sample concentrations immediately prior to use to avoid inaccurate sample input.
- Compatible DNA buffering systems are Tris HCl or TE.

## Set up and Preparations

- When working with in high throughput setting, normalizing each individual DNA sample may not be practical. However, for most genotyping applications, a starting input that falls within the range of 50-200ng will be sufficient.
- Bring Magnetic bead solution to room temperature for at least 30 minutes before use. Put back in 2-8 °C storage at the end of the day.

- Freshly prepare the appropriate amount of volume for 70% Ethanol by referring to Step 4 below in the protocol. Do not top off one liquid with the other in a volumetric container because the volumetric ratio will not be accurate. When water and ethanol are mixed, the final volume will be less than the sum of individual volumes. Lower concentration of ethanol will affect final library yield.
- For all samples to be sequenced together, assign a specific index (CleanPlex Single-Indexed PCR Primers for Ion Torrent) or index combination (CleanPlex Dual-Indexed PCR Primers for Illumina) to each sample.
- Note the safe stopping point is after 2<sup>nd</sup> PCR purification. Stopping during the workflow apart from the explicit “safe stopping points” may reduce yields outside of -20C storage. Please plan accordingly.



## 1. Multiplex PCR (mPCR) Reaction

- 1.1 Calculate the sample input volume per reaction based on sample concentration range. Calculate the sample input volume for the sample set and create a mPCR reaction mastermix for ease of processing.
- 1.2 Using an appropriately sized nuclease free tube, prepare the mPCR Reaction Master Mixture without DNA for your total sample number by adding components in the following order **on ice or a cold block**. Vortex to mix thoroughly and aliquot the appropriate volume of mastermix into each well of a 96-well PCR plate or 384-well PCR plate on ice. Then add sample to each plate according to your calculated volume.

mPCR Reaction Mixture		
Reagent	Cap Color	Volume per reaction
Nuclease-Free Water	—	7 – X $\mu$ l
5X mPCR Mix	Green	2 $\mu$ l
10X mPCR Primer Pool	Varies	1 $\mu$ l
DNA Sample	—	X $\mu$ l
<b>Total Volume per reaction</b>		<b>10 <math>\mu</math>l</b>



**Important!** mPCR Mix and mPCR Primer Pool(s) are viscous. Pipette slowly, remove any excess reagent on the outside of the pipette tip, and rinse tip in solution when handling these reagents.

- 1.3 Either pipette up and down to mix thoroughly with at least 75% of total volume and at least 10 times, or by sealing the plate securely with adhesive seal, spinning down, vortexing thoroughly until homogenous. Then spin down briefly to collect the liquid.

**Note:** It is crucial that the reaction mixture is homogeneous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase non-specific product formation.

**Note:** When using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during thermal cycling.

- 1.4 Load the plate in the thermal cycler with a heated lid at 105 °C. Ensure the plate is making complete contact with the thermocycler and run the following thermal cycling protocol to amplify target DNA regions. Use the table below to determine the mPCR thermal cycling conditions for specific AgriType Auto Custom Panels.

mPCR Thermal Cycling Protocol				
Step	Temperature	Time	Ramping*	Cycles
Initial Denaturation	95 °C	10 min	-	-
Denaturation	98 °C	15 sec	3 °C/s	13
Annealing/Extension	60 °C	See table below	2 °C/s	
Hold	4°C	∞	-	-

\* For thermal cyclers without adjustable ramp speed, the default setting can be used if max ramping speed is ≤5°C/S.

**Note:** For AgriType Auto Custom panels, use amplicon number to determine the appropriate annealing/extension times using the table below. *Please refer to the “ampinsert” file of your custom design to determine the amplicon count per pool, or contact Technical Support at support@paragongenomics.com if you have any questions.*

Panel Specific mPCR Thermal Cycling Conditions	
Panel	Annealing/Extension Time
AgriType Auto	5 min for 50–2,000 amplicons
Custom NGS Panels	8 min for 2,000–5,000 amplicons 16 min for 5,000–7,000 amplicons



**Important!** It is highly recommended to immediately proceed to Step 2, Intermediate Dilution. However, samples may be stored on ice at 4°C for up to 1h or at -20°C overnight immediately after cycle is complete. Working outside of these recommendations may result in increased non-specific products.

## 2. Intermediate Dilution

- 2.1 Remove 6 µl of mPCR product from each well.

**Note:** *The extra mPCR product that is not carried over to the next steps may be stored at -20C for 1 day if replicates are desired.*

- 2.2 Working with a cold plate or on ice, add 20 µl of cold Nuclease-Free Water into each well and mix thoroughly by either pipetting up and down with at least 75% of total volume at least 10 times, or by sealing the plate securely with adhesive seal, spinning down, vortexing thoroughly until homogenous. Then spin down briefly to collect the liquid. If possible, adjust the centrifuge to -4C during plate spinning.

**Note:** *Diluting the sample either on ice and with cold water minimize handling or timing associated nonspecific amplification, especially during long dilution processes.*

- 2.3 Remove and discard 20 µl from each reaction to retain 4 µl for each sample.

- 2.4 Dilute each well further on a cold plate or on ice by adding 20 µl of cold Nuclease-Free Water into each well and mix thoroughly by either pipetting up and down with at least 75% of total volume at least 10 times, or by sealing the plate securely with adhesive seal, spinning down, vortexing thoroughly until homogenous. Then spin down briefly to collect the liquid.

- 2.5 Finally, remove 21.5 µl to retain 2.5 µl for each sample for a final dilution of the original mPCR product.

**Note:** *Though a 36X dilution ratio has been explicitly outlined in the steps above, the assay supposes a dilution ratio between 30X-40X. Please contact [support@paragongenomics.com](mailto:support@paragongenomics.com) for additional information specific to your custom panel.*

- 2.6 Proceed to step 3 **immediately** after dilution.

### 3. Second PCR Reaction

**Note:** Remember to assign a specific index or unique combination of dual indexes to each sample before starting this step. Either Combinatorial or Unique Dual-Index primers can be used for any AgriType Auto libraries.

We do not recommend re-using single reaction index plates to minimize cross-contamination. If bulk index plates are used, smaller subplates can be made for batches of sample processing.

- 3.1 Thaw Indexed PCR Primers, vortex or pipette mix thoroughly, then spin briefly to collect the liquid. Prepare Second PCR Reaction Master Mix for your total number of samples without the index primers as detailed in the table below, and add to each diluted sample from Step 2.

Second PCR Reaction Master Mix		
Reagent	Cap Color	Volume per reaction
Nuclease-Free Water	—	15 $\mu$ l
5X 2nd PCR Mix	Blue	5 $\mu$ l
<b>Total Volume per reaction</b>		<b>20 <math>\mu</math>l</b>

- 3.2 Add a unique (combination of) Indexed PCR Primer(s) to each sample. We recommend you add the reagents in the order specified below.

**Note:** If you plan to pool these libraries during the bead purification after this PCR step, please ensure you use unique combinations of indexes.

Second PCR Reaction Mixture		
Reagent	Illumina Volume per reaction	Ion Torrent Volume per reaction
Second PCR Reaction Master Mix	20 $\mu$ l	20 $\mu$ l
Diluted Sample from Step 2	2.5 $\mu$ l	2.5 $\mu$ l
i5 Indexed PCR Primer for Illumina	1.25 $\mu$ l	—
i7 Indexed PCR Primer for Illumina	1.25 $\mu$ l	—
<b>Single-Indexed PCR Primer for Ion Torrent</b>	—	2.5 $\mu$ l
<b>Total Volume per reaction</b>	<b>25 <math>\mu</math>l</b>	<b>25 <math>\mu</math>l</b>

**Note:** For Illumina indexes in plates, the i5 and i7 indexes are already combined. Use 2.5  $\mu$ l of the pooled index per sample. For separate indexes in tubes, use 1.25  $\mu$ l each as shown above.



**Important!** When handling Indexed PCR Primer plates, take extra care when removing the seal, and change pipette tips and gloves as necessary to prevent cross contamination. Avoid touching the opening and inside of the tubes with your hands, pipette channel, or anything non-disposable.

3.3 Either pipette up and down to mix thoroughly with at least 75% of total volume and at least 10 times, or by sealing the plate securely with adhesive seal, spinning down, vortexing thoroughly until homogenous. Then spin down briefly to collect the liquid.

**Note:** It is crucial that the reaction mixture is homogeneous prior to thermal cycling. Incomplete mixing can cause decreased yield and increase non-specific product formation.

**Note:** When using a PCR plate, use an applicator tool to firmly secure each reaction well and around the perimeter of the plate to prevent evaporation during thermal cycling.

3.4 Load the plate in the thermal cycler, ensure the plate is making complete contact with the thermocycler, and run the following thermal cycling protocol to amplify and index the libraries. Use the tables below for thermal cycling protocol and cycle number suggestions for AgriType Auto Custom Panels.

Second PCR Reaction — Thermal Cycling Protocol					
Step	Temperature		Time	Ramping*	Cycles
Initial Denaturation	95 °C		10 min	-	-
Denaturation	98 °C		15 sec	3 °C/s	Refer to table below for cycle numbers
Annealing/Extension	Illumina 60 °C	Ion Torrent 68 °C	75 sec	2 °C/s	
Hold	4 °C		∞	-	-

\* For thermal cyclers without adjustable ramp speed, the default setting can be used if max ramping speed is ≤5°C/Ss..

AgriType Auto Custom Panels — Second PCR Cycle Number	
Total Number of Amplicons in Panel	50-200 ng of High Quality gDNA per Pool
200-500	12
501-3000	11
>3000	9
<b>For suspected low-quality DNA</b>	<b>+1-2 cycles to above suggestions</b>

**Note:** The Second PCR thermal cycling protocol depends on the starting DNA input amount and DNA quality. Generally, lower quality DNA, lower DNA input amount, or fewer amplicons in a panel requires more PCR cycles.

**Note:** For optimal yield, AgriType Auto NGS panels may require adjustment to Second PCR cycle numbers based on the sample quality, panel design, amplicon number, and application.

- 3.5 Proceed to Step 4. Post-Second PCR Purification immediately after cycle completion. Do not allow PCR product to hold at 10 °C for more than 30 minutes.



**Important.** Do not stop and store PCR product after Second PCR. Proceed to Step 4, Post-Second PCR purification immediately.

## 4. Post-Second PCR Purification



**Important!** Samples are to be bead purified immediately after pooling. Do not allow pooled samples to stand. This can lead to non-specific product formation or increased chances of index hopping.



**Important!** Ensure the Magnetic Bead Solution has come to room temperature before use.



**Important!** Use freshly prepared 70% ethanol. Poorly stored mixture can cause reduced ethanol composition and may result in lower yields.

- 4.1. Number of samples to be pooled can vary and can be determined for the most efficient bead purification processing based on each lab’s available equipment and preferences. Multiple 384 well plates that have finished 2<sup>nd</sup> PCR can be pooled equivolume to 96 well configurations for automated 96 well bead purification workflows. Samples can be pooled into 8 strip tubes for manual processing. Alternatively, samples can be pooled into a single tube, and the bead purification can be performed in bulk. Some suggestions are listed below.

**Optional:** All 25 µl for each sample can be pooled for maximum yield recovery, or a fraction of the final product can be pooled (at least 5 µl per well) if sufficient product is expected and sufficient number of samples are pooled to reach compatible sequencing concentration. This can save magnetic bead use and increase sample processing when working in volume-restricted vessels.

Post-Second PCR Purification — 1X Beads-to-Sample Volume Ratio

Reagent	96 Samples into single microcentrifuge tube	96 samples into 8 strip tubes	4 x 384 samples into 96 well plates
Second PCR Reaction Product	96 samples pooled at 5 µl per sample	12 sample pools at 5 µl per sample	16 sample pools at 5 µl per sample
Magnetic Beads	480 µl	60 µl per tube	80 µl per well

**Note:** To ensure accurate bead purification ratio is applied, pool libraries with added dead volume, and draw out the expected volume for 1:1 bead ratio purification. Depending on the vessel or trough used for pooling, the exact overage volume may differ. For example, if the intention is to pool 5µl per well of a 96 well plate, you may want to add 6µl per well in a tube or add 8 µl per well in a trough. Mix well until homogenous and transfer the desired 480µl (5µl \* 96 samples) from the pool and add 480µl magnetic bead solution following the next step.

- 4.2. Vortex the magnetic beads suspension to disperse beads until homogenous. Perform a **1X** bead-based purification by adding the corresponding volume of magnetic beads for each sample pool, as detailed in the table above. Mix bead and sample solution thoroughly by vortexing vigorously or pipetting up and down until homogeneous.



**Important!** Magnetic bead volume is critical to the purification process. Always dispense slowly and carefully. Keep the outside of the pipette free from droplets, **AND** make sure the entire volume is added to the sample (residual from inside pipette tips) before discarding the tip.

Ensure the bead + sample solution is thoroughly mixed before incubation, especially when working in plated format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

- 4.3. Incubate the sample + bead pool for 10 minutes at room temperature.

**Note:** For pools greater than 1mL in total volume, incubate for 15-20 minutes for improved binding efficiency.

- 4.4. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and allow the liquid to become clear. The beads will be drawn onto one side of each tube/wall. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.
- 4.5. Cap/seal the tubes/plate, and briefly spin again to bring down the remaining supernatant. Place the tubes/plate on the magnetic rack to gather the beads. Using an appropriately sized pipette tip for the residual volume, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.



**Important!** Removing all residual supernatant from the Second PCR Reaction *prior* to ethanol washing is critical to obtaining a clean, high-quality library. The above spin step ensures complete removal of supernatant.

- 4.6. Add sufficient freshly prepared 70% ethanol to each tube or well to generously cover the original volume of the total bead+sample solution (approximately 120% or more of original volume). Model examples are shown below. Allow the bead to sit for at least 30 seconds with the tube off the magnet to ensure ethanol has access to an even distribution of beads that are not clumped. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.



	96 Samples into single microcentrifuge tube	96 samples into 8 strip tubes	4 x 384 samples into 96 well plates
Total volume per tube/well	960	120 µl per tube	160 µl per well
	> 1.2 mL	> 150 µl	>190 µl

- 4.7. Repeat step 4.6 for a second ethanol wash.
- 4.8. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Carefully remove the residual ethanol left behind in each tube/well. Keeping the tubes/plate on the magnetic rack, air-dry the beads at room temperature as long as necessary until the bead is visibly dried and just starting to crack. The exact time will vary based on how pooling is performed. **Do not over or under dry.**

**Note:** Over-drying beads can lead to reduced yield and difficulty in resuspension, while under-drying beads carry over ethanol that can inhibit downstream processes.

**Note:** We recommend air drying rather than using an oven to expedite the drying process.

- 4.9. Remove tubes/plate from the magnetic rack and add TE buffer (at approximately 1 µl per 5 µl of sample pooled) directly onto the pellet for each sample pooled.

Reagent	96 samples into single microcentrifuge tube	96 samples into 8 strip tubes	4 x 384 samples into 96 well plates
Original Sample Pool Volume	96 x 5 µl	12 x 5 µl	4 x 4 x 5 µl
<b>Final Elution Volume</b> per tube/well	96 µl	12 µl	16 µl

**Note:** Total elution volume when pooling may be adjusted on a case-by-case basis to concentrate the library to the expected yield. Please contact [support@paragongenomics.com](mailto:support@paragongenomics.com) for further questions.

- 4.10. Briefly spin and vortex to resuspend the dried beads completely in TE. The beads must make contact with the solution. Spin down and incubate for 2 minutes. There is no need to remove the beads for storage. At this point the library is complete and can be stored with beads at -20°C.

**Note:** For quantification, QC, and sequencing, use a magnetic rack to separate the beads. Avoid transferring the beads when pipetting the clear supernatant for downstream processes.



**Safe Stopping Point.** Purified products may be stored with beads at -20°C until ready to sequence. When taking the library out for QC and sequencing, vortex briefly and place the tubes or plate on a magnetic rack to pull the beads to one side. Avoid pipetting the beads, which will affect QC and sequencing.

## Quality Control Prior to Sequencing

Check pooled library quality and concentration using a high sensitivity fragment analyzer such as Agilent 2100 Bioanalyzer Instrument and Agilent High Sensitivity dsDNA Kit, or a qPCR-based method. The final library can also be quantified using a Qubit Fluorometer or equivalent. However, this method will only give you the absolute yield and not differentiate potential background from the actual library. To confirm the quality of the library, it is highly recommended that a high sensitivity fragment analyzer is used to visualize the peak shape, size, and potential background concentration.

AgriType Auto NGS panels typically have a yield of 5nM to 16nM, depending on the agricultural species, DNA sample quantity/quality and PCR cycle numbers, and how the libraries were pooled and eluted. Library quantity is not indicative of library quality. Additional input or 2<sup>nd</sup> PCR cycles can be adjusted accordingly for optimal yield for your downstream processing or sequencing needs.

Example library peak size and shapes for AgriType Auto kits can be found in the troubleshooting guide at the end of this user guide. The expected amplicon size distribution for Custom Panels can be found in the design files folder, available for download via your account through our website:

**Custom panels** → **ParagonDesigner Portal** → [View Designs](#).

Please see troubleshooting guide towards the end of this document if you observe non-specific peaks or unexpected yield.

After confirmation of library quality, the libraries can be normalized using library peak(s) concentrations, not including the non-specific product(s) concentration(s). Then samples with unique index combinations can be pooled for sequencing.

For high-throughput applications with well-integrated workflow on to automation, normalization of library product after 2<sup>nd</sup> PCR is likely not necessary before loading on the sequencer. With consistent DNA input quality and quantity, intra and inter-sample yield variability likely will not affect overall coverage. For ease of processing, especially when handling thousands of samples, sequencing at 100X depth without normalization is likely sufficient for most genotyping needs.

## Recommended Sequencing Length and Depth

AgriType Auto Panels are typically designed for PE 150 bp reads (2x150 bp), unless otherwise decided and communicated between the customer and the Paragon Genomics panel design team. Panels can be designed for shorter or longer read length as appropriate.

For genotype calls, the recommended average sequencing read depth is 100X. Based on empirical data, depth can be further reduced to maintain sufficient coverage but also increase sample multiplexing efficiency.

## Terminology

Coverage	Also known as depth of coverage, is achieved with one set of paired end reads that cover the entire length of the amplicon.
Read	One continuous act of sequencing. For example, 1 million clusters on a flow cell would produce 1 million single reads and 2 million paired-end reads. Paired end reads count as two individual reads, and required for 1x coverage.
Call Rate	Percentage of markers which generate a genotype call for a given sample

When calculating how many samples can be sequenced in one chip, it is important to refer to the correct total read capacity of the chip.

### For example:

For **100X** coverage of a panel with **1000** amplicons, it requires a total of 100,000 cluster reads or 200,000 paired end reads per sample. With a **Miseq V2 kit** (15 million single reads/ 30 million paired end reads) you can sequence about **150** samples per run.

For a sample multiplexing calculator and recommended sample multiplexing on various Illumina NGS instruments, please refer to tools section and panel-specific product sheets at [www.paragongenomics.com/product\\_documents/](http://www.paragongenomics.com/product_documents/).

# Supporting Information

## Dual-Indexed PCR Primers for Illumina

### Best Practices

- Employ best laboratory practices to avoid cross contamination of indexed primers. Upon removing the primers from -20C storage, centrifuge the plate at room temperature (e.g. for 1min at 280 x g), ensure ALL the liquid is collected in the bottom of the wells, then use accordingly. We do not recommend vortexing the adapter plates as it could result in cross-contamination of the indexed primers. Pipette mix to resuspend individual adapters prior to use.
- The foil cover is pierceable and peelable. If peeling the cover off, take care to slowly remove the foil cover to avoid cross-contamination and to remove the foil evenly.
- Upon first use, carefully remove the foil cover to avoid cross-contamination. Discard the original foil cover and do not reuse. If only using a subset of the adapters, partial use of the foil is not recommended. Please replace the seal entirely once it has been opened.

### Primer Sequences

Each sample is indexed by a pair of Indexed PCR Primers for sequencing on Illumina platforms. XXXXXXXXXX denotes the index region of the primer. Index sequences are listed below.

i5 Indexed Primer

5' -AATGATACGGCGACCACCGAGATCTACACXXXXXXXXXXACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

i7 Indexed Primer

5' -CAAGCAGAAGACGGCATAACGAGATXXXXXXXXXXGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'

Set A and Set B have mutually exclusive sequences and allow use together to form 384 combinatorial index pairs for sequencing up to 384 samples on one Illumina flow cell.

Plates 1-64 combinatorial indexes yield a total of 6144 unique combination that can be run in one Illumina flowcell for ultra-high sample multiplexing. This set includes set A and B above and cannot be combined to form more unique combinations. Please contact sales@paragongenomics you need more indexes beyond the 6144 offered.

AgriType Auto Targeted Library Kits and Panels are not compatible with most other commercially available index primer kits. Please use CleanPlex PCR Primers for Illumina for best results.

## Index Sequences



Additional sequence and Sample sheet information can be downloaded from [Product Document Page](#) under Indexes and Useful Tools.

---

### **CleanPlex Dual-Indexed PCR Primers for Illumina Set T**

3 x 3 indexes, 9 reactions (SKU 716021)

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A selection of three i5 Indexes and three i7 Indexes from Illumina Set A to create 9 unique index combinations. Index selection may vary from kit to kit.

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## Single-Indexed PCR Primers for Ion Torrent

### Primer Sequences

Each sample is indexed by uniquely designed Indexed PCR Primers for sequencing on Ion Torrent platforms. Only CleanPlex Single-Indexed PCR Primers for Ion Torrent are compatible with AgriType Auto workflow for generating libraries intended for Ion Torrent sequencing.

### Index Sequences

CleanPlex Single-Indexed PCR Primers v2 for Ion Torrent Sets C, D, E, and F contain index sequences identical to the sequences of the equivalent IonCode™ barcodes. These indexed PCR primers are provided in sealed 96-well PCR plates and arrange in ascending order in columns.



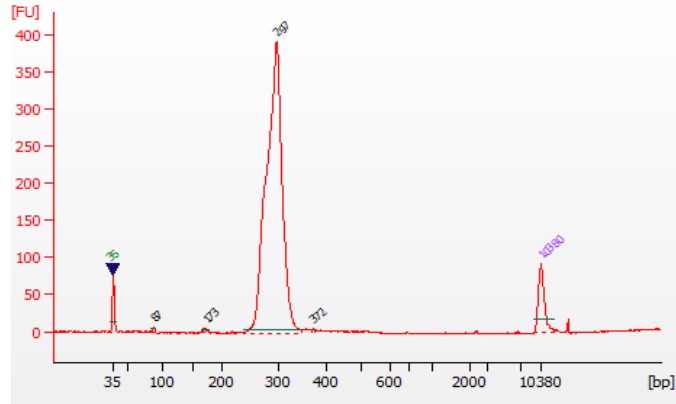
For plated indexes v2 sets C, D, E, and F, plate map and sequences information can be downloaded from [Product Document Page](#) under Indexes.

## Troubleshooting Guide

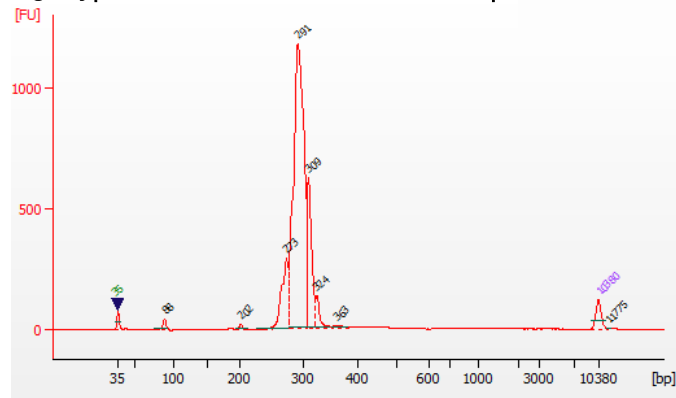
### Examples of Libraries Prepared with AgriType Auto Panels

Depending on the AgriType Auto Panel used, library peak(s) should be between 200 and 400 bp. Below are representative Agilent Bioanalyzer traces generated for various AgriType Auto Custom Panels using 50-200 ng of gDNA as input.

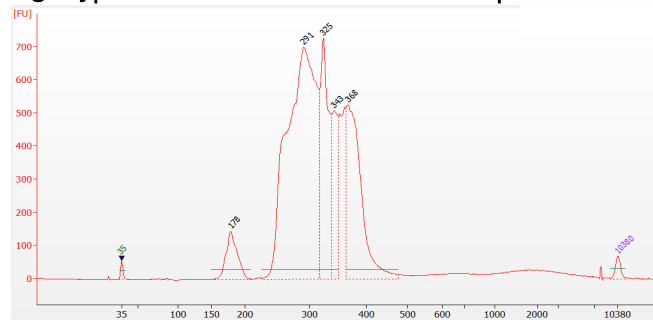
#### AgriType Auto Corn Panel – 200 amplicons



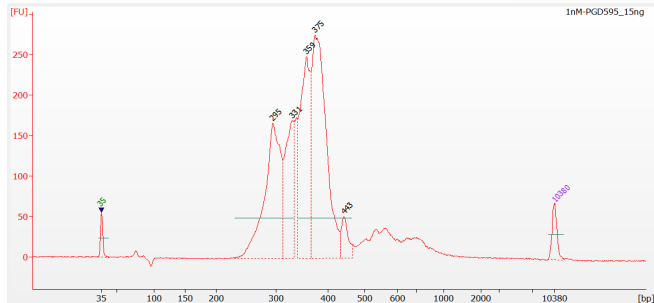
#### AgriType Auto Tomato Panel – 400 amplicon panel



#### AgriType Auto Cotton Panel – 2500 amplicon panel



## AgriType Auto Soybean Panel – 7000 amplicon panel



### Potential Causes for Extra Peaks and Suggested Solutions

**Peaks around 70–90 bp** are index primer dimers from the Second PCR Reaction and result from incomplete removal of low molecular weight DNA fragments during the final magnetic bead purification (Post-Second PCR Purification). When these peaks are high, it usually indicates one or more of the following:

- Inaccurate pipetting of magnetic beads when making a large number of libraries in a short period of time.
- Insufficient removal of supernatant and/or ethanol washes during the last purification.
- Adding more than the specified amount of indexed primers to Second PCR Reaction.

**Peaks around 150–190 bp** are residues of digested non-specific amplification products and adapter dimers. They come from non-specific products formed during or after the Multiplex PCR (mPCR) Reaction due to deviation from protocol or high concentration of the panel. These non-specific amplification products are removed during magnetic bead purification. These peaks are usually caused by one or more of the following:

- Inaccurate pipetting of magnetic bead volume.
- Insufficient mixing of reaction solutions or bead and sample mixture.
- Insufficient removal of supernatant and ethanol washes during purification.
- Allowing mPCR products to sit for too long (and form non-specific products) before purification. If there must be delay after the mPCR reaction, be sure to set samples on ice, and in -20C if the delay goes over 30 minutes.
- Inaccurate intermediate dilution. Make sure to check for evaporation following the mPCR reaction and adjust the volume input/extraction accordingly to achieve the same dilution factor.
- AgriType Auto Custom Panels may require panel titration for optimal performance. Often dimers will decrease by reducing the panel concentration in the mPCR Reaction Mixture.
- Incorrect annealing time, specifically unnecessarily longer annealing time.

**Broad peaks spread across 500 – 10,000 bp range** are non-specific products due to overamplification. Double check that the correct mPCR and Second PCR cycle numbers were used. PCR cycles are determined based on the amplicon count per pool of your panel, DNA input amount, and DNA quality. Try reducing the Second PCR cycles by 2-3 cycles if this issue persists. If you're working with a custom

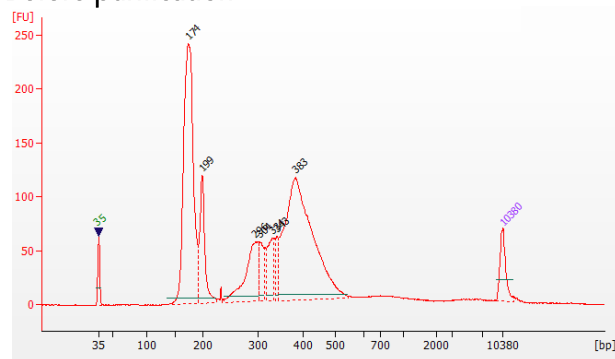


panel for organisms with a much smaller genome than human's, consider reducing the DNA input and Second PCR cycles accordingly.

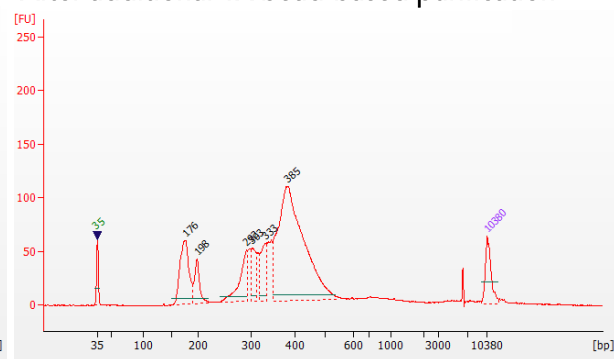
## Removing non-specific products from final libraries

If short non-specific products described above are present in significant amounts, they can be reduced by pooling indexed libraries (that will be sequenced in the same lane) and performing one additional round of 1X magnetic bead-based purification if the pooled library **concentrations is >3,000 pM** and the **total volume is >20 µL**. Low volume and low concentration libraries should not be bead purified again as it will lead to significant loss of the library of interest. In the figures below, the Agilent Bioanalyzer trace on the left shows an example CleanPlex library that contains significant adapter dimers due to poor library preparation. The Bioanalyzer trace on the right shows the same library after an additional 1X magnetic bead-based purification. The bead purification can be repeated once more if volume and concentration still meet the criteria above. We recommend keeping short adapter dimer peaks to less than 20% for best results. When possible, it's better to repeat the library preparation with the corrected steps so non-specific products do not form in the first place.

### Before purification



### After additional 1X bead-based purification



## Potential Reasons for No Peaks

- 30% ethanol instead of 70% ethanol was used in DNA purification with magnetic beads.
- Magnetic beads were not added for one or more of the purification steps.
- DNA quantification was inaccurate, especially if using spectrophotometric methods, such as the NanoDrop instrument. Try using more input DNA.
- DNA quality is extremely degraded. Try using more input DNA.
- Incompatible indexed PCR primers were used in the Second PCR Reaction. Only use CleanPlex Index primers with AgriType Auto library prep. Use the appropriate index primers for the sequencer of choice.
- A weak or incompatible magnetic rack was used to perform magnetic bead purification, resulting in significant bead loss.

## Additional Resources

Please visit [www.paragongenomics.com/product/faq/](http://www.paragongenomics.com/product/faq/) for additional troubleshooting resources.

## Data Analysis Recommendations for Illumina

We recommend on-instrument analysis using Illumina's DNA Amplicon Module for the simplest and most streamlined experience for downstream QC and variant calling. Please follow Illumina's latest user guide for step-by-step instructions, which is available in their support center. Manifest files are automatically provided for hg19 designs (in DesignDetails.zip) and upon request, Paragon Genomics can provide the manifest file for other genomes. Please contact [support@paragongenomics.com](mailto:support@paragongenomics.com) for any questions or requests.

As an alternative, the Broad Institute's GATK Best Practice (<https://software.broadinstitute.org/gatk/best-practices/>) offers general guiding principles for sequencing data analysis.

For off-instrument analysis, please refer to the following recommended steps for analyzing AgriType Auto NGS libraries sequenced on Illumina platforms.

**1. BED Files** . Visit <https://www.paragongenomics.com/my-account/downloads/> to download panel-specific amplicon insert BED file. For AgriType Prime Custom Panels, the BED file is available for download via the associated order account through our design portal: <https://www.paragongenomics.com/panel-design-my-design-requests/>

### 2. Adapter Trimming.

For panel with amplicon inserts that fall less than 150bp lengths, it is recommended to trim leftover adapter sequences for 2X150 sequencing before read mapping.

Following is an example command with open-source software cutadapt (<https://cutadapt.readthedocs.io/en/stable/>).

```
cutadapt -g CCTACACGACGCTCTTCCGATCT \  
-a AGATCGGAAGAGCACACGTCTGAA \  
-A AGATCGGAAGAGCGTCGTGTAGG \  
-G TTCAGACGTGTGCTCTTCCGATCT \  
-e 0.1 -O 9 -m 20 -n 2 \  
-o R1_out.fq.gz -p R2_out.fq.gz R1_in.fq.gz R2_in.fq.gz \  
> cutadapt_report.output.txt
```

### 3. Map reads to reference genome.

Ready to use panels are designed against GRCh37 (hg19) reference genome. For customer panels, please refer to your specified reference genome. Bwa mem is recommended for read mapping and de-duplication procedure shall be skipped.

### 4. Trim primer sequences.

Before construction of a consensus genome sequence, it is recommended to remove primer sequences. The trimming should be based primarily on reads' mapping starts/ends positions and primers 3' ends' coordinates in ampInsert.bed files. We recommend using the DNA amplicon workflow on Illumina instruments for trimming primers and calling variants. An alternative approach for primer trimming is using the software package fgbio. It requires primer genomic coordinates in a tab delimited file which can be provided by Paragon Genomics to customers.

Following is an example command:

```
java -jar fgbio-1.2.0-e7ac607-SNAPSHOT.jar TrimPrimers -i input.bam -o  
output.primerTrim.bam -p primer_info.tab -H true
```

## 5. Calculate QC metrics.

In order to assess the quality of the sequencing results, it is recommended to assign mapped reads to amplicons based on mapping position. Subsequently, the following metrics can be used to measure general performance of the panel.

- **Mapping Rate:** Percentage of reads mapped to reference genome. It assesses primer-dimers and other PCR artifacts.
- **On-Target Rate:** Percentage of mapped reads that aligned to the targeted regions. It assesses binding/amplification specificity of designed primers.
- **Coverage Uniformity:** Percentage of amplicons with read depth equal to or greater than 20% of mean read depth of all amplicons in the panel. It measures performance uniformity of amplicons in the panel.

## Data Analysis Recommendations for Ion Torrent

We recommend customers to use analysis software provided by Ion Torrent. A few suggestions specific to Paragon Genomics panels are given below.

1. **BED Files.** Visit <https://www.paragongenomics.com/my-account/downloads/> to download panel-specific amplicon insert BED file. For AgriType Prime Custom Panels, the BED file is available for download via the associated order account through our design portal: <https://www.paragongenomics.com/panel-design-my-design-requests/>
2. **Extra Trimming settings.** When working in the Ion environment, such as the Torrent Suite, Ion Reporter, or VariantCaller Plugin, discard any custom argument and revert to default arguments for your chip type and application combination, then try adding to both Pre-BaseCaller Args and BaseCaller Args : `--extra-trim-left 24 --extra-trim-right 24` , to avoid misassignment of read quality.

Setting	Description
Pre Basecaller args for calibration	<code>--extra-trim-left 24 --extra-trim-right 24</code>
Basecaller args	<code>--extra-trim-left 24 --extra-trim-right 24</code>

3. **Trim tag sequences.** If working with FASTQ directly without BaseCaller trimming setting above, tag sequences can be trimmed with the following instructions. If you're working with already trimmed BAM files, additional trimming with cutadapt is not necessary.

As shown in the diagram of library structure below, a short tag sequence (TAG-1 and TAG-2 respectively) is attached to both ends of an amplicon. The tag sequences are added during primer synthesis and they serve as priming site in second PCR reaction where Ion Torrent adapters are added. It is the best to trim those sequences from sequencing read before read mapping.



It is recommended to trim the tag sequences with open-source software cutadapt using the following options:

```
cutadapt -g CCTACACGACGCTCTTCCGATCT \  
-g TTCAGACGTGTGCTCTTCCGATCT \  
-a AGATCGGAAGAGCGTCGTGTAGG \  
-a AGATCGGAAGAGCACACGTCTGAA \  
-e 0.1 -O 9 -m 20 -n 2 \  
-o out.fq.gz in.fq.gz \  
> cutadapt_report.output.txt
```

The above command would trim left-over adapter sequences as well.

4. **Trim primer sequences.** For a single-pool panel, we don't recommend to trim primer sequences from sequencing reads. For a two-pool panel, however, trimming primer sequences with a software is recommended to avoid false positive calls from primer binding sites. The trimming should be based primarily on reads' mapping starts/ends positions and primers 3' ends' coordinates in ampInert bed files. We recommend using the Torrent Variant Caller plugin for trimming primers and calling variants. For other applications that require actual primer sequences, please contact Paragon Genomics to acquire them.

## Technical Support

For technical assistance, please contact Paragon Genomics Technical Support.

**Phone:** 650-822-7545

**Email:** [support@paragongenomics.com](mailto:support@paragongenomics.com)

**Website:** [www.paragongenomics.com](http://www.paragongenomics.com)

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