CleanPlex® for MGI NGS Panel

User Guide

This user guide is for the following products:

- CleanPlex® for MGI OncoZoom® Panel
- CleanPlex® for MGI BRCA1 & BRCA2 Panel
- CleanPlex® for MGI Custom NGS Panel

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UG1003-03
November 2021

Revision History

Document	Date	Description of Change
UG1003-01	June 2019	• Initial version
UG1003-02	July 2019	• Included Circularization to protocol and workflow
UG1003-03	November 2021	• Removed Set A-F of tubed MGI indexes and added MGI Plated PCR Primers

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Overview

Product Information

CleanPlex® for MGI NGS Panels are a fast, robust, and versatile solution for target enrichment and library preparation for next-generation sequencing (NGS) on MGISEQ sequencing platforms. CleanPlex for MGI NGS Panels generate highly accurate data from as little as 1 ng of DNA using a fast and simple workflow. CleanPlex for MGI Ready-to-Use NGS Panels are expertly optimized with predesigned primers to generate valuable insights in key cancer and disease research areas. CleanPlex for MGI Custom NGS Panels are made-to-order to target user-defined genomic regions of interest.

CleanPlex for MGI NGS Panels are powered by Paragon Genomics' proprietary CleanPlex Technology, which combines an advanced primer design algorithm and an innovative background cleaning chemistry to generate best-in-class target enrichment performance and efficient use of sequencing reads. The patented CleanPlex background cleaning chemistry effectively removes non-specific PCR products to enable ultra-high multiplexing of amplicons. More than 20,000 amplicons can be multiplexed per primer pool to build large NGS panels that can interrogate megabase-size genomic regions to unlock new applications using a streamlined workflow.

CleanPlex for MGI NGS Panels feature a fast and simple workflow that can be completed in about 3 hours from input DNA to indexed and amplified NGS libraries. The workflow involves just 3 steps, each consisting of a thermal-cycling or incubation reaction followed by a 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. A single-pool CleanPlex for MGI NGS Panel, such as the CleanPlex for MGI OncoZoom Cancer HotSpot Panel, has no tube-to-tube transfer and thus offers the many benefits of a single-tube workflow.

The first step of the CleanPlex workflow is a multiplex PCR reaction that uses target-specific primers to amplify targets of interest. The second step is a digestion reaction that performs background cleaning by removing non-specific PCR products. The third step is a PCR reaction that uses CleanPlex for MGI Indexed PCR Primers to amplify and add sample-level indexes to the NGS libraries. CleanMag® Magnetic Beads are recommended for library purification. After the third step, a circularization step is carried out using the MGIEasy Circularization Kit to generate circularized DNA libraries, which are specific to and required for sequencing on MGI's NGS instruments. See the Workflow section for a detailed depiction of the CleanPlex for MGI workflow.

CleanPlex Target Enrichment and Library Preparation Workflow

3 hours of total assay time, 75 minutes of hands-on time

Applications

The CleanPlex for MGI NGS Panels can be used to detect germline and somatic mutations in a wide variety of applications ranging from tumor profiling and early cancer detection to high-throughput genotyping and gene expression analysis. CleanPlex for MGI Custom NGS Panels can be built from 7 to 20,000 amplicons per primer pool to target either or both hotspot position and full genes. Amplicons can be designed with size ranging from 80 to 500 bp 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 CleanPlex for MGI NGS Panels. This is not meant to be an exhaustive or restrictive list.

Organisms	Sample Types	Applications
• Human • Mammals • Fish • Insects • Plants • Microorganisms	• Genomic DNA from whole blood, tissues, biofluids, buccal swab, dried blood spots (DBSs), feces, circulating cells, and cultured cells • Degraded DNA from FFPE tissues • Cell-free DNA (cfDNA) from plasma and other biofluids • cDNA from bulk or single cell RNA samples	• Tumor profiling • Variant detection and discovery • Disease predisposition • Species identification • High-throughput genotyping • Gene expression profiling

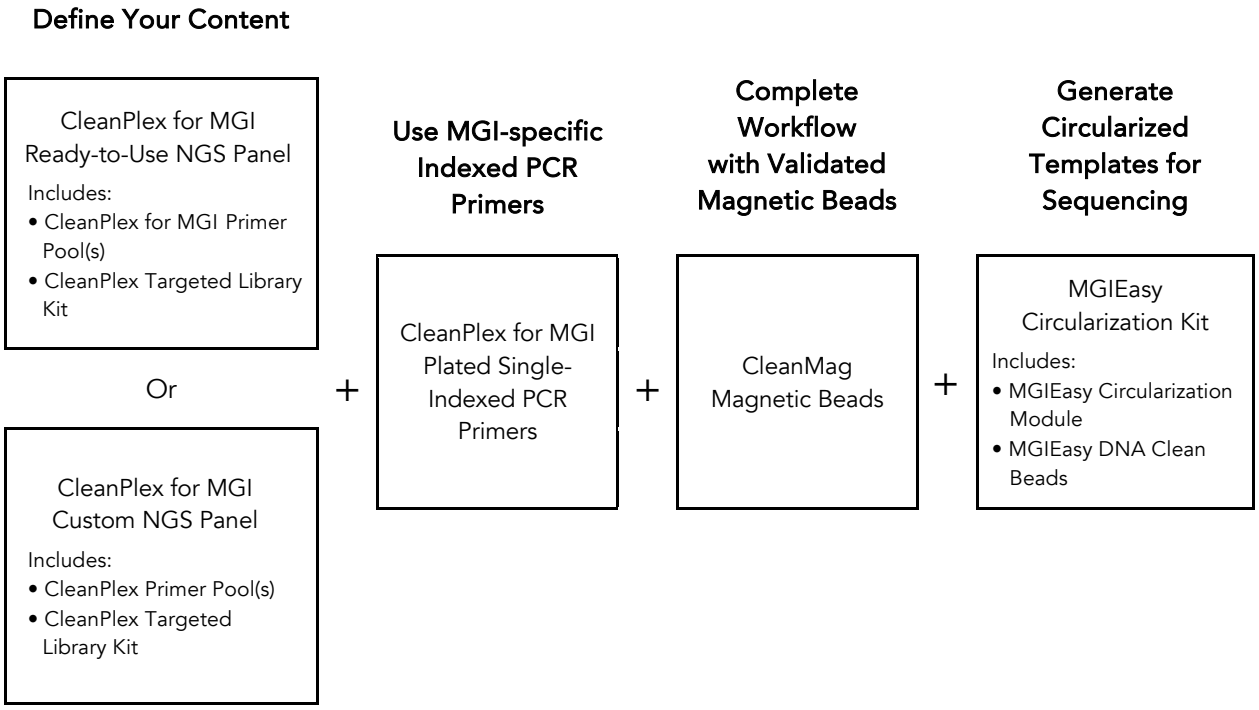
Compatible Sequencing Instruments

CleanPlex for MGI NGS Panels and CleanPlex for MGI Indexed PCR Primers are used to generate target-enriched and indexed NGS libraries that are compatible with all MGISEQ sequencing platforms, including MGISEQ-T7, MGISEQ-2000, MGISEQ-200, and BGISEQ-500 systems.

Kit Contents

The protocol outlined in the CleanPlex for MGI NGS Panel User Guide requires the following components, which need to be ordered separately:

- CleanPlex for MGI Ready-to-Use NGS Panel or CleanPlex for MGI Custom NGS Panel
- CleanPlex for MGI Indexed PCR Primers
- CleanMag Magnetic Beads (or equivalent)
- MGIEasy Circularization Kit (MGI, 1000005259)



Panel Specifications

Panel	Number of Primer Pools	Primer Pool Concentration	Number of Amplicons	Average Amplicon Length	Average Library Length
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	1	5X	601	146 bp	231 bp
CleanPlex for MGI BRCA1 & BRCA2 Panel	2	5X	218	158 bp	243 bp
CleanPlex for MGI Custom NGS Panels	Varies	5X	Varies	Varies	Varies

CleanPlex NGS Panel — Kit Contents, Store at –20°C							
Panel	SKU	Size (Reactions)	Components				
			Primer Pool 1	Primer Pool 2	Primer Pool 3	Primer Pool 4	CleanPlex Targeted Library Kit
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	317001	8	16 µl	---	---	---	1-pool, 8 rxns
	317002	96	192 µl	---	---	---	1-pool, 96 rxns
CleanPlex for MGI BRCA1 & BRCA2 Panel	317003	8	16 µl	16 µl	---	---	2-pool, 8 rxns
	317004	96	192 µl	192 µl	---	---	2-pool, 96 rxns
CleanPlex for MGI Custom NGS Panels	---	96+	Varies				Varies

A CleanPlex Targeted Library Kit is included in every CleanPlex for MGI Ready-to-Use NGS Panel and CleanPlex for MGI Custom NGS Panel. Please note that the CleanPlex Targeted Library Kit is **not** sold separately.

CleanPlex Targeted Library Kit — Kit Contents, Store at –20°C (not sold separately)								
	Configuration		1-Pool		2-Pool		4-Pool	
		Size	8 Rxns	96 Rxns	8 Rxns	96 Rxns	8 Rxns	96 Rxns
Component	Cap Color	SKU	816001	816002	816006	816007	816023	816024
5X mPCR Mix	Green		16 µl	192 µl	32 µl	384 µl	64 µl	768 µl
CP Reagent Buffer	White		16 µl	192 µl	16 µl	192 µl	16 µl	192 µl
CP Digestion Reagent	Yellow		16 µl	192 µl	16 µl	192 µl	16 µl	192 µl
Stop Buffer	Red		32 µl	384 µl	48 µl	576 µl	80 µl	960 µl
5X 2nd PCR Mix	Blue		64 µl	768 µl	64 µl	768 µl	64 µl	768 µl
TE Buffer	Clear		500 µl	4 ml	500 µl	4 ml	500 µl	4 ml

Required Materials and Equipment Not Included

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

CleanPlex for MGI Plated Single-Indexed PCR Primers— Store at –20°C			
	SKU	Size (Reactions)	Format
CleanPlex for MGI Plated Single-Indexed PCR Primers	318013 318014	96 384	96 indexes (96-well plate)
CleanPlex for MGI Single-Indexed PCR Primers T8	318015	8	8 indexes (8 tubes)

- CleanMag Magnetic Beads, or equivalent — eg. Agencourt™ AMPure™ XP Kit (Beckman Coulter, Cat. No. A63880, A63881, or A63882)

CleanMag Magnetic Beads — Store at 2–8°C			
	SKU	Size (Volume)	Reactions
CleanMag Magnetic Beads, 1 ml	718001	1 ml	~9
CleanMag Magnetic Beads, 5 ml	718002	5 ml	~45
CleanMag Magnetic Beads, 60 ml	718003	60 ml	~540
CleanMag Magnetic Beads, 450 ml	718004	450 ml	~4,050

- MGIEasy Circularization Kit (MGI, Cat. No.1000005259, 16 reactions)
- For PCR tubes or strips, CleanMag Magnetic Rack, for 0.2 ml 8/12-tube PCR Strip (SKU 719001) or equivalent magnetic racks designed for PCR strip workflows
- For 96-well PCR plates, CleanMag Magnetic Plate, for 96-well PCR plates (SKU 719002) or equivalent magnetic plates designed for PCR plate workflows

CleanMag Magnetic Rack & Plate		
	SKU	Fit
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

- 70% ethanol (freshly prepared)
- Nuclease-free water
- Nuclease-free, low bind, thin-wall PCR strip tubes with attached caps, or 96-well PCR plate with adhesive film

- Pipettors and low-retention filtered pipette tips
- Thermal cycler
- Mini-centrifuge, 96-well plate centrifuge
- Qubit® Fluorometer and dsDNA HS (high sensitivity) Assay Kit, or equivalent
- Agilent® 2100 Bioanalyzer® Instrument and Agilent High Sensitivity DNA Kit, or equivalent

Storage, Handling, and Usage

CleanPlex for MGI NGS Panels and CleanPlex for MGI Indexed PCR Primers are shipped on blue ice (ice packs). Upon receipt, immediately store CleanPlex for MGI NGS Panels and CleanPlex for MGI 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.

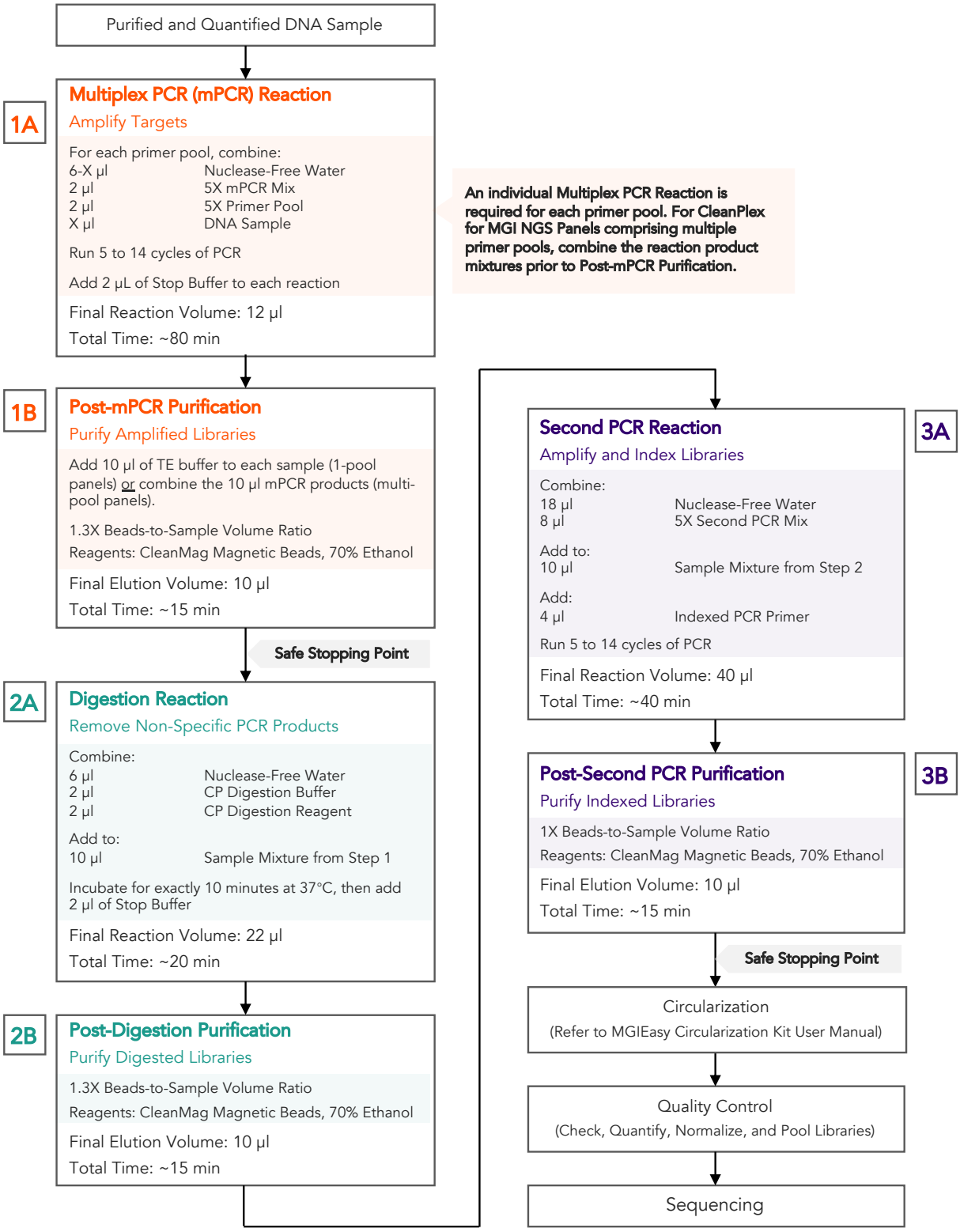
The components containing enzymes (5X mPCR Mix, CP Digestion Reagent and 5X 2nd PCR Mix) are specially formulated for storage at –20°C without freezing to avoid freeze-thaw cycles to retain their full activity. Please avoid storing these components below –23°C.

CleanPlex for MGI NGS Panels 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 CleanPlex NGS Panel's targeted NGS library preparation workflow.



Protocol

Best Practices

- When using the kit for the first time, 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, CP Digestion Reagent, 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, CP Digestion Reagent, 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. Remember to briefly spin the PCR tubes or 96-well PCR plate after mixing.

- Always keep working solutions and PCR products on ice until needed. Combine PCR mixes just immediately prior to use and do not prolong storage of combined PCR mixes and PCR products.

- The protocol is designed to minimize the number of tube-to-tube transfer in order to avoid or reduce sample loss. For a single-pool CleanPlex for MGI NGS Panel, the entire protocol is performed following a single-tube workflow, with no tube-to-tube transfers. For a multi-pool CleanPlex for MGI NGS Panel, the individual mPCR products from the primer pool-specific reactions are combined into one tube, and the remaining protocol is carried out using a single-tube workflow.

- When working with 96-well PCR plates, take extra care to ensure thorough mixing of all samples and proper sealing to avoid cross contamination between samples.

- Magnetic bead purification steps 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 manual handling of PCR tubes or 96-well PCR plates is critical for a successful bead purification

- Always pre-warm thermal cyclers, pre-warm a water bath or heat block to 37°C.

- Assign sample indexes to specific samples before starting the protocol.

- Always prepare a master mix of reagents when working with multiplex reactions. Prepare ~5% excess of each master mix to allow for pipetting losses. A master mix calculation sheet can be downloaded from the Product Documents page on the Paragon Genomics website at www.paragongenomics.com/product_documents/.

Input DNA Requirements

Refer to the following chart for the amounts of input DNA.

Panel	DNA Input Range (per pool)	Recommended gDNA Input (per pool)	Recommended FFPE DNA Input (per pool)
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	10–40 ng	10 ng	20 ng
CleanPlex for MGI BRCA1 & BRCA2 Panel	10–40 ng	10 ng	20 ng
CleanPlex for MGI Custom NGS Panels (Human genotyping)	1–40 ng	10 ng	20 ng
CleanPlex for MGI Custom NGS Panels (Human somatic mutation detection)	10–40 ng	10 ng	20 ng

- The maximum volume of DNA input per Multiplex PCR Reaction is 6 µl. For CleanPlex for MGI NGS Panels with multiple primer pools, an individual Multiplex PCR Reaction is required for each primer pool.
- Qubit dsDNA HS Assay Kit (Thermo Fisher, Cat. No. Q32851 or Q32854) or an equivalent fluorometric method is recommended for measuring DNA concentration. UV spectrophotometry methods (e.g. NanoDrop™ spectrophotometer) are not recommended because it can significantly overestimate the DNA concentration.
- After DNA extraction, avoid diluting DNA samples to < 10 ng/µl when possible for prolonged storage since DNA is less stable in solution at lower concentrations.
- Avoid freeze-thawing dilute DNA samples when possible, and measure sample concentrations immediately prior to use to avoid inaccurate sample input.
- 1–40 ng of human genomic DNA from normal or FFPE tissue is recommended for each Multiplex PCR Reaction depending on the panel and application.
- A minimum of 10 ng of human genomic DNA is recommended for detecting somatic variants with 1% frequency.
- 1–10 ng of DNA is recommended for genotyping applications that do not require low allele frequency detection. In rare cases where DNA is extremely limited and the application allows, 0.1 ng of DNA can be used.
- When DNA quality is low or unknown (such as DNA from FFPE tissues), higher DNA input can be used to produce better library quality and reach lower limits of detection.
- For panels specific to organisms other than humans, DNA input need will vary based on the genome size of the organism. For example, significantly less DNA input is needed for bacteria panels as each nanogram of DNA contains many more copies of the target DNA. An input titration can be done to determine minimum DNA input.
- Compatible DNA buffering systems are Tris HCl or TE.

Set up and Preparations

- When working with multiple samples, it is recommended to normalize all DNA samples to the same concentration, and prepare a master mix of mPCR Reaction Mixture for each primer pool. Mix well, aliquot into individual tubes, then add the recommended DNA input to each reaction.
- Bring Magnetic bead solution to room temperature for at least 30 minutes before use. Replace in 2-8 °C storage at the end of the day.
- Freshly prepare 10mL (per8 reactions) of 70% Ethanol by combining 7mL of 100% ethanol and 3mL nuclease-free water at volume ratios of 7 to 3 respectively. 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 points after mPCR purification and 2nd PCR purification. After starting Digestion Step, samples must be carried through to 2nd PCR purification without stopping. Plan accordingly.
- Upon initial receipt, Stop Buffer can be thawed and stored continuously at room temperature.

1A. Multiplex PCR (mPCR) Reaction

1A.1. Using thin-wall PCR strip tubes (or a 96-well PCR plate), prepare the mPCR Reaction Mixture by adding components in the following order on ice or a cold block. For CleanPlex for MGI NGS Panels with multiple primer pools, prepare individual reaction for each primer pool.

Note: *When working with multiple samples, normalize all DNA samples to the same concentration, and prepare a master mix of mPCR Reaction Mixture for each primer pool. Mix well, aliquot into individual tubes, then add the recommended DNA input to each reaction. For CleanPlex for MGI NGS Panels with multiple primer pools, prepare a separate mPCR Reaction Mixture for each primer pool for each sample.*

Note: *When working with multi- pool panels such as the CleanPlex for MGI BRCA1 & BRCA2 Panel, the recommended (or specified) amount of DNA is to be added to* ***each*** *pool, and not divided between the pools.*

Note: *Thaw and keep Stop Buffer and Magnetic Beads at room temperature for use at the end of step 1A and beginning of 1B.*

mPCR Reaction Mixture

Reagent	Cap Color	Volume per reaction
Nuclease-Free Water	—	6 – X µl
5X mPCR Mix	Green	2 µl
5X mPCR Primer Pool	Varies	2 µl
DNA Sample	—	X µl
Total Volume per reaction		**10 µl**

Important! 5X mPCR Mix and 5X 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.

1A.2. Close the caps of the PCR tubes or seal the PCR plate with adhesive film, spin briefly to bring down the liquid, mix thoroughly by pipetting up and down at least 5 times or by vortexing vigorously for at least 5 seconds until homogeneous. Avoid unnecessarily prolonged vortexing. Spin 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 nonspecific product formation.*

Note: *If 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.*

1A.3. Load the tubes/plate in the thermal cycler with a heated lid at 105 °C 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 CleanPlex for MGI NGS Panels.

mPCR Thermal Cycling Protocol				
Step	**Temperature**	**Time**	**Ramping***	**Cycles**
Initial Denaturation	95 °C	10 min	-	1
Denaturation	98 °C	15 sec	3 °C/s	Refer to table below for cycle numbers
Annealing/Extension	60 °C	See table below	2 °C/s	
Hold	10 °C	∞		

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

Note: For CleanPlex Custom NGS panels, use per pool amplicon number to determine the appropriate annealing/extension times using the table below. *Typically, a custom multi-pool gene panel from a single design will evenly split the total amplicons into each primer pool. Please refer to the "ampinsert" file of your custom design to determine the amplicon count per pool, or contact Technical Support if you have any questions.*

Panel Specific mPCR Thermal Cycling Conditions		
Panel	**Annealing/Extension Time**	**mPCR Cycle Number**
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	5 min	10
CleanPlex for MGI BRCA1 & BRCA2 Panel	5 min	10
CleanPlex for MGI Custom NGS Panels	5 min for 7–2,000 amplicons per pool 8 min for 2,000–5,000 amplicons per pool 16 min for 5,000–20,000 amplicons per pool	10

1A.4. Immediately add 2 µl of Stop Buffer (red cap) into each tube/well and mix by spinning briefly then vortexing. Spin again briefly to collect the liquid. The volume of each sample is approximately 12 µl.

Note: *Do not allow samples to hold at 10 °C for longer than 30 minutes before adding Stop Buffer.*

1A.5. Proceed to Step 1B. Post-mPCR Purification.

Important. Do not stop and store PCR products after multiplex PCR reaction. Add Stop Buffer then proceed to Step 1B, Post-mPCR Purification immediately.

1B. Post-mPCR Purification

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

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

1B.1. Open the tubes or carefully remove the adhesive film from the PCR plate.
- For CleanPlex for MGI NGS Panels comprising of one primer pool, add 10 µl of TE buffer to each sample.
- For CleanPlex for MGI NGS Panels with multiple primer pools, combine the 12 µl multiplex PCR products for each pool for each sample into one tube and perform purification on the combined product. See table below for examples.

1B.2. Vortex the magnetic beads suspension vigorously until homogeneous. Perform a **1.3X** bead-based purification by adding magnetic beads to the combined sample as described in the table below. Mix by pipetting up and down at least 5 times or vortex vigorously for at least ~5 seconds until homogeneous.

Note: *Accurate dispensing of magnetic beads is critical for each purification step. Aspirate and dispense slowly, taking care to completely transfer the required volume of beads and do not allow droplets of beads on the outside of the tip to be added to the sample.*

Note: *Ensure the mixture is thoroughly mixed before incubation, especially when working in a 96-well PCR plate format. Incomplete mixing can result in lowered yields and/or increased background in the final library.*

Post-mPCR Purification — 1.3X Beads-to-Sample Volume Ratio

	1-Pool	2-Pool	3-Pool	4-Pool
Volume of Combined Sample per reaction	22 µl (12 µl of sample + 10 µl of TE Buffer)	24 µl	36 µl	48 µl
Volume of Magnetic Beads per reaction	29 µl	31 µl	47 µl	62 µl

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 a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

1B.3. Incubate the mixture for 5 minutes at room temperature.

1B.4. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is clear**. The beads will be drawn onto one side of each tube/well. While keeping the tubes/plate on the magnetic rack, carefully remove and discard the supernatant without disturbing the beads.

Note: *If the magnetic beads are aspirated into the pipette tip at any point during the purification process, dispense the solution back into tube, remove the tip, allow the beads to migrate toward the magnet again, then repeat.*

Note: *When working with 96-well PCR plates, avoid touching the side of the well with the pipette tip when removing supernatant to minimize wicking up of liquid to the side of the well.*

1B.5. Cap/seal the tubes/plate, and briefly spin again to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

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

Note: *A strong compatible magnetic rack is essential. If your magnetic rack or plate cannot collect the magnetic beads effectively, please look into a replacement.*

1B.6. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

1B.7. Repeat step 1B.6.

1B.8. After the second wash, briefly spin the tubes/plate to bring down all remaining liquid. Place the tubes/plate on the magnet rack to gather the beads. Carefully remove the residual ethanol in each tube/well. Keeping the tubes/plate open on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: *Over-dried beads can dislodge from the side of the tube and lead to cross contamination. They are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.*

1B.9. Remove tubes/wells from the magnetic rack and add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads.

1B.10. Proceed to Step 2A. Digestion Reaction.

Safe Stopping Point. Purified products may be stored at 2-8°C for up to 1 hour or at –20°C for up to 24 hours.

2A. Digestion Reaction

Note: *After starting the Digestion Reaction, the samples cannot be stored and must continue to Post-Digestion Purification, then Second PCR Reaction. Plan accordingly.*

Note: *When thawing CP Reagent Buffer, bring the tube to room temperature. Ensure all visible precipitate is dissolved by vortexing as needed.*

Note: *Pre-warm a thermal cycler or heating unit to 37°C before beginning the Digestion Reaction.*

Note: If not already, keep magnetic beads and freshly prepared 70% ethanol ready at RT

2A.1. Prepare Digestion Reaction Master Mix and add directly into the same wells of each purified sample from Step 1B. No tube-to-tube transfer is needed and the magnetic beads from the previous reaction are carried onto the next steps and do not affect future reactions.

Note: *When working with multiple reactions, prepare a master mix. Mix well, then add 10 µl of master mix to each sample.*

Digestion Reaction Master Mix		
Reagent	**Cap Color**	**Volume per reaction**
Nuclease-Free Water	—	6 µl
CP Reagent Buffer	White	2 µl
CP Digestion Reagent	Yellow	2 µl
Total Volume per reaction		**10 µl**

Digestion Reaction Mixture	
Reagent	**Volume per reaction**
Digestion Reaction Master Mix	10 µl
Purified Sample from Step 1B	10 µl
Total Volume per reaction	**20 µl**

2A.2. Mix by pipetting up and down at least 5 times or vortexing vigorously for at least 5 seconds until homogeneous. Avoid unnecessarily prolonged vortexing. Spin briefly to collect the liquid.

Note: *It is crucial that the reaction mixture is homogeneous prior to incubation. Incomplete mixing can cause digestion of the library or under-digestion of nonspecific products.*

Note: *If 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 incubation.*

2A.3. Incubate at 37°C for exactly 10 minutes. **Do not** incubate shorter or longer than 10 minutes.

2A.4. Immediately add 2 µl of Stop Buffer (red cap) to each tube/well and mix by spinning briefly then vortexing. Spin again briefly to collect the liquid. The volume of each sample is approximately 22 µl.

2A.5. Proceed to Step 2B. Post-Digestion Purification immediately.

Important! Do not stop and store samples after the Digestion Reaction. Proceed to Step 2B. Post-Digestion Purification immediately.

2A.6. Incubate at 37°C for exactly 10 minutes. **Do not** incubate shorter or longer than 10 minutes.

2A.7. Immediately add 2 µl of Stop Buffer (red cap) to each tube/well and mix by spinning briefly then vortexing. Spin again briefly to collect the liquid. The volume of each sample is approximately 22 µl.

2A.8. Proceed to Step 2B. Post-Digestion Purification immediately.

Important! Do not stop and store samples after the Digestion Reaction. Proceed to Step 2B. Post-Digestion Purification immediately.

2B. Post-Digestion Purification

2C.

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

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

2B.1. Vortex the magnetic beads suspension to disperse beads. Perform a **1.3X** bead-based purification by adding **29 µl** of magnetic beads to each sample. Mix by pipetting up and down at least 5 times or vortex vigorously for at least 5 seconds until homogeneous.

Note: *Accurate dispensing of magnetic beads is critical for each purification step. Aspirate and dispense slowly, taking care to completely transfer the required volume of beads and do not allow droplets of beads on the outside of the tip to be added to the sample.*

Note: *Ensure the mixture is thoroughly mixed before proceeding, especially when working with 96-well PCR plates.*

Post-Digestion Purification — 1.3X Beads-to-Sample Volume Ratio	
Reagent	**Volume per reaction**
Digestion Reaction Product	22 µl
Magnetic Beads	29 µl

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 a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

2B.2. Incubate the mixture for 5 minutes at room temperature.

2B.3. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is 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.

2B.4. Cap/seal the tubes/plate and briefly spin again to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, carefully remove all residual supernatant from the bottom of the tube/well without disturbing the beads.

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

2B.5. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

2B.6. Repeat step 2B.5.

2B.7. 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 open on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: *Over-dried beads can dislodge from the side of the tube and lead to cross contamination, and they are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.*

2B.8. Remove tubes/wells from the magnetic rack and add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads.

2B.9. Proceed to Step 3A. Second PCR Reaction immediately.

Important! Do not stop and store samples after Post-Digestion Purification. Proceed to Step 3A. Second PCR Reaction immediately.

2B.10. 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 for 5 minutes. **Do not over or under dry.**

Note: *Over-dried beads can dislodge from the side of the tube and lead to cross contamination, and they are also more difficult to resuspend during elution. Residual ethanol inhibits PCR and will result in reduced yield. Especially when working with 96-well PCR plates, make sure all residual ethanol is dried (may take longer than 5 minutes) before going to the next step.*

2B.11. Add 10 µl TE buffer to each tube/well. Briefly spin and vortex to resuspend the beads and elute DNA from the beads (there is no need to remove the beads). Spin briefly to collect the liquid.

2B.12. Proceed to Step 3A. Second PCR immediately.

Important! Do not stop and store samples after Post-Digestion Purification. Proceed to Step 3A. Second PCR immediately.

3A. Second PCR Reaction

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

3A.1. Thaw Indexed PCR Primers, vortex thoroughly, then spin briefly to collect the liquid. Prepare Second PCR Reaction Master Mix and add to each purified sample from Step 2B. Then add a unique (combination of) Indexed PCR Primer(s) to each sample.

Note: *When working with multiple samples, prepare a master mix. Mix well, then add 26 µl of master mix to each sample.*

Second PCR Reaction Master Mix		
Reagent	**Cap Color**	**Volume per reaction**
Nuclease-Free Water	—	18 µl
5X 2nd PCR Mix	Blue	8 µl
Total Volume per reaction		**26 µl**

Second PCR Reaction Mixture	
Reagent	**Volume per reaction**
Second PCR Reaction Master Mix	26 µl
Purified Sample from Step 2B	10 µl
CleanPlex for MGI Indexed PCR Primer	4 µl
Total Volume per reaction	**40 µl**

Important! When handling Indexed PCR Primers, take extra care to prevent cross contamination by opening one tube at a time and changing pipette tips and gloves as necessary. Avoid touching the opening and inside of the tubes with your hands, pipette channel, or anything non-disposable.

3A.2. Close the caps of the PCR tubes or seal the PCR plate with adhesive film. Spin briefly to collect the liquid, then mix thoroughly by pipetting up and down at least 5 times or vortexing vigorously for at least 5 seconds until homogeneous. Avoid unnecessarily prolonged vortexing. Spin 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: *If 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.*

3A.3. Load the tubes/plate in the thermal cycler, and run the following thermal cycling protocol to amplify and index the libraries. Use the three tables below for thermal cycling protocol and cycle number suggestions for CleanPlex for MGI Ready-to-Use NGS Panels and CleanPlex for MGI Custom NGS Panels.

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*: See tables below for thermal cycling protocol and cycle number suggestions for CleanPlex for MGI Ready-to-Use NGS Panels and CleanPlex for MGI Custom NGS Panels.*

Note*: Nanogram input suggestions below are based on Human Genome DNA samples. For custom panels amplifying target regions of other organisms, use the genome size vs human to calculate nanogram equivalent as needed.*

Note*: Use the number of amplicons per pool to determine the correct Second PCR cycles for CleanPlex for MGI Custom NGS Panels. Typically, a custom multipool panel from a single design will evenly splits the total amplicons into each pool. Please contact Technical Support if you have any questions about your custom panel.*

Second PCR Reaction — Thermal Cycling Protocol

Step	Temperature	Time	Ramping*	Cycles
Initial Denaturation	95 °C	10 min	-	1
Denaturation	98 °C	15 sec	3 °C/s	Refer to table below for cycle numbers
Annealing/Extension	60 °C	75 sec	2 °C/s	
Hold	10 °C	∞		

* For thermal cyclers without adjustable ramp speed, use the default settings.

CleanPlex for MGI Ready-to-Use NGS Panels — Second PCR Cycle Number		
Panel	**10 ng of High Quality gDNA per Pool**	**10 ng of Low Quality DNA per Pool (eg. FFPE DNA)**
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	9	10
CleanPlex for MGI BRCA1 & BRCA2 Panel	13	14

CleanPlex for MGI Custom NGS Panels — Second PCR Cycle Number		
Total Number of Amplicons* in Panel	**10 ng of High Quality gDNA per Pool**	**10 ng of Low-Quality DNA per Pool (eg. FFPE DNA)**
7 - 100	**13**	**14**
101 - 200	**11**	**12**
201 - 500	**10**	**11**
501 – 1,000	**9**	**10**
1,001 – 2,000	**8**	**9**
2,001 – 5,000	**7**	**8**
5,001 – 12,000	**6**	**7**
12,001 – 20,000	**5**	**6**

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

Second PCR Reaction — Input Specific Cycle Number	
Input DNA per Pool	**Change in Cycle Number**
0.1 ng	+ 7
1 ng	+ 3 to + 4
5 ng	+ 1
10 ng	-
20 ng	– 1
40 ng	– 2
Low Quality DNA	+ 1 to + 2

3A.4. Proceed to Step 3B. 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 3B, Post-Second PCR purification immediately.

3B. Post-Second PCR Purification

3B.1. Vortex the magnetic beads suspension to disperse beads. Perform a **1X** bead-based purification by adding **40 µl** of magnetic beads to each sample. Mix by pipetting up and down at least 5 times or by vortexing vigorously for at least 5 seconds until homogeneous.

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

Reagent	Volume per reaction
Second PCR Reaction Product	40 µl
Magnetic Beads	40 µl

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 a 96-well PCR plate format. Inadequate mixing can result in lowered yields and/or increased background in the final library.

3B.2. Incubate the mixture for 5 minutes at room temperature.

3B.3. Briefly spin the tubes/plate. Place the tubes/plate on a magnetic rack and incubate for at least **2 minutes until the liquid is 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.

3B.4. Cap/seal the tubes/plate, and briefly spin again for to bring down the remaining liquid. Place the tubes/plate on the magnetic rack to gather the beads. Using a 10 µl pipette tip, 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.

3B.5. Add 180 µl of freshly prepared 70% ethanol to each tube/well. Reposition the tubes/plate on the magnetic rack by placing the clear side of the tubes/wells (the side without beads) against the magnet. Allow the beads to completely migrate through the ethanol to the other side. **Do not vortex.** Carefully remove and discard the supernatant without disturbing the beads.

3B.6. Repeat step 3B.5.

3B.7. 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 tubes/well. Keeping the tubes/plate on the magnetic rack, air-dry the beads at room temperature for 5 minutes. **Do not over or under dry.**

Note: *Over-drying and under-drying the beads can lead to reduced yield.*

3B.8. Remove tubes/wells from the magnetic rack and add 10 µl TE buffer directly onto the pellet for each tube/well. Briefly spin and vortex to resuspend the dried beads completely. The DNA will be immediately released from the beads as long as all beads are in solution. Spin briefly to collect the liquid to the bottom. There is no need to remove the beads. At this point the library is complete and can be stored with beads at –20°C.

Note: *To perform QC and sequencing, use a magnetic rack to separate the beads. Avoid transferring the beads when pipetting the clear supernatant for QC or sequencing.*

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.

Circularization

Please use MGIEasy Circularization Kit (MGI, 1000005259) or equivalent and refer to the MGIEasy Circularization Kit User Manual for instructions on circularizing the templates for sequencing on MGI platforms.

Quality Control Prior to Sequencing

Check 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 DNA, it is highly recommended that a high sensitivity fragment analyzer is used to visualize the peak shape, size, and potential background concentration.

CleanPlex for MGI Ready-to-Use NGS Panels typically have a yield of 6 pM to 30nM, depending on DNA sample quantity/quality and cycle numbers. Custom panel yield might be more variable. Library quantity is not indicative of library quality. Additional input or 2nd PCR cycles can be adjusted accordingly for optimal yield for your downstream processing or sequencing needs.

Expected library peak size and shapes for Ready-to-use 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 nonspecific peaks or unexpected yield.

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

Recommended Sequencing Length and Depth

All CleanPlex for MGI Ready-to-Use NGS Panels are designed to be compatible with PE 150 bp reads (2x150 bp). CleanPlex Custom NGS Panels also have standard 2x150 bp designs unless otherwise decided and communicated between the customer and the Paragon Genomics panel design team.

For detecting germline mutations, the recommended average sequencing read depth is 500X. For detecting somatic mutations down to 1% minor allele frequency, the recommended average sequencing read depth is 5,000X. Based on results, depth can be further reduced to maintain sufficient coverage but also increase sample multiplexing efficiency.

Recommended Sequencing Depth

Panel	Application	Average Read Depth
CleanPlex for MGI OncoZoom Cancer HotSpot Panel	Somatic Mutations	5,000X
CleanPlex for MGI BRCA1 & BRCA2 Panel	Somatic Mutations	5,000X
	Germline Mutations	500X

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.

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 **1000x** coverage of this panel with **343** amplicons, it requires a total of 343,000 cluster reads or 686,000 paired end reads per sample. With a **Miseq V2 kit** (15 million single reads/ 30 paired end reads) you can sequence about **44** samples (30 M PE reads/686 PE reads) per run.

For recommended sample multiplexing on various NGS instruments, refer to the panel-specific product sheet available for download on the product document page:
https://www.paragongenomics.com/product_documents/

A sample multiplexing calculator also be download from the product document page.

Supporting Information

CleanPlex for MGI Plated Single-Indexed PCR Primers

Primer Sequences

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

Universal primer
5'-Phos-GAACGACATGGCTACGATCCGACT*T-3'

Single- Indexed Primer
5'-TGTGAGCCAAGGAGTTG**XXXXXXXXXX**TTGTCTTCCTAAGACCGCTTGGCCTCCGACT*T-3'

Index Sequences

CleanPlex MGI Plated Single-Indexed PCR Primers contain index sequences identical to the sequences of the equivalent tubed format. These indexed PCR primers are provided in sealed 96-well PCR plates and arranged in ascending order in columns. Please refer to the excel sheet for exact barcode sequences.

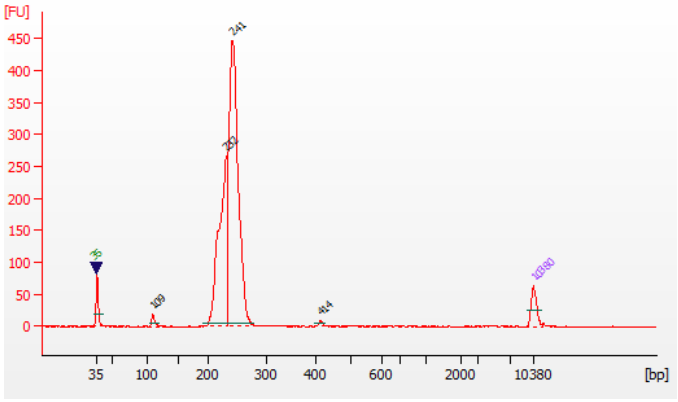
For MGI plated indexes, plate map and sequences information can be downloaded from Product Document Page under Indexes.

Troubleshooting Guide

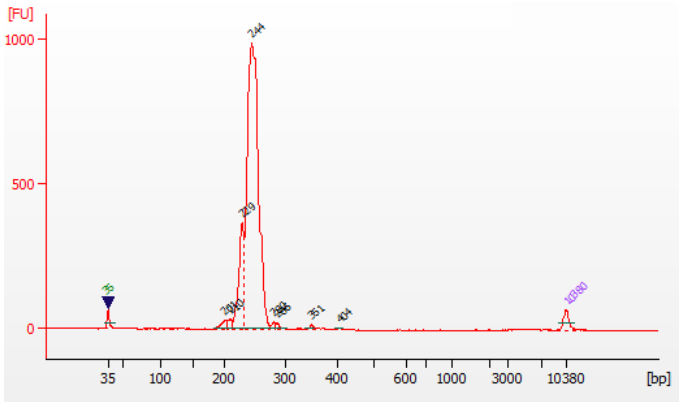
Examples of Libraries Prepared with CleanPlex for MGI NGS Panels

Depending on the CleanPlex for MGI NGS Panel used, library peak(s) should be between 200 and 400 bp. Below are representative Agilent Bioanalyzer traces generated for various CleanPlex for MGI Ready-to-Use Panels using 10 ng of gDNA as input.

CleanPlex for MGI OncoZoom Cancer HotSpot Panel



CleanPlex for MGI BRCA1 & BRCA2 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 specified amount of indexed primers to Second PCR Reaction Primer.

Peaks around 150–190 bp are residues of digested non-specific amplification products and adapter dimers. They come from incomplete removal of low molecular weight DNA fragments during the Post-Digestion Purification or nonspecific products formed during or after the Multiplex PCR (mPCR) Reaction due to deviation from protocol or high concentration of panel. The digestion reagent degrades non-specific amplification products into small pieces, which are then removed during magnetic bead purification. These peaks are usually caused by one or more of the following:

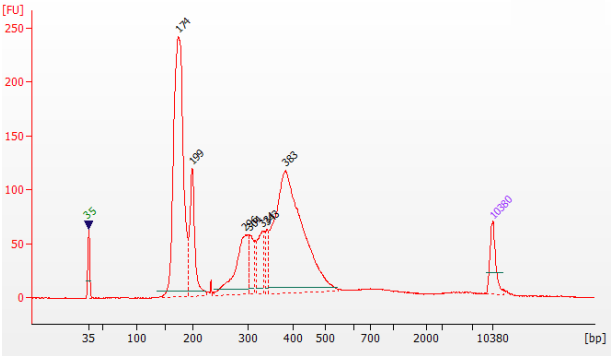
- Inaccurate pipetting of magnetic bead volume.
- Insufficient mixing of reaction solutions or bead and sample mixture.
- Insufficient removal of supernatant and ethanol washes during purification.
- Allowing mPCR products to sit for too long (and form nonspecific products) before purification.
- CleanPlex Custom NGS 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 nonspecific 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 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.

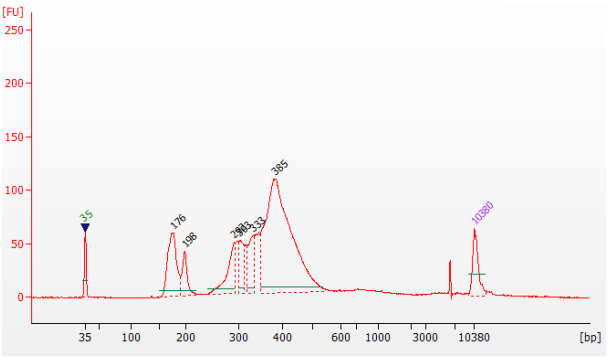
Removing nonspecific products from final libraries

If short nonspecific 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 >5 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 a CleanPlex NGS 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 5% for best results. When possible it's always better to repeat the library preparation with the corrected steps so nonspecific 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.
- Stop Buffer was not added or was added too late after Digestion Reaction, resulting in over-digestion of the samples. This may happen when handling a large number of samples.
- 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 using in the Second PCR Reaction. Only use CleanPlex for MGI Indexed PCR Primers with CleanPlex for MGI NGS Panels.
- A weak or incompatible magnetic rack was used to perform magnetic bead purification, resulting in significant bead loss. Do **not** use magnetic racks designed for 1.5 ml tubes.

Additional Resources

Please visit www.paragongenomics.com/product/faq/ for additional troubleshooting resources.

Data Analysis Recommendations for MGISEQ

For help with sequencing data analysis, please contact technical support.

Technical Support

For technical assistance, please contact Paragon Genomics Technical Support.

Phone: 650-822-7545
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