

CleanPlex[®] NGS Panel

Quick Guide



The CleanPlex[®] NGS Panel Quick Guide supplements the CleanPlex NGS Panel User Guide as a quick reference at bench side after users have become familiar with the protocol. For first time users and for detailed protocol and notes, refer to the full user guide at

www.paragongenomics.com/product_documents/.

This quick guide is for the following products:

- CleanPlex[®] OncoZoom[®] Panel
- CleanPlex[®] BRCA1 & BRCA2 Panel
- CleanPlex[®] TP53 Panel
- CleanPlex[®] Mitochondrial Disease Panel
- CleanPlex[®] Hereditary Cancer Panel
- CleanPlex[®] Custom NGS Panel

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Best Practices

Viscous Reagents

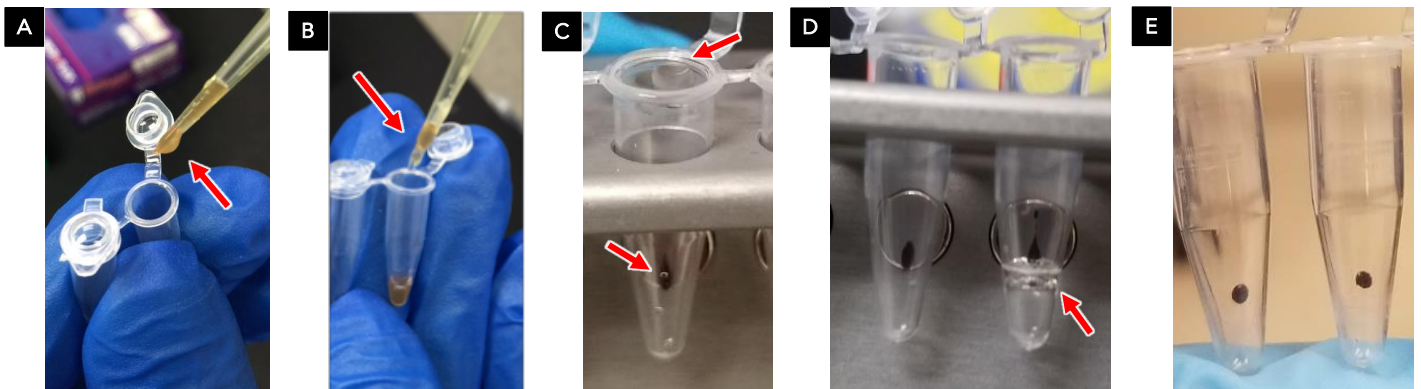
1. Take extra care in pipetting: since the reaction volumes are small, even +/- 1 μ L in volume dispensed can have a significant effect on the reaction.
2. Ensure accurate volume transfer (especially for viscous solutions such as PCR mixes, Digestion Reagent, and magnetic beads) by aspirating and dispensing slowly in addition to pipetting up and down to rinse tip as needed.
3. Always inspect the tip to avoid any liquid collecting on the outside of the tip prior to dispensing (Figure A). Also check to ensure no liquid is left on the inside of the tip after dispensing (Figure B).

Magnetic Beads

1. Warm beads to room temperature prior to use. Beads can be kept at room temperature for the entirety of the workflow and returned to 2-8 °C at the end of the day. Do NOT freeze.
2. Make small aliquots of beads for use to prevent contamination of the entire batch of bead solution.
3. Vortex to mix beads thoroughly prior to every use.
4. Use a magnetic rack/plate designed for PCR tubes or PCR plates for best results. Unnecessary tube to tube transfers result in volume loss, which can lead to incorrect beads to sample ratio and decreased yield.

Purification Steps

1. Make fresh 70% ethanol by adding 7 parts 100% ethanol to 3 parts nuclease-free water. Do not top off one liquid with the other in a volumetric container.
2. Use a 10 μ L tip for precise removal of liquids.
3. Liquids may collect on the wall of the tube (Figures C and D). Briefly spin the tube to collect liquids to the bottom of the tube.
4. Take extra care to avoid disturbing the bead pellet during the purification process. The DNA of interest is bound to the beads.
5. After the second ethanol rinse, briefly spin to collect liquids, place tube on magnetic rack to gather beads, and remove residual ethanol. Successfully collected bead pellets (Figure E) are ready to be air-dried. Do not over or under dry the bead pellets. Residual ethanol will inhibit PCR and result in reduced yield.



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Quick Protocol

1A. Multiplex PCR (mPCR) Reaction

- For each primer pool, combine:

6-X μ l	Nuclease-Free Water
2 μ l	5X PCR Mix (Green cap)
2 μ l	5X Primer Pool
X μ l	DNA Sample
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10 μ l	Total volume per primer pool
- Run 5 to 14 cycles of PCR (according to panel).
- Add 2 μ l of Stop Buffer (Red Cap). Final reaction volume is 12 μ l.

1B. Post-mPCR Purification

- For CleanPlex NGS Panels comprising of one primer pool, add 10 μ l of TE buffer to each sample. For CleanPlex NGS Panels with multiple primer pools, combine the 12 μ l multiplex PCR products for each sample.
- Perform a **1.3X** bead-based purification by adding magnetic beads to the sample.

2A. Digestion Reaction

- To each 10 μ l sample from Step 1B, add:

6 μ l	Nuclease-Free Water
2 μ l	CP Reagent Buffer (White cap)
2 μ l	CP Digestion Reagent (Yellow cap)
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10 μ l	Total volume per reaction
- Incubate at 37°C for **exactly 10 minutes**.
- Add 2 μ l of Stop Buffer (Red cap) to each sample. Final reaction volume is 22 μ l.

2B. Post-Digestion Purification

- Perform a **1.3X** bead-based purification by adding 29 μ l of magnetic beads to each sample.

3A. Second PCR Reaction

- To each 10 μ l sample from Step 2B, add:

18 μ l	Nuclease-Free Water
8 μ l	5X Second PCR Mix (Blue cap)
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26 μ l	Total volume per reaction
- For Illumina libraries, then add

2 μ l	i7 Indexed PCR Primer
2 μ l	i5 Indexed PCR Primer

- Or for Ion Torrent libraries, then add:

4 μ l	Indexed PCR Primer
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- Run 5 to 14 cycles of PCR (according to panel, DNA input amount, and DNA quality). Final reaction volume is 40 μ l.

3B. Post-Second PCR Purification

- Perform a **1X** bead-based purification by adding 40 μ l of magnetic beads to each sample.
- Completed and purified libraries can be stored with beads at -20 °C. When ready to sequence, thaw, vortex briefly to mix, quick spin, and place on magnetic rack to separate beads.

Magnetic Bead-Based Purification

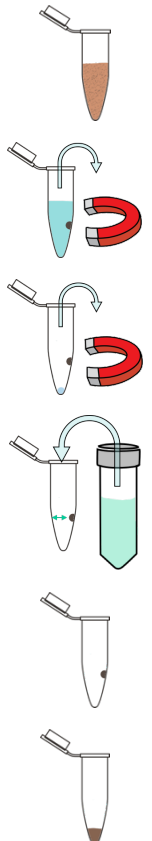
Before starting:

- Equilibrate CleanMag® Magnetic Beads at room temperature for at least 30 minutes.
- Make **FRESH** 70% ethanol. Ethanol content decreases with time as it evaporates.



Protocol:

- Add magnetic beads to each sample, vortex thoroughly, and incubate at room temperature for 5 minutes.
- Place tube on a magnetic rack/plate and discard supernatant.
- Quick spin and remove all residual supernatant.
- Wash 2 times with 70% ethanol. Move beads across the solution, through the ethanol, each time.
- Remove ethanol, then air-dry gathered beads for 5 minutes.
- Resuspend beads in TE buffer to elute DNA. There is no need to remove the beads for the next step of the protocol.



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