CleanPlex® UMI 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

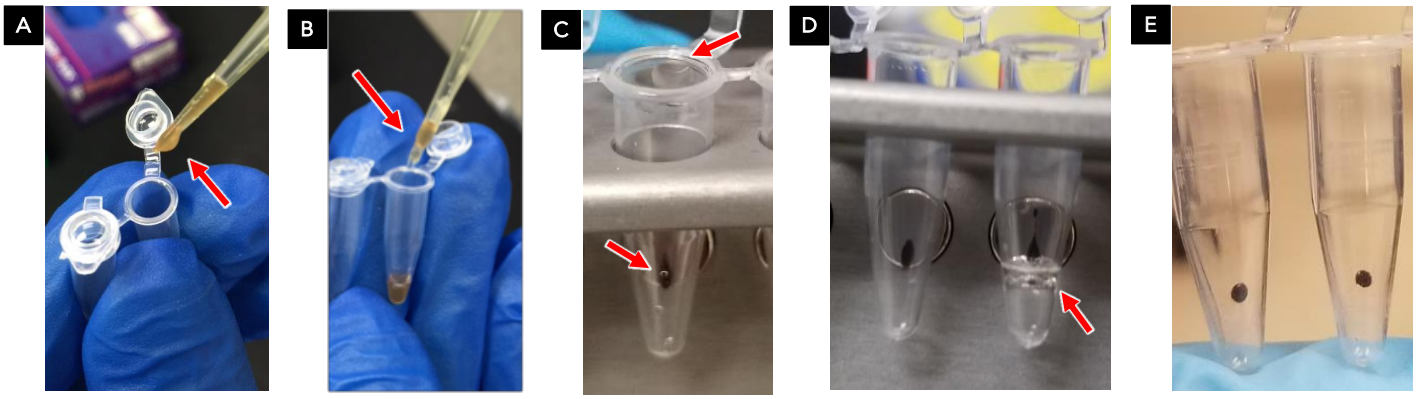
1. Take extra care in pipetting: since the reaction volumes are small, even +/- 1 µL in volume dispensed can have a significant effect on the reaction.
2. Ensure accurate volume transfer (especially for viscous solutions such as PCR mixes, Digestion Reagent, and magnetic beads) by aspirating and dispensing 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 µL 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.



QG2001-03
February 2024

Quick Protocol

1A. Barcoding Reaction

- For each primer pool, combine:

30-X µl	Nuclease-Free Water
8 µl	5X Barcoding Reagent (Green cap)
2 µl	20X Primer Pool
X µl	DNA Sample
40 µl	Total volume per primer pool

- Run 3 cycles of PCR.
- Add 2µl of Stop Buffer (red cap) into each tube/well and mix by spinning briefly, then vortexing.

1B. Post-Barcoding Purification

- Perform a **1.6X** bead-based purification by adding 67.2 µl of magnetic beads to the sample.
- Transfer the liquid of each reaction to a new tube.

2A. Resolving Reaction

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

6 µl	Nuclease-Free Water
2 µl	Resolving Buffer (Orange cap)
2 µl	Resolving Reagent (Yellow cap)
10 µl	Total volume per reaction

- Incubate at 37°C for **exactly 20 minutes**. Final reaction volume is 20 µl.
- Add 2µl of Stop Buffer (red cap) into each tube/well and mix by spinning briefly, then vortexing.

2B. Post-Resolving Purification

- Perform a **1.6X** bead-based purification by adding 35.2 µl of magnetic beads to each sample.
- Transfer the liquid of each reaction to a new tube.

3A. Amplification Reaction

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

18 µl	Nuclease-Free Water
8 µl	5X Amplifying Reagent (Blue cap)
26 µl	Total volume per reaction

- Then add

4 µl	UDI PCR Primers

- Run 18 to 21 cycles of PCR (according to DNA input amount and DNA sample type). Final reaction volume is 40 µl.

3B. Post-Amplification Purification

- Perform a **1X** bead-based purification by adding 40 µl 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.

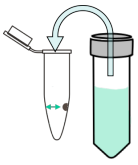
Protocol:

1. Add magnetic beads to each sample, vortex thoroughly, and incubate at room temperature for 5 minutes.

2. Place tube on a magnetic rack/plate and discard supernatant.

3. Quick spin and remove all residual supernatant

4. Wash 2 times with 70% ethanol. Move beads across the solution, through the ethanol, each time.



5. Remove ethanol, then air-dry gathered beads for 5 minutes.

6. Resuspend beads in TE buffer to elute DNA. There is no need to remove the beads for the next step of the protocol.