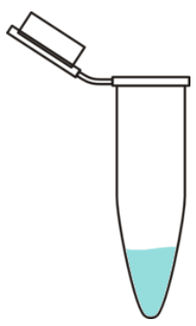
Quick Guide for CleanPlex® 3-Step Workflow

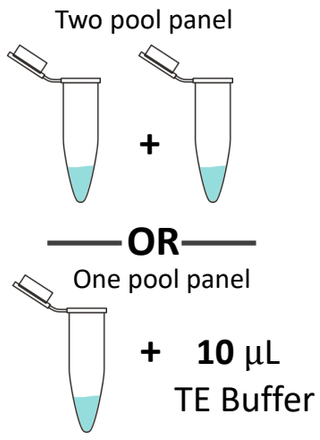
This quick guide is provided as a pictorial bench side aid to assist in daily library preparation. Please refer to the CleanPlex® user guide for the comprehensive protocol, notes, and product details. The reverse side contains the purification guide along with helpful tips for making consistently clean and high quality libraries for sequencing.

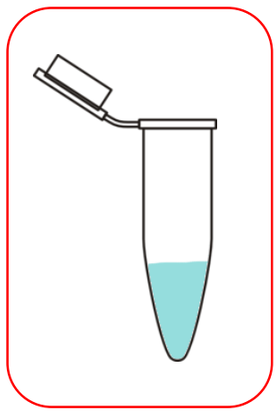
1. Multiplex PCR

For each panel pool
+ **6-X** µL Water
+ **2** µL 5X PCR Mix
+ **2** µL 5X Primer Pool
+ **X** µL DNA Sample
10 µL total per pool



10 cycles PCR
Annealing time
based on panel size



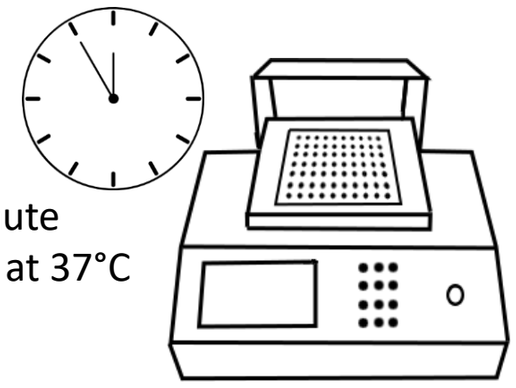


Follow with 1.3X bead purification

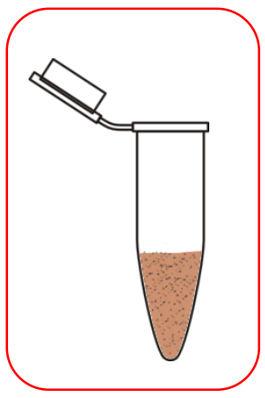
2. Digestion

Add to tube
+ **6** µL Water
+ **2** µL CP Digestion Buffer
+ **2** µL CP Digestion Reagent
= **10** µL total added per tube

10 Minute
incubation at 37°C



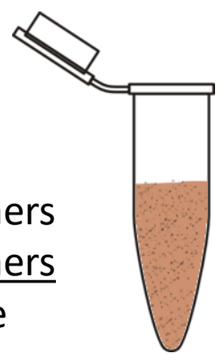
+ **2** µL Stop
Buffer



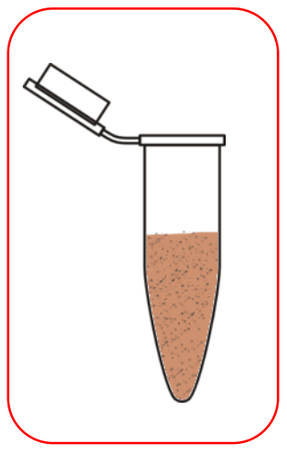
Follow with 1.3X bead purification

3. Amplification & Index addition

Add to tube
+ **18** µL Water
+ **8** µL 5X 2nd PCR Mix
+ **2** µL i7 Illumina Index Primers
+ **2** µL i5 Illumina Index Primers
= **30** µL total added per tube

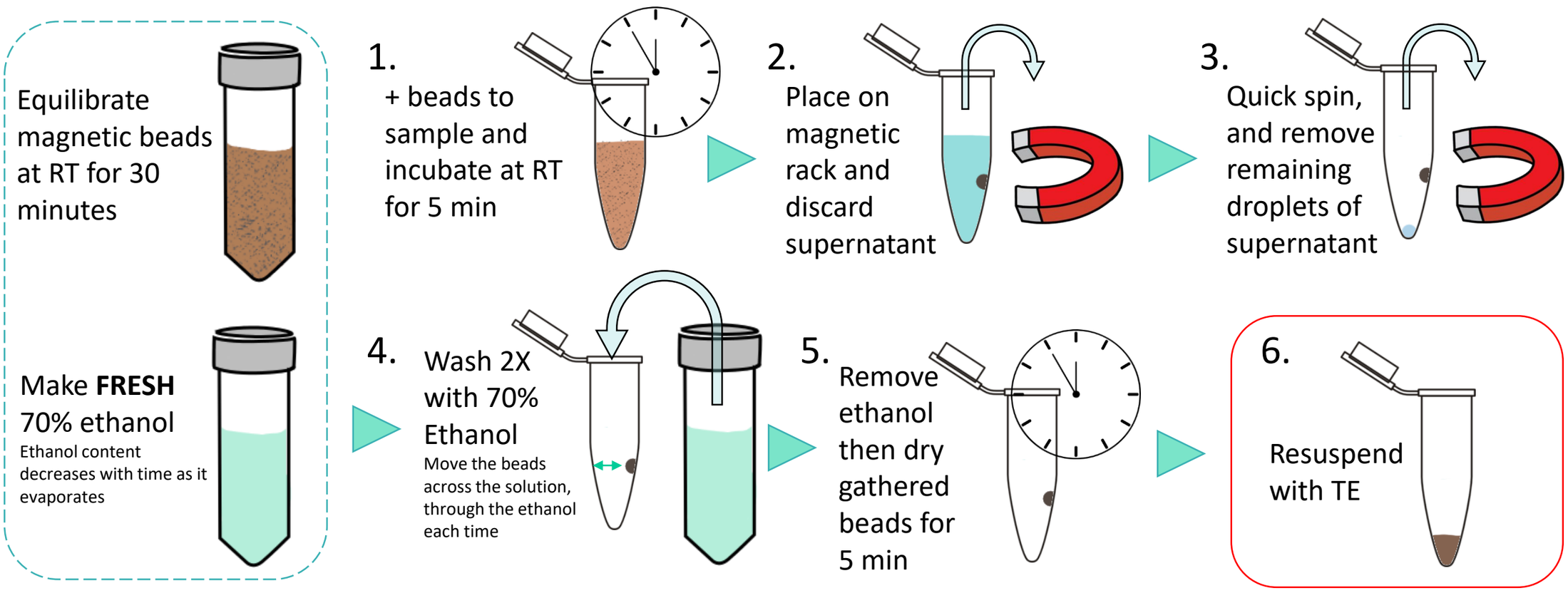


PCR cycle
depends on input
DNA quality and
amount. See
user guide.



Follow with 1X bead purification

CleanMag™ Purification

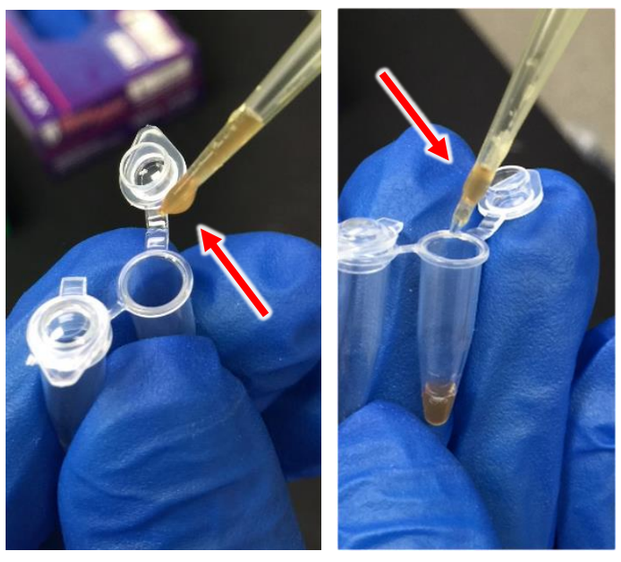


Completed libraries can be stored with beads at -20 °C. When ready to sequence, thaw, vortex briefly to mix, quick spin, and place on magnetic rack to move beads.

Tips & Notes for the Perfect Library

Viscous Reagents

1. Because reaction volumes are small, even +/- 1uL can have a large effect on the reaction.
2. Ensure accurate volume transfer (especially for viscous material such as PCR mixes, Digestion Reagent, and bead solution) by aspirating and dispensing slowly in addition to pipetting up and down to rinse tip as needed.
3. Always inspect the tip(s) to see if there is any liquid on the outside of the tip prior to dispensing, and if there is any liquid left over inside the tips after dispensing.



Magnetic Beads

1. Always vortex mix beads for 5-10 seconds prior to every use. When possible, make small aliquots of beads for use to prevent contamination of entire batch.
2. Allow beads to come to room temperature prior to use. Beads can be kept at room temperature during the entirety of the workflow and returned to 2-8°C at the end of the day. Do NOT freeze.
3. Use a magnetic rack designed for PCR tubes for best results. Unnecessary tube to tube transfers result in volume loss, which leads to inaccurate bead ratio and decrease in yield.
4. When withdrawing magnetic beads, the dripping droplets outside the tip should NOT be added into the reaction.
5. After adding magnetic beads, there will be some left over inside the tip. This bit of magnetic beads SHOULD be added into the reaction.

Purification Steps

1. When removing supernatant after each bead incubation, take care to remove as much of the supernatant as possible. Quick spin the tubes to collect droplets from walls and use a 10 uL tip for precision.
2. Make fresh 70% ethanol by adding 7 part 100% ethanol to 3 part nuclease free water. Do not top off one liquid with the other in a volumetric container.
3. After second ethanol rinse, quick spin to collect droplets to the bottom of tube, move beads with magnetic rack, and remove residual ethanol with 10 uL tip.
4. Take extra care to NOT disturb the bead pellet. The DNA of interest is bound to the beads.