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Quick Start Guide

5R-PLEX Kit

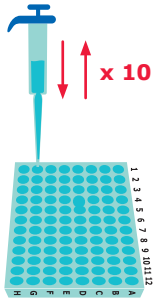
MBD6000

Prepare PCR 1 Mix

Water, microbial DNA-free	_____ µL
5X HF Buffer	10 µL
dNTPs	1 µL
5R-PLEX PCR1 Primers mix	0.25 µL
HF DNA Polymerase (add last)	0.5 µL
DNA (Add separately)	_____ µL
Total PCR1 volume	**50 µL**

1. Dispense PCR1 mix in each well.
2. Add ___ µL DNA.
3. Mix up and down 10 times.
4. Add 2 µL of diluted 5R-PLEX Positive Control.
5. Add 6 negative controls (no DNA template).

Total reaction volume/well 50 µL.



PCR1 Program

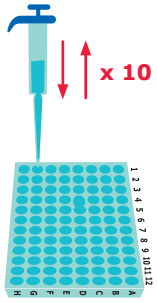
98 °C	2 minutes	
98 °C	10 seconds	x 30
62 °C	15 seconds	
72 °C	35 seconds	
72 °C	5 minutes	

Prepare PCR 2 Mix

Water, microbial DNA-free	_____ µL
5X HF Buffer	10 µL
dNTPs	1 µL
5R-PLEX PCR2 For Primers mix	0.25 µL
HF DNA Polymerase (add last)	0.5 µL
PCR1 product (Add separately)	2–5 µL
Total PCR2 volume	**50 µL**

1. Dispense PCR2 mix in each well of the 5R-PLEX index plate.
2. Add 2–5 µL of PCR1 Mix.
3. Mix up and down ten times.

Total reaction volume/well 50 µL.



PCR2 Program

98 °C	2 minutes	
98 °C	10 seconds	x 6
64 °C	15 seconds	
72 °C	25 seconds	
72 °C	5 minutes	

MilliporeSigma

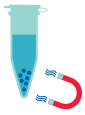
Bead Purification Process

The Bead Purification Process is performed twice, the first time is post PCR2, and the second in the Dual Pooled Library Purification Step. Note step 2 below.

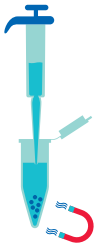
1. Vortex beads 30 seconds

2. Add 42.5 µL beads to 50 µL DNA sample (PCR2 product **OR** the purified Pooled Ligrary)

3. Mix up and down ten times.
4. Place tubes on magnetic stand for 5 minutes.



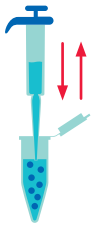
5. Remove and discard the supernatant.
6. Add 200 µL of freshly prepared 80% ethanol.
7. Incubate the tubes on the magnetic stand for 30 seconds
8. Remove and discard the supernatant



Repeat steps 6-8 x 2

9. **IMPORTANT** After the second wash, remove all traces of the ethanol wash.
   a. Briefly centrifuge.
   b. Return tubes to the magnetic stand and remove the ethanol residual with 10 µL pipette.

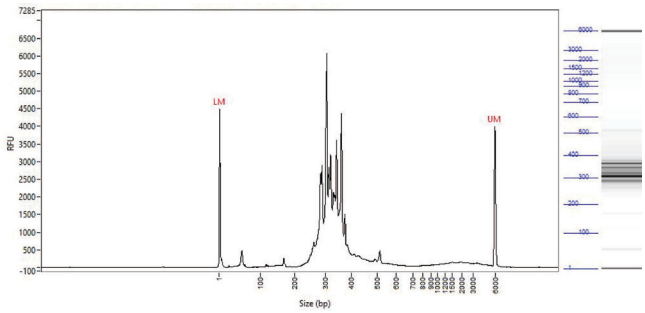
   c. Air-dry for 3–5 minutes. **Do not over-dry the bead.**
10. Remove tubes from the magnetic stand and add 20 µL of EB to each tube.
11. Mix up and down 10 times.
12. Incubate at room temperature 2 minutes
13. Place tubes on magnetic stand for 2–5 minutes or until supernatant is clear.



14. Transfer 18 µL of the supernatant (DNA) into a new tube.

Sample Library QC

Run on a Multiplexed Capillary Electrophoresis (CE) such as Bioanalyzer, TapeStation, or equivalent. and verify amplicons traces in the range size of 250–400 bp.



Quantify and Pool

1. Measure the DNA concentration of the purified libraries with a fluorometric quantification method that uses dsDNA binding dyes, such Qubit® dsDNA HS Assay Kit or equivalent.
2. Pool equal amount of each sample into a single tube (use the maximum amount possible).

3. Transfer a volume that is equivalent to up to 10 µg DNA of the pooled library in a new tube and continue to library purification.

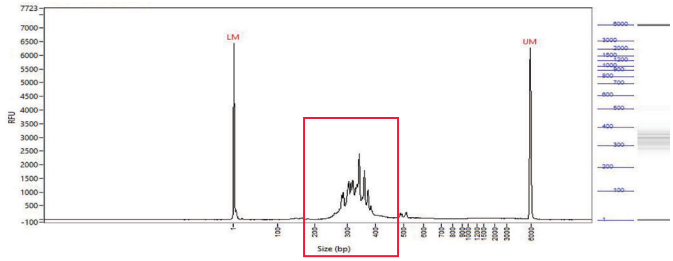
Dual Pooled Library Purification

1. Perform PCR clean up using the GenElute™ PCR Purification Kit (NA1020) or equivalent. Follow the GenElute™ PCR Clean-Up Procedure video at SigmaAldrich.com/genelute-pcr-cleanup.
2. Repeat **Bead Purification Process** on the previous page.

Pooled Library QC and Quantification

1. Perform QC for library size: Run on a Multiplexed Capillary Electrophoresis instrument and verify amplicons traces in the range size of 250–400 bp.

   IMPORTANT: If primer dimers are observed (~160–180 bp), it is highly recommended to repeat beads purification.



2. Library quantification: Measure the DNA concentration of the purified pooled library using KAPA Library Quantification Kit Illumina® Platforms (Roche, KK4873) (recommended) or Qubit® dsDNA HS Assay Kit.

Prepare the Library for NGS

Follow the Illumina® guide for preparing the library for sequencing of 150 cycles paired-end. The 5R-PLEX single index list can be found in the Appendix A in the Product Information Sheet. The Product Information Sheet can be downloaded from the 5R-PLEX product page at SigmaAldrich.com.

Bioinformatic Analysis

- Create account and log-in to m-camp.info/microbiome.
- Use the Key-code printed on the Quick Start Guide provided in the kit packaging.
- Upload fastQ in the 5R-PLEX module and follow the instructions in the Product Information Sheet.

Additional Information

The Product Information Sheet and other documents, are available on the product page at SigmaAldrich.com.

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