QUICK REFERENCE

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Ion Amplicon Library Preparation (Fusion Method)

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *Ion Amplicon Library Preparation (Fusion Method) User Guide* (Part no. 4468326). For every reagent, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended as a benchtop reference for experienced users. Refer to the *Ion Amplicon Library Preparation (Fusion Method) User Guide* for detailed instructions and additional information.

PCR amplify genomic DNA targets

Refer to the *Ion Amplicon Library Preparation (Fusion Method) User Guide* for information on PCR primer design.

1. Thaw the PCR primers, Platinum® PCR SuperMix High Fidelity, and high-quality genomic DNA on ice.
2. For each primer pair, mix equal volumes of 10 µM forward and 10 µM reverse primers for a 10-µM primer stock mix (5 µM of each primer).
3. Add the following reagents to 0.2-mL strip tubes or to the wells in a 96-well PCR plate exactly in this order:

Component	Volume
Platinum® PCR SuperMix High Fidelity	45 µL
20–50 ng genomic DNA	4 µL
10-µM primer stock mix	1 µL
Total	**50 µL**

4. Load the tubes or plates in a thermal cycler and run the program to amplify the target DNA.

   Note: Amplification conditions may vary according to primer design and DNA input. Adjust the cycling conditions and number of cycles for your specific experiment to achieve optimal results.

Stage	Step	Temperature	Time
Holding	Activate the enzyme	94°C	3 min
Cycling (40 cycles)	Denature	94°C	30 sec
	Anneal	58°C	30 sec
	Extend	68°C	1 min/kb
Holding	—	4°C	∞

Purify the amplicon libraries

IMPORTANT! If the library length, including amplicon and fusion primer sequence, is <100 bp, use a different purification method such as Qiagen® MinElute® PCR Purification Kit.

1. Resuspend the Agencourt® AMPure® XP Reagent and allow the mixture to come to room temperature (~30 minutes).
2. Prepare sufficient 70% ethanol for the purification: 60 µL per PCR reaction.

   IMPORTANT! Use *freshly prepared* 70% ethanol for the next steps.
3. Add 90 µL Agencourt® AMPure® XP Reagent to each well or tube, mix the bead suspension with the DNA by pipetting up and down several times, and incubate the samples at room temperature for 5 minutes.
4. Place each plate or tube on a magnet (such as the Agencourt® SPRIPlate 96R Magnet Plate or Magna-Sep™ 96 Magnetic Particle Separator) for 2 minutes. After the solution clears, carefully remove and discard the supernatant from each well or tube without disturbing the pellet.
5. Without removing the samples from the magnet, add 30 µL of freshly prepared 70% ethanol to each well or tube, and incubate the samples at room temperature for 30 seconds. After the solution clears, remove and discard the supernatant without disturbing the pellet.
6. Repeat step 5 for a second wash.
7. To remove residual ethanol, keep the samples on the magnet and carefully aspirate remaining supernatant with a 20-µL pipet without disturbing the pellet.
8. Air-dry the beads on the magnet at room temperature for ≤5 minutes.
9. Remove the samples from the magnet, add 20 µL of TE to each well or tube, and pipet the samples up and down to mix.

10. Place the plate or tube on the magnet for at least 1 minute until the solution clears and the beads are pelleted. Transfer each supernatant, which contains the amplicon library, to a new well or tube.

    IMPORTANT! The supernatant contains the amplicon library. Do not discard!

Prepare an equimolar pool of the amplicon libraries

1. Analyze an aliquot of each amplicon library with a Bioanalyzer™ instrument and Agilent DNA 1000 Kit or Agilent High Sensitivity Kit, as appropriate for the final library concentration.

   IMPORTANT! Ensure that excessive amounts of primer-dimers or over-amplification products (concatemers) are not present.

2. Determine the molar concentration (nmol/L) of each amplicon library using the Bioanalyzer™ software. If necessary, use manual integration to place the entire range of library fragments within a single peak.
3. Prepare an equimolar pool of amplicon libraries at the highest possible concentration.
4. Calculate the combined concentration of the pooled amplicon library stock.

   Alternatively, analyze an aliquot of the library pool on the Bioanalyzer™ with an Agilent DNA 1000 Kit, and use the Bioanalyzer™ software to determine the molar concentration of the pooled library stock.

   STOPPING POINT Store the pooled library stock at –20°C in several aliquots.

Determine the dilution required for Template Preparation

Determine the Template Dilution Factor of the library pool.

Template Dilution Factor = (Library pool concentration [pM])/26 pM

Note: You will need to prepare three serial dilutions of the library at ½× Template Dilution Factor, Template Dilution Factor, and 2× Template Dilution Factor, to ensure that one or more dilutions are in the optimal concentration range for the Template Preparation procedure.

Note: Do not quantitate fusion PCR amplicon libraries with the Ion Library Quantitation Kit (Part no. 4468802). Libraries prepared using trP1 and A sequences are incompatible with this qPCR method.

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