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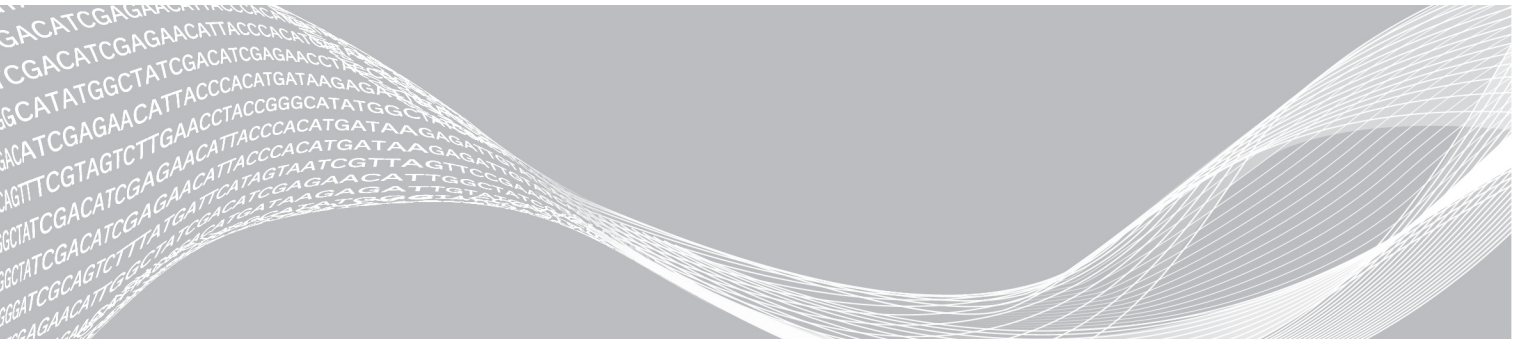
Illumina Free Adapter Blocking Reagent

Reference Guide



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Protocol

Introduction

This protocol provides information for performing a free adapter blocking treatment on libraries to block excess free adapter, minimizing potential index hopping levels and enhancing data quality.

Use this method on unpooled or pooled libraries with any NGS library prep method before the denaturation step and subsequent cluster generation using Illumina sequencers.

Sample Treatment Workflow

The following diagram illustrates the treatment workflow.

Figure 1 Sample Treatment Workflow

Sample Treatment

The following consumables and equipment are required to perform the protocol. Part numbers are provided where applicable.

Consumables and Equipment

Item	Part Number	Supplied By
Illumina Free Adapter Blocking Reagent (FAB) 12 reactions	20024144	Illumina
Illumina Free Adapter Blocking Reagent (FAB) 48 reactions	20024145	Illumina
Agencourt Ampure XP beads	A63880	Beckman Coulter Life Sciences
Library sample pool – 30 µl at concentration to be used for denaturation during clustering		User
Freshly prepared 80% ethanol (EtOH)		User
Ethanol 200 proof, for molecular biology (absolute)	E7023	Sigma-Aldrich
Magnetic Stand-96	AM10027	Thermo Fisher Scientific
Vortexer		General lab supplier
96-well thermal cycler (with heated lid)		General lab supplier

About Reagents

- Vortex Ampure XP before each use.
- Vortex Ampure XP frequently to make sure that beads are evenly distributed.
- Aspirate and dispense Ampure XP slowly due to the viscosity of the solution.

Preparation

1 Prepare the following consumables.

Item	Storage	Instructions
FAB	-25°C to -15°C	Thaw at room temperature, and then place on ice. Return to storage after use.
RSB	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.
Ampure XP beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2 Save the FAB program on the thermal cycler:
   - Choose the preheat lid option and set to 100°C.
   - 38°C for 20 minutes
   - 60°C for 20 minutes
   - Hold at 4 °C

   Each tube contains 60 µl.

Procedure

Sample Treatment

1 Mix FAB reagent by inversion.

2 Centrifuge FAB at 600 × g for 5 seconds.

3 Add 30 µl of library sample pool to PCR tube.

4 Add 30 µl of FAB to each PCR tube and then mix thoroughly by pipetting up and down.

5 Centrifuge briefly to make sure all contents are on the bottom of the tube.

6 Incubate by placing on the thermal cycler and running the FAB program.

Clean Up Pooled Sample

1 Vortex AMPure XP until well-dispersed.

2 Add 60 µl AMPure XP to each sample treatment tube and mix thoroughly by pipetting up and down.

3 Incubate at room temperature for 5 minutes.

4 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

5 Remove and discard all supernatant from each tube.

6 Wash 2 times as follows:
- Add 200 µl freshly prepared 80% EtOH to each tube.
- Incubate on the magnetic stand for 30 seconds.
- Remove and discard all supernatant from each tube.

7 Use a 20 µl pipette to remove residual EtOH from each tube.

8 Air-dry on the magnetic stand for 5 minutes.

9 Add 22.5 µl RSB to each tube.

10 Remove from the magnetic stand and then mix thoroughly by pipetting up and down.

11 Incubate at room temperature for 2 minutes.

12 Place on a magnetic stand and wait until the liquid is clear (2–5 minutes).

13 Transfer 20 µl supernatant to a new tube.

14 Quantify libraries if necessary and proceed onto standard clustering for your Illumina sequencing instrument starting with the NaOH denaturation step.

15 Store at -25°C to -15°C if not clustering immediately.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

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Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download in PDF from the Illumina website. Go to support.illumina.com, select a product, then select **Documentation & Literature**.

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