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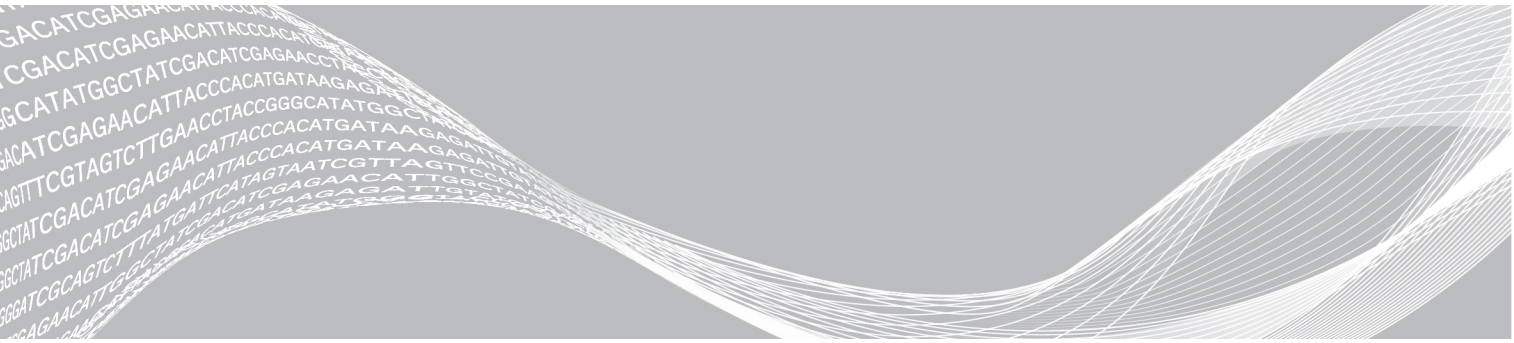
AmpliSeq Direct FFPE DNA Kit for Illumina

Reference Guide



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Kit Contents

The AmpliSeq Direct FFPE DNA Kit for Illumina provides sufficient reagents to bypass nucleic acid isolation and prepare DNA from unstained, slide-mounted, formalin-fixed, paraffin-embedded (FFPE) tissue samples.

Kit	Reagent	Volume	Storage
AmpliSeq Direct FFPE DNA Kit for Illumina (24 reactions, catalog # 20023378)	FFPE DNA Transfer Solution (purple cap)	720 µl	2°C to 8°C
	FFPE DNA Direct Reagent (orange cap)	510 µl	2°C to 8°C

About Reagents

- FFPE preservation methods can cause significant cytosine deamination within the isolated DNA, which decreases sequencing quality. Optionally, treat FFPE DNA with UDG to remove deaminated cytosines (uracil).
- Use the Qubit dsDNA HS Assay Kit for optional quantification of FFPE DNA before amplification. For detailed information and instructions for this kit, see the manufacturer documentation.

Consumables and Equipment

In addition to the AmpliSeq Direct FFPE DNA Kit for Illumina, make sure that you have the required consumables and equipment before starting the protocol.

Consumables

Item	Supplier
Eppendorf DNA LoBind Microcentrifuge Tubes, 0.5 ml or 1.5 ml	Fisher Scientific, catalog # 13-698-791
MicroAmp Clear Adhesive Film	Thermo Fisher Scientific, catalog # 4306311
One of the following 96-well PCR plates: For use with Thermo Fisher thermal cyclers: • MicroAmp EnduraPlate Optical 96-Well Clear Reaction Plates with Barcode For use with Bio-Rad thermal cyclers: • Hard-Shell 96-Well Skirted PCR Plates, low-profile, skirted	One of the following suppliers, depending on plate type: • Thermo Fisher Scientific, catalog # 4483352 or 4483354 • Bio-Rad, catalog # HSP-9601
MicroAmp Optical Film Compression Pad (required for use with Thermo Fisher thermal cyclers)	Thermo Fisher Scientific, catalog # 4312639
Pipettes, 2–200 µl, and low-retention filtered pipette tips	Fisher Scientific
[Optional] UDG (Uracil DNA Glycosylase)	Thermo Fisher Scientific, catalog # EN0361
[Optional] Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific, catalog # Q32851

Equipment

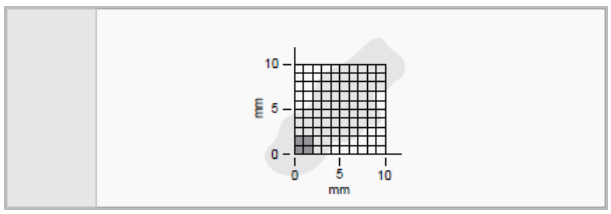
Item	Supplier
One of the following thermal cyclers. Thermo Fisher thermal cyclers: • SimpliAmp Thermal Cycler • Applied Biosystems 2720 Thermal Cycler • Veriti 96-Well Thermal Cycler • ProFlex 96-well PCR System • GeneAmp PCR System 9700* Bio-Rad thermal cyclers: • C1000 Touch Thermal Cycler • S1000 Thermal Cycler	Thermo Fisher Scientific, see web product pages for catalog numbers Bio-Rad: • Part # 1851196 • Part # 1852196

* No longer available for purchase.

Preparation

1 Prepare a 4–100 mm² tissue area from an unstained section that is 5–10 µm thick and mounted on a slide. Optionally remove any unwanted tissue from the slide.
In the following example, the shaded squares show a 4 mm² area in a 100 mm² grid.

Figure 1 Example Tissue on a Standard Microscope Slide (25 mm × 75 mm)



NOTE
Deparaffinization is not required.

2 Prepare the following consumables.

Item	Storage	Instructions
Transfer Solution	2°C to 8°C	Bring to room temperature. Vortex briefly or pipette to mix.
Direct Reagent	2°C to 8°C	Keep on ice. Vortex briefly or pipette to mix.
[Optional] UDG	-25°C to -15°C	Thaw on ice. Vortex briefly or pipette to mix.

3 Save the following PREP program on a thermal cycler:
- 65°C for 15 minutes
- Hold at 20°C for up to 30 minutes

Procedure

1 For each sample, add 30 µl Transfer Solution to one well of a 96-well plate.

2 Transfer 2–10 µl Transfer Solution from the well to the tissue region of interest.

3 Use the pipette tip to evenly spread the Transfer Solution over the region of interest, and then break up the tissue with the same pipette tip.

The resulting tissue is a slurry of fine particles in the Transfer Solution.

4 Transfer the slurry from the slide to the well containing Transfer Solution.

5 Pipette at least five times to mix. Leave as much tissue as possible in the well.

6 If necessary, repeat steps 2–5 to transfer the entire region of interest to the 96-well plate.
The final volume of Transfer Solution in the 96-well plate can vary, but adjusting the volume is not necessary.

7 Add 21 µl Direct Reagent to each well in the 96-well plate that contains sample.

8 Pipette 10 times to mix, and then seal the plate.

9 Centrifuge to collect the contents.

10 Place on the thermal cycler, cover with a compression pad (if applicable), and run the PREP program.

11 [Optional] If concerned about cytosine deamination in the isolated DNA, treat with UDG as follows.

   a Add 1–2 units UDG to the lower (aqueous) phase of each FFPE sample.
   b Pipette the lower phase of the sample 10 times to mix.
   c Centrifuge briefly to collect contents and separate the phases.
   d Seal the plate.
   e Incubate at 37°C for 5 minutes, then 65°C for 5 minutes.

12 [Optional] Analyze 2 µl library using the Qubit 2.0 or 3.0 Fluorometer with the Qubit DNA HS Assay Kit.

Remove an Aliquot for Library Preparation

1 Pipette the lower (aqueous) phase of the sample 10 times to mix.

2 Depending on the number of primer pools and reaction size, transfer 6–20 µl of the lower phase to the appropriate well of a 96-well PCR plate or to a master mix tube.

Troubleshooting

Problem	Potential Cause	Resolution
Tissue does not transfer from the slide.	Insufficient volume of Transfer Solution.	Hold the slide at a 45° angle and add Transfer Solution to the top of the slide, allowing the tissue to flow down. Remove collected tissue from the bottom of the slide, and repeat as needed.
	Tissue is clumpy.	Transfer the tissue mass to a collection tube and break it up with a pipette tip.
		Preincubate the tissue with Transfer Solution on the slide for 5 minutes before scraping.
Difficulty scraping tissue from the slide.	Tissue is fibrous.	Using a circular motion, scrape with 200 µl pipette tip before transfer, and then continue with a 20 µl pipette tip.
		Scrape and homogenize tissue with a scalpel blade, and then continue breaking up tissue with a 20 µl pipette tip.
		Repeat the transfer step with a greater volume of Transfer Solution.
	Target tissue area is surrounded by unwanted tissue.	Use a scalpel blade to remove undesired tissue or paraffin, and then use Transfer Solution to collect the desired tissue.
Direct Reagent contains excess undissolved tissue.	Reaction contains too much tissue.	Use a 4– 100 mm2 tissue section that is 5–10 µm thick.
	Digest might be incomplete.	Incubate for an additional 5–15 minutes at 65°C. The sample might be cloudy after digestion, but this cloudiness does not affect performance.
		Centrifuge at ≥ 1,000 × g for 1 minute, and then transfer 15 µl to a fresh tube. Avoid the fibrous pellet.
Transfer Solution and Direct Reagent do not separate into two phases.	Too much paraffin in sample.	Use a scalpel blade to remove unwanted paraffin before adding Transfer Solution to the desired tissue area.
		Centrifuge at ≥ 1,000 × g for 1 minute, and then transfer 15 µl to a fresh tube. Avoid the fibrous pellet and tube walls.
		Partially deparaffinize before adding Transfer Solution to tissue on the slide: • Submerge the slide in 100% xylene for 30 seconds. • Remove the slide, and then drain any excess xylene. • Submerge the slide in 100% ethanol for 30 seconds. • Remove the slide, and then air-dry.
Difficulty transferring lower (aqueous) phase to target amplification reaction.	Pipette tip contains Transfer Solution.	Return tip contents to the reaction tube, and then centrifuge at ≥ 1,000 × g for 1 minute to separate phases. Move pipette quickly through the upper phase when transferring. Transfer Solution does not interfere with target amplification.
Library concentration is low.	Insufficient tissue was used.	Use 25–100 mm² tissue section of 5–10 µm thickness. If needed, use multiple slides to obtain 25–100 mm² tissue.
	Insufficient amplifiable DNA was used.	Although the Qubit assay can detect the presence of DNA, the DNA quality might not be sufficient for generating an AmpliSeq Library PLUS library. Reprepare FFPE DNA from a 100 mm² tissue section that is 5–10 µm thick. If needed, use multiple slides to obtain 100 mm² tissue.
Qubit result indicates a high concentration (> 10 ng/ µl).	FFPE tissue has high DNA content.	Reduce the volume of input sample going into the target amplification reaction by one-half to one-quarter.

Problem	Potential Cause	Resolution
Qubit result indicates low concentration (< 0.5 ng/µl).	FFPE tissue has low DNA content.	Increase the number of target amplification cycles by two or three. Samples with low DNA yield can be sufficient to generate an AmpliSeq Library PLUS library.

Revision History

Document	Date	Description of Change
Document # 1000000056164 v00	July 2018	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
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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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