FFPE DNA QC Kit

RK20229

www.abclonal.com

Version：N16F15v1.0

Contents

1. Introduction

FFPE DNA QC Kit is designed for the evaluation of the integrity and quality of FFPE samples by multiplex PCR. This kit contains 4 pairs of specific primers and high-fidelity DNA polymerase which enables users to define the quality of FFPE sample DNA via multiplex PCR methodology. An optimal PCR cycle number for NGS library amplification is determined by the number amplified bands.

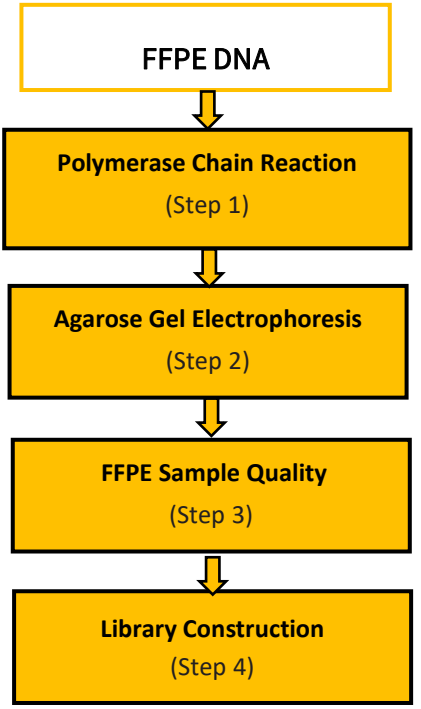

Figure 1. Overall sample preparation workflow.

2. Components

All components should be stored at -20°C. The shelf life of each reagent is one year when stored properly.

Table 1. Components supplied with this product

Components	8 RXN	24 RXN	96 RXN
Gloria Nova HS 2X HF Master Mix	100 µL	300 µL	1200 µL
Primers FIL	4 µL	12 µL	48 µL

3. Storage

The FFPE DNA QC Kit must be stored at -15°C to -25°C. Dry ice or dry ice combined with ice packs should be used for long-distance transportation.

4. Notes

Input DNA should be quantified using Qubit® or other fluorometric quantification kits. Impurities in DNA samples, such as trace amounts of residual RNA, nucleotides, single-stranded DNA, and other contaminants may have an impact on library construction. Avoid vortexing the supplied enzyme; to mix, use a pipette.

5. Protocol

Step 1. Polymerase Chain Reaction

1.1 Preheat PCR instrument to 98°C.

1.2 Prepare a sample tube for each FFPE sample alongside a positive control reaction containing 5 ng Human gDNA. Arrange the following reaction mixtures on ice:

Table 2. Polymerase Chain Reaction Setup (per sample)

Component	Volume
Gloria Nova HS 2X HF Master Mix	12.5 µL
Primers FIL (10 µM)*	0.5 µL
FFPE DNA	5 ng
ddH_2O	to 25 µL

Note: Primers FIL is a mixture of 4 primers that amplify 100 bp, 200 bp, 300 bp, and 400 bp fragments. FFPE DNA samples can amplify 0-4 bands according to their quality.

1.3 Place the tube on the thermocycler and run the reaction program described in Table 3. Set the temperature of the heated lid to 105℃.

Table 3. Thermal cycler program for Library Amplification

Temperature	Time	Cycles
98°C	45 s	1
98°C	10 s	30
60°C	30 s	
72°C	30 s	
72°C	1 min	1
4°C	∞	1

Step 2. Agarose Gel Electrophoresis

Visualize the PCR amplification products via agarose gel electrophoresis on a 4% agarose gel using an appropriate dye.

Step 3. FFPE Sample Quality Analysis

FFPE samples are graded according to the PCR product amplification results visualized via gel electrophoresis according to the grading criteria shown in Table 4.

Table 4. Grading Standards for FFPE Sample Quality

Number Of Product Bands	Grading Standard
4	1
3	2
2	3
1	4
0	5

Note: To ensure experimental reliability, ensure that the control produces the four expected bands at 100 bp, 200 bp, 300 bp, and 400 bp. FFPE DNA samples can amplify 0-4 bands according to their quality. See the Appendix for more information.

Step 4. Library Construction

Refer to Table 5 for the recommended kits suitable for subsequent library construction.

Table 5. Recommended kits for Library Construction

Recommended Kit	Catalog Number
Rapid Plus DNA Lib Prep Kit for Illumina V2	RK20255
FS Pro DNA Lib Prep Kit for Illumina	RK20261

Refer to Table 6 for the recommended amplification cycles to obtain 1 μg final NGS library when using FFPE input DNA with the Rapid Plus DNA Lib Prep Kit for Illumina V2 (ABclonal, Cat. No. RK20255) or the FS Pro DNA Lib Prep Kit for Illumina (ABclonal, Cat. No. RK20261).

Table 6. Recommended FFPE Amplification Cycles for RK20255/RK20261

FFPE Sample Quality	Number of cycles required to generate 1 μg library*	
	Input DNA 200 ng	Input DNA 30-200 ng
1	6	6-9
2	7	7-10
3	9	9-12
4	11	11-14
5	/	/

6. Appendix

Figure 2: A 4% agarose gel is used to qualify FFPE samples for NGS library construction. Human gDNA template serves as positive control and is shown in lane 1. The samples in lanes 2, 3, and 4 are three different FFPE samples of differing quality and are Grade 1, Grade 1, and Grade 3, respectively.

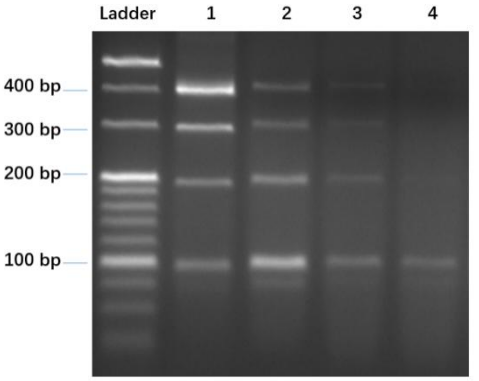

Figure 2. FFPE Sample Grading

Companion Library Construction Products:

DNA can be fragmented enzymatically using DNA Frag Module (ABclonal, Cat. No. RK20260).

For DNA fragmented using mechanical shearing or restriction enzyme cleavage, use Rapid Plus DNA Lib Prep Kit for Illumina V2 (ABclonal, Cat. No. RK20255).

United States

www.abclonal.com

Address: 500 W. Cummings Park Dr, Woburn, MA 01801, United States

Phone: 888.754.5670, +1 857.259.4898 (Int'l)

Email: service@abclonal.com