

# KAPA Frag Kit

## for Enzymatic Fragmentation

KR1141 – v4.17

This Technical Data Sheet provides product information and a detailed protocol for the KAPA Frag Kit for Enzymatic Fragmentation.

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Kapa/Roche Kit Codes and Components		
<b>KK8600</b> 7962495001 8 reactions	KAPA Frag Enzyme	100 µL
	KAPA Frag Buffer (10X)	50 µL
	Conditioning Solution	580 µL
	Stop Solution	580 µL
<b>KK8601</b> 7962509001 24 reactions	KAPA Frag Enzyme	270 µL
	KAPA Frag Buffer (10X)	140 µL
	Conditioning Solution	580 µL
	Stop Solution	580 µL
<b>KK8602</b> 7962517001 96 reactions	KAPA Frag Enzyme	1,270 µL
	KAPA Frag Buffer (10X)	640 µL
	Conditioning Solution	580 µL
	Stop Solution	580 µL

Quick Notes
<ul style="list-style-type: none"> <li>• This kit is designed for the enzymatic fragmentation of 1 ng – 1 µg of double-stranded DNA, for the construction of high-quality NGS libraries.</li> <li>• The fragmentation parameters provided in this protocol are a starting point, and may have to be optimized for your specific sample type and input. Please refer to <b>Reaction Optimization</b> for guidelines.</li> <li>• A bead-based cleanup or size selection is required after fragmentation. KAPA Pure Beads are recommended for this application and are sold separately.</li> <li>• For DNA preparations containing EDTA we recommend a 3X KAPA Pure Beads cleanup prior to enzymatic fragmentation. Alternatively, Conditioning Solution may be used. Please refer to <b>Important Parameters: Input DNA</b> for details.</li> <li>• The KAPA HyperPlus Kit combines the KAPA Frag and KAPA Hyper Prep chemistries in a streamlined, one-tube fragmentation/library construction protocol that offers the highest library yields from your available sample. For more information, please visit <a href="http://www.sequencing.roche.com">www.sequencing.roche.com</a>.</li> </ul>

### Product Description

The KAPA Frag Kit for Enzymatic Fragmentation provides for robust and reproducible enzymatic fragmentation of double-stranded DNA (dsDNA) across a wide range of sample types and inputs (1 ng – 1 µg), and may be incorporated in any NGS library construction workflow that requires fragmented dsDNA as the input.

The workflow is automation-friendly, and unlike mechanical shearing, does not require any specialized equipment or consumables. The degree of fragmentation (mode size and size distribution of DNA fragments) is controlled by fragmentation time and temperature. Optimal fragmentation parameters are somewhat dependent on the amount and nature of input DNA, but the KAPA Frag system is much less sensitive to DNA input and quality, and significantly more reproducible than other enzymatic fragmentation technologies, including tagmentation.

Libraries produced from DNA fragmented with the KAPA Frag system are functionally equivalent to libraries prepared from Covaris-sheared DNA.

### Product Applications

The KAPA Frag Kit is ideally suited for low- and high-throughput fragmentation of dsDNA for NGS library construction. It is compatible with complex, genomic DNA; low-complexity samples such as small viral genomes, plasmids, cDNA and long amplicons; and low-quality DNA such as FFPE samples. Fragmented DNA may be used in a variety of NGS applications, including:

- whole-genome shotgun sequencing
- whole exome or targeted sequencing, using Roche SeqCap EZ, Agilent SureSelect, Illumina TruSeq, IDT xGen Lockdown™ Probes, or other hybridization capture systems
- sequencing of long amplicons
- selected RNA-seq applications.

The **KAPA HyperPlus Kit** combines the KAPA Frag and KAPA Hyper Prep chemistries in a streamlined, one-tube fragmentation/library construction protocol that offers the highest library yields from your available sample. Please visit [www.sequencing.roche.com](http://www.sequencing.roche.com) for more information.

### Product Specifications

#### Shipping and Storage

The enzymes provided in this kit are temperature sensitive, and appropriate care should be taken during shipping and storage. KAPA Frag Kits for Enzymatic Fragmentation are shipped on dry ice. Upon receipt, immediately store enzymes and reaction buffers at -15°C to -25°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the kit components will retain full activity until the expiry date indicated on the kit label.

#### Handling

Always ensure that components have been fully thawed and thoroughly mixed before use. Keep all enzyme components and master mixes on ice as long as possible during handling and preparation.

#### Quality Control

All kit components are subjected to stringent functional quality control, are free of detectable contaminating exo- and endonuclease activities, and meet strict requirements with respect to DNA contamination. Please contact Technical Support at [sequencing.roche.com/support](http://sequencing.roche.com/support) for more information.

## Important Parameters

### Input DNA

- This protocol is suitable for fragmentation of 1 ng – 1 µg of double-stranded DNA.
- The enzymatic fragmentation reaction is very sensitive to the presence of EDTA, which must be removed or neutralized prior to fragmentation.* EDTA in DNA preparations is usually introduced via elution buffers used in the final stages of the DNA extraction or purification process.
- Removal of EDTA from DNA samples prior to fragmentation is recommended to ensure consistent results. This may be achieved by means of a 3X bead-based cleanup with KAPA Pure Beads. Please refer to the **KAPA Pure Beads Technical Data Sheet** for a detailed DNA cleanup protocol. For optimal fragmentation results, elute DNA in 10 mM Tris-HCl (pH 8.0 – 8.5) after the cleanup.
- DNA isolated from blood samples has been reported to contain inhibitors, which can affect the efficiency of fragmentation. Performing a 3X bead-based cleanup prior to fragmentation is recommended.
- Bead-based cleanups to remove EDTA from FFPE DNA samples may not yield comparable results. Recovery of FFPE DNA may be low, and not always proportional to DNA quality. For FFPE DNA, neutralization of EDTA with the Conditioning Solution (see below) is recommended as a first approach.
- If a DNA cleanup is not feasible, the inhibitory effect of the EDTA can be mitigated by the inclusion of Conditioning Solution at the appropriate final concentration in the fragmentation reaction.
- To facilitate reaction setup, the Conditioning Solution is pre-diluted to the appropriate working concentration as outlined in Table 1, and a fixed volume (5 µL) is included in the fragmentation reaction. **Please note that dilution of the Conditioning Solution is based on the final concentration of EDTA in the fragmentation reaction** (once input DNA has been diluted in a volume of 50 µL), and not on the EDTA concentration in the DNA preparation.
- Prepare a minimum of 100 µL of diluted Conditioning Solution (as indicated in Table 1), or calculate the volume needed using the following formula:  

$$(\text{number of reactions} \times 5 \mu\text{L}) + 10\% \text{ excess}$$
- The addition of Conditioning Solution to fragmentation reactions will lead to suboptimal results if your DNA does not contain EDTA, or if the final concentration of the Conditioning Solution is not matched to the final EDTA concentration in the reaction.*
- Please refer to **Reaction Optimization** (p. 5) if you are unsure about the presence or concentration of EDTA in your DNA samples.

Table 1. Conditioning Solution dilutions for DNA samples containing EDTA

Final EDTA concentration in 50 µL rxn	Dilution factor	Volume of Conditioning Solution (per 100 µL)	Volume of PCR-grade water (per 100 µL)
0.02 – 0.05 mM	32.0	3.1 µL	96.9 µL
0.1 mM	15.4	6.5 µL	93.5 µL
0.2 mM	7.4	13.5 µL	86.5 µL
0.3 mM	4.8	21.0 µL	79.0 µL
0.4 mM	3.3	30.0 µL	70.0 µL
0.5 mM	2.6	38.8 µL	61.2 µL
0.6 mM	2.2	46.5 µL	53.5 µL
0.7 mM	1.8	56.0 µL	44.0 µL
0.8 mM	1.6	64.0 µL	36.0 µL
0.9 mM	1.4	72.0 µL	28.0 µL
1.0 mM	1.3	80.0 µL	20.0 µL

### Fragmentation Parameters

- The fragmentation parameters provided in **Fragmentation Protocol** (step 1, p. 8) apply to the fragmentation of high-quality genomic DNA.
- The degree of fragmentation (mode size and size distribution of DNA fragments) is controlled by fragmentation time and temperature, and both factors may be modulated to achieve the desired results.
- DNA quality impacts the fragmentation of FFPE DNA. The guidelines in **Fragmentation Protocol** (step 1, p. 8) are a good starting point for FFPE samples with a Q129/Q41 ratio of ~0.4 or higher (as determined with the KAPA hgDNA Quantification and QC Kit). However, slightly longer fragmentation times may improve results for lower-quality FFPE samples. Longer fragmentation times typically increase the proportion of input DNA converted to fragments in the 150 – 250 bp range, reduce residual high-molecular weight DNA, and correlate with higher yields during library construction.
- Standard fragmentation parameters may result in over-fragmentation of low-complexity samples, such as small viral genomes, plasmids, long amplicons and cDNA. For these sample types, the fragmentation time may have to be reduced to 5 min or less to achieve the desired mode fragment length. This makes control over the reaction difficult, particularly when a large number of samples are processed manually. To enable more robust and reproducible results, the fragmentation temperature may be decreased (to 30°C or 25°C) to reduce enzymatic activity, thus increasing the time needed to achieve the desired fragment length.
- Please refer to **Reaction Optimization** (p. 5) for guidelines on how to systematically optimize the fragmentation parameters for your specific samples.

- Different devices (e.g., a thermocycler vs. heating block, or different Peltier devices integrated into automated liquid handling systems) may not yield identical fragmentation profiles for the same sample and input, and fragmentation times may have to be modified slightly when switching between devices. The relative impact of the device used for the fragmentation incubation is likely to be less significant as fragmentation time increases.

### Post-fragmentation Processing

- Fragmentation reactions are terminated with Stop Solution. The Stop Solution contains EDTA, which is incompatible with the initial enzymatic step (end repair and/or A-tailing) in library construction protocols for Illumina and Ion Torrent sequencing. Residual fragmentation enzyme may also interfere with the efficiency of library construction. For this reason, fragmentation reaction products are purified using a bead-based cleanup or double-sided size selection with KAPA Pure Beads prior to library preparation.
- The 2X bead-based cleanup described in **Fragmentation Protocol** (step 2, p. 8) is designed for optimal recovery (70 – 80%) of fragmented DNA, and will only exclude very small fragments (~50 bp or less). The bead to DNA ratio may be decreased to exclude a wider range of small fragments prior to library construction.
- The double-sided size selection described in **Fragmentation Protocol** (step 3, p. 9) is designed to exclude both small and large fragments, and is recommended when the input into fragmentation is >100 ng, the desired mode fragment length is ≥350 bp and/or when a narrower size distribution is optimal for your sequencing application. The standard protocol yields DNA fragments in the range of 250 – 450 bp, but may be tailored to obtain a population of shorter or longer fragments. Guidelines for modifying the protocol are included.
- Electrophoretic size selection methods or instrumentation (e.g., the Sage Science Pippin Prep) may be employed instead of the bead-based size selection described in the protocol.
- Size selection inevitably leads to a significant loss of material. Typically, 60 – 95% of input DNA can be lost as the result of intentional exclusion of small and large molecules, and the inherent inefficiencies of size selection techniques. The potential advantages of size selection should therefore be weighed against the potential loss of sample complexity, especially when input DNA is limited.
- Post-fragmentation procedures outlined in this document do not apply when the KAPA Frag system is used in the integrated KAPA HyperPlus workflow. Please refer to the **KAPA HyperPlus Kit Technical Data Sheet** for details.

### Reaction Cleanups/Size Selection

- This Protocol has been validated for use with either KAPA Pure Beads or Agencourt Ampure XP (Beckman Coulter). Solutions and conditions for DNA binding and size selection may differ if other beads are used.
- Observe all the storage and handling recommendations for KAPA Pure Beads or AMPure XP. Equilibration to room temperature is essential to achieve specified size distribution and yield of DNA.
- Beads will settle gradually; always ensure that they are fully resuspended before use.
- *To ensure optimal DNA recovery, it is critical that the DNA and the KAPA Pure Beads are thoroughly mixed* (by vortexing or extensive up-and-down pipetting) before the DNA binding incubation.
- Bead incubation times are guidelines only, and may be modified/optimized according to current protocols, previous experience, specific equipment and samples in order to maximize library construction efficiency and throughput.
- The time required for complete capture of beads varies according to the reaction vessel and magnet used. It is important not to discard or transfer any beads with the removal or transfer of supernatant. Capture times should be optimized accordingly.
- The volumes of 80% ethanol used for bead washes may be adjusted to accommodate smaller reaction vessels and/or limited pipetting capacity, but it is important that the beads are entirely submerged during the wash steps. **Always use freshly prepared 80% ethanol.**
- It is important to remove all ethanol before proceeding with subsequent reactions. However, over-drying of beads may make them difficult to resuspend, resulting in a dramatic loss of DNA. With optimized aspiration of ethanol, drying of beads for 3 – 5 min at room temperature should be sufficient. **Drying of beads at 37°C is not recommended.**
- Where appropriate, DNA should be eluted from beads in elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5). Elution of DNA in PCR-grade water is not recommended, as DNA is unstable in unbuffered solutions. Purified, fragmented DNA in elution buffer should be stable at 2°C to 8°C for 1 – 2 weeks, or at -15°C to -25°C for long-term storage. The long-term stability of DNA at -15°C to -25°C depends on a number of factors, including concentration. Always use low DNA-binding tubes for long-term storage, and avoid excessive freezing and thawing.



## Reaction Optimization

Fragmentation guidelines provided in **Fragmentation Protocol** (step 1, p. 8) may not result in the optimal fragmentation profile for your specific DNA samples. For this reason, precious samples should not be used when evaluating this kit for the first time. Instead, fragmentation parameters should be optimized with a non-precious, bulk DNA sample that is representative of the actual samples to be processed.

The information in this section should be considered during the experimental design for your evaluation of the KAPA Frag Kit for Enzymatic Fragmentation.

### Quantification of Input DNA

Although the KAPA Frag enzymatic fragmentation system is less sensitive to DNA input than tagmentation-based library construction methods, it is recommended that input DNA be quantified. PicoGreen/Qubit is recommended for the quantification of high-quality DNA, whereas the KAPA Human gDNA Quantification and QC Kit provides both concentration and quality information for FFPE DNA.

### Handling of DNA Samples Containing EDTA

If the DNA samples contain EDTA, perform a 3X bead-based cleanup with KAPA Pure Beads to remove EDTA prior to fragmentation. Please refer to the **KAPA Pure Beads Technical Data Sheet** for a detailed DNA cleanup protocol.

Alternatively, the Conditioning Solution may be used to neutralize EDTA prior to fragmentation. This strategy is recommended as a first approach for precious FFPE DNA samples of variable quality.

Since EDTA in DNA preparations is usually introduced via elution buffers used in the final stages of the DNA extraction or purification process, the concentration of EDTA is typically known (e.g., 1 mM for standard TE buffer and 0.1 mM for “low-EDTA” TE buffer). If this is the case, and your samples are of similar concentration (i.e., a constant volume of DNA is used for library construction), simply refer to Table 1 (p. 3) for the appropriate dilution of Conditioning Solution, and follow **Fragmentation Protocol** (step 1).

If you know the composition of the EDTA-containing buffer used for DNA purification, but your samples span a wide concentration range (i.e., variable volumes will be used to achieve the desired input into library construction), samples should be normalized in the same EDTA-containing buffer used for DNA purification.

For example:

- If your DNA samples are in TE Buffer, and your input into library construction is 100 ng, dilute 100 ng of each sample into a final volume of 30  $\mu$ L (i.e., to 3.33 ng/ $\mu$ L) using TE Buffer.
- All samples will now contain the same final EDTA concentration once diluted to 50  $\mu$ L for fragmentation.

This concentration is:

$$\begin{aligned} &\text{EDTA concentration in TE Buffer} \times (30 \mu\text{L}/50 \mu\text{L}) \\ &= 1 \text{ mM} \times (30 \mu\text{L}/50 \mu\text{L}) = 0.6 \text{ mM} \end{aligned}$$

- Make a 2.2-fold dilution of the Conditioning Solution (as per Table 1 on p. 3), and follow the **Fragmentation Protocol** (second half of step 1.1).

If you are unsure about the presence or concentration of EDTA in your DNA samples:

- Set up a series of test reactions with the appropriate amount of input DNA, and different final concentrations of Conditioning Solution.
- Include at least one reaction with the same input of control DNA known to be EDTA-free. The control DNA should preferably be of the same type and quality as the test samples.
- Fragment the DNA using the appropriate parameters, as outlined in **Fragmentation Protocol** (step 1, p. 8). Perform a **Post-fragmentation Cleanup** (step 2), and compare fragmentation profiles for the test and control samples using an electrophoretic system—see **Analysis of Fragmentation Results** (p. 7).
- Titrate the final concentration of Conditioning Solution in the reaction until the test samples yield similar fragmentation profiles as the EDTA-free control sample.
- A two-step strategy may be the best. Start with 3 – 4 test samples covering a broad range of final Conditioning Solution concentrations, then perform a finer titration over a narrower concentration range.

### Optimization of Fragmentation Time

The fragmentation guidelines in **Fragmentation Protocol** (step 1, p. 8) are a good starting point for high-quality genomic DNA. When evaluating the KAPA Frag Kit for the first time, it is recommended that you proceed as follows:

- Set up at least three replicate reactions with the desired input of a non-precious, bulk sample that is representative of the actual samples to be processed.
- Select the most appropriate fragmentation time (for the desired mode fragment length) from the third table in **Fragmentation Protocol** (step 1, p. 8). Perform one reaction with that time, and one reaction each with either a slightly shorter or slightly longer fragmentation time within the optimization range. Increments of 3 – 5 min are recommended.
- Perform a **Post-fragmentation Bead-based Cleanup** (step 2 of the **Fragmentation Protocol**), and evaluate fragmentation profiles electrophoretically.
  - If the mode fragment length is too long, increase the fragmentation time in increments of 2 – 5 min until the optimal size distribution is achieved.
  - If the mode fragment length is too short, reduce the fragmentation time in increments of 2 – 5 min until the optimal size distribution is achieved.

- Further fine-tuning (plus or minus 1 – 2 min) may be necessary if the fragmentation time is relatively short ( $\leq 10$  min). If this is the case, consider optimizing the fragmentation temperature (see below).

A similar strategy may be employed to optimize the fragmentation time for FFPE samples, remembering that lower quality samples may benefit from slightly longer fragmentation times. For FFPE samples:

- Set up 4 – 5 replicate reactions with the desired input of a non-precious, bulk sample that is representative of the actual samples to be processed. This sample may have to be generated by pooling a few individual samples.
- Select the fragmentation time corresponding to the desired mode fragment length from the third table in **Fragmentation Protocol** (step 1, p. 8). Use that as the minimum fragmentation time, and increase the incubation time at 37°C by 5 min for each additional replicate.
- Perform a **Post-fragmentation Cleanup** (step 2 of the **Fragmentation Protocol**), *continue with library construction using your preferred reagents and protocol*, and evaluate the outcome of the fragmentation reaction in the context of the size distribution of the final libraries. Fine-tune fragmentation time if needed, as described above.

With respect to FFPE samples, please note the following:

- Electrophoretic profiles of FFPE samples, generated during sample QC prior to fragmentation, are not always good predictors of library and sequence quality. Samples that appear to consist of high-molecular weight DNA may not yield libraries of significantly better quality than samples that appear to be degraded. The KAPA hgDNA Quantification and QC Kit provides a qPCR-based assay for assessment of FFPE DNA quality. Quality scores (Q-ratios) determined with this assay have been shown to correlate with the success of library construction.
- The mode fragment length of an amplified FFPE library is typically shorter than than expected based on the size distribution after fragmentation and adapter length. This is a common phenomenon, attributable to the inability of high-fidelity DNA polymerases used in library amplification to efficiently amplify damaged DNA, particularly templates that contain deaminated or oxidized bases. For this reason, it is not productive to try and optimize fragmentation parameters independently of the rest of the library construction process when using the KAPA HyperPlus workflow.

### Optimization of Fragmentation Temperature

The standard fragmentation temperature is 37°C. If you are fragmenting high-quality genomic DNA, any other high-complexity DNA sample, or FFPE DNA to a mode fragment length  $< 500$  bp, it is unlikely that you will have to change or optimize the fragmentation temperature.

Low-complexity samples (e.g., small viral genomes, plasmids, long amplicons and cDNA) may, however, be over-fragmented at 37°C, even with short incubation times. The likelihood of over-fragmentation depends on the nature, molecular weight/length of the input DNA, the desired size distribution after fragmentation and, to a lesser degree, the input into fragmentation. For example, 100 ng of a 1.8 kb PCR product will yield a similar mode fragment length ( $\sim 300$  bp) as 100 ng *E. coli* or human genomic DNA when fragmented at 37°C for 10 min, whereas 1 ng of a 1 kb PCR product will be fragmented to a mode size  $< 250$  bp using the same parameters.

To determine the optimal fragmentation parameters for low-complexity samples, or high-complexity samples when the desired mode fragment length is  $> 500$  bp:

- Set up four replicate reactions with a non-precious, bulk sample that is representative of the actual samples to be processed.
- Fragment two of the samples at 37°C, for 5 min and 10 min, respectively. Repeat these fragmentations with the other two samples, but at 30°C.
- Perform a **Post-fragmentation Cleanup** (step 2 of the **Fragmentation Protocol**) and evaluate the fragmentation profiles electrophoretically.
  - If the mode fragment length obtained with a 10 min incubation at 37°C is too long, continue optimizing (increasing) the fragmentation time at 37°C.
  - If the mode fragment length obtained with a 10 min incubation at 30°C is too long, but 5 min at 37°C resulted in over-fragmentation, continue optimizing (increasing) the fragmentation time at 30°C.
  - If a 5 min incubation at 30°C resulted in over-fragmentation, perform a second set of reactions (e.g., for 5 min, 10 min, 15 min, and 20 min) at 25°C, and fine-tune the fragmentation time if needed.

### Post-fragmentation Processing and Library Prep

Once fragmentation parameters have been optimized, review fragmentation profiles to confirm whether a post-ligation cleanup, or size selection (double-sided bead-based or other method) is most appropriate for your specific samples and sequencing application. Please refer to **Important Parameters: Post-fragmentation Processing** (p. 4) for guidelines, or contact Technical Support at [sequencing.roche.com/support](http://sequencing.roche.com/support).

Fragmented DNA can be used for NGS library construction with the KAPA Hyper Prep Kit, KAPA HTP or LTP Library Preparation Kits for Illumina platforms, or the KAPA Library Preparation Kit for Ion Torrent platforms.

For optimal library construction efficiency in Illumina pipelines (across all DNA sample types and inputs), the **KAPA HyperPlus Kit** is highly recommended. This kit combines the KAPA Frag and KAPA Hyper Prep chemistries in a streamlined, one-tube fragmentation/library construction protocol. For more information, visit [www.sequencing.roche.com](http://www.sequencing.roche.com).

### Analysis of Fragmentation Results

Fragmentation results are analyzed electrophoretically. Instruments such as a Bioanalyzer or TapeStation (Agilent Technologies), or LabChip GX, GXII, or GX Touch (PerkinElmer) are recommended over conventional gels.

- The post-fragmentation cleanup or size selection must be performed before reaction products are analyzed. If the fragmentation enzyme is not completely removed from the reaction product, it will degrade molecular weight markers, and cause other artifacts during analysis.
- The amount of purified, fragmented DNA needed for the analysis depends on the system used, and can be limited to 1 – 2  $\mu$ L by using a high-sensitivity assay. If the input into fragmentation was >500 ng, the sample may have to be diluted prior to analysis to avoid over-loading.
- Fragmentation profiles for low-input samples (<10 ng into fragmentation) may not be highly informative, even when a high-sensitivity assay is used. In such cases, the outcome of fragmentation is best evaluated in terms of the size distribution of amplified libraries produced from the fragmented DNA.

- DNA fragmented with the KAPA Frag Kit typically yields profiles with a slightly positive skew. The mode (most frequent) fragment length referenced throughout this document corresponds to the “top of the peak” (as shown in Figure 1), and can differ significantly from the median (middle) or mean (average) fragment length.
- Examples of fragmentation profiles generated with the KAPA Frag Kit are given in the **Appendix**.

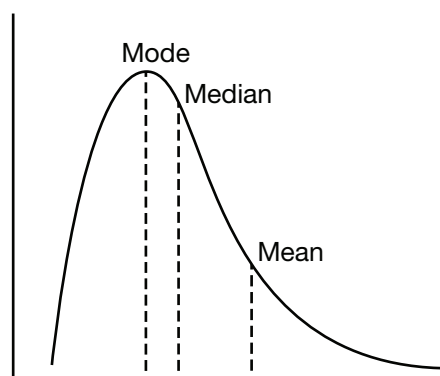


Figure 1. Mode vs. median and mean fragment lengths

## Fragmentation Protocol

### 1. Enzymatic Fragmentation

*If the DNA samples contain EDTA, perform a 3X bead-based cleanup with KAPA Pure Beads to remove EDTA prior to fragmentation.* Please refer to the KAPA Pure Beads Technical Data Sheet for a detailed DNA cleanup protocol.

Alternatively, prepare a sufficient volume of appropriately diluted Conditioning Solution (5 µL per DNA sample, plus excess). Refer to Table 2 (p. 4) for guidelines on the dilution of the Conditioning Solution.

- 1.1 Dilute the amount of dsDNA to be used for library construction as follows:
  - If the DNA preparation **does not** contain EDTA, dilute in 10 mM Tris-HCl (pH 8.0 – 8.5) in a total of 35 µL
  - If the DNA preparation **does** contain EDTA, dilute in the EDTA-containing buffer in which samples are currently suspended, in a total of 30 µL. To each reaction with 30 µL of EDTA-containing DNA, add 5 µL of diluted Conditioning Solution.
- 1.2 Mix by gentle vortexing or pipetting up and down
- 1.3 Assemble each fragmentation reaction **on ice** by adding the rest of the components in the order shown below:

Component	Volume
Double-stranded DNA (with Conditioning Solution, if needed)	35 µL
KAPA Frag Buffer (10X)*	5 µL
KAPA Frag Enzyme*	10 µL
<b>Total volume:</b>	<b>50 µL</b>

\*The KAPA Frag Buffer and Enzyme may be pre-mixed and kept on ice prior to reaction setup, and dispensed as a single solution. Please note the enzyme volume is greater than the buffer volume in this reaction.

- 1.4 Vortex gently and spin down briefly. Return the plate/tube(s) to ice. Proceed immediately to the next step.

- 1.5 Incubate in a thermocycler, pre-cooled to 4°C and programmed as outlined below. Set the lid temperature to ≤50°C.

Step	Temp	Time
Pre-cool block	4°C	N/A
Fragmentation	37°C	See table below
HOLD	4°C	∞

Mode fragment length	Incubation time at 37°C*	Optimization range
600 bp	5 min	3 – 10 min
350 bp	10 min	5 – 20 min
200 bp	20 min	10 – 25 min
150 bp	30 min	20 – 40 min

\*These parameters are a good starting point for high-quality genomic DNA. Please refer to Reaction Optimization (p. 5) for guidelines on how to optimize fragmentation time and temperature. If incubation times longer than the recommended range are needed, samples likely contain inhibitors which impact the fragmentation efficiency. Bead-based DNA cleanup, prior to fragmentation, is recommended over longer fragmentation times.

- 1.6 Transfer reactions to ice, and immediately add 5 µL of Stop Solution. Vortex gently and proceed directly to the **Post-fragmentation Cleanup** (step 2) or **Post-fragmentation Size Selection** (step 3).

### 2. Post-fragmentation Cleanup

- 2.1 Perform a bead-based cleanup by combining the following:

Component	Volume
Fragmentation reaction product (with Stop Solution)	55 µL
KAPA Pure Beads	110 µL
<b>Total volume:</b>	<b>165 µL</b>

\*The standard protocol provides for a 2X bead to DNA ratio. Please refer to Important Parameters (pp. 3 – 4) for more details, and guidelines on how to modify the cleanup parameters.

- 2.2 Mix thoroughly by vortexing and/or pipetting up and down multiple times.
- 2.3 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind DNA to the beads.
- 2.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
- 2.5 Carefully remove and discard the supernatant.
- 2.6 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.
- 2.7 Incubate the plate/tube(s) on the magnet at room temperature for ≥30 sec.
- 2.8 Carefully remove and discard the ethanol.
- 2.9 Keeping the plate/tube(s) on the magnet, add 200 µL of 80% ethanol.



- 2.10 Incubate the plate/tube(s) on the magnet at room temperature for  $\geq 30$  sec.
- 2.11 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
- 2.12 Dry the beads for 3 – 5 min at room temperature, or until all of the ethanol has evaporated. **Caution: over-drying the beads may result in reduced yield.**
- 2.13 Remove the plate/tube(s) from the magnet.
- 2.14 Resuspend the beads in the appropriate volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5):
  - Resuspend in 10 – 25  $\mu\text{L}$  if the input into fragmentation was in the range of 1 – 100 ng. The minimum volume will depend on the properties of the magnet used.
  - Resuspend in 30 – 55  $\mu\text{L}$  if the input into fragmentation was  $>100$  ng.
- 2.15 Mix thoroughly by vortexing and/or pipetting up and down.
- 2.16 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.
- 2.17 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
- 2.18 Transfer the clear supernatant to a new plate/tube(s). Store fragmented DNA at  $2^{\circ}\text{C}$  to  $8^{\circ}\text{C}$  for 1 – 2 weeks, or at  $-15^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$ .

### 3. Post-fragmentation Double-sided Size Selection

To achieve a narrower size distribution of fragmented DNA, particularly when the input into fragmentation is  $>100$  ng, and/or the desired mode fragment length exceeds 350 bp, any commonly used size selection technique (e.g., the double-sided size selection described here, or an electrophoretic method) is recommended after fragmentation.

The double-sided size selection protocol outlined in this section is designed for the selection of DNA fragments in the range of 250 – 450 bp. To obtain a population of shorter or longer fragments, the protocol may be modified as follows:

Upper size limit	Modification
Increase	Decrease ratio of the first cut
Decrease	Increase ratio of the first cut

Lower size limit	Modification
Increase	Decrease ratio of the second cut*
Decrease	Increase ratio of the second cut*

\*The second size cut should be performed with at least 0.2 volumes of AMPure XP.

**NOTE: the volume of KAPA Pure Beads needed for the second cut is calculated relative to the volume of the DNA at the start of the size selection procedure, not the volume of the DNA-containing supernatant transferred after the first cut.** DNA recovery is dramatically reduced if the difference between first and second cuts are less than  $\sim 0.2$  volumes. To increase the amount of DNA recovered,  $>0.2$  volumes of KAPA Pure Beads may be used for the second cut, but note that this may result in the recovery of smaller library fragments and/or a broader size distribution.

For more information on double-sided size selection, please refer to the **KAPA NGS Library Preparation Technical Guide**, or contact Technical Support at [sequencing.roche.com/support](http://sequencing.roche.com/support).

- 3.1 Perform the first (0.6X) size cut (to exclude fragments larger than  $\sim 450$  bp) by combining the following:

Component	Volume
Fragmentation reaction product (with Stop Solution)	55 $\mu\text{L}$
KAPA Pure Beads	33 $\mu\text{L}$
<b>Total volume:</b>	<b>88 <math>\mu\text{L}</math></b>

- 3.2 Mix thoroughly by vortexing and/or pipetting up and down multiple times.
- 3.3 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind DNA fragments larger than  $\sim 450$  bp to the beads.
- 3.4 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
- 3.5 Carefully transfer  $\sim 85$   $\mu\text{L}$  of supernatant containing DNA fragments smaller than  $\sim 450$  bp to a new plate/tube(s). It is critical that no beads are transferred with the supernatant. Discard the plate/tube(s) with the beads to which DNA fragments larger than  $\sim 450$  bp were bound.
- 3.6 Perform the second size cut (0.8X, to retain fragments  $>250$  bp) by combining the following:

Component	Volume
Supernatant from first size cut	85 $\mu\text{L}$
KAPA Pure Beads	11 $\mu\text{L}$
<b>Total volume:</b>	<b>96 <math>\mu\text{L}</math></b>

- 3.7 Mix thoroughly by vortexing and/or pipetting up and down multiple times.
- 3.8 Incubate the plate/tube(s) at room temperature for 5 – 15 min to bind DNA fragments larger than  $\sim 250$  bp to the beads.

- 3.9 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
- 3.10 Carefully remove and discard the supernatant, which contains DNA fragments smaller than ~250 bp.
- 3.11 Keeping the plate/tube(s) on the magnet, add 200  $\mu$ L of 80% ethanol.
- 3.12 Incubate the plate/tube(s) on the magnet at room temperature for  $\geq 30$  sec.
- 3.13 Carefully remove and discard the ethanol.
- 3.14 Keeping the plate/tube(s) on the magnet, add 200  $\mu$ L of 80% ethanol.
- 3.15 Incubate the plate/tube(s) on the magnet at room temperature for  $\geq 30$  sec.
- 3.16 Carefully remove and discard the ethanol. Try to remove all residual ethanol without disturbing the beads.
- 3.17 Dry the beads for 3 – 5 min at room temperature, or until all of the ethanol has evaporated. **Caution: over-drying the beads may result in reduced yield.**
- 3.18 Remove the plate/tube(s) from the magnet.
- 3.19 Thoroughly resuspend the beads in the required volume of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5).
- 3.20 Incubate the plate/tube(s) at room temperature for 2 min to elute DNA off the beads.
- 3.21 Place the plate/tube(s) on a magnet to capture the beads. Incubate until the liquid is clear.
- 3.22 Transfer the clear supernatant with size-selected DNA to a new plate/tube(s) and store DNA at 2°C to 8°C for 1 – 2 weeks, or at -15°C to -25°C.

Appendix: Examples of Fragmentation Profiles

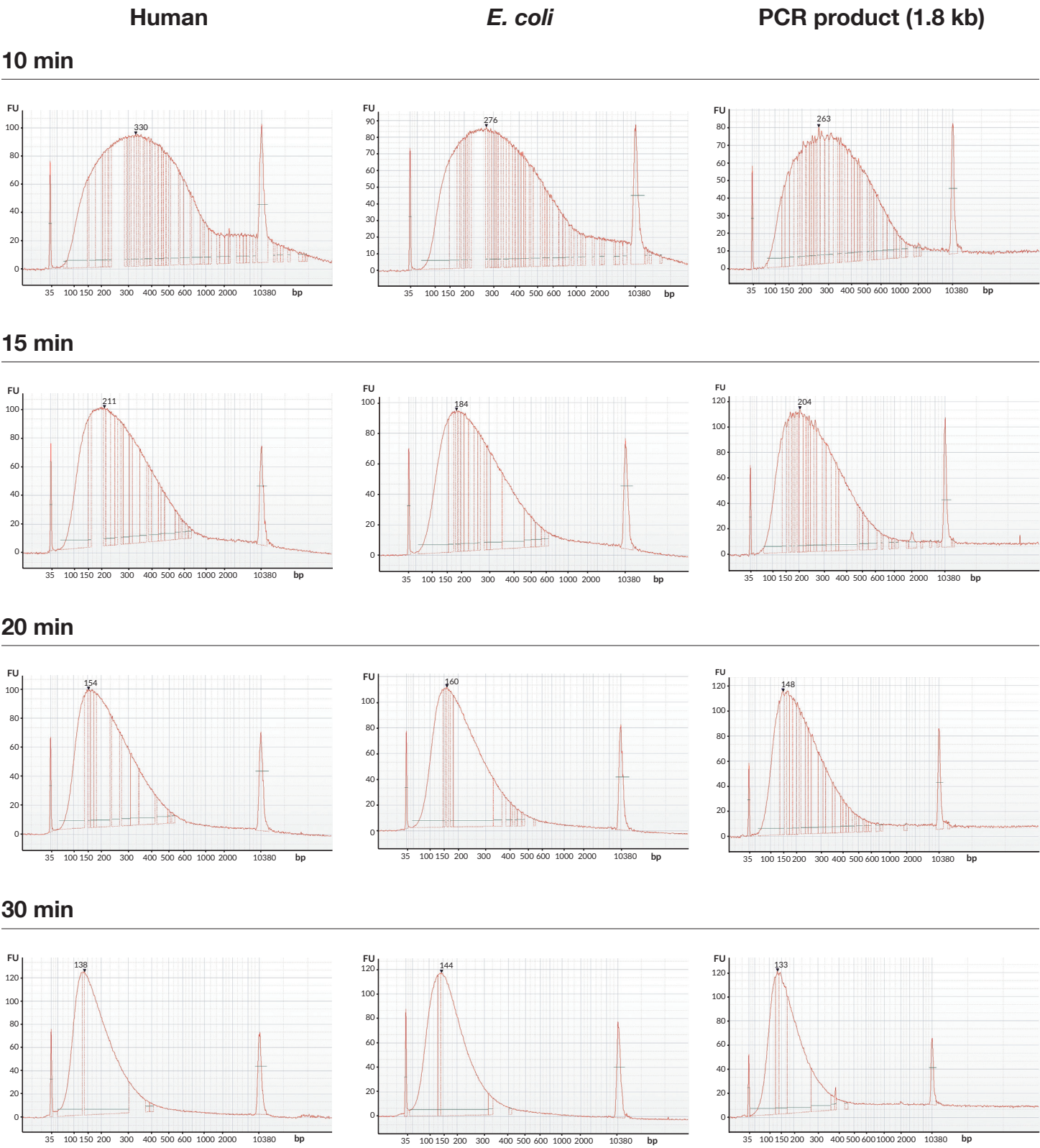


Figure 2. Examples of Fragmentation Profiles. Fragmentation profiles for 100 ng human genomic DNA (left), 100 ng *E. coli* genomic DNA (middle), and 100 ng of a 1.8 kb PCR product (right), incubated at 37°C for 10 min, 15 min, 20 min, or 30 min, respectively. Reactions were stopped with Stop Solution, after which DNA was cleaned up as described in Fragmentation Protocol (step 2). Purified DNA was resuspended in 20 µL of elution buffer (10 mM Tris-HCl, pH 8.0 – 8.5). Samples (1 µL each) were analyzed without further dilution using a Bioanalyzer 2100 High Sensitivity DNA Assay (Agilent Technologies).

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