xGen™ Deceleration Module

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OVERVIEW

The xGen™ Deceleration Module offers reagents to construct libraries with an average aligned insert size of 500 bp. Reagent DE in the xGen Deceleration Module is used in place of Reagent K2 (from the **xGen DNA Library Prep EZ and xGen DNA Library Kit EZ UNI kit**) to construct these libraries.

Note: Actual insert size may vary and should be assessed after sequencing. Variations are a result of BioAnalyzer® (Agilent Technologies) traces that depict a broader size distribution than the aligned insert size. This is due to shorter library molecules, which cluster more efficiently on Illumina® instruments.

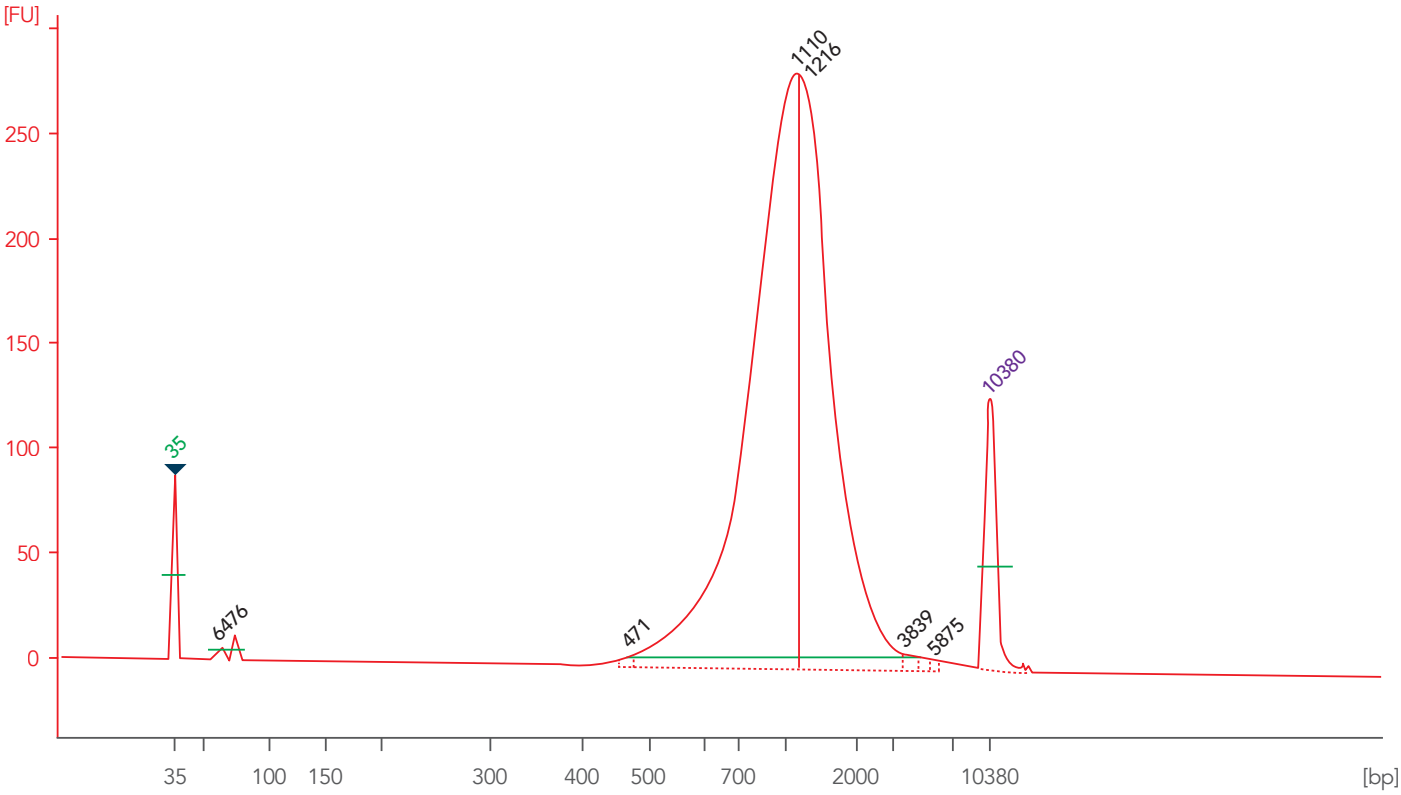

Figure 1. xGen DNA Library Prep EZ libraries with 1110 bp mode and average size of 1180 bp on the BioAnalyzer produce a mean aligned insert size of 552 bp on an Illumina® MiSeq®.

FRAGMENTATION TIME

Note: Refer to the certificate of analysis (CoA) for specific fragmentation time recommendations for the lot number of your product. To find the CoA, enter the lot number of your kit (found on the bottom of the packaging) at idtdna.com/COA. Alternatively, read the CoA provided with your kit, on the label inside the product box, for specific fragmentation parameters.

If using a DNA suspension buffer other than xGen Low EDTA TE Buffer (0.1 mM), it may be necessary for you to adjust fragmentation time since different resuspension buffers can affect the rate of fragmentation.

Refer to the table below, for recommended fragmentation times when using Reagent DE:

	>25 ng gDNA	<25 ng gDNA
Mean aligned insert size of 550 bp	Reduce 350 bp fragmentation time* by 1–2 minutes	Use fragmentation time* listed for 350 bp for >25 ng inputs

*350 bp fragmentation time as listed on your xGen DNA Library Prep EZ and xGen DNA Library EZ UNI CoA.

Further adjustments may be necessary to obtain aligned insert sizes >550 bp.

This can be achieved by:

- Decreasing fragmentation time
- Increasing volume of Reagent DE (maximum of 10 µL)
- Applying a more stringent SPRI size selection after ligation or PCR (Note: A more stringent SPRI size selection will reduce library complexity).

CONSUMABLES AND EQUIPMENT

Consumables from IDT—kit contents

The xGen Deceleration Module (Cat. No. 10009823) is supplied with excess reagent volume for processing a minimum of 96 libraries (10% overage included).

xGen Deceleration Module components		Volume (µL)	Storage
End prep	Reagent DE	1000	Room temperature

Consumables from IDT—reagents

The xGen Deceleration Module is used with the following xGen DNA library prep kits:

Workflow component	Product name	Index number	Reaction size	Catalog number
xGen core reagents	xGen DNA Library Prep EZ Kit	N/A	16 rxn	10009863
	xGen DNA Library Prep EZ Kit	N/A	96 rxn	10009821
	xGen DNA Library Prep EZ Kit	N/A	4x96 rxn	10010150
	xGen DNA Library Prep EZ UNI Kit	N/A	16 rxn	10009864
	xGen DNA Library Prep EZ UNI Kit	N/A	96 rxn	10009822
	xGen DNA Library Prep EZ UNI Kit	N/A	4x96 rxn	10010151

PROTOCOL

Perform enzymatic preparation

Follow this protocol alongside the xGen DNA Library Prep EZ and xGen DNA Library Prep EZ UNI protocol. Please visit www.idtdna.com/protocols for the most up-to-date versions.

Note: This protocol has been developed with high-quality genomic DNA (gDNA) inputs of 1 to 250 ng in low EDTA TE (0.1 mM). Please review the entire xGen DNA Library Prep EZ and xGen DNA Library Prep EZ UNI protocol, and accompanying Certificate of Analysis (CoA), prior to performing library preparation.

Important: Keep the Enzymatic Prep Master Mix and the DNA samples on ice until they are loaded in the thermal cycler to safeguard against fragmentation. Enzymes are active at room temperature and may fragment DNA to undesired sizes.

1. Transfer DNA sample to a 0.2 mL PCR tube and adjust the volume of the sample to a total of 16 µL by adding Low EDTA TE, if necessary.

Component	Volume per sample (µL)
Low EDTA TE	16 – x
DNA sample	x
Total volume	16

2. Pre-program the thermal cycler for Enzymatic Prep, as described below. Confirm lid heating is turned ON and is set to 70°C.

Enzymatic Prep program		
Step	Temperature	Time
1	4°C	Hold
2	32°C	Variable*
3	65°C	30 min
4	4°C	Hold

* The incubation time at 32°C is determined by consulting the CoA for the specific lot of xGen DNA Library EZ or xGen DNA Library EZ UNI and incorporating the adjustments listed in the Fragmentation Time section on page 4 in this protocol.

Important: Fragmented samples can be kept at 4°C no more than 1 hour.

3. Begin the Enzymatic Prep thermal cycler program. Allow the block to reach 4°C.
4. Prepare the Enzymatic Prep Master Mix by adding the components in the order shown:

Component	Volume per reaction (µL)
Buffer K1	3
Reagent DE	5
Enzyme K3	6
Total Master Mix volume (µL):	**14**

5. Vortex the Enzymatic Prep Master Mix for 5 seconds, then briefly centrifuge. Keep mix on ice until ready to use.

   Important: Ensure that the Enzymatic Prep Master Mix is mixed thoroughly before and after adding DNA samples to prevent incomplete fragmentation.

6. Add 14 µL of pre-mixed Enzymatic Prep Master Mix to each tube containing DNA sample and low EDTA TE to reach a final volume of 30 µL.

7. Thoroughly vortex to mix for 5 seconds.
8. Briefly centrifuge sample tubes, then *immediately* place in the chilled thermal cycler. Advance the Enzymatic Prep program to the 32°C fragmentation step.

   While the Enzymatic Prep program runs, prepare the Ligation Master Mix as directed in the xGen DNA Library Prep EZ or xGen DNA Library Prep EZ UNI protocol.

ADDITIONAL INSTRUCTIONS

1. Follow directions in the xGen DNA Library Prep EZ and xGen DNA Library Prep EZ UNI protocol, but make the following modifications:
   a. Use a 0.6X SPRI ratio to purify the Ligation reaction, instead of the 0.8X SPRI suggested in the standard protocol.

SPRI ratio	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
0.6X	60	36	22

2. Perform three or more additional PCR cycles than the number of cycles suggested in the Indexing PCR step of the conventional protocol.
3. After the Indexing PCR, perform size selection using either a 0.5X SPRI (for inputs >25 ng) or a 0.55X SPRI (for inputs <25 ng), as shown below:

DNA input	SPRI ratio	Sample volume (µL)	Bead volume (µL)	Elution volume (µL)
>25 ng	0.5X	50	25	22
<25 ng	0.55X	50	27.5	22

4. Libraries are now ready for quantification and fragment analysis.

For additional support, please contact us at applicationsupport@idtdna.com

xGen™ Deceleration Module

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