Optimization Guide

sparQ Frag & Library Prep – Easy as 1, 2, 3 …

Before you start

Check the buffer

- Remove all cations and chelators from DNA preparations
- Ideal buffers:
  10 mM Tris·HCl, buffer EB, nuclease-free water, or LoTE (0.1x TE)
- For 1x TE or unknown buffers:
  purify with 1.8X sparQ PureMag Beads before use

Sample type	Application	Recommended input
Complex gDNA	WGS WGS PCR-free Targeted sequencing	50 ng – 1 µg ≥500 ng 1 ng – 1 µg
Microbial gDNA	WGS WGS PCR-free	1 ng – 500 ng ≥100 ng
FFPE DNA	WGS/targeted sequencing	≥50 ng
Other	e.g. ChIP-Seq, methyl-Seq	≥50 ng

1 Single Tube, One-Step Frag & DNA Polishing

Optimize fragmentation time

- Enzyme based DNA fragmentation is sensitive to many factors e.g. reaction temperature, time and set up conditions
- Single Tube, one-step DNA fragmentation and polishing reaction is fully tunable based on DNA inputs
- Enhancer solution for low inputs – no loss of fragmentation efficiency

Choose time from table below based on standard fragment size.

Fragmentation time (min) at 32°C				
Fragment Peak size	250 bp	350 bp	450 bp	550 bp
10 ng input DNA	24	16	14	10
100 ng input DNA	16	10	5	6
1000 ng input DNA	14	8	6	4

2 Adapter Ligation

Select adapter

- Streamlined workflow – just add ligation reagents to fragmented DNA
- Adapter ligation efficiency is maximized with correct adapter concentration for your input
- Validated with popular adapter types follow guide for optimized concentrations

For full confidence and simplicity, choose sparQ UDIs and use the table below.

Input DNA	Dilution of Adapter stock solution	Adapter Concentration in Ligation
500 ng	None	500 nM
100 ng	1:2 (2-fold)	250 nM
10 ng	1:25 (25-fold)	20 nM

Post-Ligation cleanup (0.8X) **sparQ PureMag Beads**

Optional size selection

3 Library Amplification

Optional PCR

- Recommended for low input workflows (≤100 ng)
- sparQ HiFi PCR Master Mix for high fidelity amplification to maintain library representation

Input DNA	Number of cycles*
100 ng	4 – 5
50 ng	5 – 6
10 ng	8 – 10
1 ng	13 – 15

* To yield 500 ng DNA Library

Post-Amplification cleanup (0.8X) **sparQ PureMag Beads**

Library Quantification

- For accurate quantification, sparQ Universal Library Quant Kit is recommended using gold standard qPCR
- Faster time to results: 40 min
- Easy to use master mix formulation
- Accurate over wide range of GC-contents
- Wide dynamic range, quantify even low concentration libraries

40 min

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For more information and ordering instructions, visit: www.quantabio.com