seqWell

FAQs - MosaiX™ DNA Library Prep Kit

1. What is MosaiX and what applications is it best for?
    - MosaiX is a transposase-based DNA library preparation kit that combines tagmentation and ligation in a single workflow to generate highly complex, directional, Illumina-compatible libraries.
    - MosaiX has been optimized for ≥30X coverage human, plant, animal whole-genome sequencing and hybrid capture. This kit is also compatible with a broad range of applications, including low-pass whole-genome sequencing, de novo assembly of microbial/small whole-genome sequencing, and metagenomics sequencing.

2. Is MosaiX automation-friendly?
    - Yes. MosaiX uses a master-mix workflow and plate-based indexing format that is compatible with manual or automated liquid-handling setups. Total hands-on time is ~35 minutes, with ~90 minutes total workflow time.

3. What DNA input range is supported?
    - DNA input required is 10µL of 0.1 ng/µL to 4 ng/µL (1 ng to 40 ng). For hybrid capture, it is recommended to start with ≥10 ng to ensure ample library yield and complexity.

4. Are all required purification beads, indices, polymerases, and amplification primers included in the MosaiX Kit?
    - The MosaiX Kit includes enzymes, adapters, PCR master mix, and purification beads.
    - Indexing primers for library amplification are not included and must be purchased separately (from seqWell or compatible third-party primers). Hybrid capture reagents are also not included.

5. Do samples need to be normalized before starting?
    - To minimize sample-to-sample variation, we recommend normalizing input DNA to a defined concentration before beginning library preparation. When multiple input amounts are needed, samples should be grouped into input-based batches; however, keeping the number of batches to a minimum will help simplify the workflow.
    - If normalization is difficult to implement or not readily streamlined within your workflow, MosaiX with the Auto-Normalization Module may provide an alternative solution. This module is currently available through an alpha testing program. To learn more, please contact alpha@seqwell.com

6. What buffer should DNA be in?
    - DNA can be in 1× TE, low-TE, Tris buffer, or molecular-grade water.

7. Is MosaiX tolerant of common inhibitors?
    - MosaiX is generally robust in the presence of common inhibitors encountered in blood, plant, microbial, and environmental DNA extractions based on internal testing. Performance may vary depending on extraction method. To learn more please contact support@seqwell.com

8. My library fragment size is too large or too small. What can I do to optimize library sizing?
    - Library fragment size can be tuned by adjusting the post-PCR bead cleanup ratio.
        - Lower ratios (e.g., 0.75–0.70×) → larger fragments
        - Higher ratios (e.g., 0.80–0.85×) → smaller fragments
    - For more information contact support@seqwell.com

9. Should I use CDI's or UDI's?
    - While seqWell suggests and sells UDI primers for MosaiX, if a customer is ordering custom primers then the choice of CDI vs UDI depends on the particular application. Please see the blog post below for thoughts on indexing strategies:

      https://seqwell.com/indexing-with-intention-matching-indexing-strategies-to-your-projects-and-experiments/

10. For hybrid capture, which adapter-blocking oligos should be used with MosaiX libraries?
    - To prevent high carryover of off-target material, xGen Universal Blockers-NXT Mix must be used. Reaction number and corresponding part numbers are below:

      xGen™ Universal Blockers NXT, 16 rxn (IDT,1079584)
      xGen™ Universal Blockers NXT, 96 rxn (IDT, 1079585)

11. Does MosaiX incorporate end-repair or are native ends maintained?
    - MosaiX does not require 5' phosphorylation or end-repair. The method does not use any polymerase or exonuclease before PCR, which maintains native ends generated during tagmentation.

12. What is the expected chimera rate of MosaiX libraries?
    - MosaiX does not use blunt or AT ligation chemistry, leading to lower rates of chimerization during ligation. Observed chimera rates may vary by sample, but typically range from 1-2%

13. How do I optimize PCR cycles for samples with lower DNA quality (DIN <6)?
    - To generate sufficient material for hybrid capture or whole-genome sequencing, we recommend starting with an addition of 3 cycles to the standard cycle number for the given DNA input. Adjust as needed based on the yield obtained; additional cycles may be required for highly degraded samples.

14. Does adapter concentration need to be adjusted based on input?
    - For inputs below 5ng, adapters should be diluted 4X in Tris-HCl to avoid adapter dimer generation.

15. What indexes come with the kit? What indexes are compatible if I need more than 96?
    - Currently 96 UDI primers are available to order as a kit add-on. We plan on expanding to 384 later in 2026. You may design and validate any primers based on information in Appendix B of the user guide. If assistance on designing more indexes is needed, reach out to support@seqwell.com.

16. How many freeze-thaws is the kit stable for?
    - We recommend minimizing freeze–thaw cycles. Most components are expected to tolerate ~5 freeze–thaw cycles without significant impact, but for best performance we recommend aliquoting reagents upon first use.