PhiRx™ Indexed Control FAQs

1. What is PhiRx Indexed Control? Which sequencers should I use it on?

PhiRx Indexed control is a dual-indexed control library, made from phiX174 genomic DNA, optimized for Illumina sequencing platforms, particularly two-color systems like XLEAP SBS chemistry on NextSeq 1000/2000 and NovaSeq X. By improving color balancing for low-plexity libraries our PhiRx Indexed Control ensures cleaner, more accurate sequencing results.

2. If I use PhiRx Indexed Control, do I still need to also spike-in PhiX Sequencing Control V3?

PhiRx Indexed Control should be added to runs for color-balancing both the reads and barcodes. Other sequencing spike-ins should not be required for most use cases.

3. Is PhiRx Indexed Control already denatured?

PhiRx Indexed Control is a non-denatured, double-stranded library that is ready to be mixed with non-denatured target libraries. For applications requiring a denatured format, PhiRx Indexed Control can also be denatured following standard procedures and mixed exclusively with other denatured libraries.

4. How much PhiRx Indexed Control do I add to my sequencing run?

Check Illumina recommendations specific to your sequencing platform and analysis software version to determine the appropriate PhiRx Indexed Control spike-in percentage.

Recommended Spike-In Ratios by Application:

a. **For Sequencing Quality Monitoring**: Add 1–2% of PhiRx Indexed Control to the target library.

b. **For Color Balancing of Index Reads with XLEAP-SBS**: Begin with 5–10% PhiRx Indexed Control. Per Illumina's recommendation for their PhiX Sequencing Control V3, using a higher percentage of PhiRx (up to 40%) may improve performance, especially with XLEAP-SBS 600-cycle kits.

c. **For Low-Diversity Libraries (Color Balancing of Insert Reads)**: Start with 15–20% PhiRx Indexed Control and adjust upward if needed to enhance sequencing performance.

5. How do I dilute and load the PhiRx Indexed Control for sequencing?

Follow Illumina's recommendations for diluting and loading PhiX Sequencing Control V3. Use the buffers recommended by Illumina for diluting and dentaturing. If diluting below 1 nM, store and use active aliquots for up to 2 weeks at 4°C. Aliquots not currently in use should be kept at -20°C for long-term storage.

6. How do I determine if PhiRx Indexed Control is working appropriately?

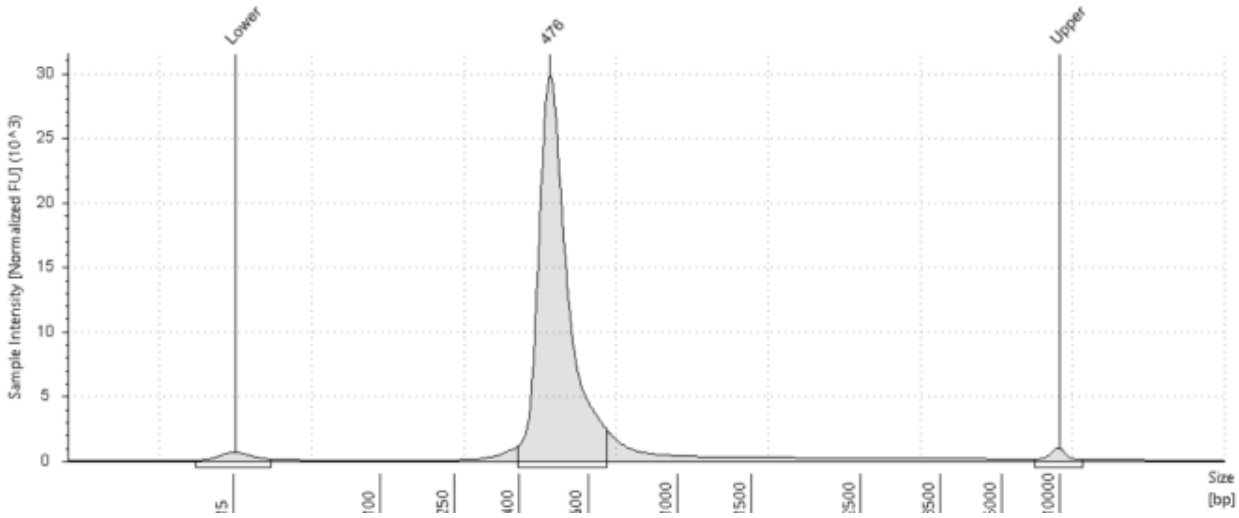
Successful use of PhiRx Indexed Control is defined by seeing a decrease in the %unassigned reads minus the %PhiX aligned in your sequencing run metrics.

7. How many freeze-thaw cycles is PhiRx Indexed Control stable for?

PhiRx Indexed Control is stable for at least 8 freeze-thaw cycles.

8. What's the fragment size of PhiRx Indexed Control?

Below is a TapeStation trace of the PhiRx Indexed Control run undiluted on a High Sensitivity D5000 ScreenTape. The mean fragment size is ~476bp.



9. What is the index structure of PhiRx Indexed Control, and how do we prevent barcode clashing or conflicts with sample barcodes?

PhiRx Indexed Control uses dual 10 bp indexes (10 bp i7 + 10 bp i5), with each position composed of randomly mixed bases. This design results in a very low probability of any specific ten-base index sequence matching a sample barcode.

When ExpressPlex kits are used, the mismatch rate is approximately 0.0001%, meaning about 1 mismatched read per 1 million reads when allowing for one mismatch per index read during demultiplexing. If unique dual indexes (UDIs) are used, the mismatch rate is expected to be even lower.

10. What is the PhiRx Indexed Control sequence?

Please refer to Illumina's FAQ on the PhiX Sequencing Control V3 reference sequence: https://knowledge.illumina.com/library-preparation/general/library-preparation-general-faq-list/000005582