TECHNICAL NOTE

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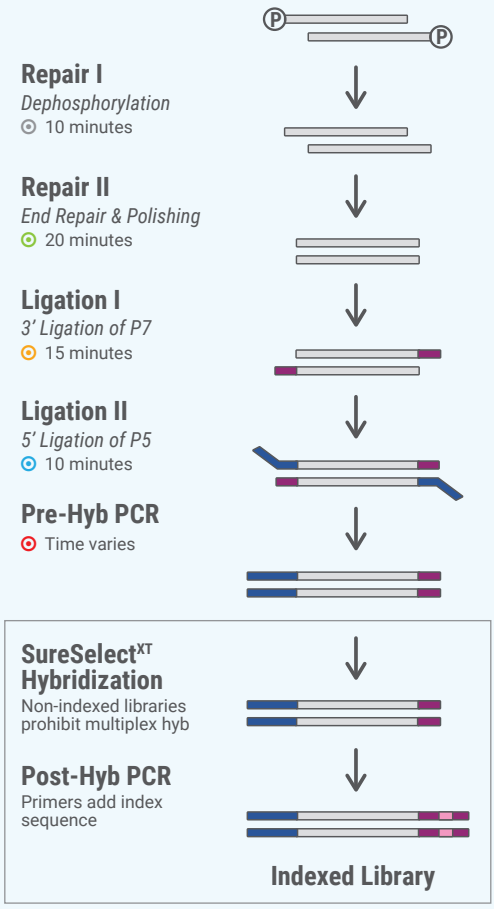
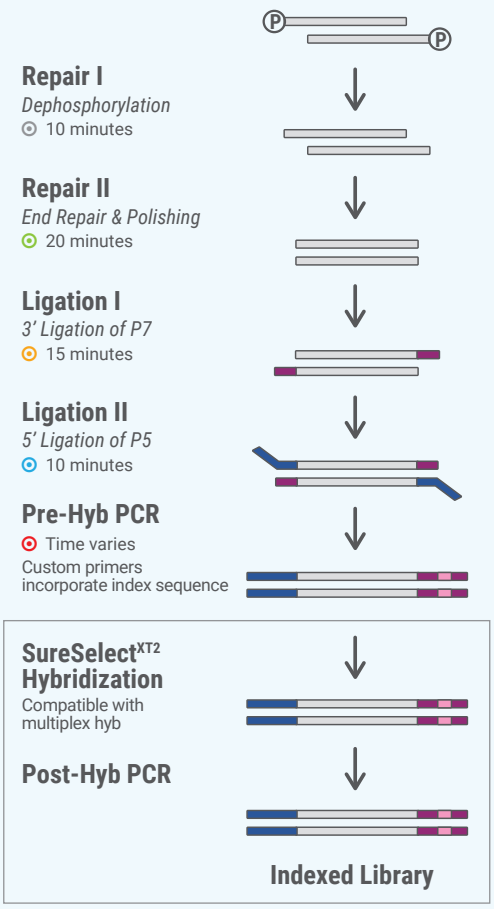
SURESELECT HYBRIDIZATION CAPTURE COMPATIBILITY WITH ACCEL-NGS® 2S HYB LIBRARY KIT

The Accel-NGS 2S Hyb DNA Library Kit (23024/23096), used together with the SureSelect Compatibility Module (26424/26496), constructs non-indexed library molecules that are compatible with SureSelect^{XT} target enrichment. For compatibility with SureSelect^{XT2} target enrichment, custom amplification primers are also required. This Technical Note describes the custom primer sequence and concentration recommendations to construct indexed library molecules compatible with SureSelect^{XT2} hybridization capture.

SURESELECT^{XT} WORKFLOW



SURESELECT^{XT2} WORKFLOW



Libraries prepared with the Accel-NGS 2S Hyb Kit and Swift's SureSelect Compatibility Module will add a non-indexed P7 adapter and a full length, universal P5 adapter during the Ligation I and Ligation II steps. These adapters are readily compatible with the blockers included in the SureSelect hybridization capture reagents. For compatibility with SureSelect^{XT2} blockers, pre-hybridization custom primers (supplied by the user) will add an 8 bp index sequence and complete the library molecules. In the above diagram, the first five colored steps utilize Accel-NGS 2S Hyb reagents while the last two steps are specific to the hyb panel. For pre-hybridization PCR, please use the polymerase supplied with the hybridization capture reagents.

The PCR primers in Reagent R-XT are at a concentration of 6 µM. In place of SureSelect Primer and SureSelect ILM Indexing Pre-Capture Reverse Primer, users constructing libraries for SureSelect^{XT} hybridization should use Reagent R-XT at 600 nM (final concentration in the PCR reaction) with the polymerase supplied with the hybridization capture reagents.

In place of SureSelect Primer and SureSelect ILM Indexing Pre-Capture Reverse Primer, users constructing libraries for SureSelect^{XT2} hybridization should use the following primers at 600 nM (final concentration in the PCR reaction) with the polymerase supplied with the hybridization capture reagents:

Primer 1: 5'-AATGATACGGCGACCACCGAGATC-3'

Primer 2: 5'-CAAGCAGAAGACGGCATACGAGAT**XXXXXXXX**GTGACTGGAGTTCAGACGTG -3'

where **XXXXXXXX** indicates the 8 bp index sequence. SureSelect^{XT2} index sequences can be found in the Reference Chapter of the SureSelect^{XT2} protocol. Please keep in mind that the reverse complement of the index sequence should be used in the custom primer. For example, to label a library with the A01 index sequence, ATGCCTAA, you would replace **XXXXXXXX** in the primer sequence with TTAGGCAT.

To keep cross-contamination of primers during synthesis to a minimum, please discuss purification options with your oligonucleotide supplier.

Contact the Applications Team at **TechSupport@swiftbiosci.com** or **734.330.2568** for further explanation.

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