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\textsuperscript{XT} target enrichment. For compatibility with SureSelect\textsuperscript{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\textsuperscript{XT2} hybridization capture.

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\textsuperscript{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 SureSelectXT 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 SureSelectXT2 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’-AATGATACGCGACCCGAGATC-3’
Primer 2: 5’-CAAGCAGAAGACGGCATACGAGATXxxxxxxxxGTTGACTGAGTTCAGACGTG -3’

where Xxxxxxxxx indicates the 8 bp index sequence. SureSelectXT2 index sequences can be found in the Reference Chapter of the SureSelectXT2 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 Xxxxxxxxx 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.