NGS Constitutional Disease Research Workflow

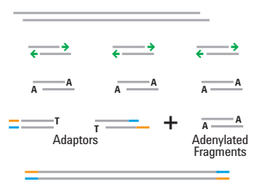
1 Ordering a Catalog Bait Library or Creating a Custom Panel

Order an NGS catalog panel or easily design a custom panel in minutes using Agilent's SureDesign web application

SureSelect Hybridization-Based Target Enrichment

Ligation-based

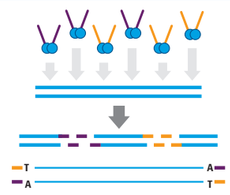
2 Preparation of DNA Libraries



1. Shear DNA into smaller fragments
2. Repair fragment ends
3. Adenylate fragments
4. Ligate adaptors
5. Amplify pre-capture library

Transposase-based

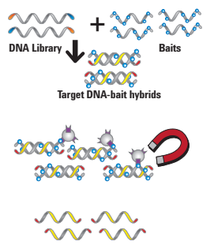
2 Preparation of DNA Libraries



Genomic DNA is enzymatically fragmented and tagged with the adaptors

Adaptor-tagged fragments are amplified to generate the pre-capture libraries

3 Hybridization & Capture



1. DNA library is allowed to hybridize with biotinylated SureSelect RNA baits (90 min for QXT; 16 hr for XT or XT2)
2. DNA-RNA hybrids are pulled down using streptavidin-coated magnetic beads
3. Stringent washes are applied to eliminate non-specific targets

HaloPlex Next-Generation PCR

2 Digest & Denature Sample DNA

Digest genomic DNA with 16 restriction enzymes and QC the reactions using a 4200 TapeStation or 2100 Bioanalyzer System

3 Hybridize Probes to DNA Targets

Mix the HaloPlex probes, indexing primer cassettes and digested DNA; hybridize for 3 hours (up to 500 kb, if >500 kb hyb time = 16 hours)

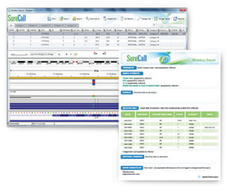
4 Amplify Targets

Amplify enriched and ligated DNA fragments using a single primer pair

5 Final Quality Control

Quantify and QC prepared libraries using a 4200 TapeStation or 2100 Bioanalyzer System

6 Sequence & Analysis



Sequence libraries, analyze and obtain report of mutations using SureCall data analysis software

Agilent Technologies

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