Data Sheet

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Magnis SureSelect XT HS2 RNA Reagent Kit

Automation of the Agilent SureSelect XT HS2 RNA library preparation workflow on the Agilent Magnis NGS Prep system

Key Attributes

- **Productivity**: Minimize multiple hands-on pipetting steps with automation that only requires approximately 15 minutes of preparation.
- **Performance**: Generate strand-specific libraries to detect gene fusions and profile gene expression levels including low-expressed transcripts.
- **Sensitivity and input flexibility**: Use as little as 10 to up to 200 ng total RNA, including FFPE samples to enable profiling of more samples*
- **Throughput**: Increase downstream sequencing pooling capacity with 192 dual sample indexes that maximize throughput and reduce sequencing costs.
- **Complementary to DNA workflow**: In conjunction with the Agilent Magnis SureSelect XT HS2 DNA workflow, both DNA and RNA workflows obtain genomic alterations of SNVs, indels, fusions, CNVs, TMB, MSI, and gene expression level.

Introduction

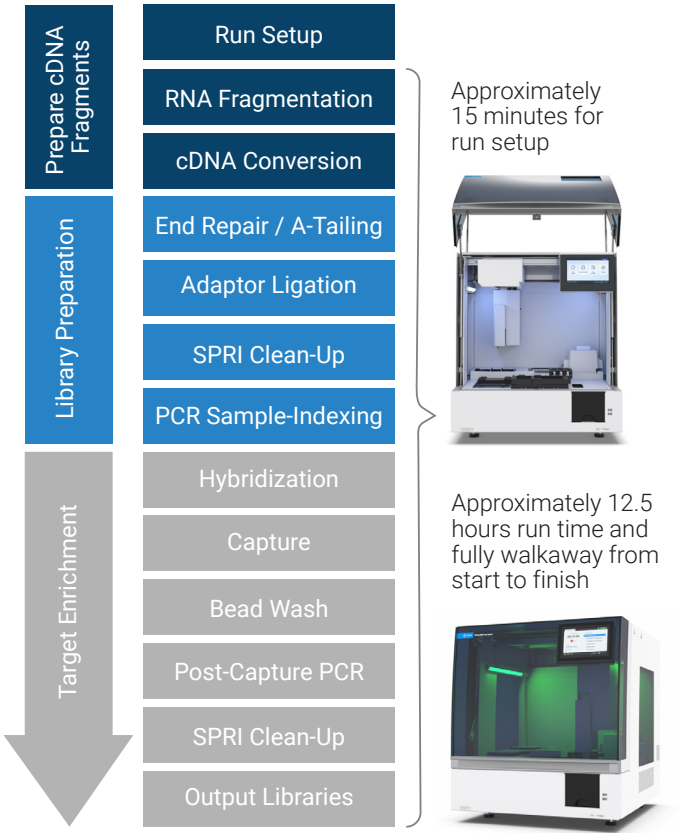
Targeted RNA-Seq is a cost-effective and accurate method to profile the RNA transcripts of interest from the transcriptome. It enables the selective capture and in-depth sequencing of a focused set of transcripts and is widely used to detect gene fusion and splicing variants, and measure gene expression levels. The Agilent SureSelect XT HS2 RNA Reagent kit generates strand-specific libraries for detecting both known and novel fusion partners with low sample input, providing sensitive transcript quantification including low-expressed genes. The workflow is optimized for many sample types including FFPE tissues and has been adopted in many molecular labs running translational and clinical research.

The Agilent Magnis NGS Prep system is a benchtop automation platform dedicated for NGS library preparation and target enrichment. It enables an automated workflow with only 10 to 15 minutes of hands-on time, compared with up to three hours needed in a manual workflow. Now the entire SureSelect XT HS2 RNA manual process including cDNA conversion, library prep, and target enrichment is automated into a fully walkaway workflow on the Magnis NGS Prep system. The user will benefit from simple automation, high-quality, reproducible libraries, and performance comparable to the manual SureSelect XT HS2 RNA assay.

** For high quality FFPE RNA, a DV200 of at least 50%, the input range is validated from 10 ng to 200 ng. For poor quality FFPE RNA, a DV_{200} between 20% to 50%, the input range is validated from 50 ng to 200 ng.*

Ease-of-Use

The Agilent SureSelect XT HS2 RNA reagent kit and workflow is a proven library preparation reagent system for targeted RNA-Seq. While this SureSelect reagent kit delivers high-quality, strand-specific libraries from low-input total RNA, the manual protocol involves a number of interventions. These steps require approximately three hours of direct hands-on time for eight-sampled batched runs going through cDNA conversion, library preparation, and target enrichment. The automated workflow on the Magnis NGS Prep system offers a significant benefit since the entire workflow only requires 15 minutes of hands-on time to set up the run, and then it is fully walkaway. Sequencing-ready libraries are ready in 12.5 hours.



Efficiency

For laboratories that routinely analyze samples by NGS, pooling multiple libraries in the same sequencing run increases throughput and reduces per-sample costs. With the Magnis SureSelect XT HS2 RNA Reagent kit, up to 192 libraries can be pooled in the same flow cell on low-, medium-, and higher-throughput Illumina NGS sequencing platforms. Moreover, many genomics laboratories rely on higher-throughput sequencers to keep up with demand for NGS data. Sample misassignment, or index hopping, has been occasionally observed on the patterned flow cell used by Illumina medium- and high-throughput sequencing platforms and can adversely affect data quality. The Magnis SureSelect XT HS2 RNA Reagent kit produces libraries with dual sample indexes to minimize index hopping and ensure high data quality.

Performance

The Magnis SureSelect XT HS2 RNA assay generates strand-specific libraries which are optimized to reliably detect known and novel fusions and quantify gene expression levels from the selected gene panels in blood, fresh frozen tissue, and formalin-fixed paraffin-embedded (FFPE) samples.

The sample input amount for the Magnis SureSelect XT HS2 RNA reagent kit is validated from 10 to 200 ng total RNA for FFPE RNA with a DV_{200} of at least 50%. The optimized input range is sufficient to produce robust results for a variety of RNA analysis workflows commonly used by research and clinical genomics labs.

The Magnis SureSelect XT HS2 RNA reagent kit generates libraries with high complexity, high strand specificity, high mapping rate, but low duplicate rate and low rRNA rates. High-complexity libraries deliver increased sensitivity of detecting known and novel fusion partners and quantification of low-expressed genes. The incorporated dual molecular barcodes allow the effective removal of PCR duplicates and provide more consistent, accurate gene expression quantification, particularly for low-expressors and/or low-input samples.

The workflow for the Magnis SureSelect XT HS2 RNA reagent kit enables precision oncology research applications by supporting automation of the workflow for the Agilent SureSelect Cancer CGP Assay. The RNA module of the SureSelect Cancer CGP Assay comprises an 80-gene panel designed to detect known and de novo gene fusion transcripts. This fully automated workflow on the Magnis NGS Prep system can reliably detect all fusions from the reference FFPE samples illustrated in Figure 1.

Junction-spanning reads	Replicate 1	Replicate 2	Replicate 3	Replicate 4
CCDC6--RET	33	34	23	31
CD74--ROS1	136	128	80	74
EGFR--AC069287.1	62	53	59	56
EML4--ALK	70	103	67	81
ETV6--NTRK3	163	213	195	189
FGFR3--BAIAP2L1	292	319	356	381
FGFR3--TACC3	417	404	489	485
KIF5B--RET	251	263	206	216
LMNA--NTRK1	160	171	155	172
NCOA4--RET	181	192	166	190
PAX8--PPARG	121	124	108	119
SLC34A2--ROS1	86	81	71	67
SLC45A3--BRAF	55	43	39	43
TFG--NTRK1	80	80	84	64
TMPRSS2--ERG	370	481	411	473
TPM3--NTRK1	167	180	149	139

Figure 1. Magnis SureSelect XT HS2 RNA reagent kit offers highly robust fusion detection demonstrated by using the SureSelect Cancer CGP panel. 50 nanograms of total RNA from SeraSeq FFPE Tumor Fusion RNA V4 reference material (material number 0710-0496, SeraCare) was used as input. Library construction and target enrichment were performed using the Magnis SureSelect Cancer CGP XT HS2 RNA kit and standard protocol (SSEL-RNA-XTHS2-ILM). Libraries were sequenced on Illumina HiSeq 4000 by 2 x 150 bp, sequencing reads were normalized to 10 M for downstream analysis. EGFR variant III and Met Exon 14 skipping in the reference SeraCare FFPE V4 sample are identified as splicing variants by in-house bioinformatics analysis pipeline.

Targeted RNA-Seq is a cost-effective tool for measuring transcript abundance by deep sequencing specific regions of interest. Global gene expression profiling of universal human reference RNA (UHRR) material is a widely used proxy to gauge the accuracy of gene expression analysis. Herein, we demonstrate high gene expression correlations using human entire exome in 10 to 200 ng input range, between Magnis and manual workflows as shown in Figure 2. The data show that libraries created by the Magnis SureSelect XT HS2 RNA Reagent kit are highly reproducible and consistent with the SureSelect XT HS2 RNA manual workflow. The SureSelect XT HS2 RNA manual workflow showed high correlation of global gene expression profiling with whole transcriptome and mRNA methods[1] and the result infers that gene expression levels measured by the Magnis SureSelect XT HS2 RNA reagent kit is in line with the standard method.

Covering Comprehensive Genomic Alterations

Facilitated with capture panel design by the Agilent SureDesign online design tool, the Magnis SureSelect XT HS2 RNA reagent kit introduced here is well-suited to detect known and novel fusion transcripts and measure gene expression levels. The Magnis SureSelect XT HS2 DNA reagent kit, currently on the market, detects single nucleotide variants (SNPs), insertions/deletions (indels), copy number variants (CNVs), tumor mutational burden (TMB), and microsatellite instability (MSI). Both the Magnis SureSelect XT HS2 RNA and DNA workflows automated on the Magnis NGS Prep system are capable of detecting genomic alterations commonly observed in translational and clinical research.

The Magnis SureSelect XT HS2 RNA reagent kit serves as an advanced, automated solution for targeted RNA-Seq, providing ease-of-use, efficient and reliable gene fusion detection, and gene expression level quantification.

200 ng		Manual Workflow							
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Replicate 8
Magnis Workflow	Replicate A	0.993	0.993	0.983	0.991	0.988	0.988	0.990	0.986
	Replicate B	0.991	0.991	0.982	0.989	0.986	0.986	0.986	0.982
	Replicate C	0.993	0.993	0.984	0.991	0.989	0.989	0.990	0.987
	Replicate D	0.991	0.990	0.981	0.988	0.985	0.985	0.985	0.981
	Replicate E	0.993	0.992	0.983	0.991	0.988	0.988	0.989	0.986
	Replicate F	0.994	0.994	0.985	0.993	0.990	0.990	0.991	0.988
	Replicate G	0.993	0.993	0.984	0.992	0.988	0.989	0.990	0.986
	Replicate H	0.992	0.992	0.982	0.991	0.987	0.988	0.988	0.985

10 ng		Manual Workflow							
		Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Replicate 6	Replicate 7	Replicate 8
Magnis Workflow	Replicate A	0.991	0.990	0.994	0.991	0.991	0.993	0.993	0.988
	Replicate B	0.989	0.988	0.992	0.989	0.989	0.990	0.992	0.983
	Replicate C	0.990	0.990	0.993	0.990	0.991	0.992	0.993	0.987
	Replicate D	0.988	0.987	0.991	0.988	0.989	0.990	0.991	0.983
	Replicate E	0.990	0.990	0.993	0.991	0.992	0.993	0.993	0.989
	Replicate F	0.991	0.991	0.994	0.991	0.992	0.993	0.994	0.988
	Replicate G	0.991	0.991	0.994	0.991	0.992	0.993	0.994	0.989
	Replicate H	0.991	0.990	0.994	0.991	0.992	0.993	0.993	0.988

Figure 2. Correlation coefficient R of global gene expression profiling between Magnis and manual workflows. For the upper table, 200 ng of total RNA from universal human reference RNA (UHRR) was used as input for each of the eight samples in the Magnis run and manual workflow, respectively. For the lower table, 10 ng of total RNA from UHRR was used as input for each of the eight samples in the Magnis run and manual workflow, respectively. For the Magnis workflow, library preparation and target enrichment was performed using the Magnis SureSelect XT HS2 RNA Exome V8 kit and SSEL-RNA-XTHS2-ILM protocol. For the manual workflow, library preparation and target enrichment were performed using the SureSelect XT HS2 RNA Exome V8 kit following procedures recommended in the user manual. Libraries were sequenced on the Illumina HiSeq 4000 by 2 x 150 bp. Sequencing reads were normalized to 20 M for downstream analysis. identified as splicing variants by in-house bioinformatics analysis pipeline.

Part Number	Product Name and Description
32 Reaction Kits	
G9751C	Magnis SureSelect XT HS2, RNA, Tier 1 (1 to 499 kb), Illumina, 32
G9752C	Magnis SureSelect XT HS2, RNA, Tier 2 (0.5 to 2.9 Mb), Illumina, 32
G9753C	Magnis SureSelect XT HS2, RNA, Tier 3 (3 to 5.9 Mb), Illumina, 32
G9754C	Magnis SureSelect XT HS2, RNA, Tier 4 (6 to 11.9 Mb), Illumina, 32
G9755C	Magnis SureSelect XT HS2, RNA, Tier 5 (12 to 24 Mb), Illumina, 32
G9756C	Magnis SureSelect XT HS2, RNA, 24 to 50 Mb, Illumina, 32
G9773C	Magnis SureSelect XT HS2, RNA, Exome V7, Illumina, 32
G9774C	Magnis SureSelect XT HS2, RNA, Exome V8, Illumina, 32
G9777C	Magnis SureSelect Cancer CGP, XT HS2 RNA, Illumina, 32
96 Reaction Kits	
G9751D	Magnis SureSelect XT HS2, RNA, Tier 1 (1 to 499 kb), Illumina, 96
G9752D	Magnis SureSelect XT HS2, RNA, Tier 2 (0.5 to 2.9 Mb), Illumina, 96
G9753D	Magnis SureSelect XT HS2, RNA, Tier 3 (3 to 5.9 Mb), Illumina, 96
G9754D	Magnis SureSelect XT HS2, RNA, Tier 4 (6 to 11.9 Mb), Illumina, 96
G9755D	Magnis SureSelect XT HS2, RNA, Tier 5 (12 to 24 Mb), Illumina, 96
G9756D	Magnis SureSelect XT HS2, RNA, 24 to 50 Mb, Illumina, 96
G9773D	Magnis SureSelect XT HS2, RNA, Exome V7, Illumina, 96
G9774D	Magnis SureSelect XT HS2, RNA, Exome V8, Illumina, 96
G9777D	Magnis SureSelect Cancer CGP, XT HS2 RNA, Illumina, 96

References

1. Agilent Technologies. SureSelect XT HS2 RNA Reagent Kit: One Easy, Parallel Workflow for RNA- and DNA-Seq to Simplify Your Work; **2020**. Data Sheet, 5994-2314EN.

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