illumina®

# TruSight™ Autism Sequencing Panel

Using expert-defined content and proven next-generation sequencing technology to efficiently and cost-effectively assist in the evaluation of characteristics associated with Autism Spectrum Disorders (ASDs).

## Highlights

- **Expert-Defined Content**
  Targeting 101 genes implicated in ASDs
- **Low Input DNA Requirement**
  Excellent data quality with as little as 50 ng DNA to preserve precious samples
- **Fast, Simple Workflow**
  Library preparation and enrichment completed in 1.5 days

## Introduction

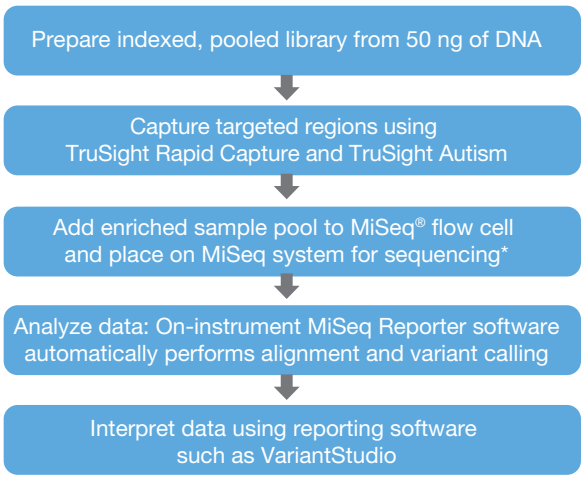
Autism Spectrum Disorders (ASDs) are developmental disorders that usually appear in young children under the age of three. It is the fastest growing serious developmental disability in the United States, affecting 1 in 88 children[1]. ASDs are typically characterized by social/communication deficits and repetitive behaviors. Early intervention with behavioral therapies has been proven to improve learning, communication, and social skills. This makes it essential to find a method for evaluating characteristics associated with ASDs as early as possible.

Although the exact causes of ASDs are unknown, risk factors include a genetic component. The TruSight™ Autism Sequencing Panel provides pre-designed, ready-to-use oligos targeting 101 developmental delay genes implicated with ASDs. The sequencing panel is compatible with TruSight Rapid Capture that takes advantage of Nextera® Rapid Capture technology to offer a single, integrated library preparation and enrichment workflow that can be completed in just 1.5 days (Figure 1). Delivering excellent data quality from low sample input (50 ng), TruSight Autism and TruSight Rapid Capture enable efficient and reliable analysis of precious samples, while retaining sufficient material for future analyses.

## Content Design Strategy

Developed in collaboration with Dr. Jonathan Pevsner and the team at Kennedy Krieger Institute (KKI), TruSight Autism targets genes implicated with ASDs. The sequencing panel includes genes reported in the Online Mendelian Inheritance in Man (OMIM) database on autism; genes with recurrent mutations suspected in developmental delays[2–4]; genes with reported mutations as found in case studies involving developmental delay characteristics; genes from other publicly available ASD panels; and genes from summaries of ASD-relevant genes (e.g., ASDKB[4]). Genes neighboring strong association signals were excluded in the absence of published reports of mutations.

**Figure 1: Integrated Workflow**




TruSight Autism is compatible with the TruSight Rapid Capture method, which integrates library preparation and enrichment steps to offer a fast, streamlined, and optimized workflow, delivering fully enriched libraries for up to 96 samples in just 1.5 days.

* For higher throughput, use NextSeq™ flow cells on the NextSeq system or HiSeq® flow cells on the HiSeq system.

## Superior Coverage

TruSight Autism features a highly optimized probe set designed to assist in the investigation of genomic features associated with ASDs. The kit includes > 4,500 80-mer probes, each constructed against the human NCBI37/hg19 reference genome. The probe set was designed to enrich for 1,728 exons, spanning 101 genes of interest (Table 1).

TruSight Autism targets a total of 328 Kb of the human genome. The 80-mer probes target libraries of approximately 500 bp (insert size of 300 bp), enriching 350–650 bases centered symmetrically around the midpoint of the probe (Figure 2)[5]. This means that the kit provides coverage of exonic and non-coding DNA in exon-flanking regions, on average 50 bp.

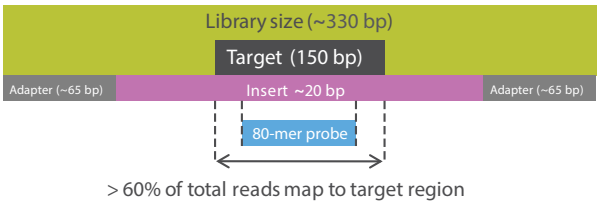
## Integrated Library Preparation and Enrichment Workflow

TruSight Autism and TruSight Rapid Capture leverage the speed of Nextera library preparation technology. By eliminating the need for mechanical DNA fragmentation and introducing a unique multiplex

## Table 1: Coverage Details

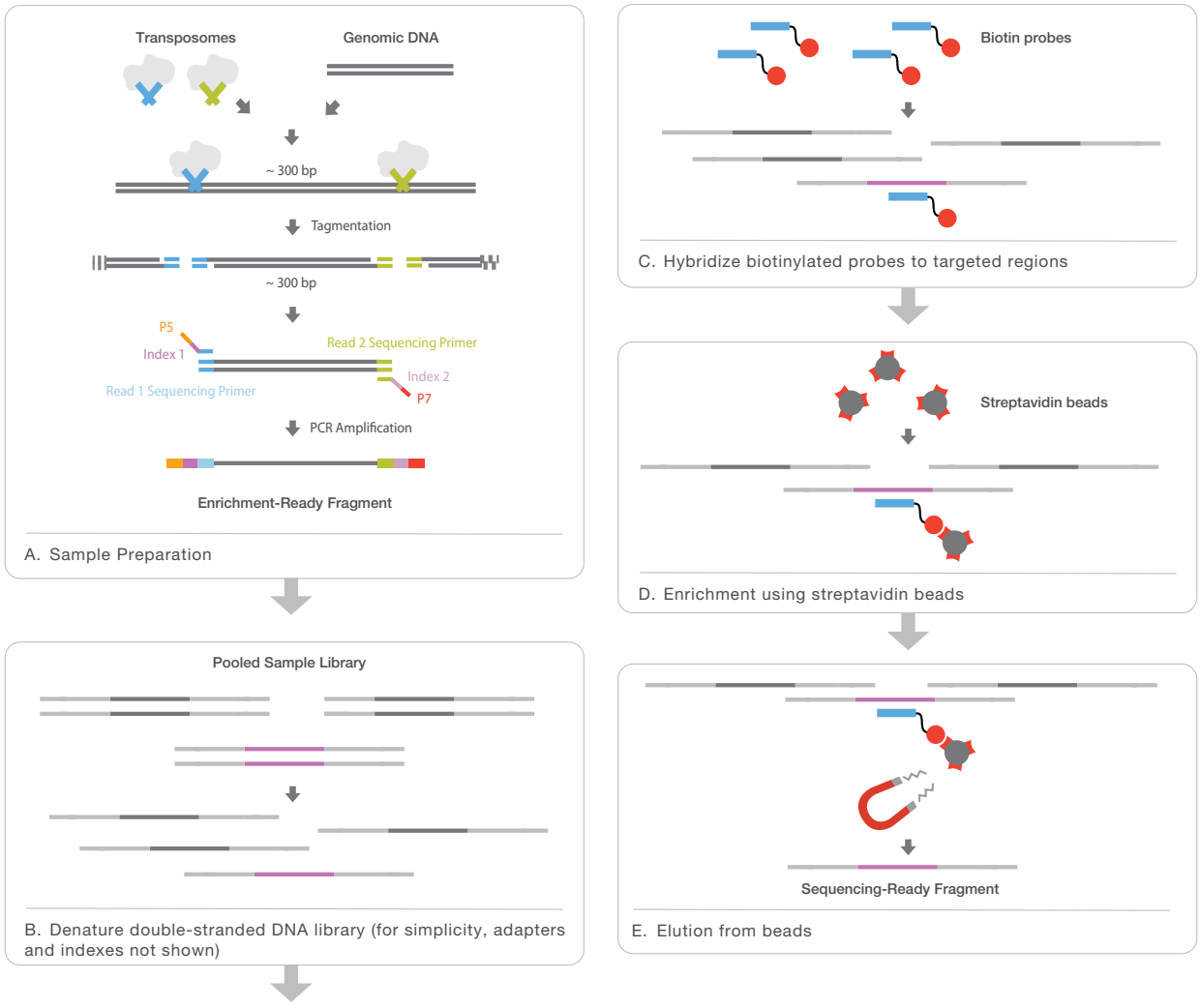
| | |
|---|---|
| Cumulative target region size | 328 Kb |
| Number of target genes | 101 |
| Number of target exons | 1,728 |
| Probe size | 80-mer |
| Number of probes | > 4,500 |
| Recommended mean coverage | 100× |
| Target minimum coverage | 20× |
| Percent exons covered based on coverage metrics | ≥ 95% |

## Figure 2: Probe Footprint



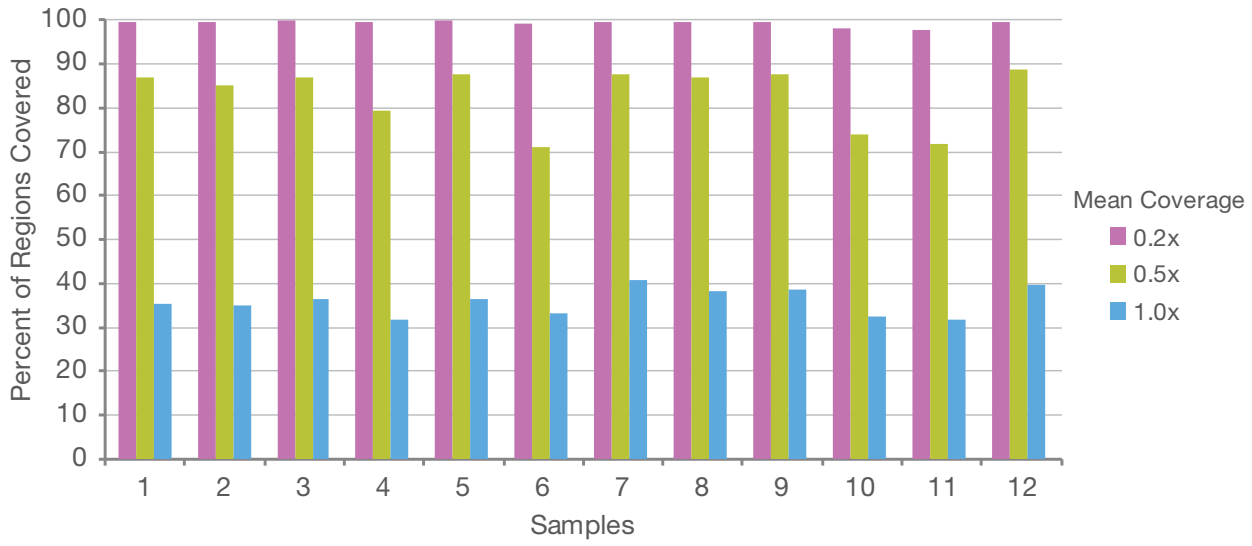

With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 350–650 bp centered around its midpoint.

## Figure 3: Integrated TruSight Rapid Capture Workflow




The TruSight Rapid Capture workflow provides a fast, simple method for isolating the genes targeted using TruSight Autism. The streamlined, automation-friendly workflow combines library preparation and enrichment steps, and can be easily completed in 1.5 days with minimum hands-on time.

Figure 4: High Coverage Uniformity




Coverage uniformity is given for 12 samples with respect to the percentage of targeted regions at varying mean normalized read depths. The 12 samples were prepared and then simultaneously enriched using the TruSight Rapid Capture Kit along with the TruSight Autism Sequencing Panel. Samples were sequenced across MiSeq flow cells, generating mean read depths of 100–300× (varying for each sample). Over 95% of bases (> 300 Kb) were covered at 0.2× mean coverage.

pre-enrichment sample pooling, the TruSight Rapid Capture method reduces hands-on time for a high-throughput workflow that saves at least one full day over all other currently available enrichment workflows (Figure 1). Furthermore, master-mixed reagents are coupled with a plate-based protocol for simultaneous processing of up to 24 enrichment reactions (288 total samples).

Flexible kit configurations enable labs to readily meet their sample throughput needs. For those requiring higher throughput, kit reagent volumes are optimized for liquid handlers to make an automation-friendly workflow. TruSight Rapid Capture kits supporting lower throughput options are also available, allowing labs to cost-effectively run samples immediately instead of waiting to batch.

Following the TruSight workflow, the process starts with rapid Nextera-based library prep to convert input genomic DNA into adapter-tagged libraries (Figure 3A). This rapid prep requires only 50 ng of input DNA and takes less than 3 hours for a plate of 96 samples. Nextera tagmentation of DNA simultaneously fragments and tags DNA without the need for mechanical shearing. Integrated sample barcodes then allow the pooling of up to 96 samples for a single Rapid Capture pull down. Next, libraries are denatured into single-stranded DNA (Figure 3B) and biotin-labeled probes specific to the targeted region are used for the Rapid Capture hybridization (Figure 3C). The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes (Figure 3D). Biotinylated DNA fragments bound to the streptavidin beads are magnetically pulled down from the solution (Figure 3E). The enriched DNA fragments are then eluted from the beads and hybridized for a second Rapid Capture. This entire process is completed in only 1.5 days, enabling a single researcher to efficiently process up to 288 samples at one time—all without automation.

## Data Analysis

Sequence data generated from TruSight Autism–enriched libraries are analyzed by the on-instrument MiSeq Reporter (MSR) software. After demultiplexing and FASTQ file generation, the software uses the Burrows-Wheeler Aligner (BWA) to align the reads against the hg19 homo sapiens reference genome to create BAM files. The Genome Analysis Toolkit (GATK) is then used to perform variant analysis for the target regions specified in the manifest file. The output of GATK are VCF files, which are text files that contain SNPs, indels, and other structural variants.

## High Data Quality

With TruSight Autism and TruSight Rapid Capture, researchers can be confident in the quality of sequencing data generated from pooled multisample libraries. Each sample is sequenced with high coverage uniformity across the target region, with ≥ 95% of exons covered at a minimum coverage of 0.2× of the mean coverage (Figure 4). This uniformity applies to smaller exons (< 150 bp) as well as long coding exons.

## Summary

The TruSight Autism Sequencing Panel enables researchers to access an expert-defined sequencing panel for investigating genomic features associated with ASDs. The optimized probe set provides comprehensive coverage of the targeted regions with high coverage uniformity for identifying variants. Combining this content with the TruSight enrichment method enables a fast, easy workflow, requiring low sample DNA input, generating a highly efficient targeted resequencing solution for early detection of ASD genetic characteristics.

## Ordering Information

| Product | Catalog No. | TG Catalog No.* |
|---|---|---|
| TruSight Autism Sequencing Panel (4 enrichments) | FC-121-0203 | TG-141-1003 |
| **Rapid Capture Kits** | | |
| TruSight Rapid Capture Kit (1 index, 8 samples) | FC-140-1101 | TG-140-1101 |
| TruSight Rapid Capture Kit (2 indices, 8 samples) | FC-140-1102 | TG-140-1102 |
| TruSight Rapid Capture Kit (4 indices, 16 samples) | FC-140-1103 | TG-140-1103 |
| TruSight Rapid Capture Kit (24 indices, 48 samples) | FC-140-1104 | TG-140-1104 |
| TruSight Rapid Capture Kit (24 indices, 96 samples) | FC-140-1105 | TG-140-1105 |
| TruSight Rapid Capture Kit (96 indices, 288 samples) | FC-140-1106 | TG-140-1106 |

* TG-labeled consumables include features intended to help customers reduce the frequency of revalidation. They are available only under supply agreement and require customers to provide a binding forecast. TruSight sequencing panels are available for evaluation purposes prior to executing a supply agreement. Please contact your account manager for more information.

## Learn More

To learn more about the TruSight Autism Sequencing Panel, TruSight Rapid Capture kits, and Illumina next-generation sequencing technology, visit www.illumina.com/trusightautism.

## References


1. Autism Speaks (www.autismspeaks.org/what-autism/facts-about-autism)
2. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, et al. (2008) Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet **82(2):** 477–488.
3. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, et al. (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell 149(3): 525–537.
4. Vendeweyer G and Kooy RF (2009) Balanced translocations in mental retardation. Hum Genet 126(1): 133–147.
5. Optimizing Coverage for Targeted Resequencing Technical Note. (www.illumina.com/documents/products/technotes/technote_optimizing coverage_for_targeted_resequencing.pdf)





**Illumina** • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com


FOR RESEARCH USE ONLY




Pub. No. 670-2012-014 Current as of 19 November 2014


illumina®


AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGAGC
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA