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TruSeq™ Neurodegeneration Sequencing Panel

A targeted sequencing solution for investigation of candidate genes associated with major neurodegenerative diseases.

Highlights

- **Expert-selected panel targets genomic regions of interest**
  Targets 118 genes with over 8.7 Mb of content, including exons, introns, untranslated regions (UTRs), and promoter regions
- **Broad coverage of neurodegenerative diseases**
  Covers Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, and others
- **Integrated, streamlined workflow**
  Includes library prep with minimal hands-on time, reliable sequencing, and user-friendly data analysis

Introduction

The TruSeq Neurodegeneration Sequencing Panel enables systematic investigation of neurodegenerative disease–associated genes. This targeted panel combines Nextera™ library preparation and target enrichment techniques with proven Illumina next-generation sequencing (NGS) technology and user-friendly data analysis for an integrated, streamlined sequencing workflow (Figure 1). The TruSeq Neurodegeneration Sequencing Panel facilitates fine-mapping validation and identification of rare, putative functional alleles and additional risk alleles. It offers the ability to survey both protein-coding and noncoding regulatory regions of candidate genes for efficient, cost-effective investigation of major neurodegenerative diseases.

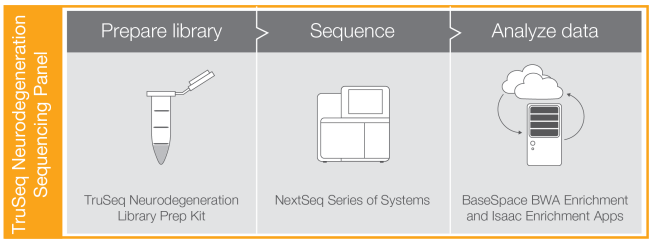

Figure 1: Overview of the TruSeq Neurodegeneration Sequencing Panel workflow—The TruSeq Neurodegeneration Sequencing Panel is part of an integrated, streamlined workflow that includes library preparation, sequencing, and data analysis.

Expert-selected panel content

The TruSeq Neurodegeneration Sequencing Panel covers 118 genes associated with major neurodegenerative diseases. These include Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, Frontotemporal Dementia, Dementia with Lewy Body, Dystonia, and Early Onset Dementia. Target genes included on the panel were selected with input from the scientific community based on recent scientific findings as either risk-validated genes or genes in loci found via Genome-Wide Association Studies (GWAS).[1–9]

The TruSeq Neurodegeneration Sequencing Panel features a highly optimized probe set of ~ 43,600 80mer probes, each constructed against the human NCBI37/hg19 reference genome. The probe set covers 8.7 Mb of genomic content, including exons, introns, UTRs, and promoter regions within 1300 bp upstream of the transcriptional start site (TSS) of candidate genes (Table 1). Probes included on the TruSeq Neurodegeneration Sequencing Panel were made using an iterative process of design and functional testing to ensure excellent performance and uniformity.

Table 1: Coverage details

Parameter	Value
Cumulative target region size	8.7 Mb
No. of genes in panel	118
Probe size	80 bp
No. of probes	~ 43,600
Target content	Exons, introns, UTRs, promoter regions (1300 bp)
Coverage uniformity (0.2× mean)	> 85%
Samples in pre-enrichment pooling	Up to 12
Sample input	50 ng
Library insert size	230 bp
Recommended sequencing run	2 × 150 bp
Quality control (QC) markers	57 on Y chromosome and 7 on X chromosome

Streamlined library preparation

The TruSeq Neurodegeneration Sequencing Panel takes advantage of the Nextera Rapid Capture assay for simple, streamlined preparation of sequencing-ready libraries. The library preparation process starts with Nextera tagmentation, which simultaneously fragments and tags DNA to generate adapter-tagged libraries without the need for mechanical shearing (Figure 2). Next, libraries are denatured (Figure 2) and biotin-labeled probes specific to the targeted region are used for hybridization (Figure 2). The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes (Figure 2). Biotinylated DNA fragments bound to the streptavidin-coated beads are magnetically pulled down from the solution

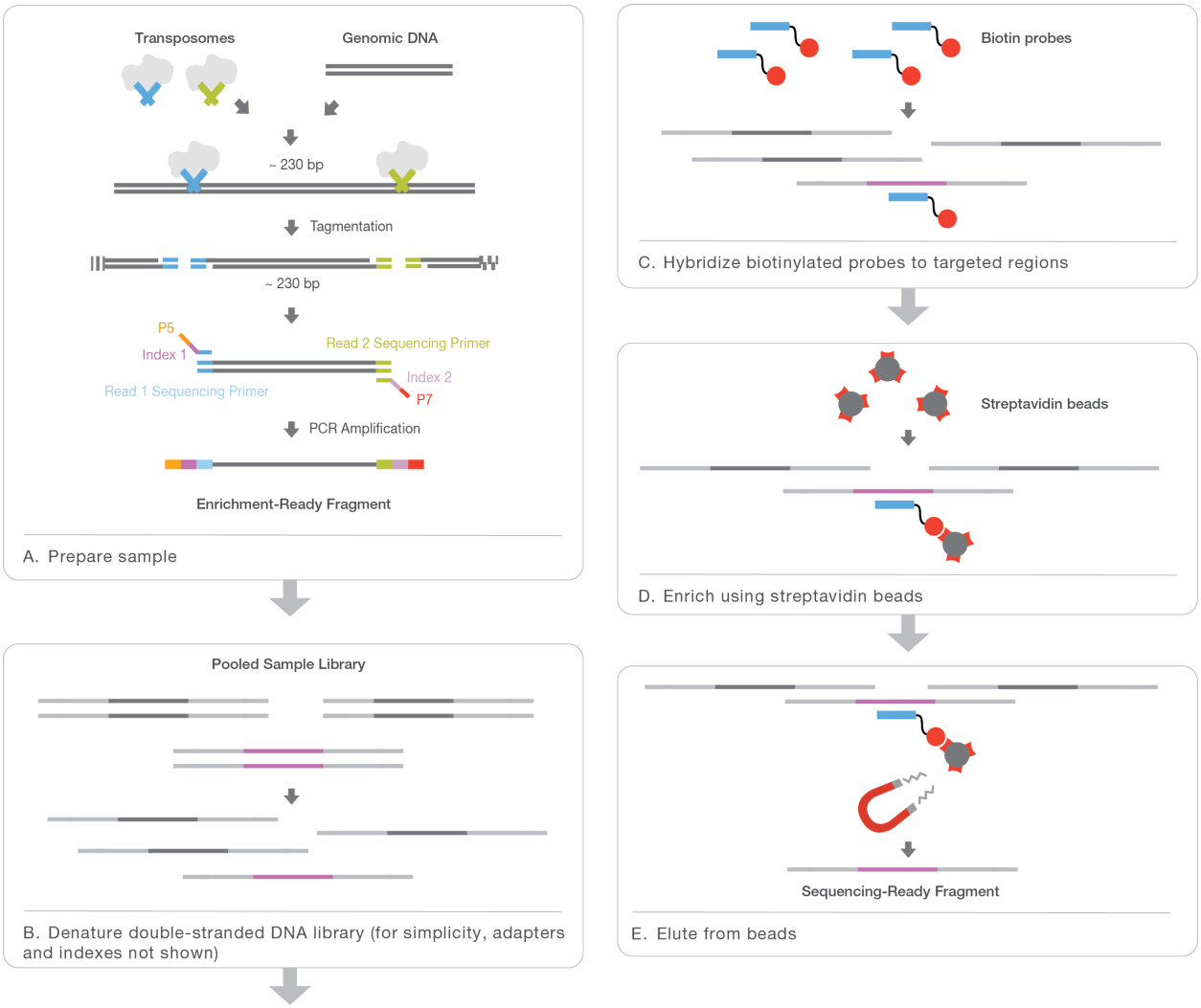

Figure 2: Nextera Rapid Capture chemistry—The TruSeq Neurodegeneration Sequencing Panel harnesses Nextera tagmentation technology that combines library preparation and enrichment steps to provide a streamlined workflow that can be completed in 1.5 days with 5 hours of hands-on time.

(Figure 2). Integrated sample barcodes allow for simultaneous capture of 3 to 12 samples in a single tube. The enriched DNA fragments are then eluted from the beads and hybridized for a second capture. This method requires 50 ng of input DNA and takes less than three hours for a plate of 3 to 12 samples.

Proven Illumina sequencing

Prepared libraries are loaded directly onto an Illumina NGS platform for sequencing. These sequencing systems harness industry-leading Illumina sequencing by synthesis (SBS) chemistry. More than 90% of the world's sequencing data are generated by Illumina SBS chemistry.[10] Illumina sequencing systems offer flexibility for a broad range of applications and scalable throughput to support various study sizes.

Intuitive data analysis

Sequencing data can be instantly transferred, stored, and analyzed securely in BaseSpace™ Sequence Hub, the Illumina cloud-based genomics computing environment. BaseSpace Sequence Hub provides a large collection of BaseSpace Apps that include commercial and open-source tools to support a range of common data analysis needs, such as alignment, variant calling, and more. These Apps feature intuitive push-button user interfaces that do not need bioinformatics expertise.

TruSeq Neurodegeneration Sequencing Panel data are analyzed using two Enrichment Core Apps. The BWA Enrichment App v2.0 provides industry-standard Burrows-Wheeler Alignment (BWA) and Genome Analysis Toolkit (GATK) variant calling. The Isaac™ Enrichment App v2.0 is the Illumina pipeline optimized for rapid analysis and variant calling.

High-quality data

Six sample libraries, prepared with the TruSeq Neurodegeneration Library Prep Kit as 6-plex pools, were sequenced on the NextSeq™ 500 System, and analyzed in BaseSpace Sequence Hub following the described workflow. The mean coverage for the sequencing

run was 155×. The percent of targeted bases that were covered at >31× show that >90% of bases are covered for all samples (Figure 3).

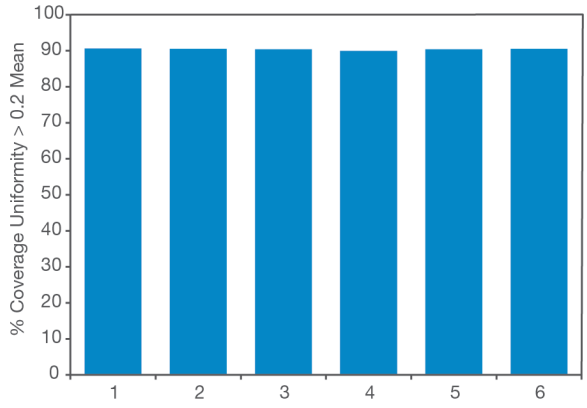

Figure 3: Coverage uniformity across 6-plex pools—Six sample libraries were prepared with the TruSeq Neurodegeneration Library Prep Kit. Libraries were sequenced on the NextSeq 500 System with a mean coverage of 155× for the sequencing run. The percent of targeted bases that were covered at > 31× are shown for each sample.

Summary

The TruSeq Neurodegeneration Sequencing Panel offers an integrated workflow solution for research of rare putative functional and high–risk alleles associated with major neurodegenerative diseases. Developed with input from the scientific community, including recent scientific findings, the panel enables systematic investigation of 118 neurodegenerative disease–associated genes, including protein-coding and regulatory regions. Libraries with target regions are prepared efficiently with minimal hands–on time using Nextera technology. Sequencing on Illumina systems combined with data analysis using intuitive BaseSpace Apps provides a streamlined solution for neurodegenerative disease research.

Ordering information

For more information about the TruSeq Neurodegeneration Sequencing Panel or to place an order, contact a local sales representative:

North America: 800.809.4566

Europe, Middle East, Africa: +44.1799.534000

Other regions: www.illumina.com/company/contact-us.html

Learn more

To learn more about solutions for targeted resequencing, visit www.illumina.com/techniques/sequencing/dna-sequencing/targeted-resequencing.html

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