Targeted Gene Panels

Overview

- Sequence specific set of genes related to condition
- Highly focused - typically 10-500 genes
- Very high coverage for selected regions (>500X)

Common Panel Types

Cancer	Cardio	Neuro
50-500 genes	**50-200 genes**	**100-300 genes**
Oncology hotspots	Heart conditions	Epilepsy, ataxia

Advantages

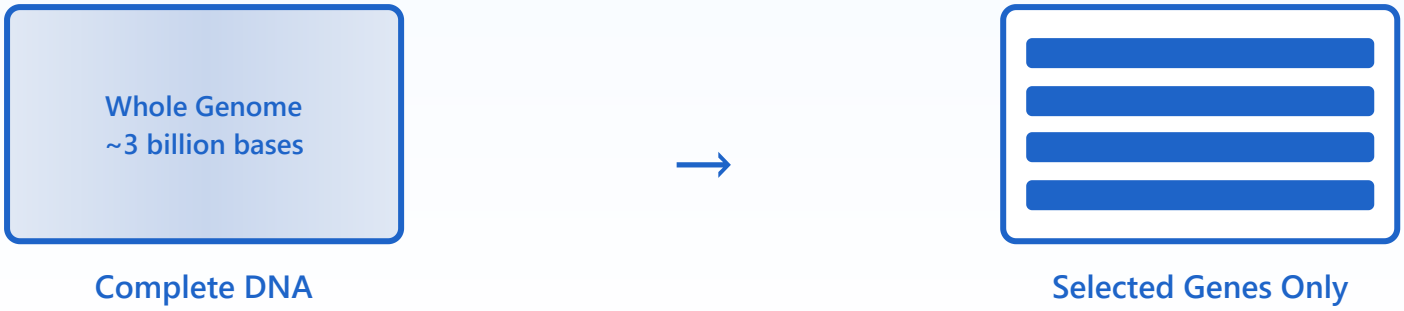
- Cost-effective ($100-300)
- Very high depth
- Faster turnaround
- Detect low-frequency variants

Use Cases

- Hereditary cancer screening
- Pharmacogenetic testing
- Carrier screening
- Targeted diagnostics

Best for known genes associated with specific phenotypes

Principle: Selective Enrichment



- Target enrichment focuses sequencing on specific genomic regions of interest
- Reduces sequencing cost by 10-1000x compared to whole genome sequencing
- Increases depth of coverage for better variant detection
- Enables detection of low-frequency somatic variants (as low as 1-5%)

Capture Methods

1. Hybridization Capture (Solution-based)

Custom oligonucleotide probes (baits) complementary to target regions are mixed with fragmented DNA library. Target fragments hybridize to biotinylated probes and are captured using streptavidin-coated magnetic beads. Non-target DNA is washed away.

Examples: Agilent SureSelect, IDT xGen, Twist Bioscience
Best for: Larger panels (>100 genes), exome sequencing

2. Amplicon-based Sequencing (PCR)

Multiple primer pairs designed to amplify specific target regions simultaneously in a single multiplex PCR reaction. Amplified products are pooled and sequenced directly.

Examples: Illumina AmpliSeq, Ion Torrent AmpliSeq
Best for: Small-medium panels (10-200 genes), hotspot regions

3. Molecular Inversion Probes (MIPs)

Single-stranded DNA probes with sequences complementary to regions flanking the target. After hybridization, the probe circularizes around the target sequence, which is then amplified.

Best for: SNP genotyping, copy number variation detection

Targeted Panel Sequencing Workflow

1

DNA Extraction & QC

Extract high-quality genomic DNA from sample (blood, tissue, saliva). Assess quantity (10-500 ng typically required) and quality (DIN/RIN score).

2

Library Preparation

Fragment DNA to optimal size (150-300 bp). Attach adapters and unique molecular identifiers (UMIs) to enable sequencing and reduce PCR duplicates.

3

Target Enrichment

Apply hybridization capture or amplicon-based enrichment to isolate genomic regions of interest. Wash away non-target DNA sequences.

4

PCR Amplification

Amplify enriched library to generate sufficient material for sequencing. Typical: 8-12 PCR cycles to minimize amplification bias.

5

Next-Generation Sequencing

Sequence enriched library on NGS platform (Illumina, Ion Torrent, MGI). Generate paired-end reads (typically 2×150 bp) with high depth (>500X average coverage).

6

Bioinformatics Analysis

Align reads to reference genome, call variants (SNVs, indels, CNVs), filter artifacts, annotate variants, and interpret clinical significance using databases (ClinVar, COSMIC, gnomAD).

Method Comparison

Feature	Hybridization Capture	Amplicon-based
Input DNA	50-500 ng	10-50 ng
Uniformity	Excellent across targets	Variable (primer efficiency)
Target Size	Best for large panels (>1 Mb)	Best for small panels (<500 kb)
Workflow Time	2-3 days	1 day

Feature	Hybridization Capture	Amplicon-based
Sensitivity	5-10% allele frequency	1-5% allele frequency
Cost per Sample	$150-400	$100-250
Best Application	Hereditary disease panels, exomes	Cancer hotspots, pharmacogenetics

Key Performance Metrics

Coverage Metrics

- **Mean coverage depth:** >500X
- **Target coverage:** >95% at 100X
- **Uniformity:** >80% bases within 0.2x mean
- **On-target rate:** >50% reads

Variant Detection

- **SNV sensitivity:** >99%
- **Indel sensitivity:** >95%
- **CNV detection:** Exon-level resolution
- **Somatic VAF:** As low as 1-5%

High depth sequencing enables confident detection of both germline and somatic variants with clinical-grade accuracy

Clinical Applications

- **Oncology:**Somatic mutation profiling for targeted therapy selection (e.g., EGFR, KRAS, BRAF in solid tumors)
- **Hereditary Cancer:**BRCA1/2, Lynch syndrome genes (MLH1, MSH2, MSH6, PMS2), Li-Fraumeni (TP53)
- **Cardiovascular:**Cardiomyopathy genes (MYH7, MYBPC3, TTN), arrhythmia panels (SCN5A, KCNQ1)
- **Neurology:**Epilepsy genes (SCN1A, KCNQ2), intellectual disability panels, muscular dystrophy genes

- **Pharmacogenomics:** Drug metabolism genes (CYP2D6, CYP2C19, TPMT, SLCO1B1) for personalized medication