Genomic Technologies for Cancer Research

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I. Introduction: Genomic Technologies for Cancer Research

In recent years, genomic technologies have emerged as invaluable tools in cancer research (Figure 1). International projects such as the International Cancer Genome Consortium (ICGC)[1] and The Cancer Genome Atlas (TCGA)[2], tasked with mapping the biology of dozens of tumor types, would not have been possible without these tools. Next-generation sequencing (NGS) and high-density microarrays are used to study the biology of cancer. Both provide the cancer research community with a growing body of knowledge that may lead to more effective drug design, better patient treatment options, and more accurate prognoses.[3]

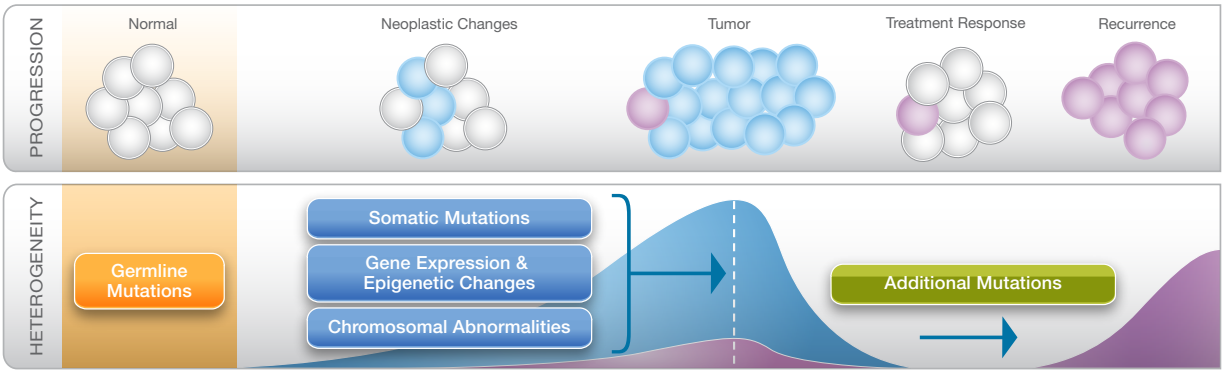

Figure 1: The Tumor Progression Pathway—Genomic technologies are helping researchers achieve a deeper understanding of the tumor progression pathway. Much of the research thus far has focused on the study of basic tumor biology and the identification of variants in germline DNA that influence cancer risk and susceptibility. However, there has been a shift over the past few years to more translational study designs, linking genetic information with phenotypes to understand the clinical significance.[4,5]

NGS is suited to the study of cancer biology.

- Paired tumor-normal (T/N) whole-genome sequencing (WGS) or T/N whole-exome sequencing (WES) have emerged as ideal methods for the discovery of somatic mutations.
- NGS-based RNA sequencing (RNA-Seq) has revolutionized gene expression studies by enabling researchers to measure relative expression changes across the whole genome in a single experiment and to identify novel transcripts.
- RNA-Seq has emerged as a leading method for identifying gene fusions, a critical class of somatic driver mutations in tumor cells.
- Chromatin immunoprecipitation sequencing (ChIP-Seq), bisulfite sequencing, or methylation-targeting microarrays, can be used to investigate the role of epigenetic factors in the biology of tumorigenesis.

The adoption of these genomic approaches in cancer research has led to a deeper understanding of tumor biology and is establishing the foundation necessary to support the long-term goals of personalized medicine. Researchers are also using NGS and microarray-based genotyping of germline DNA to identify inherited variations that influence cancer susceptibility. While NGS is an excellent tool for assessing the germline status of known cancer predisposition genes and identifying novel loci, microarrays are ideal for large-scale population studies, where large sample numbers are required to identify weakly predisposing genes.[6]

This primer describes the broad portfolio of genomic technologies offered by Illumina that are directly applicable to cancer research.

II. Approaches for Detecting Somatic Mutations

Depending on the experimental goals, desired throughput, and available budget, researchers can choose a comprehensive, whole-genome approach or a focused, targeted approach to somatic variant detection (Table 1). WGS offers a hypothesis-free method for discovering somatic changes across the genome, while targeted sequencing allows researchers to focus their investigation on specific regions of interest based on *a priori* criteria. For a given budget, targeted sequencing enables higher coverage across specific regions of interest, enabling the detection of low frequency subclones within heterogeneous tumors or detection of rare somatic variants.

Targeted Sequencing Solutions for Somatic Mutation Detection

Targeted sequencing offers several key advantages compared to whole-genome approaches:

- Narrows the scope of a sequencing project significantly
- Reduces the overall data analysis burden
- Lowers the cost of sequencing per sample
- Reduces the turnaround time

These advantages also enable researchers to perform *deep sequencing*, which is critical for identifying rare mutations or subclonal detection in heterogenous tumor samples. The trade-off with targeted sequencing is that it can miss key mutations, such as those found in intergenic or previously unknown regions. Therefore, choosing between the comprehensive view offered by WGS or the focused power of targeted sequencing depends on the specific research goals and available resources.

Exome Sequencing

Many oncogenic variants are found within exons (protein coding regions), which comprise approximately 1% of the genome.[7] Exome sequencing offers a cost-effective, efficient approach for analyzing T/N cohorts. T/N exome sequencing has been the preferred method for large projects, such as The Cancer Genome Atlas (TCGA), because it offers an attractive combination of efficient processing time and price and because it focuses on easy-to-interpret genomic regions.

Focused Sequencing Panels

Focused cancer panels are predesigned targeted sequencing panels with content selected by leading oncology experts and offer high levels of sensitivity for low-frequency somatic variants. For sequence-specific content, Illumina offers a choice of 2 different chemistries:

- Amplicon-based targeted sequencing involves a preliminary amplification step using predesigned primer mixtures that target specific regions of interest. The amplified targets are then purified and sequenced.
- Target-capture involves a preliminary DNA capture or enrichment step using predesigned capture probes conjugated to magnetic beads.

Illumina focused sequencing panels are compatible with various tissue types including solid or liquid tumor samples, and fresh-frozen or formalin-fixed, paraffin-embedded (FFPE) tissue.

Custom Targeted Sequencing

For researchers or consortia interested in designing their own targeted sequencing panel, probe design software and custom targeted sequencing kits are available for both amplicon-based and target-capture approaches. Both custom capture probes or custom amplicon primers can be designed and ordered through Illumina DesignStudio™ Software. For more on DesignStudio, visit www.illumina.com/designstudio.

Whole-Genome Sequencing Solutions

Through WGS, researchers can compare T/N sample pairs to identify somatic mutations in coding and noncoding regions across the entire genome. As a hypothesis-free approach, WGS is well suited for the discovery of novel driver mutations.

For researchers who prefer a T/N WGS service, the Illumina Genome Network™ (IGN) Cancer Analysis Service performs medium- to large-cohort T/N sequencing studies for researchers looking for a cost-effective solution. To learn more about IGN Cancer Analysis Services, visit www.illumina.com/ign.

Data Analysis Tools for Somatic Variant Detection

Whether performing whole-genome, exome, or targeted sequencing, Illumina offers seamless workflow solutions that include library preparation kits, sequencing platforms, and data analysis software packages (Figure 2).

Figure 2: Illumina Seamless Workflow Solutions—Illumina sequencing solutions are fully integrated, DNA-to-data solutions from library preparation to final data analysis. Prepare T/N paired libraries with optimized kits for whole-genome, exome, transcriptome, or targeted sequencing. Perform sequencing using the MiniSeq™, MiSeq®, NextSeq®, or HiSeq® sequencing systems. Access Illumina data analysis tools for alignment, variant calling, T/N comparative analysis, or expression profiling.

Data analysis, the final step in the workflow, is essential to experimental success and must be tailored to the research question at hand. For example, it is important that the variant calling method properly model the complexities of multiple cancer subclones versus normal sample contamination. Illumina primary data analysis and somatic variant detection software solutions are easily accessible through on-instrument analysis software, such as MiSeq Reporter Software and Local Run Manager, or through BaseSpace® Sequence Hub, the Illumina cloud-based genomic computing environment. Data are streamed from the MiniSeq, MiSeq, NextSeq, or HiSeq Systems directly and seamlessly into BaseSpace Sequence Hub, which offers a suite of apps tailored to various data analysis needs. These tools are packaged into a user interface designed to be accessible to any user, regardless of bioinformatics experience. Simple prompts guide users through the entire process, starting from selecting the files generated by the sequencer to data filtration and analysis.

For somatic variant detection, the BaseSpace Tumor Normal App can be used to report single-nucleotide polymorphisms (SNPs), indels, copy number variants (CNVs), and structural variations found only within the tumor sample. For amplicon-based sequencing panels, MiSeq Reporter with Somatic Variant Caller, or the TruSeq® Amplicon BaseSpace App can be used. Depending on the panel used, Illumina also offers VariantStudio, an annotation and filtering software that allows customers to create customized reports of variant data. For a comprehensive view of Illumina informatics solutions for variant detection, visit www.illumina.com/informatics.html.

Table 1: Illumina DNA and RNA Sequencing Solutions in Cancer

Product	Key Features/Advantages	Genomic Content	DNA/RNA Input	Sequencing Depth	Data Analysis Tools
Targeted Sequencing					
TruSight® Tumor 15	• FFPE-compatible • Minimal DNA input • Detect variants as low as 5% allelic frequency < 1 day library prep	44 kb 250 amplicons 15 genes[a]	20 ng	≥ 500× minimum coverage	• MiSeq Reporter • BaseSpace TruSight Tumor 15 App • VariantStudio
TruSight Myeloid Panel	• Identify somatic mutations in myeloid malignancies • Detect variants as low as 5% allele frequency • 1.5 day library prep	141 kb 568 amplicons 54 genes[b]	50 ng	500× for > 95% of amplicons	• MiSeq Reporter TruSeq Amplicon Workflow • BaseSpace TruSeq Amplicon Core App
TruSeq Amplicon Cancer Panel	• FFPE-compatible • Targets mutational hotspots in frequently mutated cancer genes • < 1 day library prep	> 35 kb 212 amplicons 48 genes	150 ng (250 ng for FFPE)	1000× mean coverage	• MiSeq Reporter TruSeq Amplicon Workflow • BaseSpace TruSeq Amplicon Core App
TruSight Tumor 170	• FFPE-compatible • Low DNA and RNA input • Comprehensive panel detects SNVs, amplifications and fusions contributing to tumorigenesis	533 kb, 166 genes 12,000 probes (DNA) 358 kb, 55 genes 8000 probes (RNA)	40 ng DNA, 40 ng RNA	≥ 250× minimum coverage	• BaseSpace TruSight Tumor 170 App
TruSight RNA Pan-Cancer Panel	• FFPE-compatible • Focused on oncology-specific coding regions • Optimized for degraded samples or limited starting materials	1385 genes	10 ng total RNA (20–100 ng FFPE RNA)	3M reads per sample	• BaseSpace RNA-Seq Alignment App
TruSight RNA Fusion Panel	• FFPE-compatible • Detects known and novel gene fusions • Optimized for degraded samples or limited starting materials	507 genes	10 ng total RNA (20–100 ng FFPE RNA)	3M reads per sample	• Local Run Manager RNA Fusion Module • BaseSpace RNA-Seq Alignment App
Custom Targeted Sequencing					
Nextera® Rapid Capture Custom Kits	• Custom genomic content • 0.5–25 Mb of target sequence • 1.5 day library prep	Custom	10 ng	Custom	• MiSeq Reporter Enrichment Workflow • BaseSpace Enrichment Core Apps
TruSeq Custom Amplicon Low Input	• FFPE-compatible • 16–1536 amplicons • < 1 day library prep	4–650 kb	10 ng total RNA (10-50 ng for FFPE)	Custom	• MiSeq Reporter TruSeq Amplicon Workflow • BaseSpace TruSeq Amplicon Core App
Exome Sequencing					
TruSeq Exome Library Prep Kit	• Includes coding exons • 2.5 day library prep	45 Mb 214,405 exons	100 ng	100× Normal/ 130× Tumor	• BaseSpace Enrichment Apps
TruSeq Rapid Exome Library Prep Kit	• Includes coding exons • 2.5 day library prep	45 Mb 214,405 exons	50 ng	100× Normal/ 130× Tumor	• BaseSpace Enrichment Apps
Nextera Rapid Capture Custom Expanded Exome Kits	• Includes coding exons, untranslated regions, miRNA • 1.5 day library prep	62 Mb 201,121 exons	50 ng	100× Normal/ 130× Tumor	• BaseSpace Enrichment Apps
Whole-Genome Sequencing					
TruSeq DNA PCR-Free Library Prep Kit	• Greatly reduced library bias • High coverage of challenging regions such as high GC/AT regions, promotors, and repetitive regions	Whole-genome	1–2 µg	Minimum 40× Normal/ 60× Tumor	• BaseSpace Core WGS or T/N Apps
TruSeq Nano DNA Library Prep Kit	• Optimized for low-quantity DNA samples • Greatly reduced library bias • Improved coverage uniformity	Whole-genome	100–200 ng	Minimum 40× Normal/ 60× Tumor	• BaseSpace Core WGS or T/N Apps

a. Genes informed by guidelines published by organizations such as the National Comprehensive Cancer Network (NCCN)[8] and European Society for Medical Oncology (ESMO)[9], and late-stage pharmaceutical research.

b. Designed by recognized experts in blood cancers, the panel targets loci with known association to acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myelogenous leukemia (CML), chronic myelomonocytic leukemia (CMML), and juvenile myelomonocytic leukemia (JMML).

III. Evaluating Germline Mutations in Cancer

Genomic technologies can be used to detect germline mutations associated with cancer (Table 2). Array-based studies can be used for the identification of weakly predisposing variants, because they offer a cost-effective method for genotyping large numbers of samples with an experimental design similar to a genome-wide association study (GWAS). Targeted sequencing studies, however, allow researchers to focus on previously identified genes known or suspected to play a role in cancer susceptibility.

Targeted Sequencing to Detect Common Germline Mutations

Targeted sequencing panels enable researchers to rapidly sequence known or suspected cancer-related genes for common germline mutations. As with all sequencing panels, the targeted sequencing approach provides a rapid and cost-effective alternative to single-gene testing, and allows sequencing to higher coverage levels compared to WGS.

Microarray-Based Approaches

Microarrays are a powerful, high-throughput method for researching cancer risk. Current microarray formats support up to 24 samples per BeadChip for genotyping studies with large sample sizes. Illumina high-density BeadChips are designed to interrogate hundreds of thousands of SNPs associated with breast, colorectal, lung, ovarian, and prostate cancers in addition to traits associated with ancestry and pharmacogenetics. Custom content can be added onto existing BeadChips, giving researchers the option to investigate their specific variants of interest.

Table 2: Illumina Sequencing and Microarray-Based Solutions for Evaluating Cancer Predisposition

Product	Key Features/Advantages	Genomic Content	DNA Input	Sequencing Depth	Data Analysis Tools
Targeted Sequencing					
TruSight Cancer Sequencing Panel	• Provides comprehensive coverage of genes associated with cancer predisposition • 1.5 day library prep	255 kb ~4000 probes 94 genes[a, b]	50 ng	20×	MiSeq Reporter Enrichment Workflow or BaseSpace Enrichment Apps
Illumina Microarrays					
Infinium® OncoArray-500K BeadChip	• 120,000 custom markers possible • 24 samples per microarray	~500,000 SNPs from breast, colorectal, lung, ovarian, and prostate cancer[c]	200 ng	NA	GenomeStudio® Genotyping Module
Infinium iSelect® HD Custom Genotyping BeadChips	• Interrogate virtually any SNP from any species • Interrogate SNPs, CNVs, or indels • 24 samples per microarray	3072–1,000,000 attempted bead types	200 ng	NA	GenomeStudio Genotyping Module

a. The content was designed by Professor Nazneen Rahman, a recognized expert in the field of genetic susceptibility, and targets 94 genes associated with inherited cancers, plus 284 SNPs found through GWAS studies.
b. Researchers can also add on content to TruSight Cancer to create a custom panel using the Illumina DesignStudio tool.
c. Developed in collaboration with leading experts from the OncoArray Consortium,[10] an international collaboration of leading experts from the NCI-supported Genetic Association and Mechanisms in Oncology (GAME-ON) consortium, Genome Canada, Genome Quebec, and Cancer Research UK.

IV. Structural Variant Detection in Cancer

Many cancer types carry large structural aberrations, such as CNVs, translocations, and inversions, that provide insight into cancer etiology. It is essential to use techniques that identify these aberrations accurately and efficiently. Illumina offers both array and sequencing-based approaches to identify gross chromosomal changes (Table 3).

DNA and RNA Sequencing for Translocation Detection

Translocations are important driver mutations in many cancer types, and can be detected using whole-genome T/N approaches (Table 1), along with the BaseSpace Tumor Normal App. However, detecting structural variants with targeted DNA sequencing approaches or microarrays can be difficult. Many researchers have turned to RNA-based sequencing approaches, which allow detection of expressed fusion genes as an alternative approach.

Copy Number Variation Arrays

Accurate profiling of chromosomal aberrations, such as amplifications, deletions, rearrangements, and copy-neutral loss of heterozygosity (LOH) events, is crucial for the investigation of cancer genetics. Whole-genome SNP microarrays, with enriched coverage for genes of known cytogenetic relevance, are an ideal tool for the detection of CNVs, copy-neutral LOH, low-level mosaicism, and sample heterogeneity.

Table 3: Illumina Sequencing and Array-Based Solutions for Structural Variant Detection

Products	Key Features/Advantages	Genomic Content	DNA/RNA Input	Data Analysis Tools
RNA Sequencing				
TruSight RNA Pan-Cancer Panel	• FFPE-compatible • Focused on oncology-specific coding regions • Optimized for degraded samples or limited starting material • Ideal for gene-fusion detection	1385 target genes	10 ng total RNA 20–100 ng FFPE RNA	• BaseSpace RNA-Seq Alignment App
TruSight RNA Fusion Panel	• FFPE-compatible • Focused on oncology-specific coding regions • Optimized for degraded samples or limited starting material • Detects known and novel gene fusion partners	507 target genes	10 ng total RNA 20–100 ng FFPE RNA	• Local Run Manager RNA Fusion Module • BaseSpace RNA-Seq Alignment App
TruSight Tumor 170	• FFPE-compatible • Low DNA and RNA input • Comprehensive panel detects SNVs, amplifications and fusions that contribute to tumorigenesis	DNA: 533 kb, 12,000 probes, 166 genes RNA: 358 kb, 8000 probes, 55 genes	40ng DNA, 40 ng RNA	• BaseSpace TruSight Tumor 170 App
TruSeq RNA Access Library Preparation Kit	• FFPE-compatible • Detects known and novel gene fusions • Optimized for degraded samples or limited starting material	21,415 target genes[a]	10 ng total RNA 20–100 ng FFPE RNA	• BaseSpace Sequence Hub Apps for RNA
TruSeq Stranded Total RNA Library Preparation Kit	• FFPE-compatible • Precise detection of strand orientation • Whole-transcriptome analysis	Coding and multiple forms of noncoding RNA (eg, miRNA, snRNA, lincRNA, snoRNA, and more)[b]	0.1–1 µg total RNA	• BaseSpace Core Apps for RNA
Illumina Microarrays				
Infinium CytoSNP-850K BeadChip	• FFPE-compatible • More than 850,000 markers • Higher detection sensitivity for low-level mosaics • 8 samples per microarray	3262 genes[c]	200 ng total DNA	• BlueFuse® Software

a. 98.3% of RefSeq exome regions covered.
b. miRNA = microRNA. snRNA = small nuclear RNA. lincRNA = long noncoding RNA. snoRNA = small nucleolar RNA.
c. Includes cytogenomic-relevant genes for constitutional and cancer studies with content from the International Collaboration for Clinical Genomics (ICCG)[11], the Cancer Cytogenomics Microarray Consortium (CCMC)[12], and the Sanger Institute.[13]

V. Investigating Gene Regulation in Cancer

NGS and microarray technology can be used to monitor changes in the transcriptome and epigenome (Figure 3). Protein–DNA interactions that play a role in cancer-related gene regulation can be assayed at the whole-genome level. Both sequencing and microarray options are available for researchers to detect these regulatory mechanisms (Table 4).

Protein–DNA Interactions

ChIP-Seq provides hypothesis-free information on the regulation of gene expression and gives a complete snapshot of DNA-associated protein activity across the genome. Whole-genome ChIP-Seq offers a simple, cost-effective solution for obtaining visibility into the mechanics of protein-mediated gene regulation. Samples can be multiplexed for high-throughput processing, and deep sequencing enables detection of lower abundance protein–DNA interactions, such as those associated with transcription factor studies.

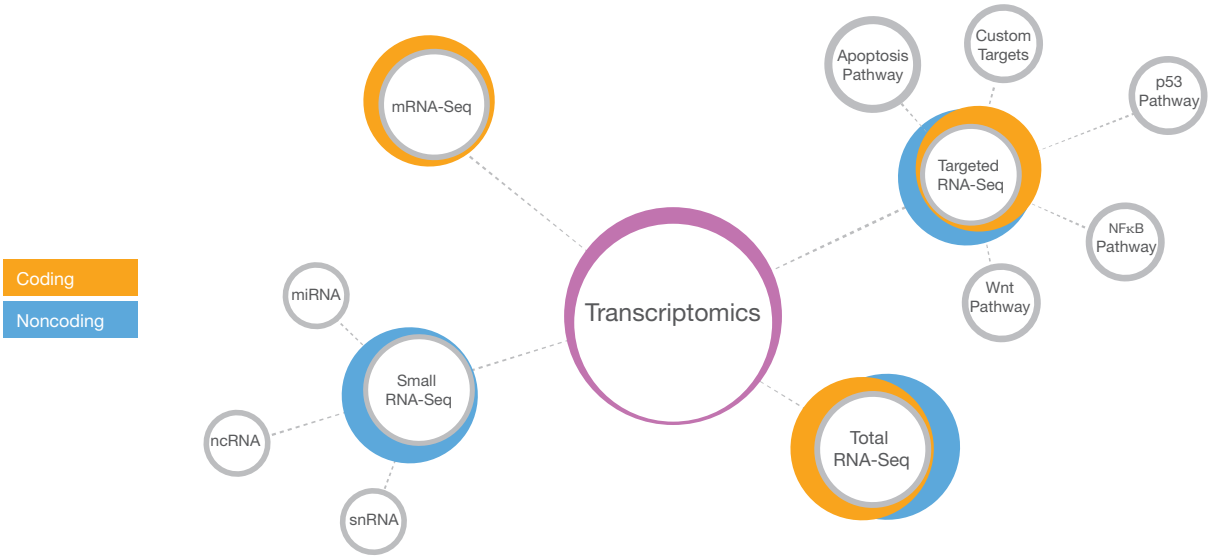

Figure 3: NGS Transcriptomics Application—Transcriptomics includes a spectrum of methods from total RNA-Seq to small RNA-Seq. Illumina provides library preparation kits for both coding and noncoding RNA sequencing applications such as mRNA-Seq, small RNA-Seq, and total RNA-Seq. Illumina also offers targeted RNA sequencing panels for apoptosis, NFkB pathway, p53 pathway, stem cells, the Wnt pathway, and more. Targeted sequencing panels can be custom designed for specific regions of interest.

DNA Methylation

Cancer progression is often caused or influenced by epigenetic changes that alter gene expression. Assessing DNA methylation status can provide insight into the regulatory drivers of gene expression, affecting cancer development and progression. Sequencing-based methylation analysis enables researchers to identify and track methylation profiles across the entire genome, detecting variations in methylation signatures at single-base resolution. With methylation microarrays, researchers can quantitatively interrogate expert-selected methylation sites while balancing throughput capacity and affordability.

RNA Sequencing

RNA-Seq allows researchers to understand the functional effects of DNA mutations, enabling the detection of gene fusions, novel alternative transcripts, and other forms of cancer-associated transcriptome variation. The broad dynamic range of next-generation RNA-Seq allows the reliable detection and quantification of transcripts even at low levels of expression. Such research data can be used to inform more effective treatments and support the development of diagnostics and biomarkers. Illumina RNA library preparation kits are available to support a broad range of RNA-Seq applications, including whole-transcriptome sequencing, strand-specific mRNA sequencing, detection of antisense expression, and detection of coding and noncoding RNA (Figure 3). Illumina also offers kits optimized for customers working with limited amounts of FFPE tissue, or low-quality/low-quantity samples.

Targeted RNA Sequencing

Similar to targeted DNA sequencing for somatic variant detection, analyzing a focused set of RNA sequences allows sequencing to greater depth and supports rapid, cost-effective sample processing. Panels with preselected, expert-defined content are available for several areas of interest. A comprehensive list of 1385 cancer-related genes enables the detection of different types of variants in numerous cancer types. Focusing in further on genes that are implicated in gene fusion events enables deeper coverage for more sensitive fusion detection, and is provided with simplified analysis software. Fixed panels are also available that cover specific cancer-related pathways, such as the p53, NF-kB, or Wnt pathway. User-defined sequencing panels can be designed and ordered through DesignStudio Software to create a fully customized targeted sequencing assay or fixed RNA panels can be semicustomized to address a unique hypothesis.

Small RNA Sequencing

Small RNAs or miRNAs can regulate gene expression through various mechanisms. Genomic regions containing deleted or amplified miRNA loci have been linked to multiple forms of cancer. The relative abundance and size of miRNAs makes them difficult to assay with standard RNA-Seq methods. Small RNA-Seq specifically targets RNAs in the size range of miRNAs, making it a powerful and effective approach for miRNA analysis.

Data Analysis Tools for the Study of Gene Regulation

Illumina BaseSpace TopHat Alignment and Cufflinks Assembly and Differential Expression (Cuffdiff) Apps provide a widely adopted suite of RNA data analysis tools in a simple click-and-go user interface. These apps include the tools required for a range of common transcriptome data analysis needs. TopHat provides high-confidence alignment for abundance measurement, and the detection of splice junctions, gene fusions, and cSNPs. Cuffdiff enables sensitive transcript discovery and differential expression analysis. TopHat Fusion delivers robust, high-confidence detection of gene fusions, while Isaac™ Variant Caller delivers reliable variant calling.

VI. Summary

Recent advances in genomic technologies have led to an accelerated pace of discovery in cancer research. NGS methods for genome, exome, epigenome, and transcriptome analysis have charted multiple research paths and are leading to a deeper understanding of cancer etiology and progression. The scale of data delivered by the HiSeq, NextSeq, MiSeq, and MiniSeq platforms supports a range of cancer research goals, including identification of recurrent somatic mutations, discovery of novel cancer-driving genes, and the analysis of epigenetic alterations. For certain types of cancer, such as breast and gastric cancer, these advances in genomic technology have already brought cancer diagnosis and treatment into the realm of personalized medicine.[14–16] Since the introduction of the first BeadChip in 2003, and the first sequencing system in 2007, Illumina has accelerated the pace of research through continuous innovation. Through partnerships with leading cancer experts and collaboration with national and international cancer organizations,[1,2,9-13] Illumina continues to expand the portfolio of cancer focused NGS and microarray solutions.

Table 4: Illumina Solutions for Investigation of Gene-Regulation in Cancer

Product	Key Features/Advantages	Genomic Content	DNA or RNA Input	Data Analysis Tools
RNA Sequencing				
TruSeq Stranded Total RNA Library Preparation Kit	• FFPE-compatible • Precise detection of strand orientation • Whole-transcriptome analysis	Coding and multiple forms of noncoding RNA (eg, miRNA, snRNA, lincRNA, snoRNA, and more)	0.1–1 µg total RNA	• BaseSpace Core Apps for RNA and RNA Express
TruSeq Stranded mRNA Library Preparation Kit	• FFPE-compatible • Precise detection of strand orientation • Captures mRNA via the polyA tail	coding RNA/mRNA	0.1–4 µg total RNA	• BaseSpace Core Apps for RNA and RNA Express
TruSeq RNA Access Library Preparation Kit	• FFPE-compatible • Focused on RNA coding regions • Optimized for degraded samples or limited starting material	21,415 target genes[a]	10 ng fresh/frozen RNA 20–100 ng FFPE RNA	• BaseSpace Core Apps for RNA and RNA Express
Targeted RNA Sequencing				
TruSight RNA Pan-Cancer Panel	• FFPE-compatible • Focused on oncology-specific coding regions • Optimized for degraded samples or limited starting material	1385 target genes	10 ng fresh/frozen RNA 20–100 ng FFPE RNA	• BaseSpace RNA-Seq Alignment App
TruSight RNA Fusion Panel	• FFPE-compatible • Detects known and novel gene fusions • Optimized for degraded samples or limited starting material	507 target genes	10 ng fresh/frozen RNA 20–100 ng FFPE RNA	• Local Run Manager RNA Fusion module • BaseSpace RNA-Seq Alignment App
TruSeq Targeted RNA Expression Kits-Custom	• FFPE-compatible • User-defined content	12–1000 targets per custom panel	50 ng intact total RNA ≥ 200 ng degraded RNA	• MiSeq Reporter Targeted RNA Workflow
TruSeq Targeted RNA Expression Kits - Fixed Panels	• FFPE-compatible • Preselected content for focused studies • Expandable with custom content	Preselected genes for apoptosis, cardiotoxicity, and more[b] 12–1000 target capacity for add-on content	50 ng	• MiSeq Reporter Targeted RNA Workflow
Small RNA Sequencing				
TruSeq Small RNA Library Preparation Kit	• Enables discovery and profiling of miRNAs at high coverage and sensitivity • Investigate small RNA between 17–35 nucleotides	small RNAs/miRNAs	1 µg total RNA 10–50 ng small RNA	• Local Run Manager MiSeq Reporter Small RNA Workflow
ChIP Sequencing				
TruSeq ChIP Library Preparation Kit	• Determine distribution and abundance of DNA bound proteins	Whole-genome	5–10 ng ChIP-derived DNA	• Third-party software[c]
TruSeq DNA Methylation Library Preparation Kit (formerly EpiGenome MethylSeq Kit)	• Detect CpG, CHH, and CHG regions[d] • Protocol includes bisulfite conversion and Illumina library preparation	Whole-genome	50–100 ng DNA	• Third-party software[c,e]
Methylation Array				
HumanMethylation450 BeadChip	• > 485,000 methylation sites • 12 samples per BeadChip	99% of RefSeq genes 96% of CpG islands[f]	500 ng DNA	• GenomeStudio Methylation Module

a. 98.3% of RefSeq exome regions covered.
b. Fixed panels available for apoptosis, cardiotoxicity, cell cycle, hedgehog pathway, neurodegeneration, NFkB pathway, p450 pathway, p53 pathway, stem cells, and the Wnt pathway.
c. There are several third-party software solutions available for ChIP-Seq data analysis, including Model-based Analysis for ChIP-Seq (MACS), an open-source solution available through Galaxy, and Avadis NGS.
d. CpG = cytosine–guanine dinucleotide. CHH and CHG are trinucledotides in which C is cytosine and H is adenine, cytosine, or thymine.
e. Coming soon, Bismark in BaseSpace.
f. The Infinium HumanMethylation BeadChip has an average of 17 CpG sites per gene region distributed across the promoter, 5′ UTR, first exon, gene body, and 3′ UTR. Contains additional coverage in island shores and the regions flanking them, CpG sites outside of CpG islands, non-CpG methylated sites identified in human stem cells, differentially methylated sites identified in T/N (multiple forms of cancer) and across several tissue types, CpG islands outside of coding regions, and miRNA promoter regions.

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