

# RNA-SEQ SERVICES COMPARISON GUIDE



## PLATFORM COMPARISON

| Parameter                        | Illumina NovaSeq X Plus<br>(Short-Read) | PacBio Revio<br>(Long-Read · Full-Length) | Admera Health Notes                                          |
|----------------------------------|-----------------------------------------|-------------------------------------------|--------------------------------------------------------------|
| Technical Specifications         |                                         |                                           |                                                              |
| Read Length                      | 150 bp (paired-end)                     | Full-length transcripts (1–10+ kb)        | Long reads span full mRNA from 5' cap to poly(A) tail        |
| Read Accuracy                    | >99.9% per base                         | >99.9% (CCS / HiFi)                       | Both achieve high accuracy via different methods             |
| Target Insert Size               | ~200 bp (library ~400–430 bp)           | 1–2 kb bins; up to 10 kb bins             | Size selection optimized per short-reads experiment          |
| Transcript Assembly Required     | Yes — computational assembly            | No — reads span full transcript           | Iso-Seq eliminates assembly ambiguity                        |
| Input Requirements & RNA Quality |                                         |                                           |                                                              |
| Min. RIN — Poly-A Selection      | RIN ≥ 7                                 | RIN ≥ 7                                   | Higher integrity needed for full-length recovery             |
| rRNA Depletion (degraded RNA)    | RIN ≥ 5 supported                       | Not recommended                           | Short-read handles low-RIN and FFPE samples                  |
| FFPE Samples                     | Supported                               | Not recommended                           | rRNA depletion protocol required for FFPE                    |
| Low-Input / Low Cell Count       | 200–500 cells (SMART kits)              | Higher input needed                       | Ultra-low input available for Illumina only                  |
| Library Preparation Options      |                                         |                                           |                                                              |
| Bulk mRNA (Poly-A Selection)     | Yes                                     | Yes                                       | Highest % protein-coding reads                               |
| Total RNA / rRNA Depletion       | Yes                                     | Less common                               | Required for lncRNA; DNase treatment recommended             |
| Small RNA / miRNA                | Yes                                     | No                                        | Far below effective size range for Iso-Seq                   |
| Circular RNA (circRNA-seq)       | Yes (2 µg min input)                    | Emerging                                  | CIRCexplorer2/3 pipeline available for Illumina              |
| High-Throughput (DRUG/BRB-seq)   | Yes                                     | No                                        | Drug discovery & perturbation screening                      |
| Strand Specificity               | Yes                                     | Yes                                       | NEBNext / TruSeq (Illumina); native directionality (Iso-Seq) |



## PLATFORM COMPARISON continued

| Parameter                        | Illumina NovaSeq X Plus<br>(Short-Read · 2×150bp) | PacBio Iso-Seq / Revio<br>(Long-Read · Full-Length) | Admera Health Notes                                                 |
|----------------------------------|---------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------|
| Sequencing Depth & Scale         |                                                   |                                                     |                                                                     |
| Poly-A Read Depth                | 40M paired-end reads / sample                     | 50-80M segmented reads                              | Iso-Seq depth affects isoform completeness, not just quantification |
| rRNA Depletion Read Depth        | 80M paired-end reads / sample                     | N/A                                                 | Higher depth required without poly-A enrichment                     |
| Small RNA Read Depth             | 10M paired-end reads / sample                     | N/A                                                 | Illumina only for miRNA / small RNA applications                    |
| Sample Throughput                | High — many samples per run                       | Lower per-run capacity                              | Multiplexing available for both platforms                           |
| Relative Cost / Sample           | Lower                                             | Higher                                              | Hybrid strategy often most cost-effective                           |
| Analytical Capabilities          |                                                   |                                                     |                                                                     |
| Gene Expression Quantification   | Primary strength                                  | With short-read integration                         | Depth drives quantification accuracy                                |
| Differential Expression Analysis | Excellent                                         | Optional downstream                                 | MA-plot, PCA, heatmaps, volcano plots                               |
| Novel Isoform Discovery          | Inferred / computationally assembled              | Direct & unambiguous                                | Single read spans entire transcript structure                       |
| Alternative Splicing Analysis    | Computational inference                           | AS events, intron retention, exon-skipping          | Alt. start/end sites reliably captured by Iso-Seq                   |
| Gene Fusion Detection            | Assembly-dependent                                | Single-read confidence                              | Definitive tumor fusion detection via Iso-Seq                       |
| Allele-Specific Isoforms         | Limited                                           | Full phasing across transcript                      | Variants phased across full-length transcripts                      |
| ORF / Functional Prediction      | GO / KEGG enrichment                              | ORF prediction + GSEA                               | Functional impact assessment of novel isoforms                      |
| Metatranscriptomics              | Yes (de novo)                                     | No                                                  | Host-microbe interactions; Trinity assembly pipeline                |

## WHEN TO CHOOSE EACH PLATFORM

## Short-Read Sequencing

- Gene expression quantification across samples
- Differential gene analysis
- Degraded RNA, FFPE, or low-input samples
- Small RNA, miRNA, or circRNA sequencing
- High-throughput drug screening (DRUG/BRB-seq)
- Metatranscriptomics
- High sample throughput at lower cost

## Long-Read Sequencing

- Full-length isoform characterization
- Alternative splicing analysis
- Gene fusion detection in tumor samples
- Allele-specific isoform identification
- ORF prediction & novel isoform functional impact
- Poorly annotated or non-model organisms
- Reference isoform catalog generation

