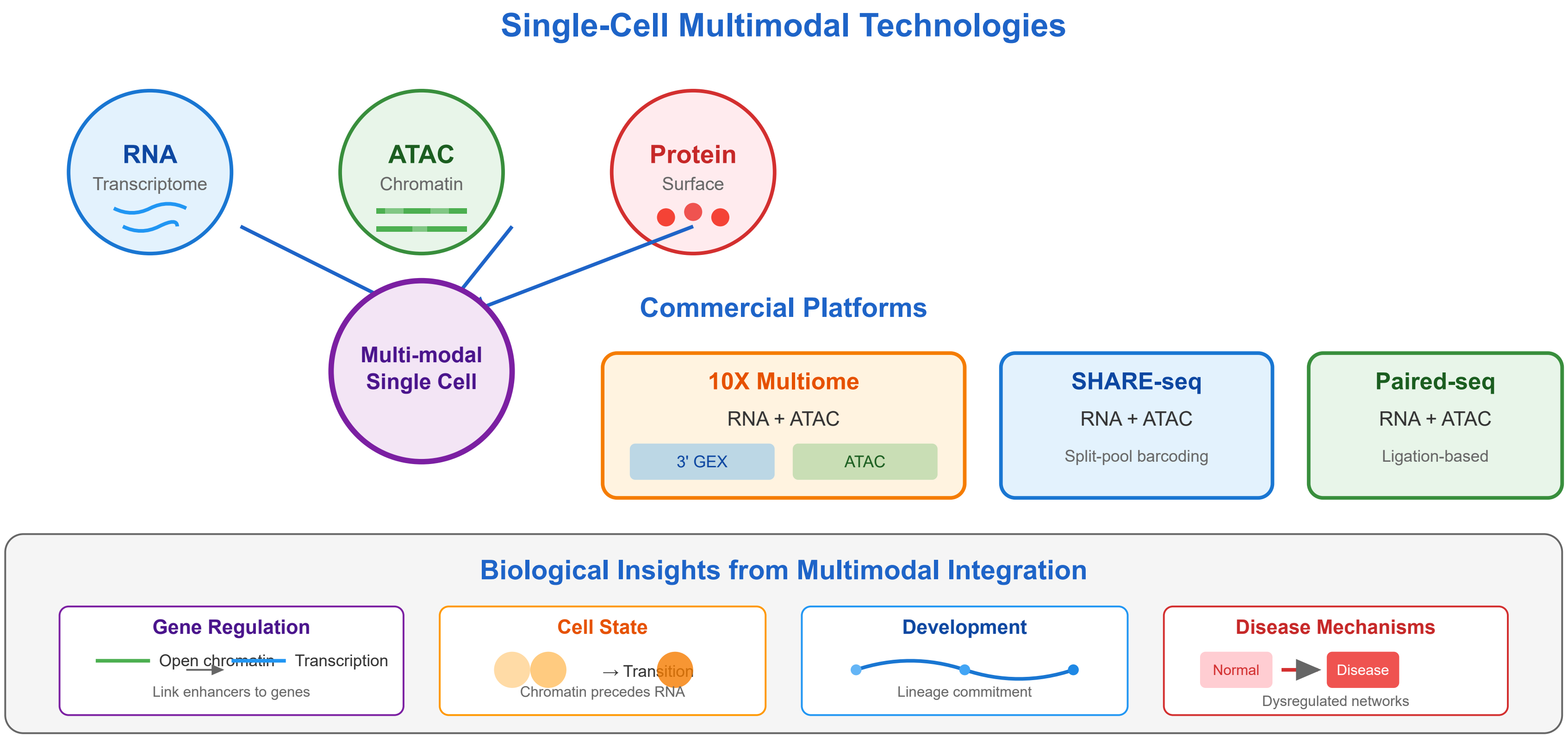
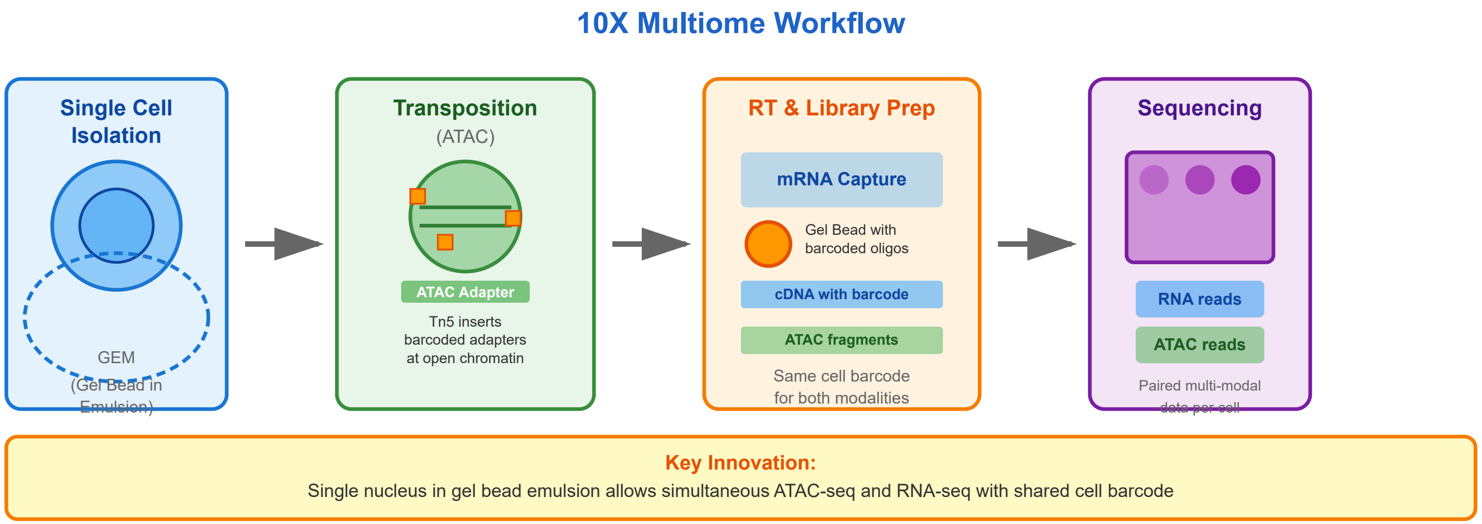
Multimodal Omics



💡 Multi-omics reveals regulatory mechanisms

Methodological Principles & Examples

1. 10X Genomics Multiome ATAC + Gene Expression



The 10X Genomics Multiome platform enables simultaneous profiling of gene expression and chromatin accessibility from the same single cell. The workflow begins with single-cell or single-nucleus isolation into gel bead-in-emulsion (GEM) droplets containing barcoded oligonucleotides. Within each droplet, Tn5 transposase performs ATAC-seq by inserting sequencing adapters at accessible chromatin regions, while poly(A)-tailed mRNAs are captured by barcoded oligonucleotides on the gel bead. Both modalities share the same cellular barcode, enabling direct linkage of chromatin accessibility and gene expression profiles.

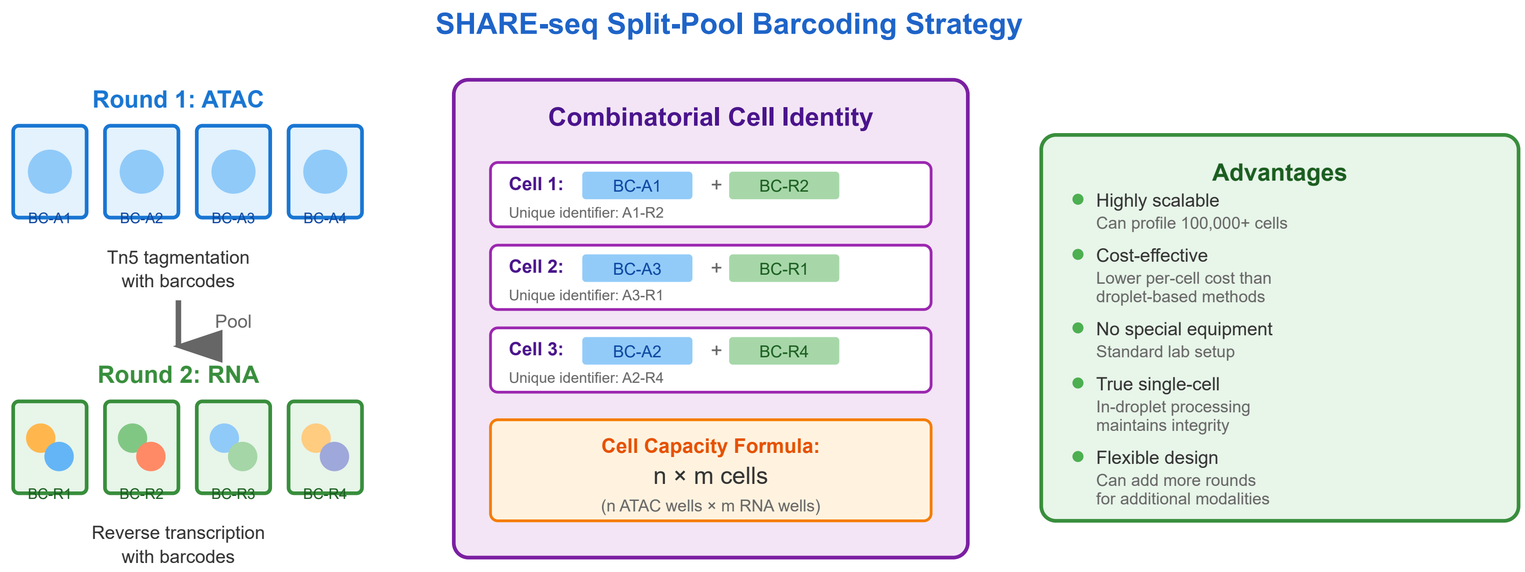
Key Features:

- **High throughput:** Process thousands of cells in parallel using microfluidic technology
- **Linked modalities:** Same cell barcode for RNA and ATAC data ensures accurate pairing
- **Single-nucleus compatible:** Works with frozen samples and difficult-to-dissociate tissues
- **Automated workflow:** Standardized protocol with commercial reagents reduces technical variability
- **Data integration:** Built-in bioinformatics tools for joint analysis of chromatin and transcription

🎯 **Applications:**

Ideal for studying gene regulatory mechanisms, cell differentiation trajectories, and linking genetic variants to gene expression through integrated chromatin accessibility and transcriptome analysis.

2. SHARE-seq (Shared Chromatin and RNA seq)



SHARE-seq employs a split-pool combinatorial indexing strategy to jointly profile chromatin accessibility and gene expression. Cells are first distributed across wells for ATAC-seq with well-specific barcodes, then pooled and redistributed for RNA-seq with a second round of barcoding. Each cell receives a unique combination of ATAC and RNA barcodes, enabling bimodal data pairing without physical cell isolation. This approach dramatically increases throughput while reducing per-cell costs.

Technical Details:

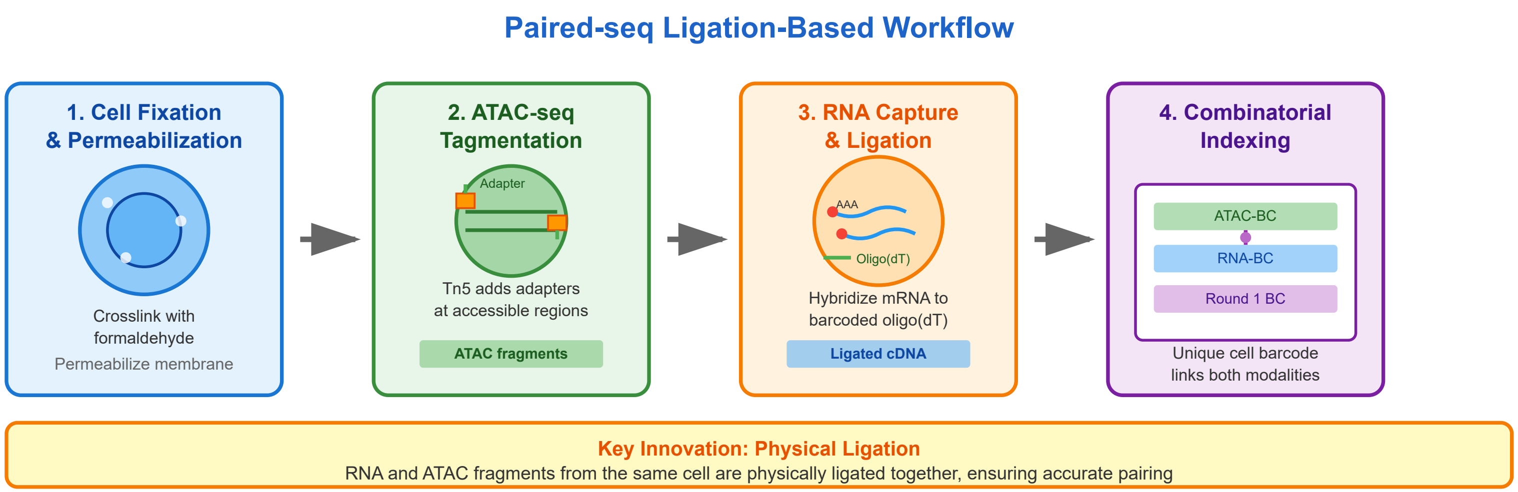
- **Split-pool strategy:** Multiple rounds of barcoding create unique cell identifiers through combinatorial indexing

- **Ultra-high throughput:** Can process >100,000 cells in a single experiment
- **In-droplet processing:** Maintains single-cell resolution without microfluidic devices
- **Scalable barcoding:** n wells in round 1 × m wells in round 2 = n×m unique cell barcodes
- **Adaptable protocol:** Can be extended to include additional modalities with extra barcoding rounds

🎯 **Best Use Cases:**

Large-scale population studies, atlas generation, rare cell type discovery, and experiments requiring cost-effective profiling of tens of thousands of cells.

3. Paired-seq (Paired-end sequencing)



Paired-seq uses a ligation-based approach to physically link RNA and ATAC-seq libraries from the same cell. After fixation and permeabilization, Tn5 transposase tags accessible chromatin regions. Subsequently, poly(A) mRNAs are captured by barcoded oligo(dT) primers. A key innovation is the physical ligation of ATAC and RNA fragments, followed by combinatorial indexing rounds. This physical linkage ensures robust pairing of both modalities from individual cells.

Distinguishing Features:

- **Ligation-based pairing:** Physical linkage of ATAC and RNA fragments from the same cell ensures high confidence in data pairing
- **Fixed cells:** Formaldehyde crosslinking preserves cellular structure during processing
- **Nucleus-friendly:** Works well with both single cells and isolated nuclei
- **Sequential processing:** ATAC and RNA steps performed on the same cell population
- **High data quality:** Ligation step reduces barcode collisions and improves data reliability

> 🎯 **Optimal Applications:**
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> Studies requiring high-confidence multimodal pairing, archived tissue samples (compatible with fixed samples), and experiments where data quality is prioritized over throughput.

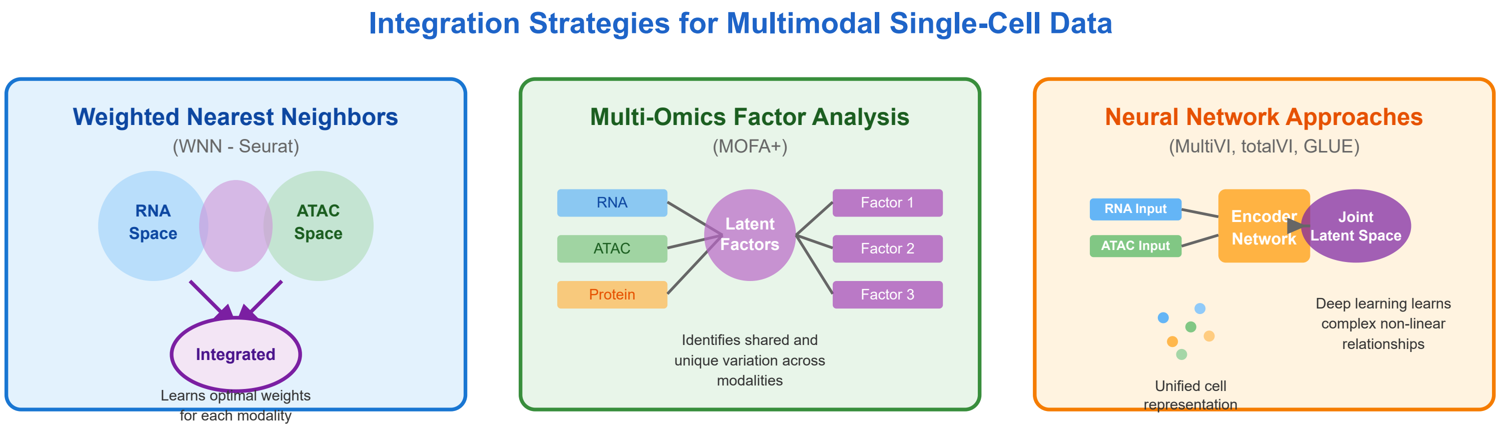
Platform Comparison

Feature	10X Multiome	SHARE-seq	Paired-seq
Barcoding Strategy	Droplet-based (GEM)	Split-pool combinatorial	Ligation + combinatorial
Throughput	1,000-10,000 cells	100,000+ cells	10,000-50,000 cells
Equipment Required	Chromium Controller	Standard lab equipment	Standard lab equipment
Cost per Cell	$$$ (Higher)	$ (Lower)	$$ (Medium)
Sample Type	Fresh/frozen nuclei	Fresh cells/nuclei	Fixed or fresh cells

Feature	10X Multiome	SHARE-seq	Paired-seq
Protocol Complexity	Low (automated)	Medium (manual steps)	High (multi-step)
Data Quality	High consistency	Good with higher noise	High with physical linkage
Cell Barcode Collisions	Very low	Low (combinatorial)	Very low (ligation)
Scalability	Limited by device	Highly scalable	Moderately scalable
Best Application	Routine multimodal analysis	Large-scale atlas projects	High-confidence pairing

Computational Data Integration

Multimodal Analysis Approaches



Integration of multimodal single-cell data requires sophisticated computational approaches that can handle the distinct characteristics of each data type. Modern methods range from weighted neighbor graphs to deep learning architectures, each with strengths for different biological questions.

Common Integration Challenges:

- **Scale differences:** RNA and ATAC data have vastly different dynamic ranges and sparsity levels
- **Feature spaces:** Different modalities measure fundamentally different molecular features
- **Missing data:** Dropout events and technical noise vary across modalities
- **Batch effects:** Technical variation can confound biological signals
- **Interpretation:** Balancing complexity with biological interpretability

Research Applications & Biological Insights

Real-World Applications

🧬 Development & Differentiation

Track chromatin remodeling during cell fate decisions. Multimodal data reveals how enhancer accessibility precedes gene activation during embryonic development and organogenesis.

🏥 Disease Mechanisms

Identify dysregulated gene regulatory networks in cancer, autoimmune diseases, and neurological disorders by linking genetic variants to altered chromatin states and gene expression.

🧪 Drug Response

Understand heterogeneous drug responses by profiling epigenetic and transcriptional changes across cell populations, identifying resistance mechanisms and therapeutic targets.

🔬 Cell Atlas Projects

Generate comprehensive reference maps of tissues and organs with integrated chromatin and transcriptome profiles, enabling discovery of rare cell types and transitional states.

🌟 Future Directions:

Emerging technologies aim to add more modalities (methylation, protein, spatial information) to create truly comprehensive single-cell profiles. Integration with spatial transcriptomics and live-cell imaging will provide temporal and spatial context to regulatory mechanisms.