

Expanded View Figures

Figure EV1. Vesalius methods visualized.

- A Vesalius converts the transcriptome of cells by using a normalized latent space (either UMAP or PCA) and using embedding values as RGB color values.
- B After image segmentation, Vesalius further isolates territories by pooling barcodes that are close to each other in 2D space. Vesalius finds all barcodes that are within a capture distance of each other (represented by red circle) and assigns a unique territory to all these barcodes. The same process is applied to all beads of that color segment until all beads have been pooled into a distinct spatial territory.
- C Simulation regimes used to benchmark Vesalius in high-resolution ST data. The pure regime only contains one cell type per territory. The uniform regime contains n different cell types in equal proportion in each territory. The exponential regime contains n cell types in varying proportions between territories. The cell types are the same between territories. The dotted regime is made of a background territory with five circular territories of random size. The number of cell types between each territory is also randomized. Circular territories may overlap.
- D Territory layering uses images representation of territory to iteratively select the edge of a territory and assign a layer value to that edge. Once all barcodes have been assigned to a layer, the number of layers can be reduced by merging neighboring layers.

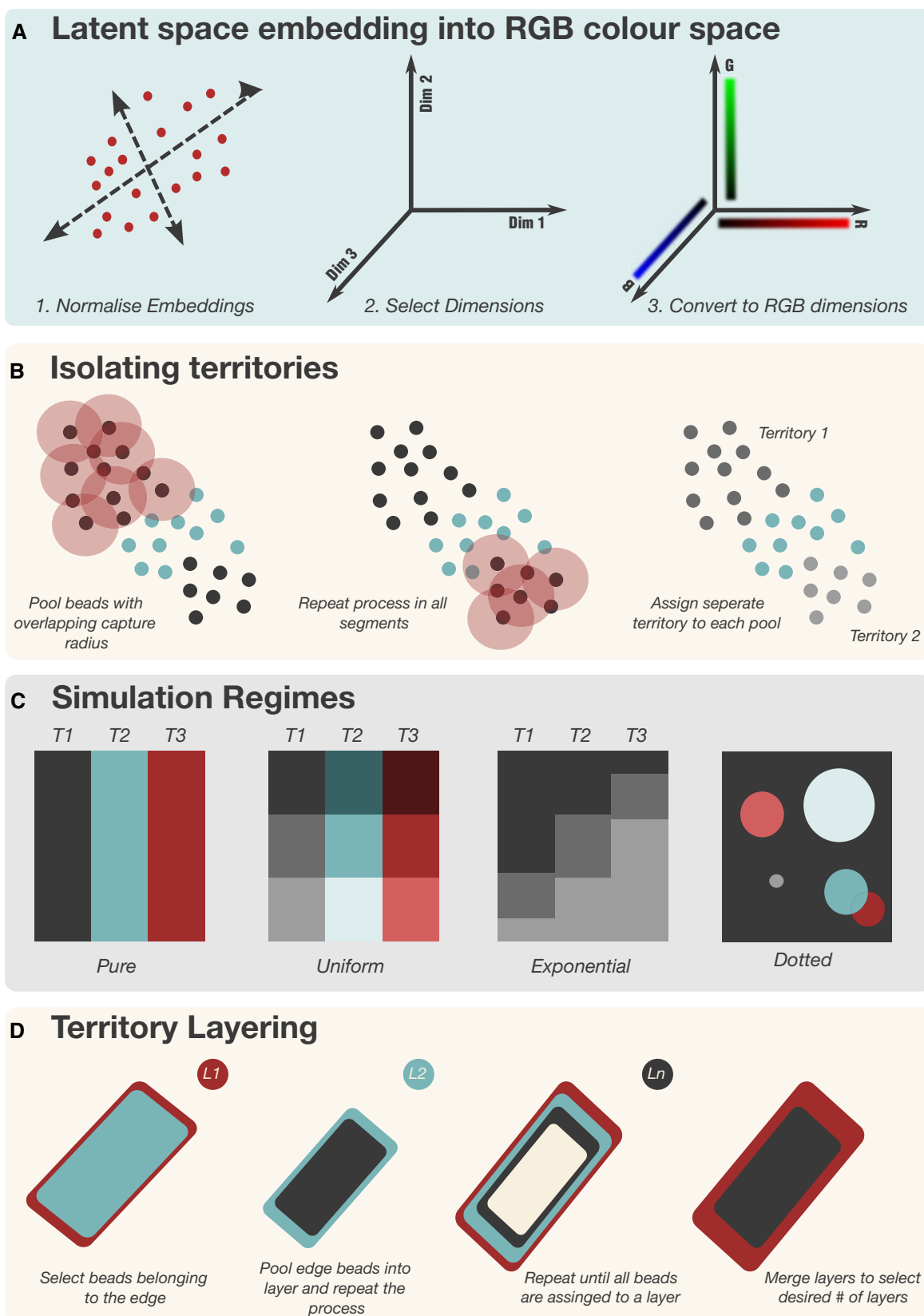


Figure EV1.



Visualizing BayesSpace clusters separately emphasizes that many of the spatial domains do not represent a specific anatomical structure. In this context, selecting a specific location in the tissue is impossible without either missing barcodes or by including unwanted barcodes. This also demonstrates why cluster sub-clustering does not equate to territory isolation and clustering.

Figure EV3. Seurat clusters in mouse hippocampus.

Visualizing Seurat clusters separately emphasizes that many of the clusters do not represent a specific anatomical structure. In this context, selecting a specific location in the tissue is impossible without either missing barcodes or by including unwanted barcodes. This also demonstrates why cluster sub-clustering does not equate to territory isolation and clustering. Seurat is not designed to recover spatial domains but rather cluster transcriptional similarity.

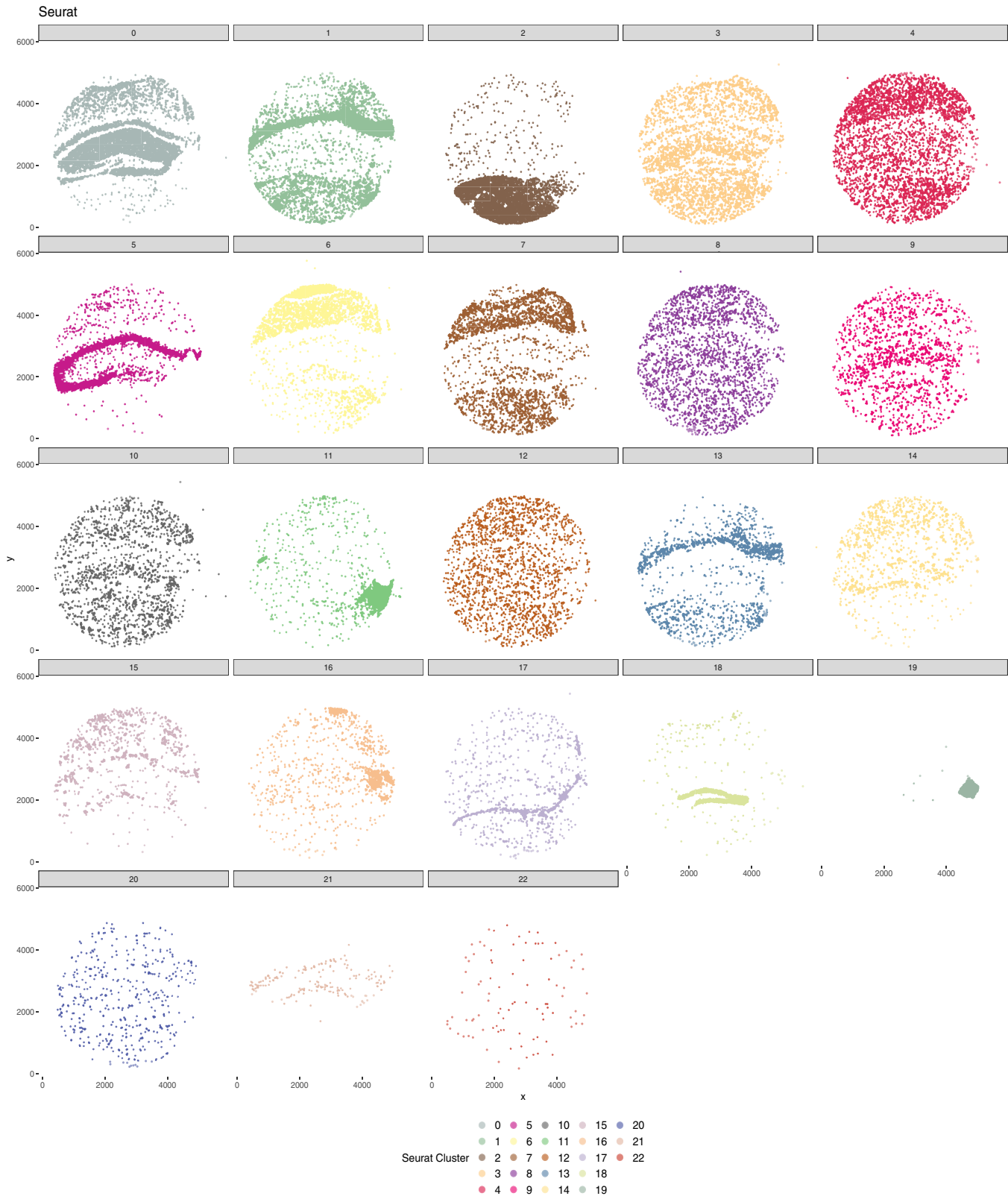


Figure EV3.

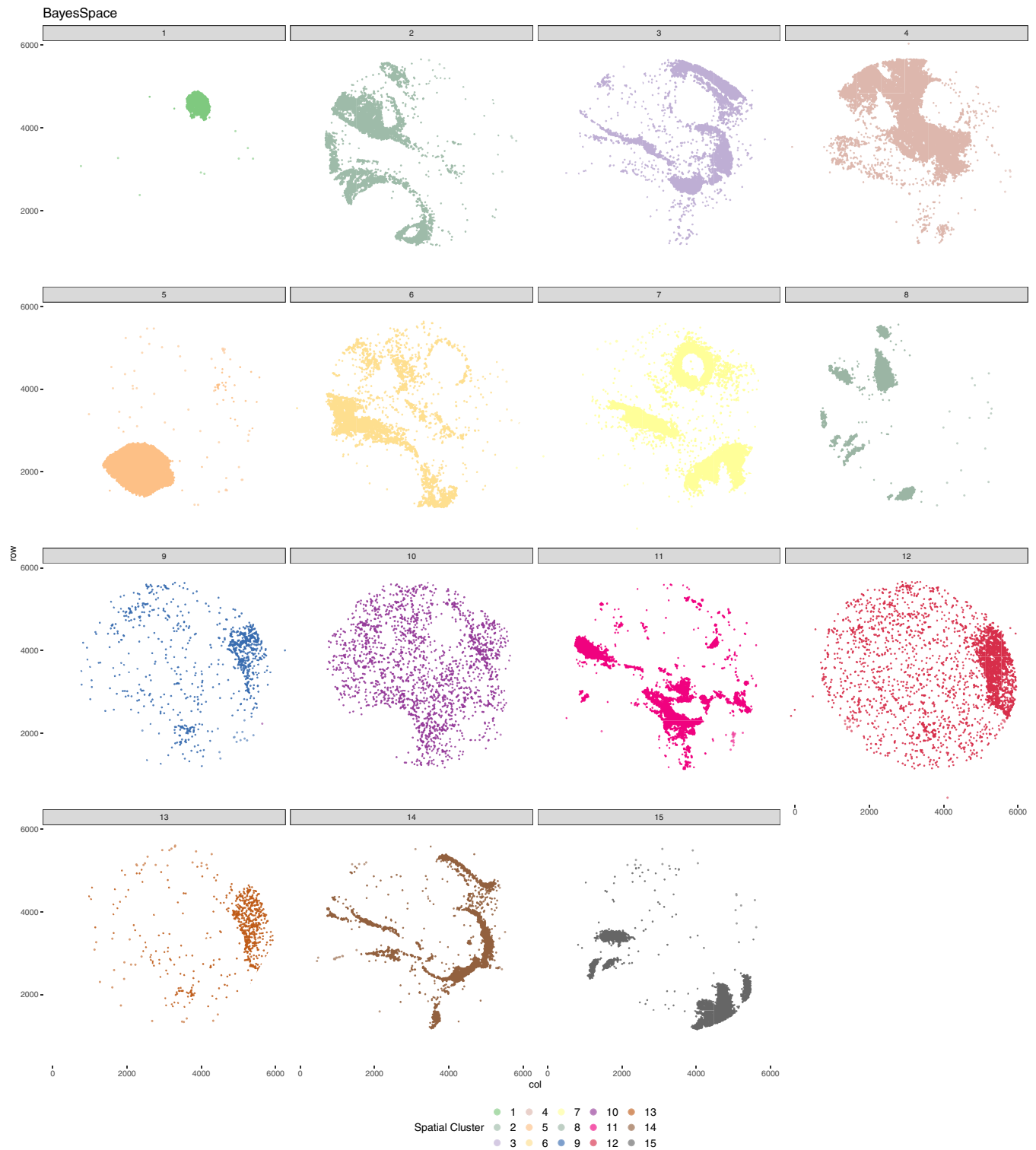


Figure EV4. BayesSpace spatial cluster in mouse embryo.

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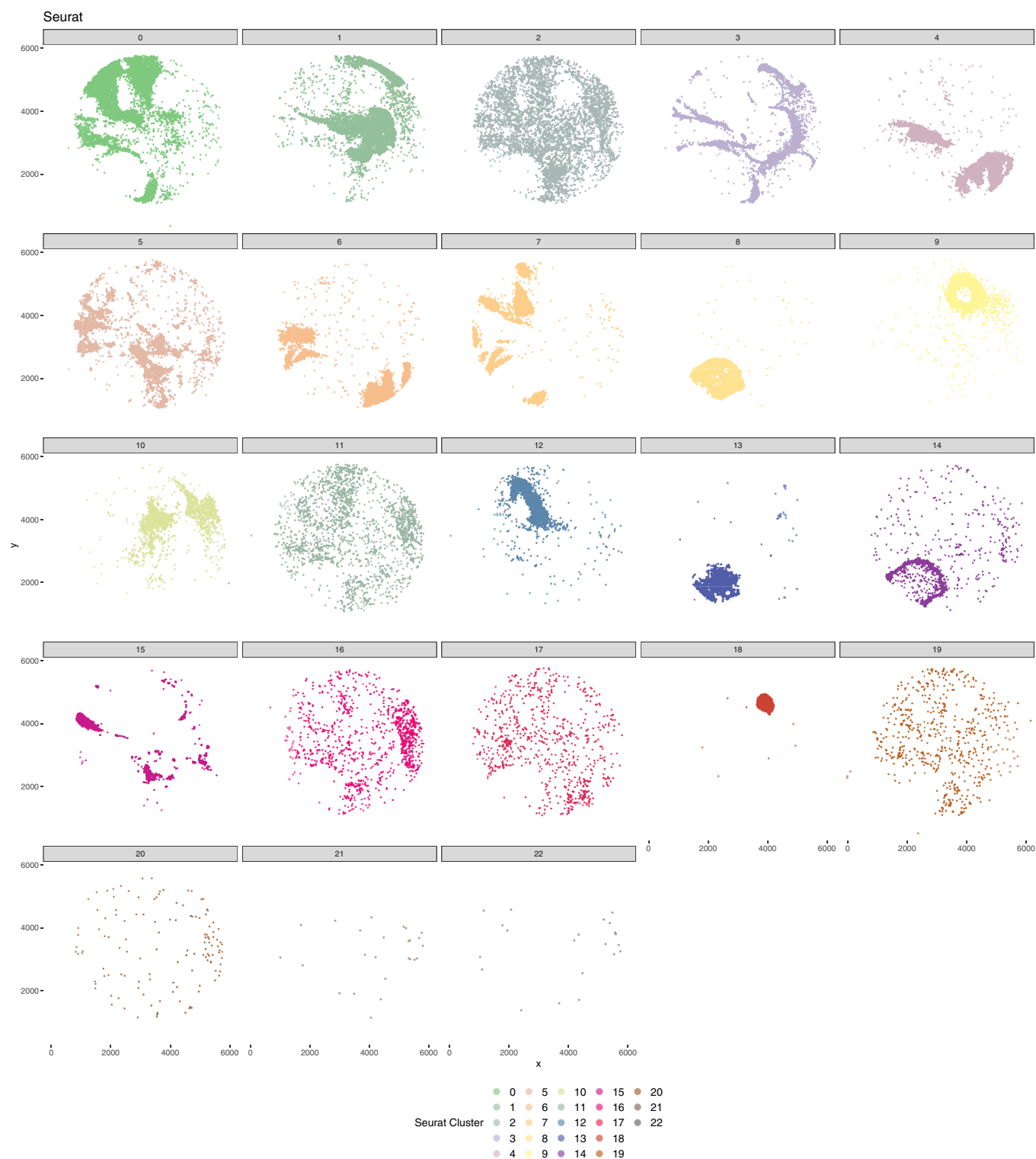


Figure EV5. Seurat clusters in mouse embryo.

Visualizing Seurat clusters separately emphasizes that many of the clusters do not represent a specific anatomical structure. In this context, selecting a specific location in the tissue is impossible without either missing barcodes or by including unwanted barcodes. This also demonstrates why cluster sub-clustering does not equate to territory isolation and clustering. Seurat is not designed to recover spatial domains but rather cluster transcriptional similarity.