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COMPUTATIONAL BIOMEDICINE
HARVARD MEDICAL SCHOOL

scDiagnostics: Diagnostic Tools to Assess the Cell Type Assignment Quality in Single-Cell RNA-Seq

scDiagnostics Package Authors

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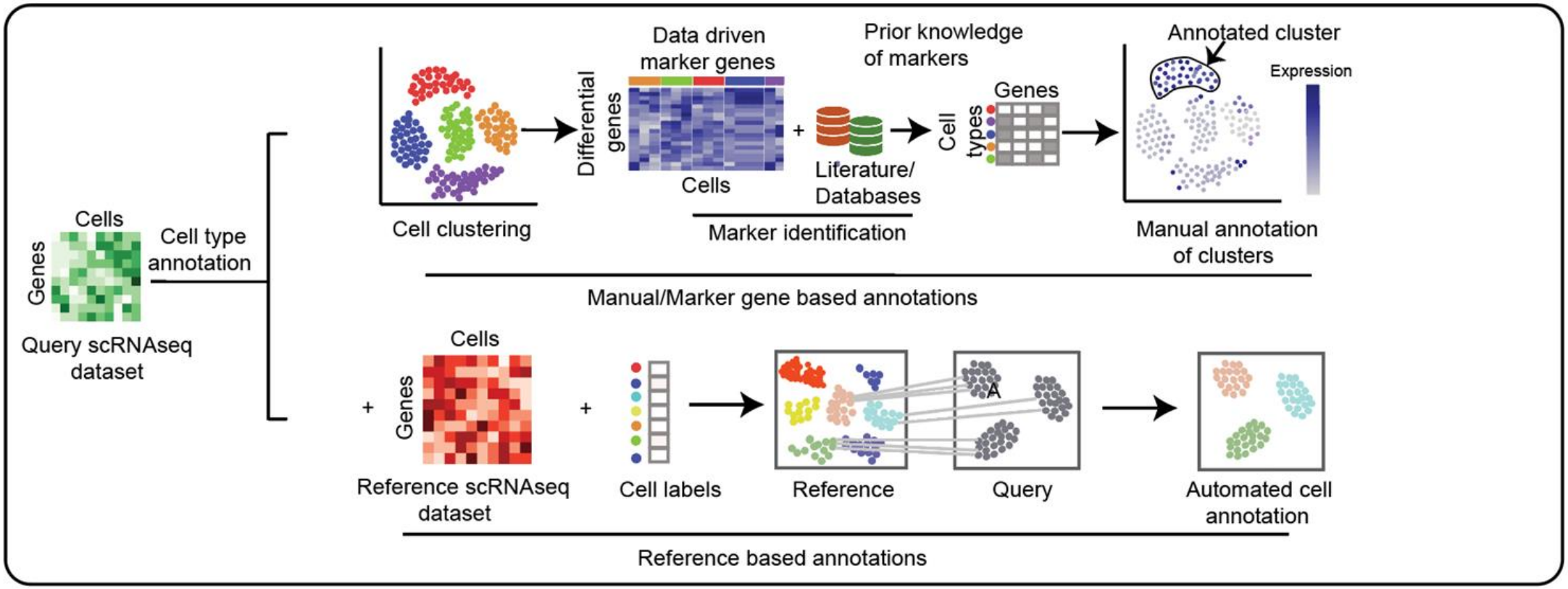
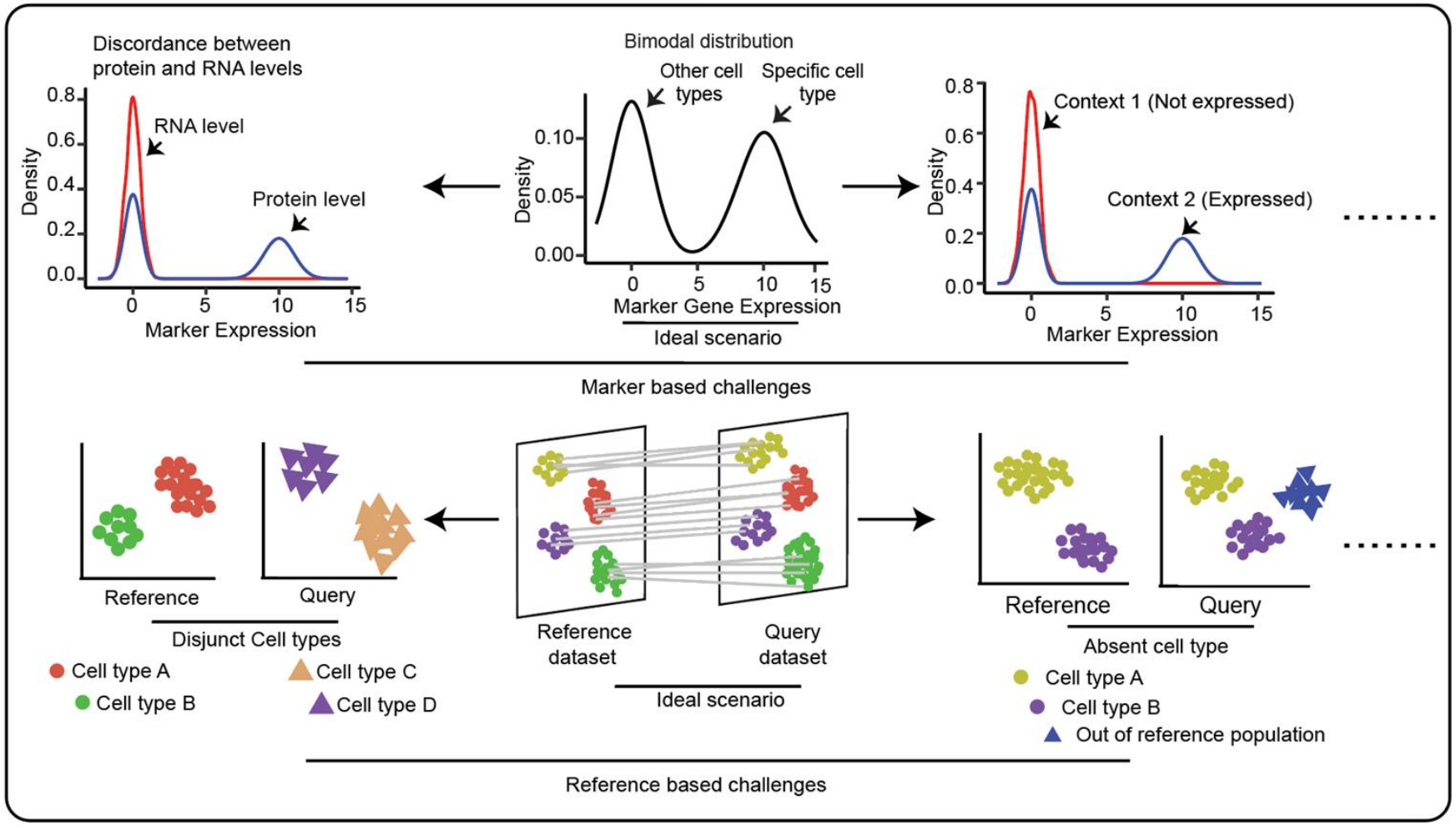
Background and Motivation

Two Main Approaches to Cell Type Annotation



Background and Motivation

Statistical Challenges in Cell Type Annotation



Previous Work in Annotation Diagnostics

Annotation Diagnostics in SingleR

- **SingleR Annotation Diagnostics:**
  - Chapter 4 of SingleR book
- **Methodological Limitations:**
  - **Uncertain Accuracy:** The diagnostics methods may not always accurately reflect true cell types, leading to potential misassignments.
  - **Limited Scope:** The available methods often fail to address the full complexity of biological variability and technical artifacts.
- **Visualization Limitations:**
  - **Inadequate Tools:** SingleR lacks comprehensive visualization options to effectively interpret annotation results.
  - **Poor Clarity:** Current visualization methods do not provide clear insights into annotation quality or highlight potential issues effectively.

Context and Data Overview

The Reference and Query Datasets

- **Reference Data:**
  - **Description:** The reference dataset should be a well-curated, expertly annotated collection of single-cell RNA-seq data.
  - **Expert Annotations:** Cells in this dataset have been accurately identified and labeled by domain experts using known marker genes and experimental validation (sometimes…).
  - **High-Quality Data:** This dataset serves should serve as ground truth for cell annotation transfer.
  - **Usage:** Used to train models, identify cell types, and serve as a benchmark data for new annotation methods.
  - **Example References Datasets in Bioconductor package celldex:**
    - **Human Cell Atlas (HCA):** This dataset contains scRNA-seq data from over 100,000 cells from 15 human tissues, including blood, bone marrow, brain, kidney, liver, lung, pancreas, and more.
    - **Mouse Cell Atlas (MCA):** Similar to HCA, this dataset contains scRNA-seq data from over 100,000 cells from 14 mouse tissues.
    - **Human Peripheral Blood Mononuclear Cells (PBMCs):** This dataset contains scRNA-seq data from over 20,000 PBMCs from healthy donors, covering various immune cell types.
    - And many more…

Context and Data Overview

The Reference and Query Datasets

- **Query Data:**
  - **Description:** The query dataset consists of new single-cell RNA-seq data that needs to be analyzed.
  - **Annotations:** The query cells have been annotation by some method (e.g. annotation transfer), but their accuracy as not been assessed.
  - **Analysis Goals:**
    - **Alignment Check:** Ensure that the new data aligns well with the reference data.
    - **Annotation Validation:** Assess to which extent the query cells have been well annotated.
    - **Anomalous Cell Detection:** Identify any potentially anomalous cells that may indicate issues with data quality or annotation.

Introducing scDiagnostics

Enhancing the Standard scRNA-seq Cell Annotation Pipeline

- **Core Functionality:**
  - **Data Alignment Checking:** Ensures that the query dataset is well aligned with the reference dataset, verifying the consistency of gene expression patterns.
  - **Annotation Validation:** Confirms the accuracy of cell type annotations in the query dataset, using statistical and computational methods.
  - **Anomalous Cell Detection:** Identifies potentially anomalous cells, ensuring robust and accurate results.
- **Impact:**
  - Enhances the reliability and accuracy of scRNA-seq data analysis.
  - Provides confidence in the results obtained from downstream analysis.

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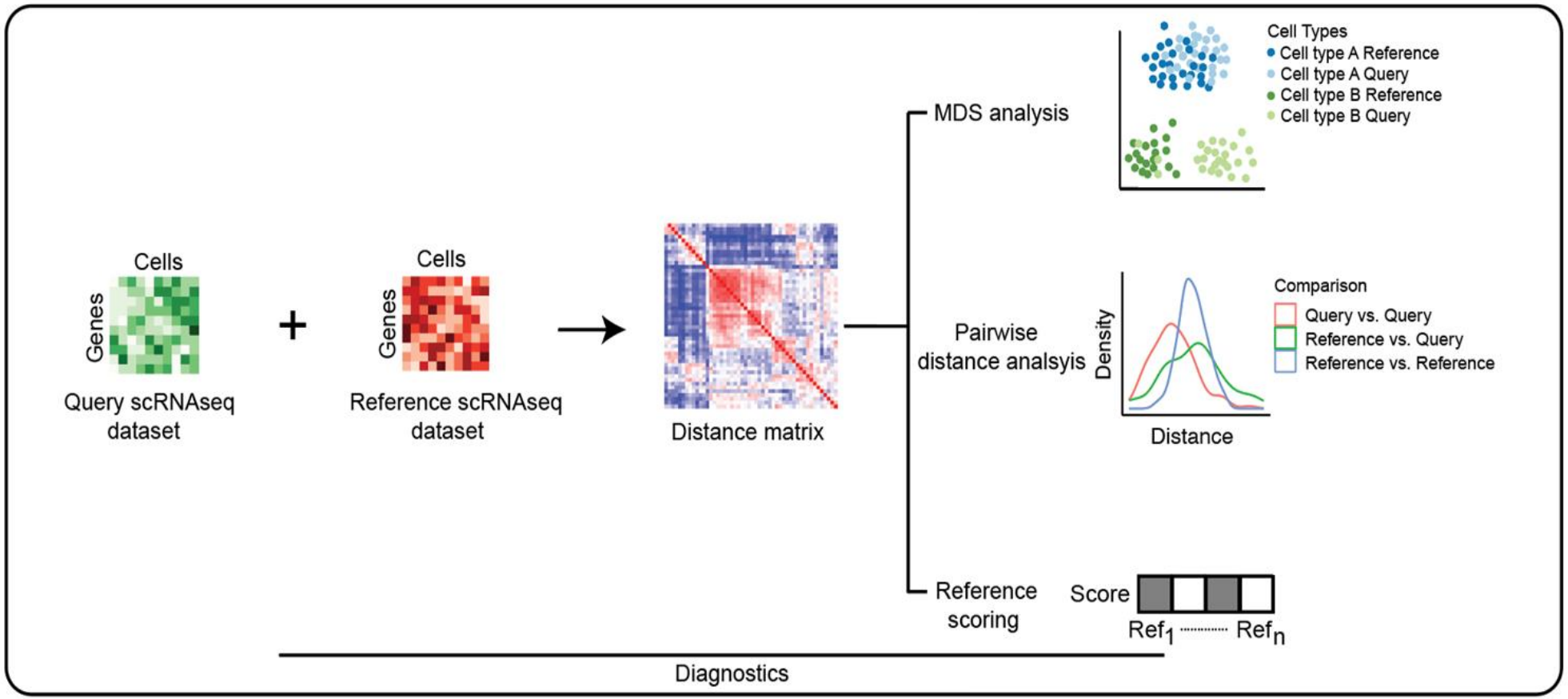


Example Application

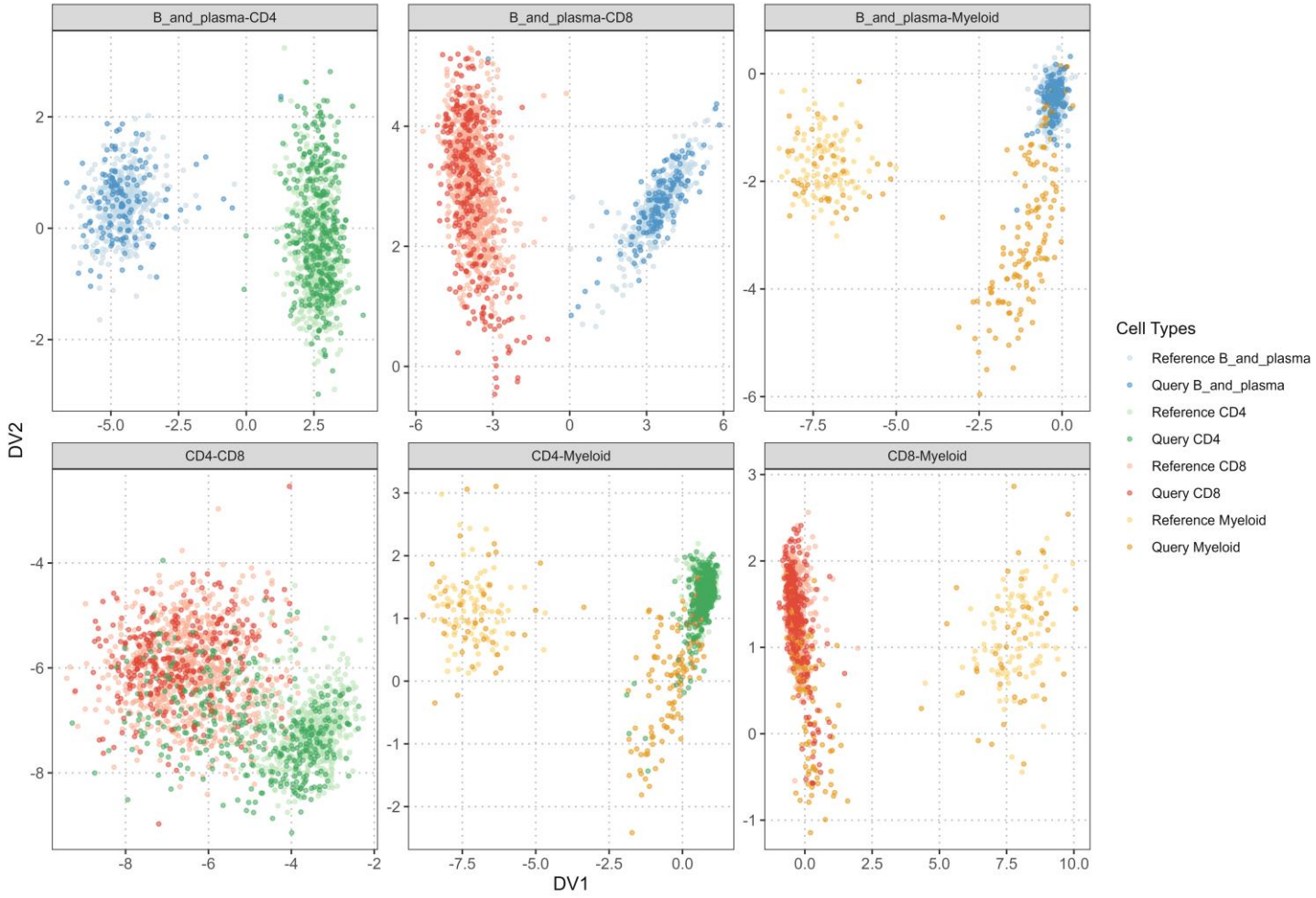
SingleR Annotation Diagnostics

- HeOrganAtlasData(tissue = c("Marrow")) Data in scRNAseq.
- Curation/processing of data (QC, normalization, etc.).
- Take 70% of data as “reference” and remaining 30% as “query” data.
- Annotation transfer from reference to query via SingleR.

	SingleR Annotation			
Expert Annotation	B and Plasma	CD4	CD8	Myeloid
B and Plasma	139	0	0	10
CD4	0	190	1	13
CD8	0	134	196	29
Myeloid	0	0	0	40

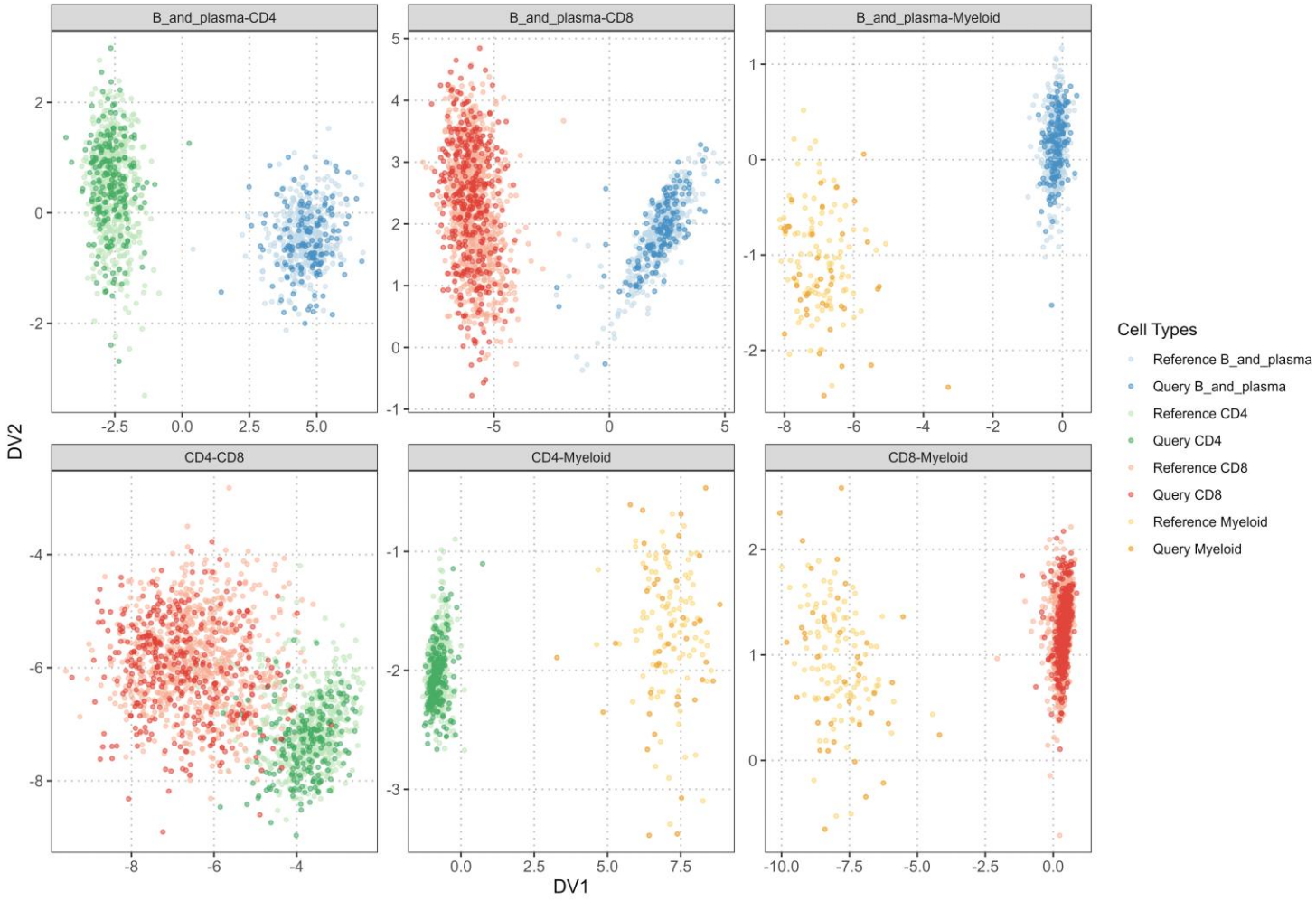
Visualization of SingleR Annotation

“Poor” Annotation Diagnostics



Visualization of Expert Annotation

“Good” Annotation Diagnostics



Workshop Materials

Material Information and Links

- **Package (development) GitHub repository**
- **Package website**
- **Workshop Materials:**
  - Repository
  - Slides
  - Vignette
  - Docker image
  - Galaxy workshop
- **Package will soon be available on Bioconductor.**

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Live Workshop Session