

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

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Background and Motivation

Understanding the Importance of Accurate scRNA-seq Analysis

- **Introduction to scRNA-seq:**
 - Single-cell RNA sequencing (scRNA-seq) enables the study of gene expression at the individual cell level.
 - It provides high-resolution insights into cellular diversity, states, and functions.
- **Importance of Data Quality:**
 - Proper alignment and accurate cell annotation are critical for meaningful biological interpretations.
 - Errors in these steps can lead to incorrect conclusions and wasted resources.
- **Common Challenges:**
 - **Batch effects:** Variability introduced during sample processing and sequencing.
 - **Misannotation:** Incorrect identification of cell types due to overlapping gene expression profiles or manual errors.

Integration into Current Workflow

Enhancing the Standard scRNA-seq Analysis Pipeline

- **Current Workflow Steps:**
 - **Quality Control:** Identifies and removes low-quality cells and potential technical artifacts.
 - **Normalization:** Adjusts for differences in sequencing depth and other technical variations.
 - **Feature Selection:** Selects the most informative genes (features) for downstream analysis.
 - **Dimensionality Reduction:** Transforms into lower-dimensional space (e.g., PCA, t-SNE, UMAP).
 - **Clustering:** Groups cells into clusters to identify distinct cell populations.
 - **Marker Gene Detection:** Identifies genes that are uniquely or highly expressed in specific clusters.
 - **Cell Type Annotation:** Assigns labels to cell clusters based on known cell type signatures.
 - **(New Step) Annotation Diagnostics:** Ensure cell type annotation is accurate.

Context and Data Overview

Understanding the Reference and Query Datasets

- **Reference Data:**
 - **Description:** The reference dataset is 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 rigorous validation techniques.
 - **High-Quality Data:** This dataset serves as a gold standard for comparison due to its high quality and reliability.
 - **Usage:** Used to train models, identify cell types, and serve as a benchmark for new data.

Load library

```
library(scDiagnostics)
```

Load reference data (processed HeOrganAtlasData(tissue = c("Marrow"))) dataset)

```
data("reference_data")
```

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Understanding the Reference and Query Datasets

- **Query Data:**
 - **Description:** The query dataset consists of new single-cell RNA-seq data that needs to be analyzed.
 - **Unknown Annotations:** Initial annotations are provided, but their accuracy has not been confirmed.
 - **Analysis Goals:**
 - **Alignment Check:** Ensure that the new data aligns well with the reference data.
 - **Annotation Validation:** Verify that the cell type annotations in the query data are accurate.
 - **Anomalous Cell Detection:** Identify any potentially anomalous cells that may indicate issues with data quality or annotation.

```
# Load query data
```

```
data("query_data")
```

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Understanding the Reference and Query Datasets

```
# Compare expert and SingleR annotation
```

```
table(Expert_Annotation = query_data$expert_annotation,  
      SingleR = query_data$SingleR_annotation)
```

SingleR

Expert_Annotation	B_and_plasma	CD4	CD8	Myeloid
B_and_plasma	136	0	0	10
CD4	0	190	1	13
CD8	0	134	196	29
Myeloid	0	0	0	40

Integration into Current Workflow

Enhancing the Standard scRNA-seq Analysis Pipeline

- **New Step: Introducing scDiagnostics**
- **Fits into the workflow after cell type annotation.**
 - **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.

Context and Data Overview

Understanding the Reference and Query Datasets

Visualize cell types in (binary) discriminant spaces

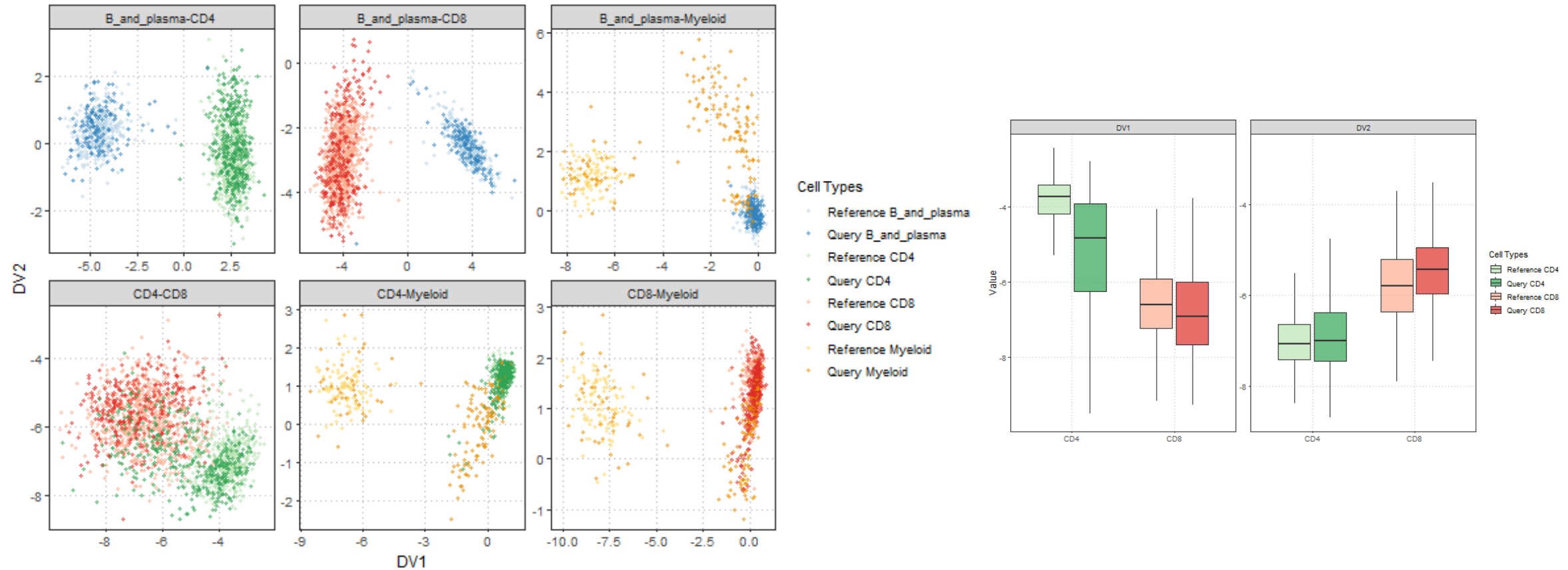
```
disc_output <- calculateDiscriminantSpace(reference_data = reference_data,  
                                          query_data = query_data,  
                                          ref_cell_type_col = "expert_annotation",  
                                          query_cell_type_col = " SingleR_annotation ")
```

Visualize output via scatterplot

```
plot(disc_output, plot_type = "scatterplot")  
plot(disc_output, cell_types = "CD4-CD8", plot_type = "boxplot")
```


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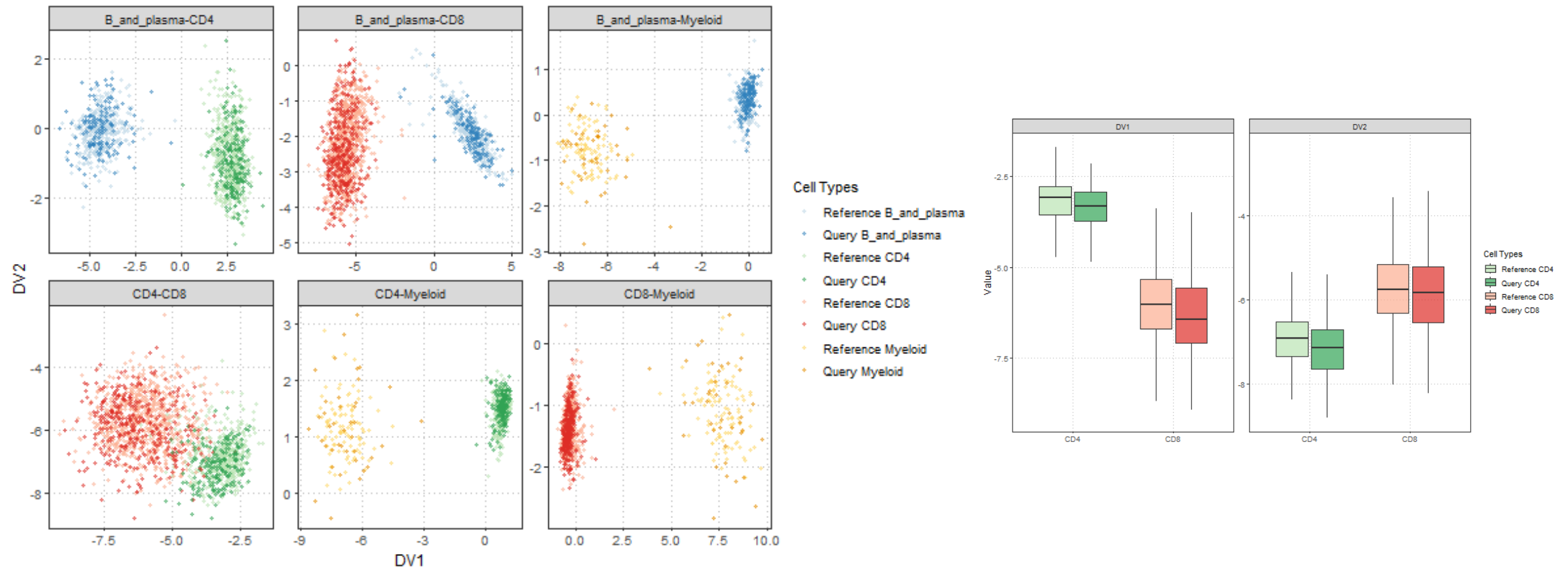
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Key Features of scDiagnostics

Enhancing Data Quality and Annotation Accuracy

- **Core Functionalities:**
 - **Alignment Checking:** Assess how well the query dataset matches the reference dataset in terms of gene expression patterns.
 - **Annotation Validation:** Use statistical and computational methods to confirm the accuracy of cell type labels.
 - **Anomalous Cell Detection:** Identify potentially anomalous cells at the global level (e.g., outliers) and cell-specific level (e.g., misclassified cells).
 - **Quality Control Measures:** Additional checks to ensure data integrity and reliability.
- **Innovative Aspects:**
 - Unique algorithms for alignment assessment.
 - Advanced validation techniques that go beyond simple cross-checks.
- **Relevance:**
 - Helps researchers maintain high standards in data analysis.
 - Facilitates accurate biological discoveries and insights.

Workshop Materials

Material Information and Links

- **Link to development repository:** <https://github.com/ccb-hms/scDiagnostics>
- **Link to pkgdown development website:** <https://ccb-hms.github.io/scDiagnostics/index.html>
- **Workshop Materials:** <https://github.com/AnthonyChristidis/scDiagnosticsBioc2024Demo>
 - Slides available [here](#).
 - Vignette available [here](#).
- **Galaxy workshop (with Docker container):** <https://workshop.bioconductor.org/>
- **Package will soon be available on [Bioconductor](#).**