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")
```

Context and Data Overview

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")
```

Context and Data Overview

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

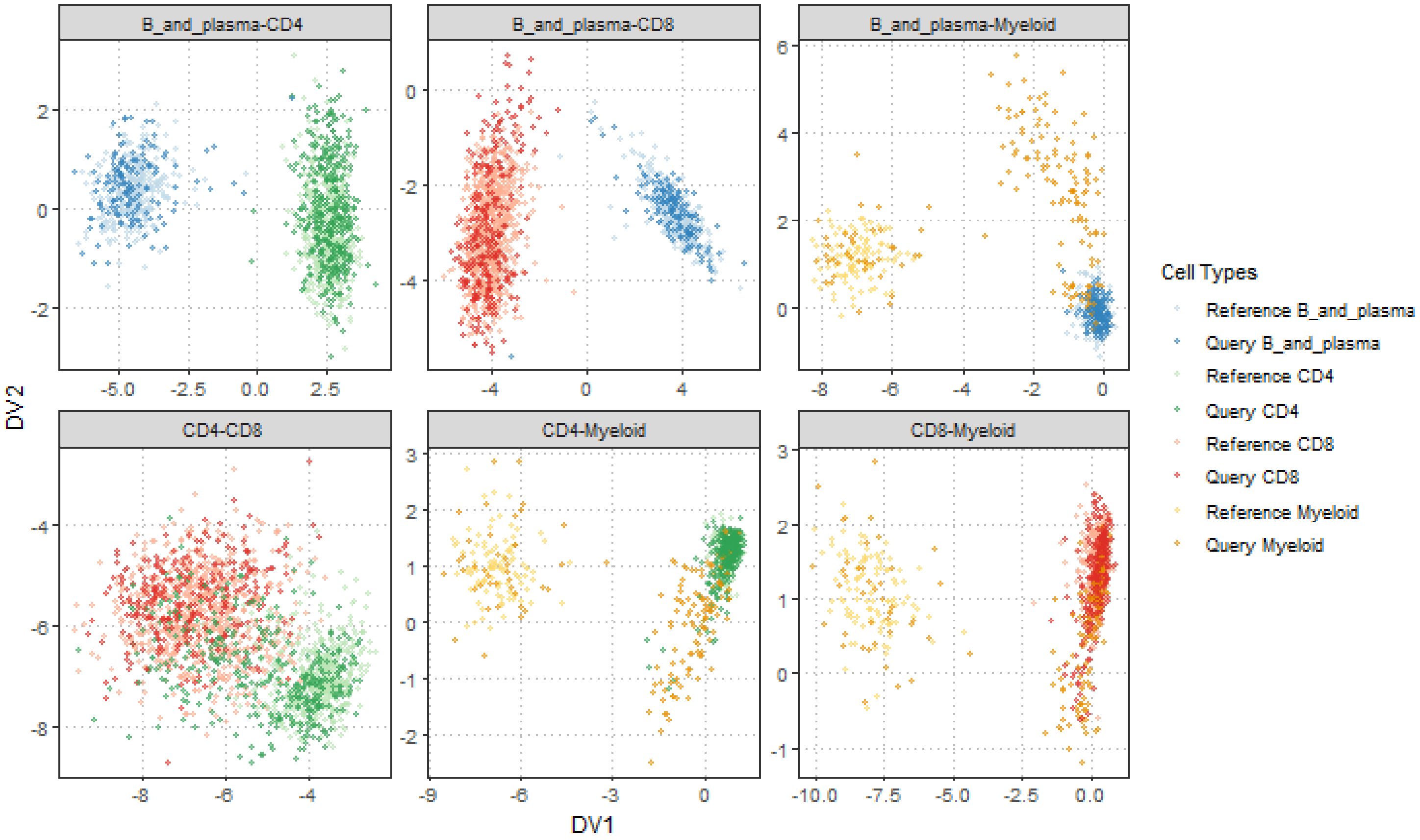
Understanding the Reference and Query Datasets

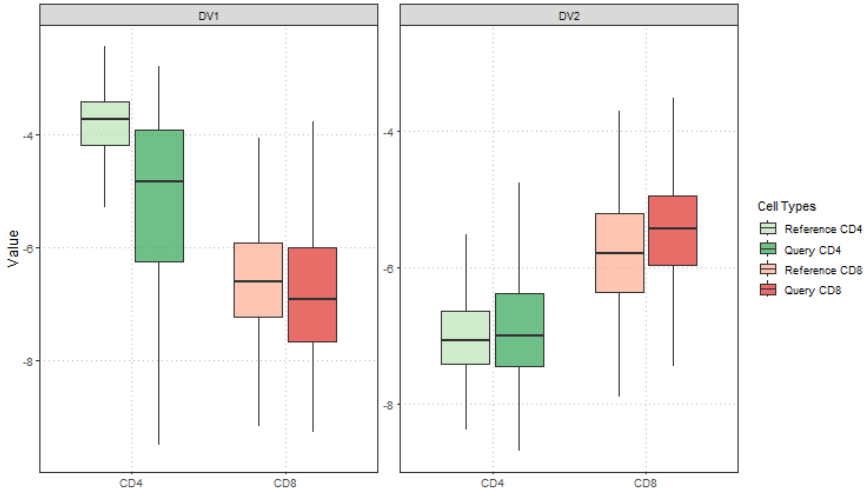
```
# Visualize cell types in (binary) discriminant spaces
disc_output <- calculateDiscriminantSpace(reference_data = reference_data,
                                          query_data = query_data,
                                          ref_cell_type_col = "expert_annotation“,
                                          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")
```

Context and Data Overview

Understanding the Reference and Query Datasets





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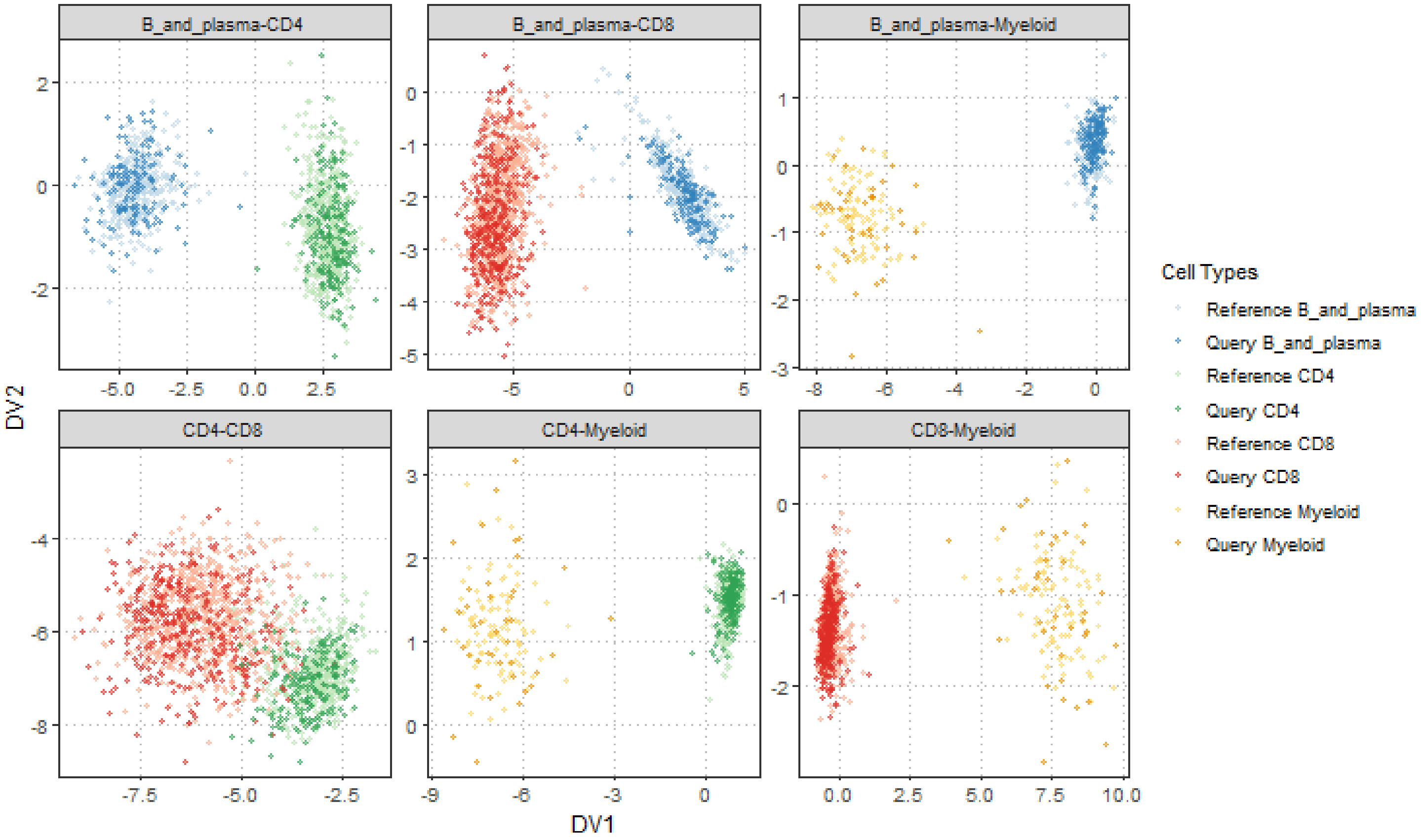

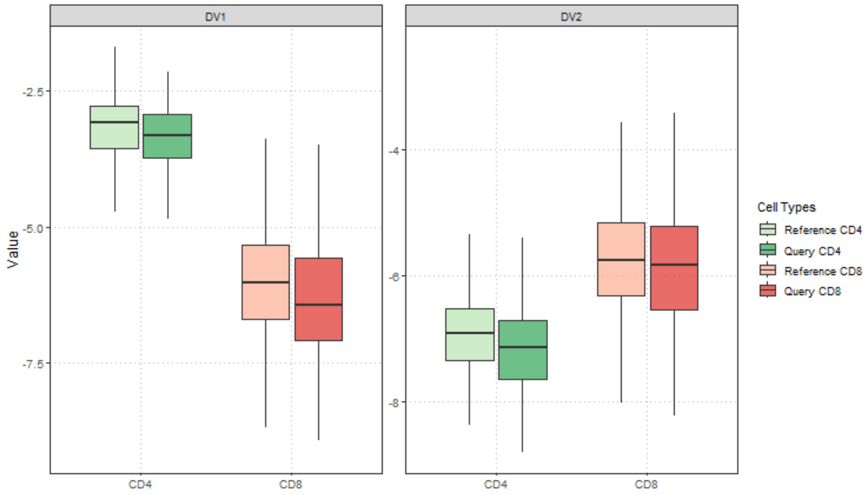

Key Features of scDiagnostics

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.**