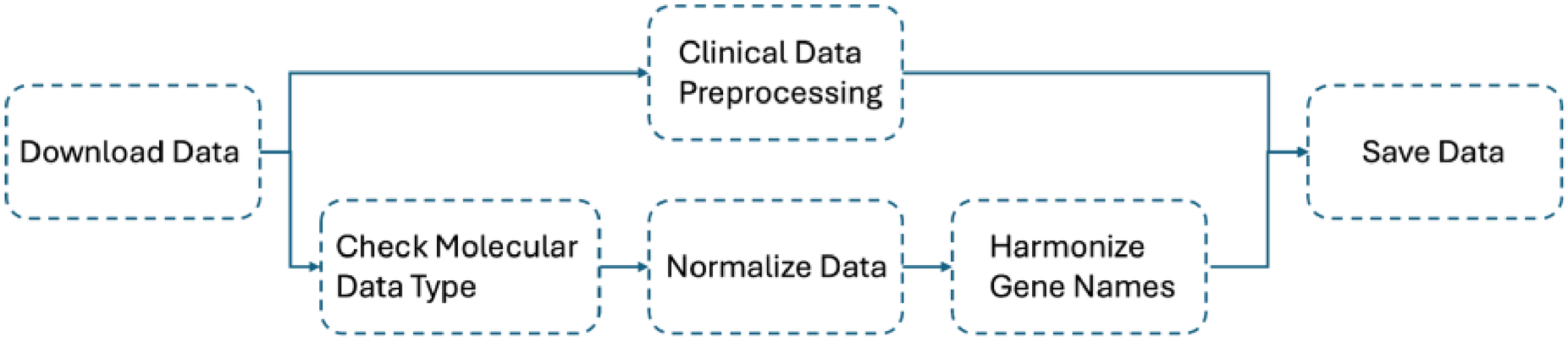
EPIGENE LABS

COMPUTATIONAL BIOLOGY INTERNSHIP-CHALLENGE

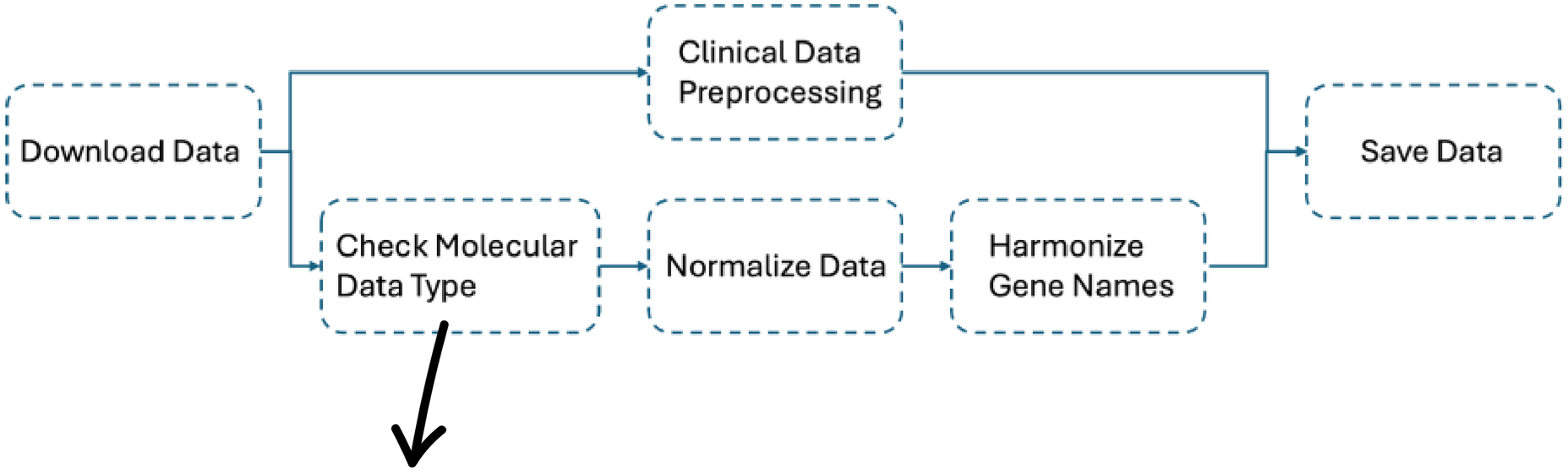
JHONATAN FELIX

Task 2 : Improve the RNAseq data integration pipeline



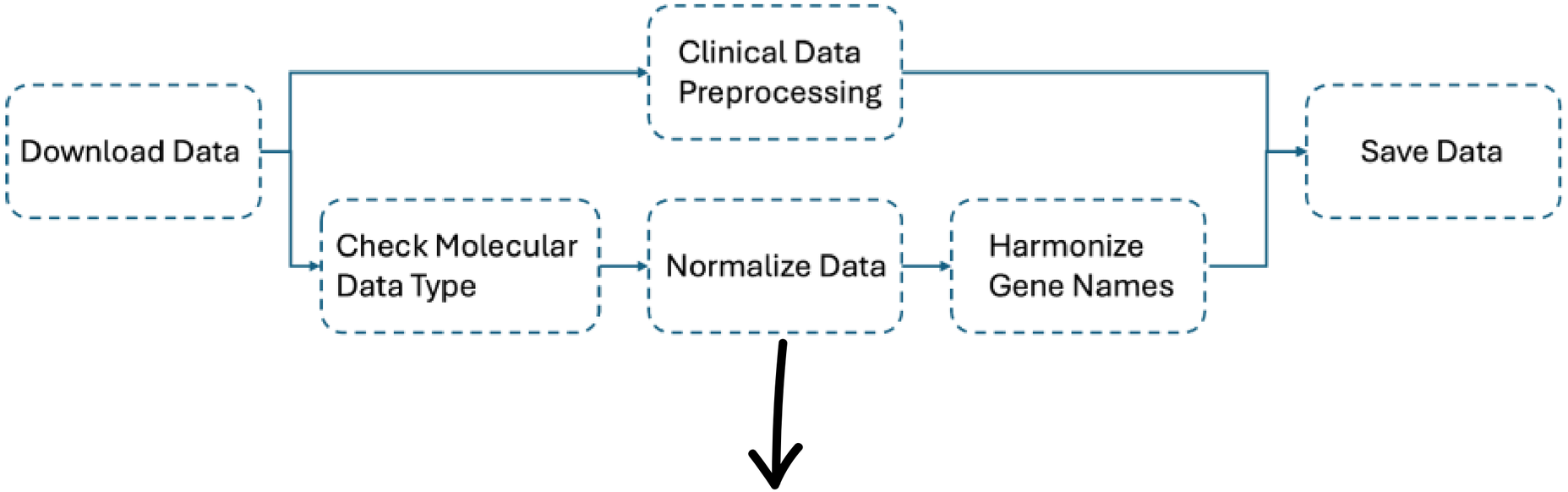
FIRST PART:
COMMENT ON THE PIPELINE

UPDATING THE WORKFLOW



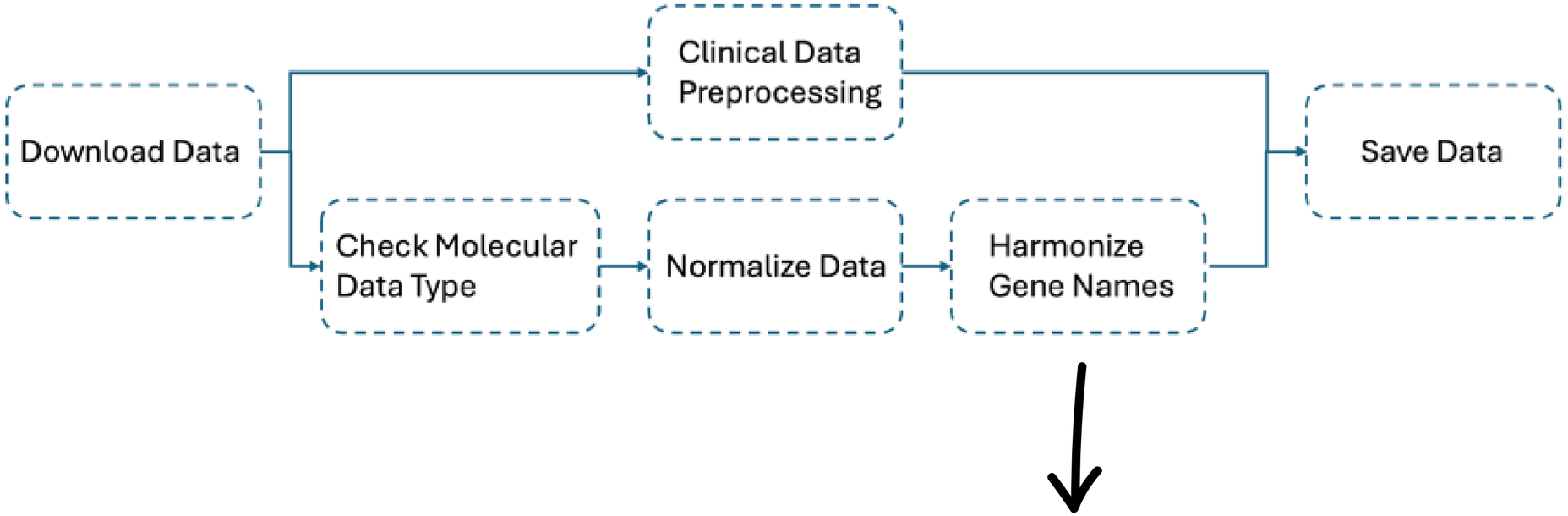
- Verify the datatype such as raw counts, TPM, TPKM
- Identify and flag missing or inconsistent data
- Validate the structure of the dataset , avoid duplicates

UPDATING THE WORKFLOW



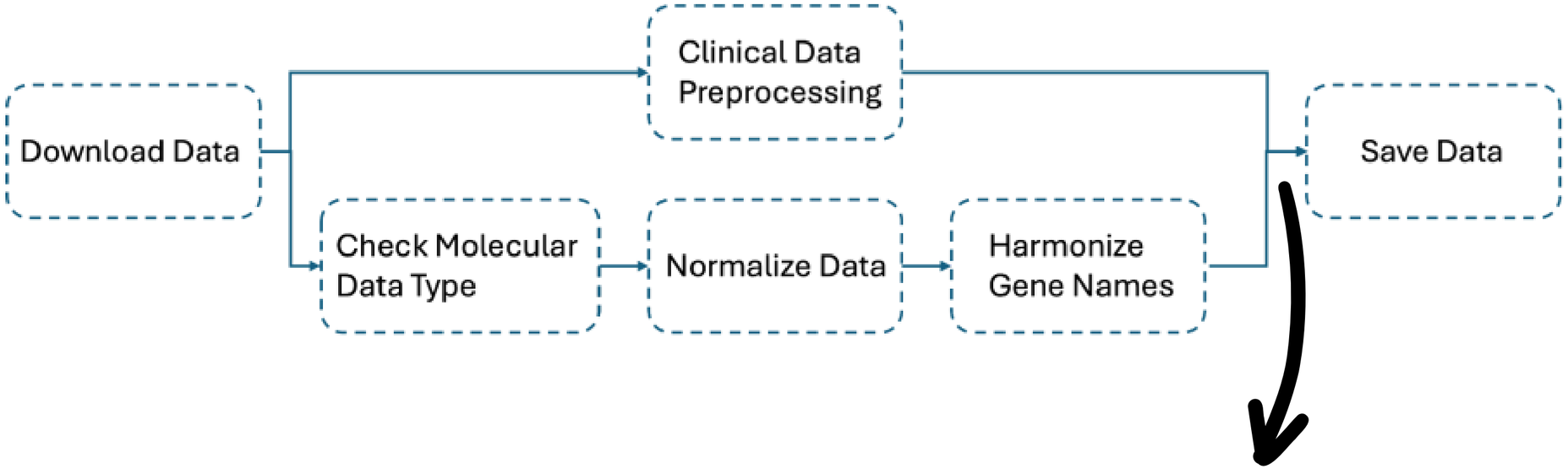
- Normalize the data either to TPM or FPKM
- Using appropriate scaling techniques to ensure values are comparable accross datasets
- Handle batch effects or technical biases

UPDATING THE WORKFLOW



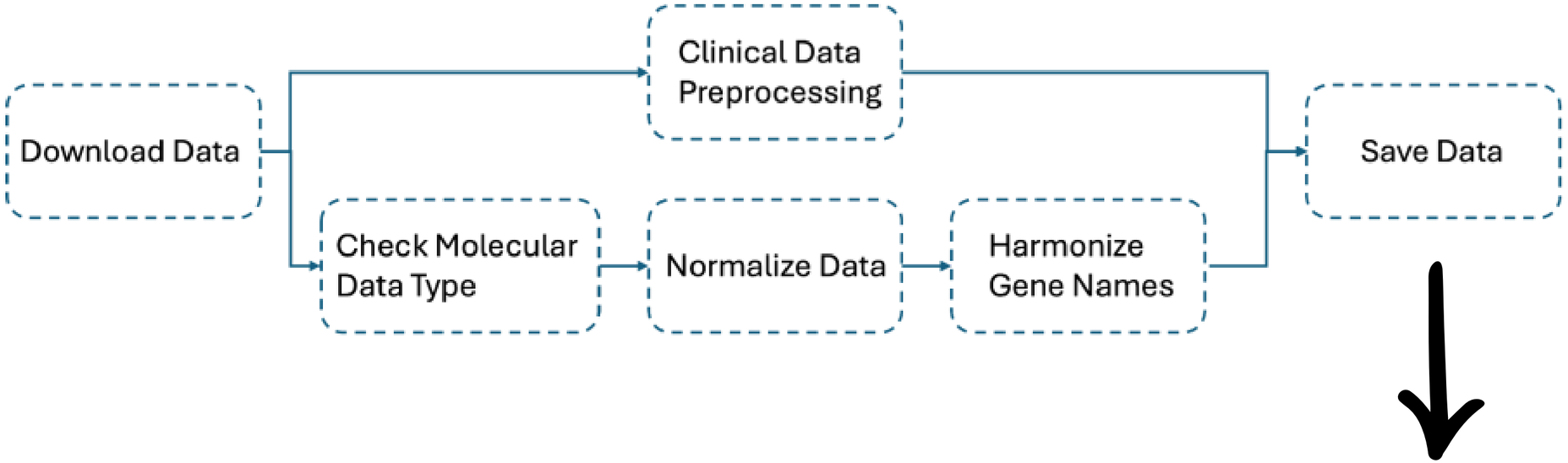
- Standardize gene identifiers across datasets (Ensembl)
- Remove or flag genes with mismatched or missing identifiers
- Map aliases or outdated gene names to the latest standards using reference database (optional)

UPDATING THE WORKFLOW



- Map molecular data to clinical features with patient metadata for instance
- Ensure a one-to-one relationship between molecular samples and clinical entries
- Perform QC to verify consistent sample annotations

UPDATING THE WORKFLOW

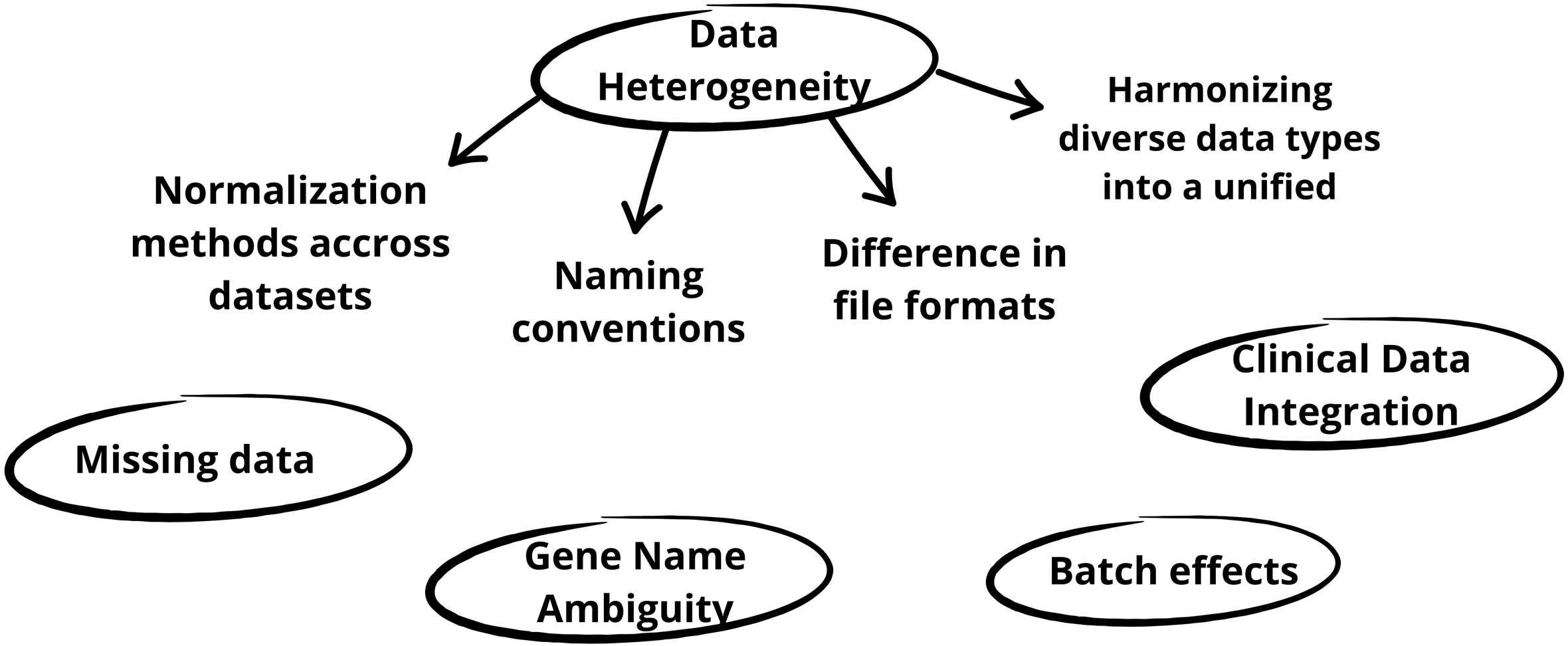


- Ensure formats are consistent for downstream analysis

ENHANCING THE PIPELINE TO ENSURE CONSISTENCY OF DATA

Automated QC	→	**Verify data completennes, formating and scaling consistency**
Use External Gene Databases	→	**Integrating tools like Ensembl API or UniProt to ensure a standard format**
Missing data Handling	→	**Specify a uniform strategy for missing values (imputation, exclusion, flagging)**
Batch Effect correction	→	**Using tools to correct batch effects when integrating datasets from different experiments**
Document each steps	→	**Generate logs for each step**

POSSIBLE CHALLENGES



SECOND PART:
COMMENT ON THE DIFFERENT OUTPUT FILES

WHY DO WE HAVE DIFFERENT FILES FOR ONE DATASET?

Because each output serves a specific analytical purpose

Raw Counts → **The unprocessed data from sequencing typically used for DEA**

Transcripts per million (TPM → **Used for cross-sample comparisons since it accounts for both sequencing depth and gene length**

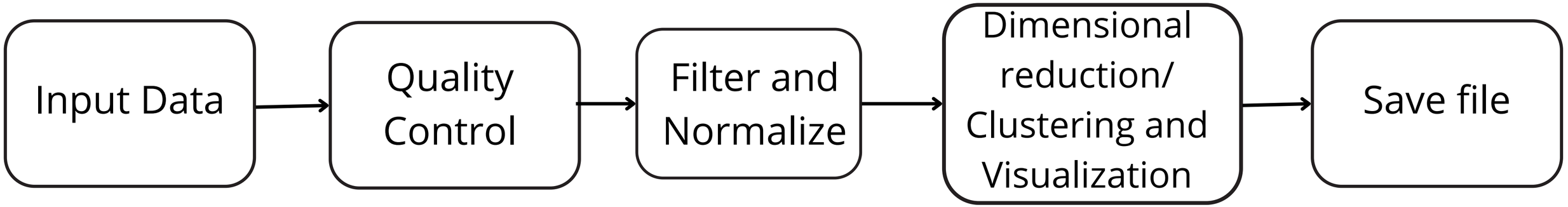
Fragments Per Kilobase Per Million (FPKM) → **Used for within-dataset comparisons or visualization**

And also we need flexibility for Downstream Analysis and to preserve biological information

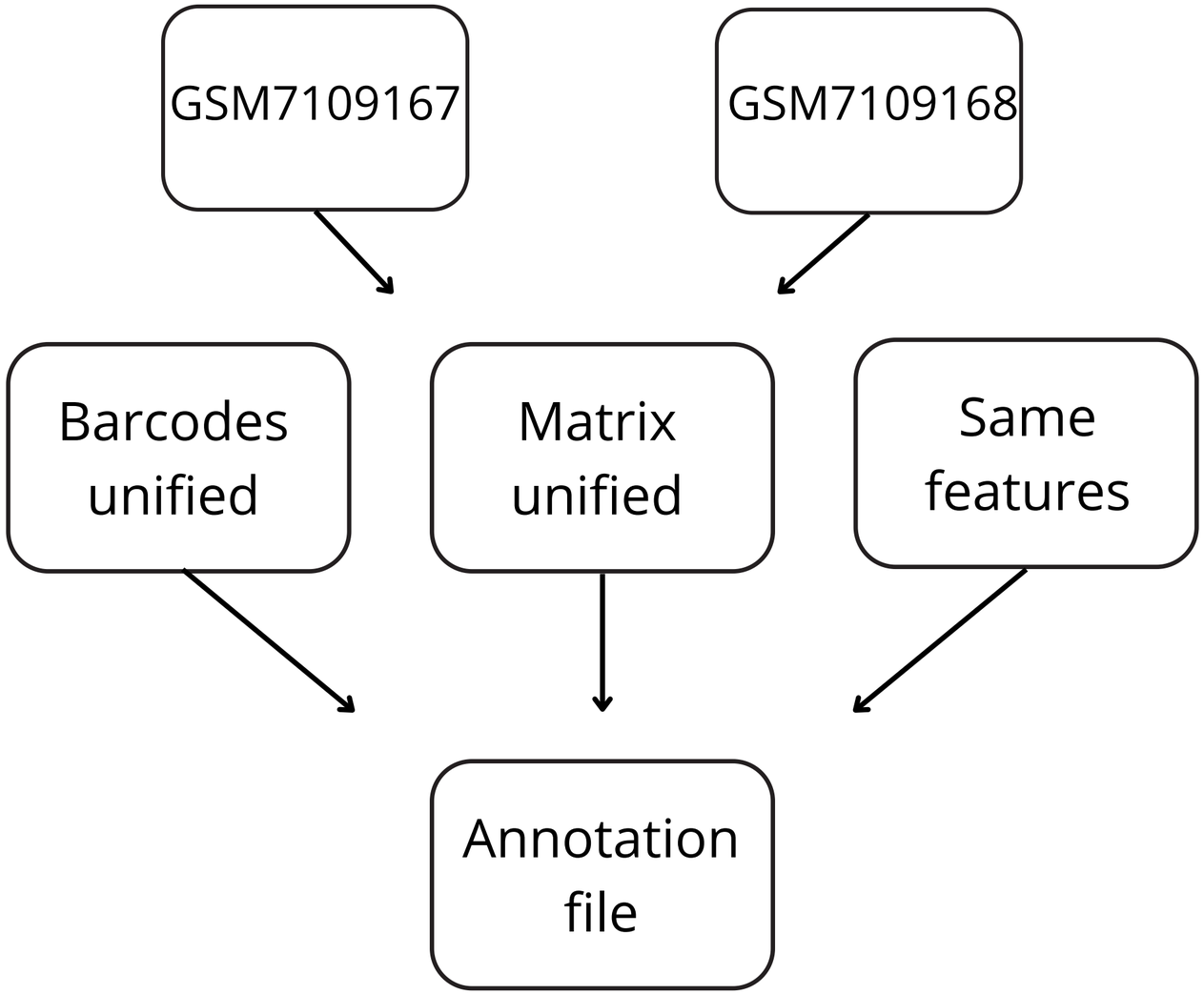
DIFFERENT FILES FOR DOWNSTREAM ANALYSIS

Raw Counts	→	**Differential expression analysis**
Transcripts per million (TPM)	→	**Cross-sample comparison, clustering and visualization (e.g., heatmaps and PCA)**
Fragments Per Kilobase Per Million (FPKM)	→	**Within-dataset comparison, exploratory analysis and visualizations**
Preprocessed Data	→	**Machine learning, pathway analysis, and integration with clinical data**

Task 1 : Single-cell RNAseq data integration



INPUT DATA AND UNIFYING FILES



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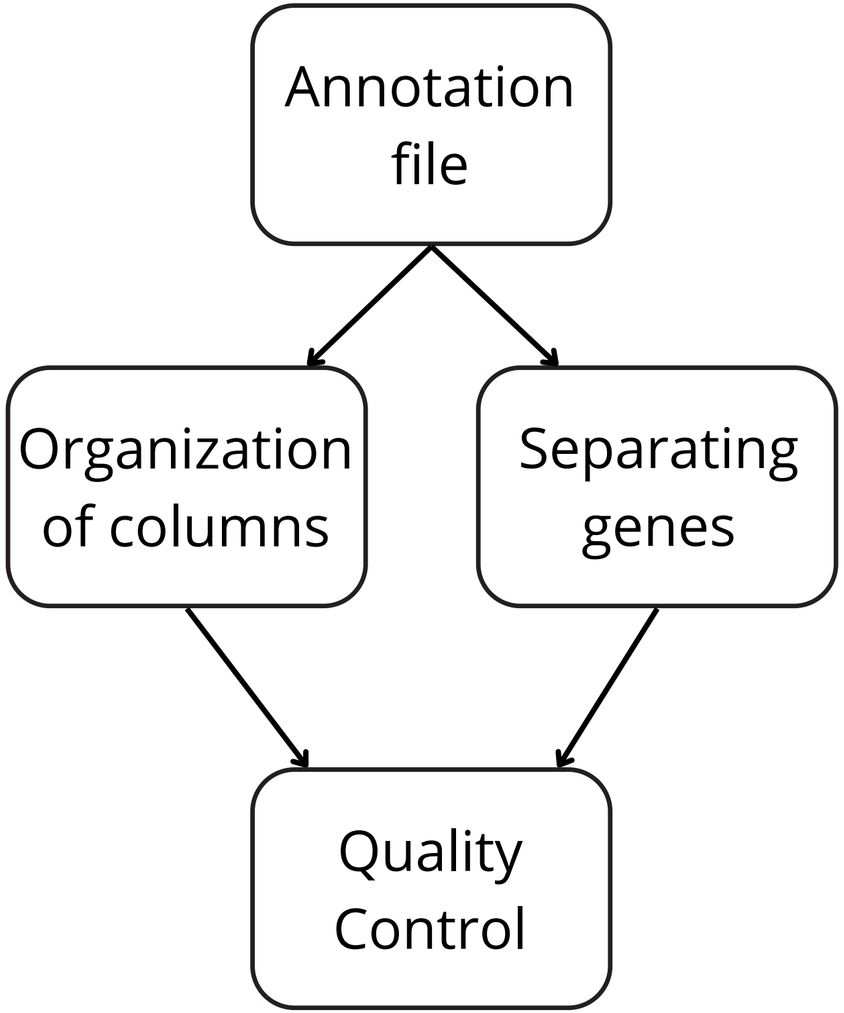


```python
def organizing_and_QC(matrix_all, barcodes_all, features):
  adata = ad.AnnData(X= matrix_all, var=features ,obs=barcodes_all.iloc[:,[0]])

  adata.obs = adata.obs.rename(columns = {0: 'barcodes'})
  adata.obs['sample_id'] = barcodes_all['sample_id'].astype(str).tolist()
  adata.obs['cell_id']   = adata.obs.sample_id + '_'+ adata.obs.barcodes
  adata.obs = adata.obs.set_index('cell_id')
  adata.obs['dataset'] = barcodes_all['dataset'].astype(str).tolist()
  adata.obs = adata.obs.drop(columns=  'barcodes')

  #. Organizing the var dataset also
  #adata.var = adata.var.rename(columns = {1: '',2: 'feature_types'})
  adata.var = adata.var.set_index('code')

  # Separating in different genes:
  # mitochondrial genes
  adata.var["mt"] = adata.var_names.str.startswith("MT-")
  # ribosomal genes
  adata.var["ribo"] = adata.var_names.str.startswith(("RPS", "RPL"))
  # hemoglobin genes.
  adata.var["hb"] = adata.var_names.str.contains(("^HB[^(P)]"))

  #### Calculating quality control metrics
  sc.pp.calculate_qc_metrics(
      adata, qc_vars=["mt", "ribo", "hb"], inplace=True, percent_top=[20,30,50],
      log1p=True
  )
  return adata
```

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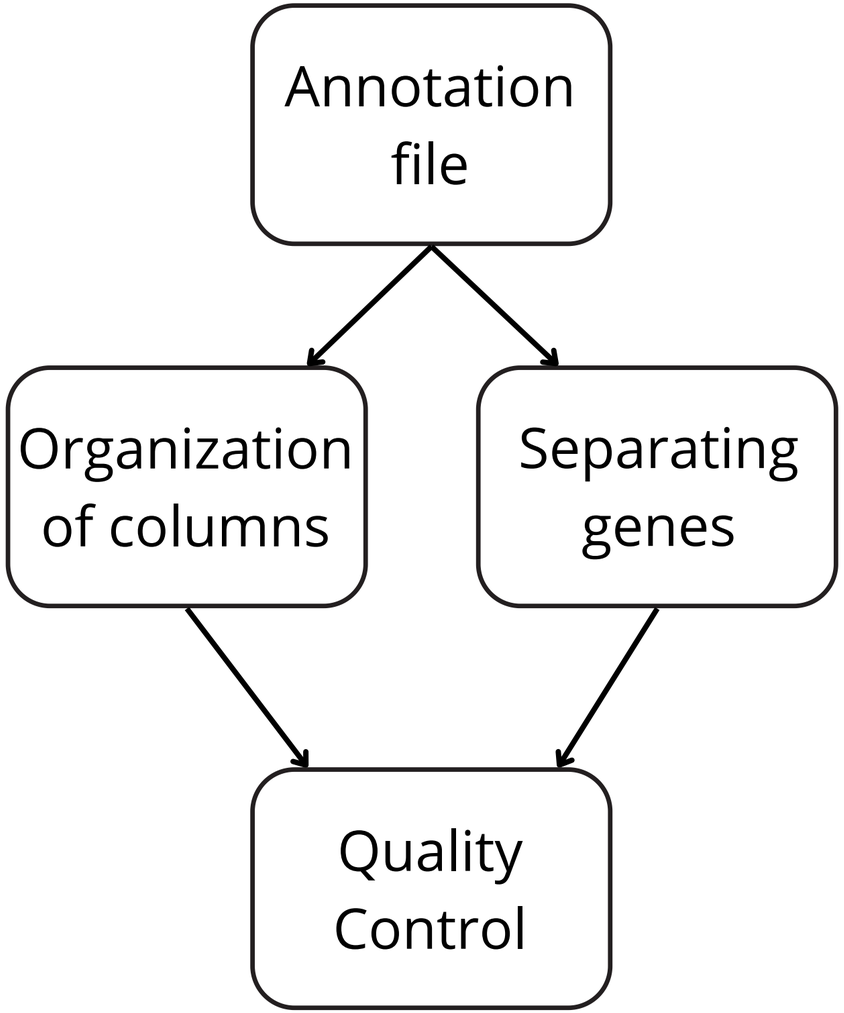


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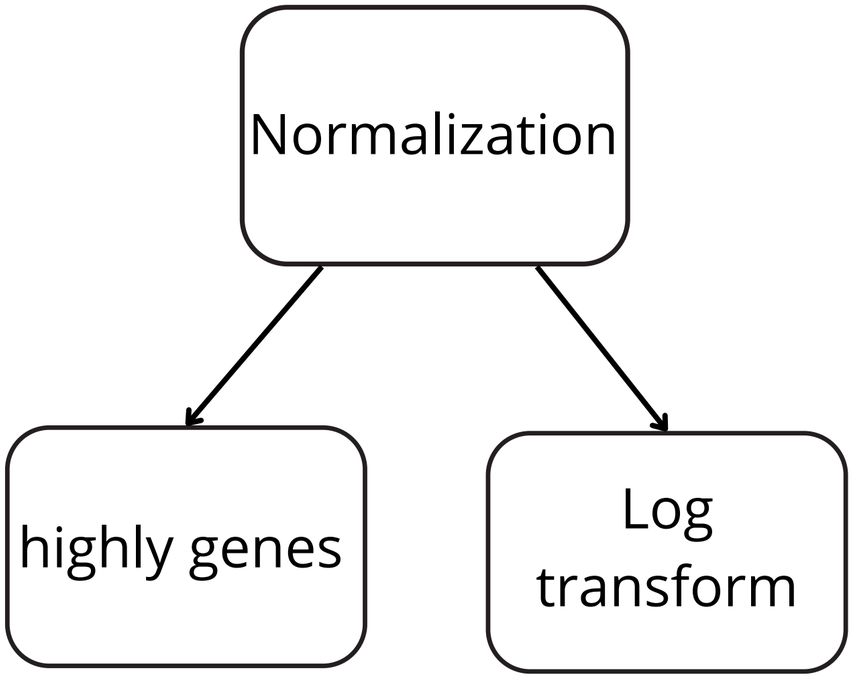
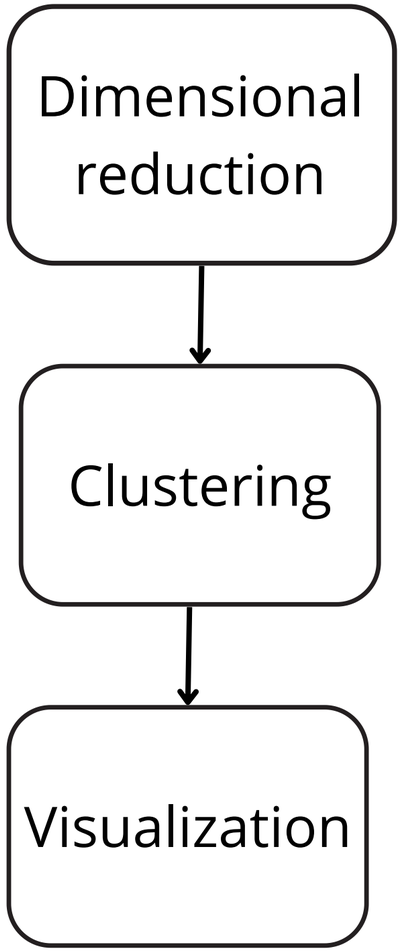


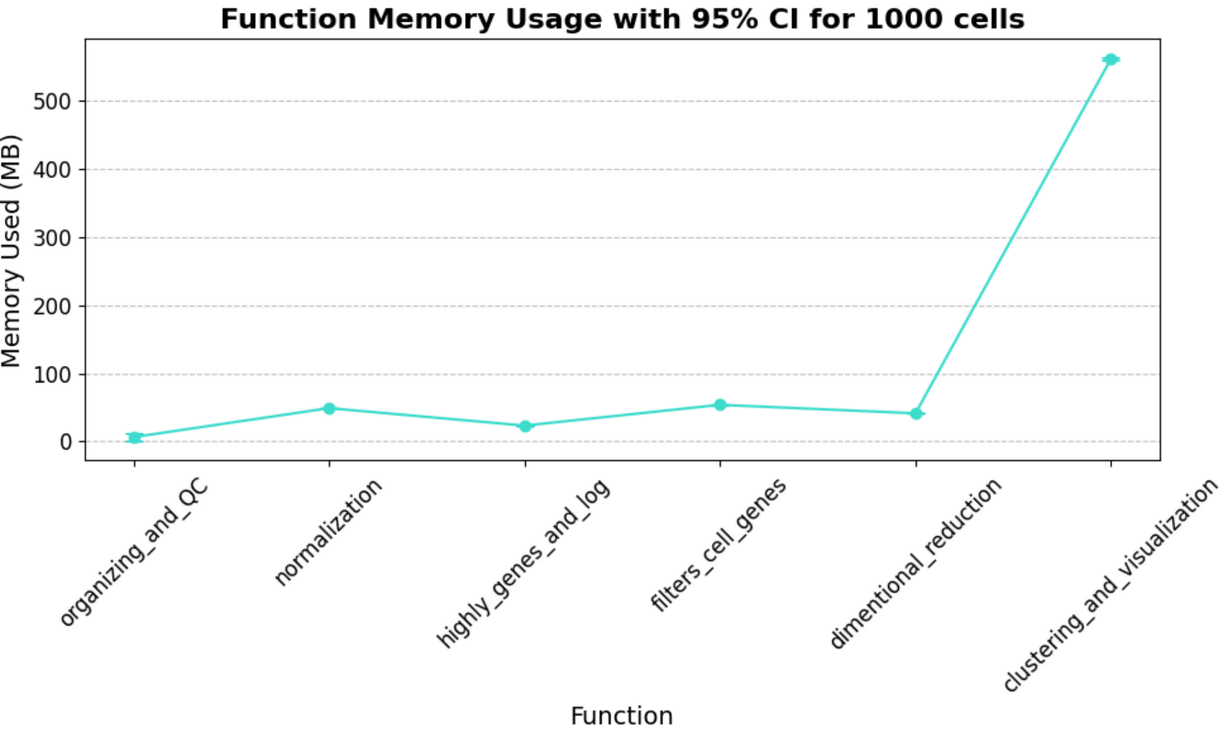

```python
# Part 2 ##############################################################################
# Initial normalization
def normalization(adata, target_sum = 1e4):
  adata.layers["raw_counts"] = adata.X.copy()
  sc.pp.normalize_total(adata, target_sum= target_sum)
  adata.layers['norm_counts'] = adata.X.copy()

  return adata

# Selection of highly variable genes
def highly_genes_and_log(adata, n_top_genes = 2000, subset = False):
  sc.pp.highly_variable_genes(
      adata, flavor="cell_ranger", n_top_genes= n_top_genes, subset= subset
  )  ### It could be setted subset= True to really filter this dataset

  # Logaritmization
  sc.pp.log1p(adata)
  adata.layers["log1p_norm"] = adata.X.copy()

  return adata
```

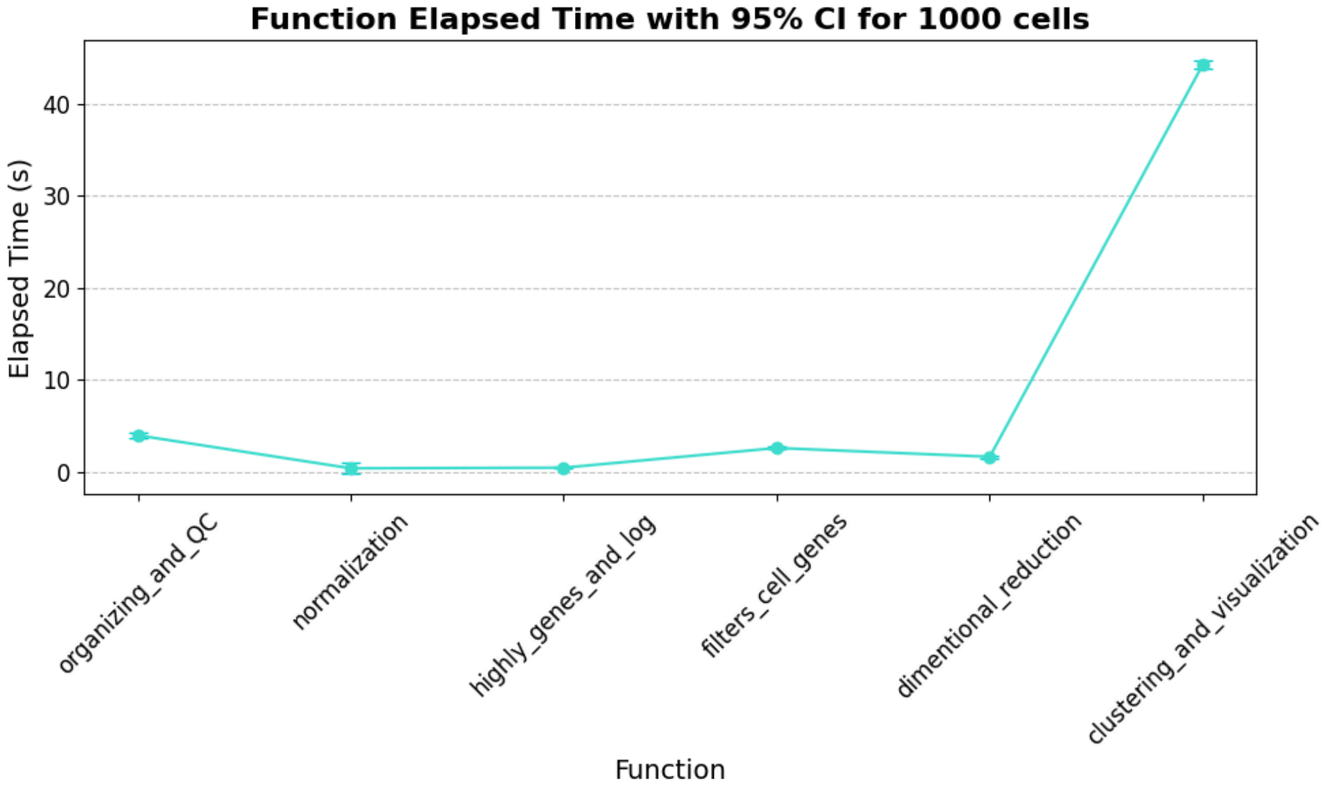
INPUT DATA AND UNIFYING FILES




```python
# Part 3 ######################################################################
# dimentional reduction
def dimentional_reduction(adata, n_comps = 50):
  sc.tl.pca(adata, n_comps= n_comps)

  return adata

# Part 4 ######################################################################
# Clustering and Visualization

def clustering_and_visualization(adata, n_neighbors = 15, n_pcs = 30,
                                 early_exaggeration = 12, learning_rate = 1000,
                                 metric = 'euclidean', n_jobs = 1, perplexity = 30,
                                 use_rep = 'X_pca', resolution = 1.0,
                                 key_added = 'leiden_res_1'):

  sc.pp.neighbors(adata, n_neighbors=15, n_pcs=30)
  sc.tl.tsne(adata, n_pcs=50, early_exaggeration = 12, learning_rate = 1000,
             metric = 'euclidean', n_jobs = 1, perplexity = 30, use_rep = 'X_pca')
  sc.tl.umap(adata)
  sc.tl.leiden(adata, resolution=1.0, key_added = 'leiden_res_1')
  return adata
```

BENCHMARKS AND EFFICIENCY





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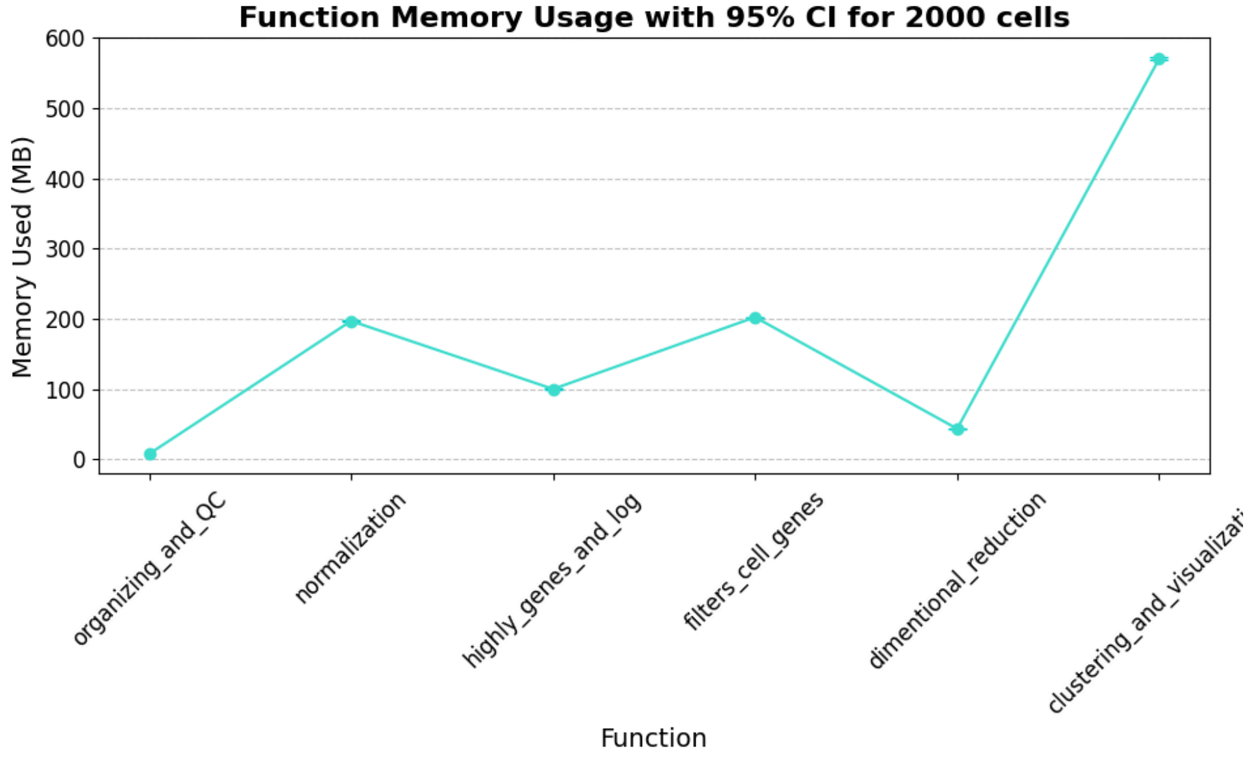

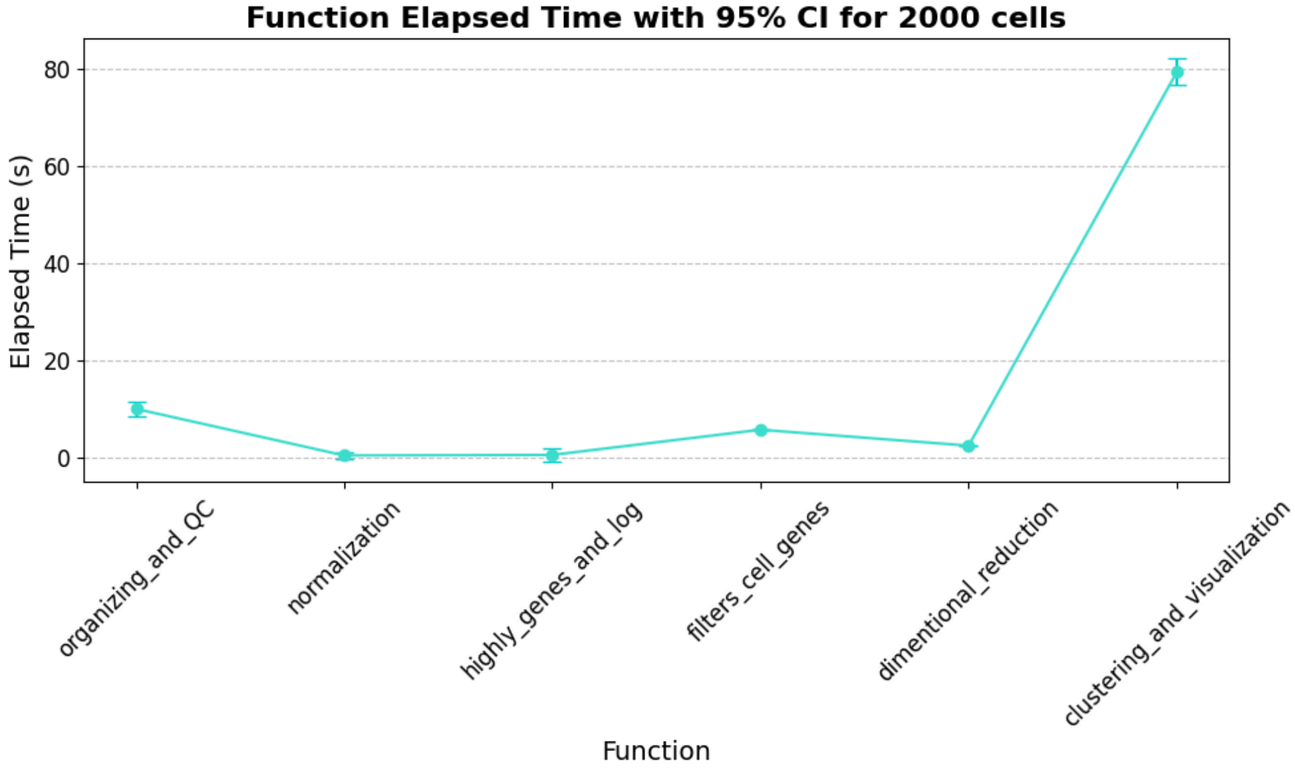

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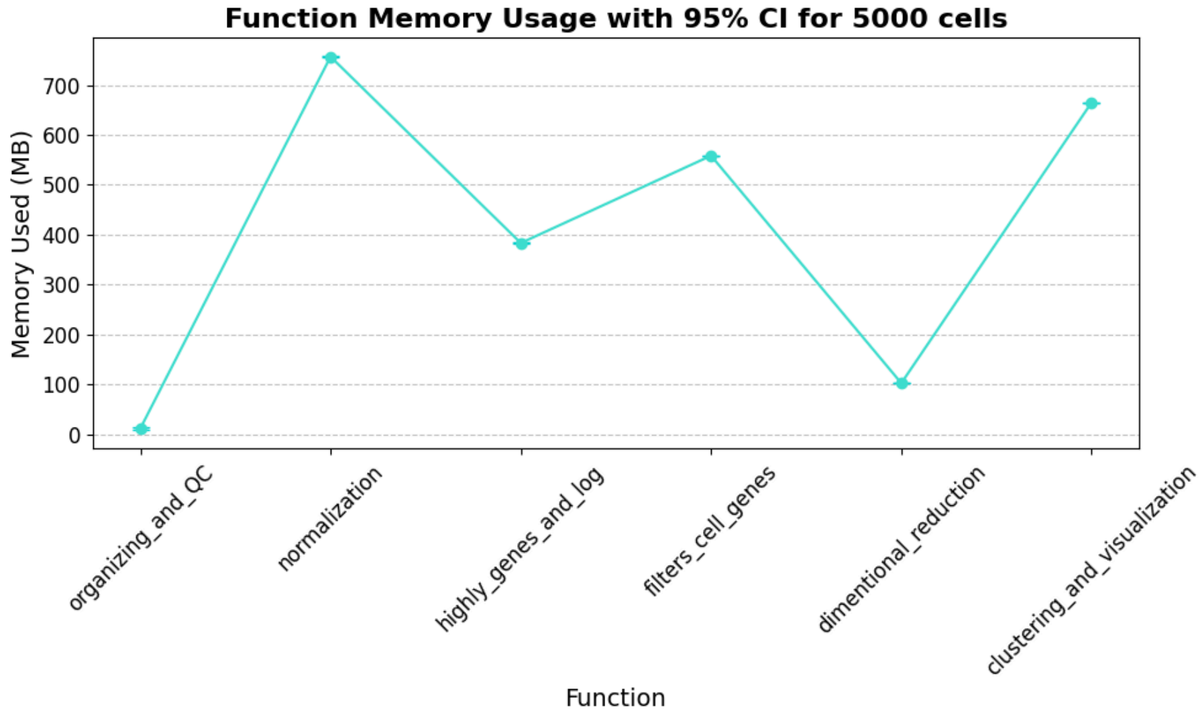
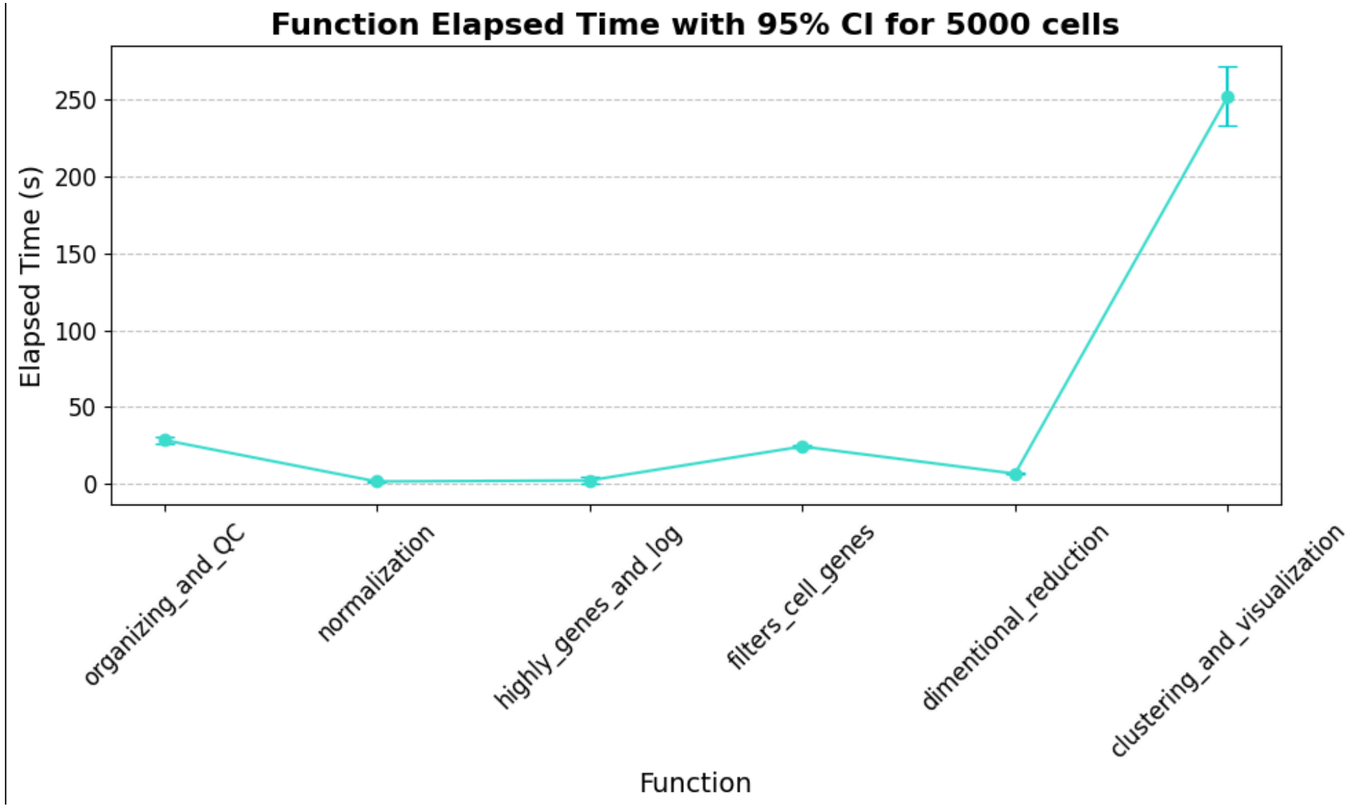
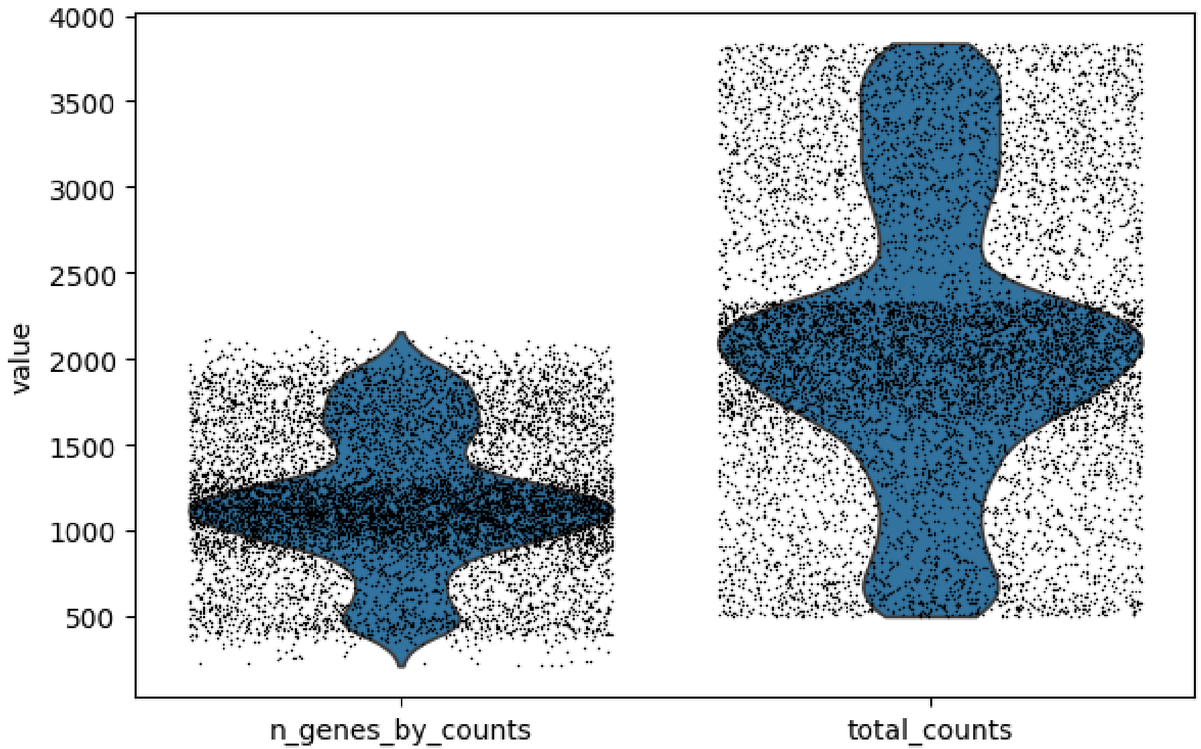
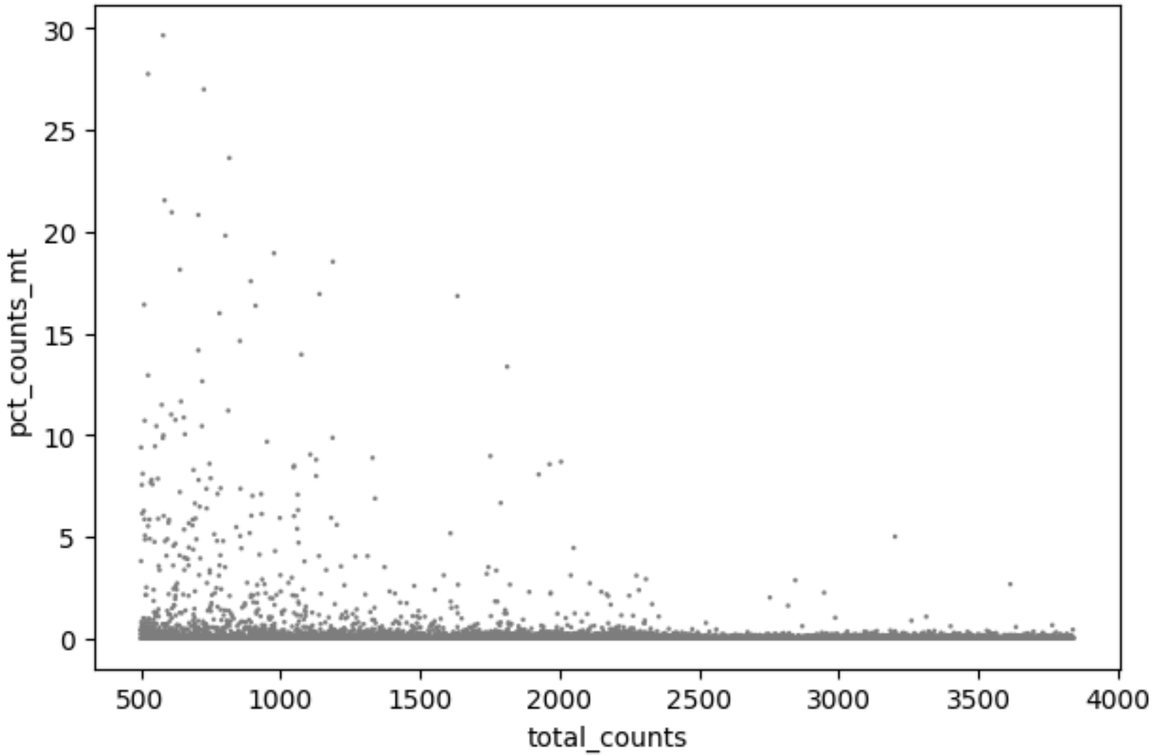




RESULTS OF THE PIPELINE





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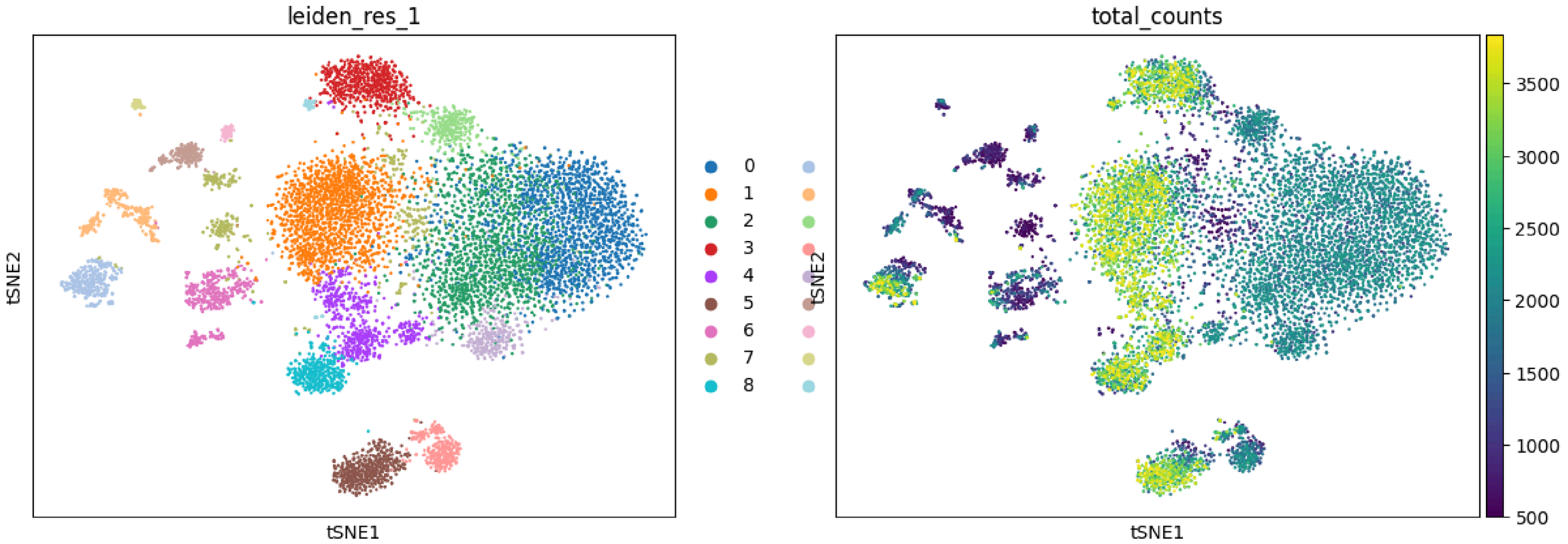

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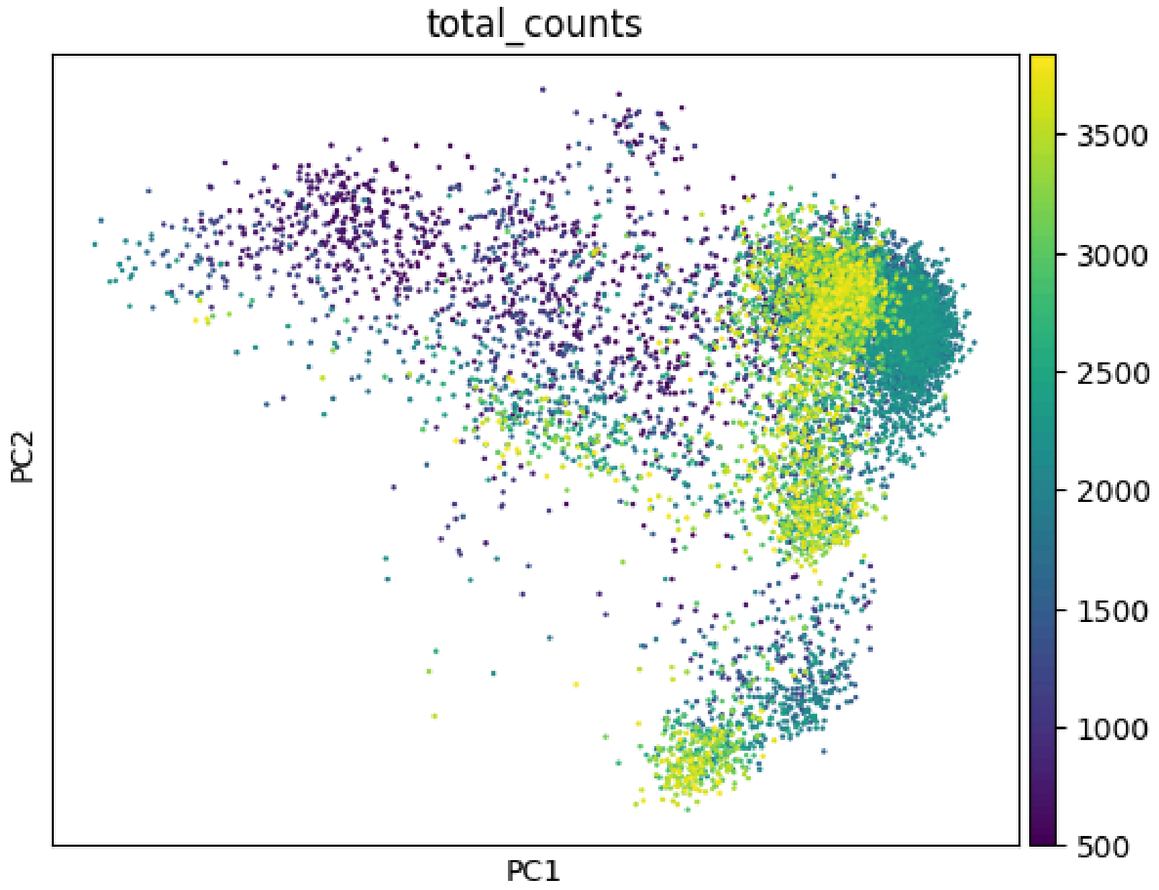

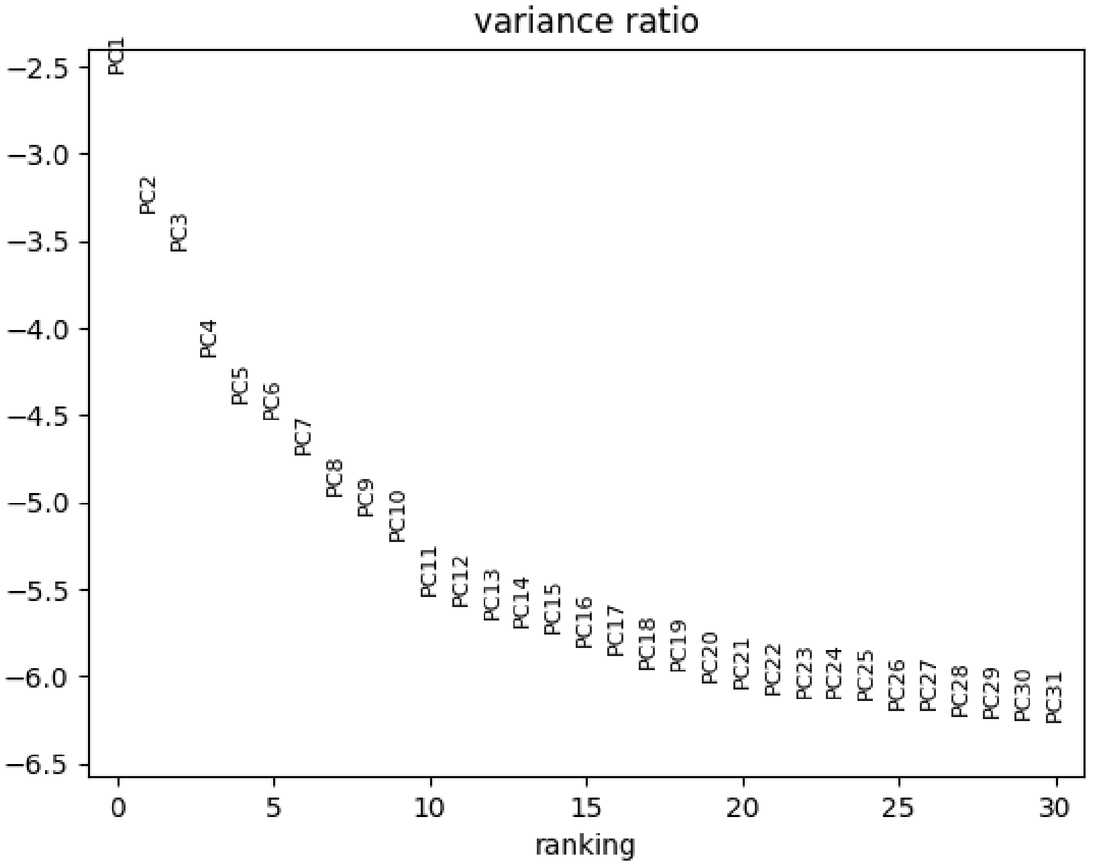

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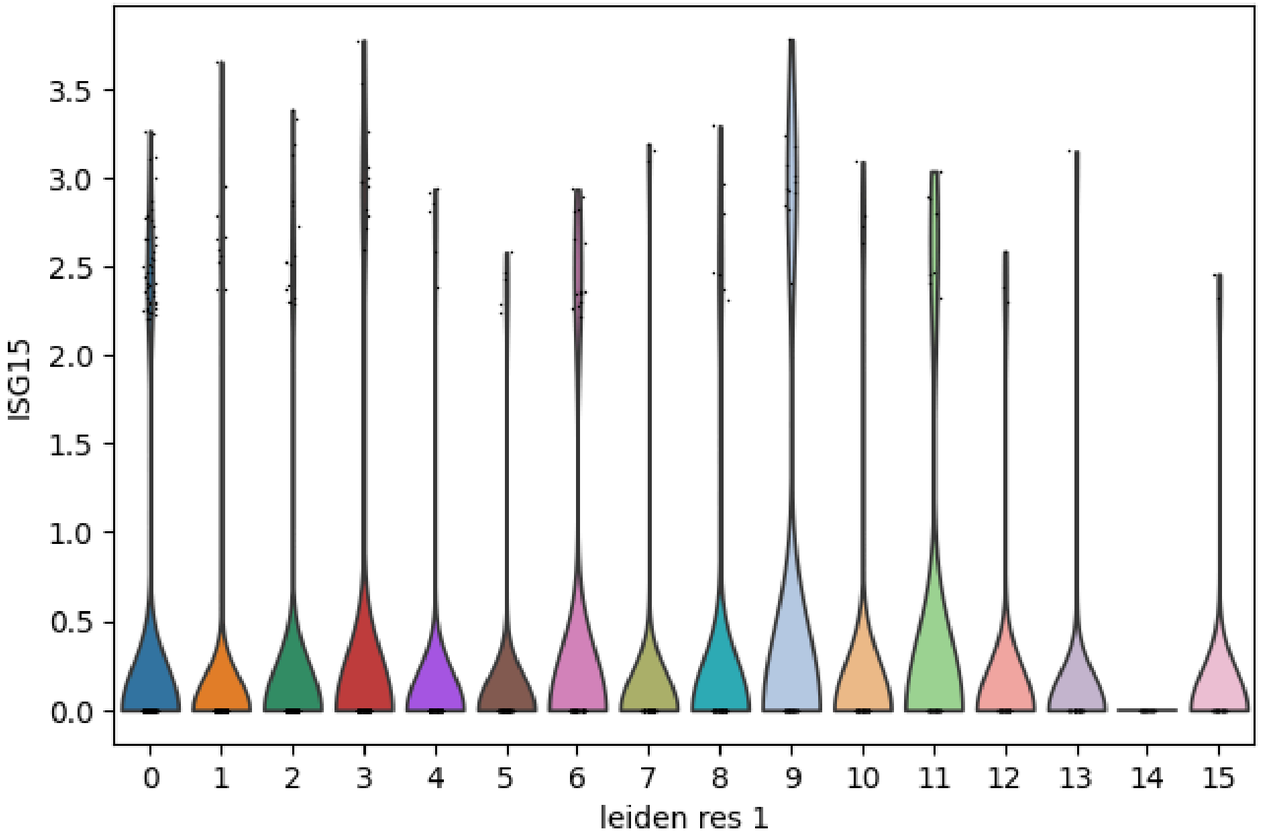

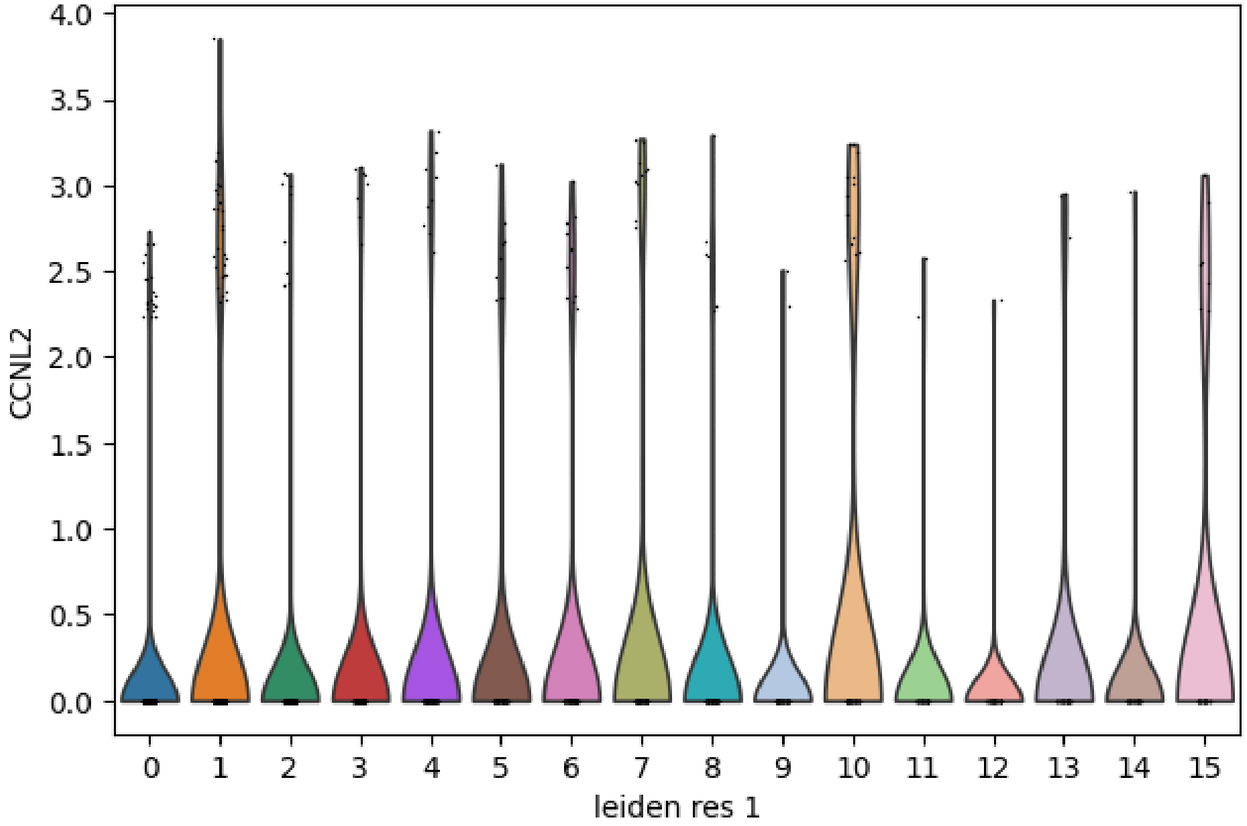

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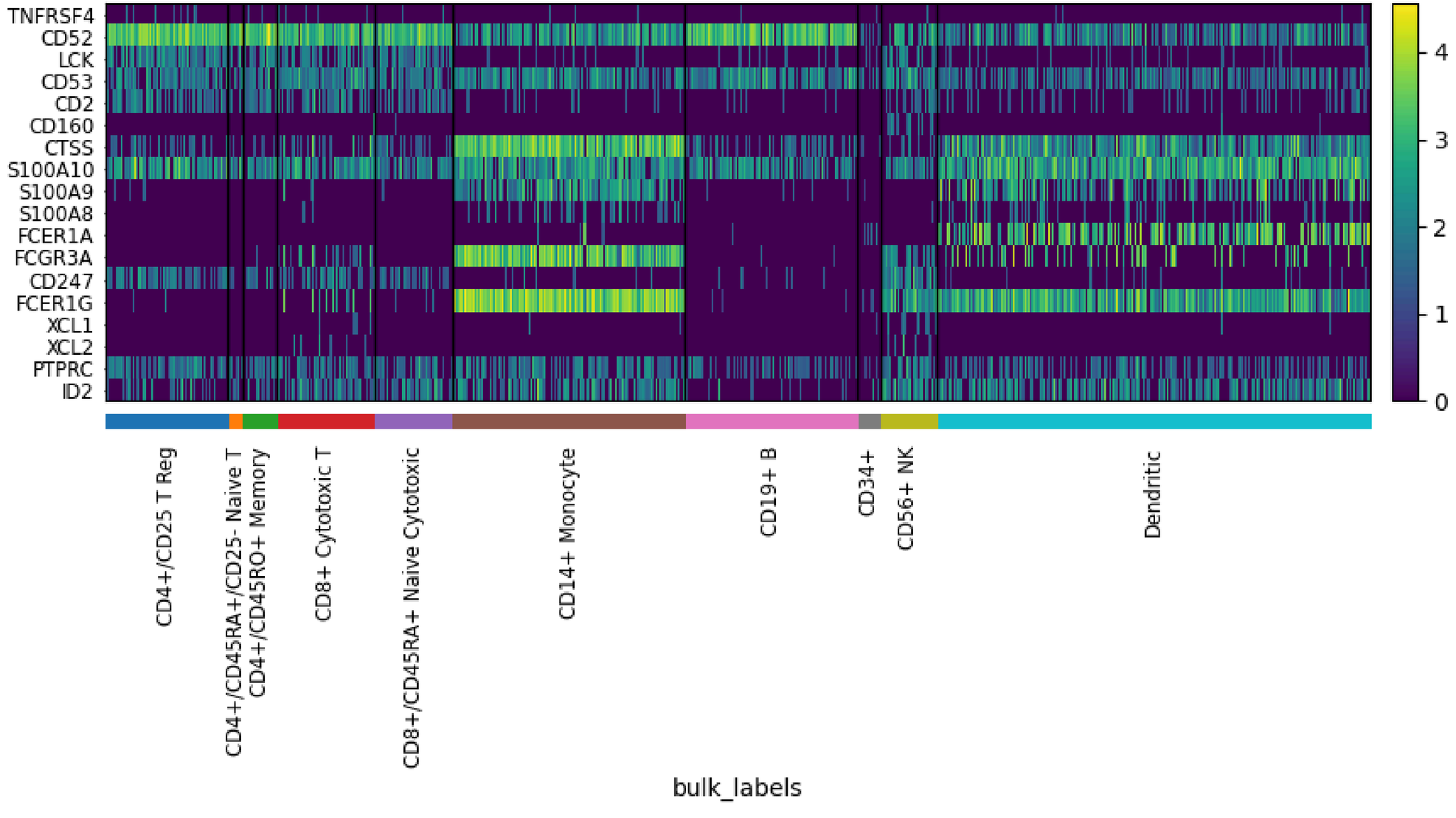

ANY QUESTIONS?

THANK YOU!