
ember Documentation

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GENERATING ENTROPY METRICS

```
ember.light_ember.light_ember(h5ad_dir, partition_label, save_dir, sampling=True, sample_id_col=None,
                              category_col=None, condition_col=None, num_draws=100,
                              save_draws=False, seed=42, partition_pvals=True, block_pvals=False,
                              block_label=None, n_pval_iterations=1000, n_cpus=1)
```

Runs the ember entropy metrics and p-value generation workflow on an AnnData object.

This function loads an AnnData *.h5ad* file, optionally performs balanced sampling across replicates, computes entropy metrics for the specified partition, and generates p-values for Psi and Zeta and optionally Psi_block for a block of choice.

Entropy metrics generated:

- Psi : Fraction of information explained by partition of choice
- Psi_block : Specificity of information to a block
- Zeta : Specificity to a partition/ distance of Psi_blocks distribution from uniform

Parameters

- **h5ad_dir** (*str*, *Required*) – Path to the *.h5ad* file to process. Data should be log1p and depth normalized before running ember. Remove genes with less than 100 reads before running ember.
- **partition_label** (*str*, *Required*) – Column in *.obs* used to partition cells for entropy calculations (e.g., “celltype”, “Genotype”, “Age”). Required to run process. If performing calculation on interaction term, first create a column in *.obs* concatenating the two columns of interest with a semicolon (:).
- **save_dir** (*str*, *Required*) – Path to directory where results will be saved. Required to run process.
- **sampling** (*bool*, *default=True*) – Whether to perform balanced sampling across replicates before entropy calculation. If True, *sample_id_col*, *category_col*, and *condition_col* must be provided. Sampling should only be False if fast intermediate results are desired or if there are no replicates to sample over. If sampling is set to False but either *partition_pvals* or *block_pvals* are set to True then the *sampling=False* will be overridden as pval generation requires sampling.
- **sample_id_col** (*str*, *default = None*) – The column in *.obs* with unique identifiers for each sample or replicate (e.g., ‘sample_id’, ‘mouse_id’).
- **category_col** (*str*, *default = None*) – The column in *.obs* defining the primary group to balance across in order to generate a balanced sample of the experiment. (e.g., ‘disease_status’, ‘mouse_strain’). Refer to readme for further explanation on how to select category and condition columns. *category_col* and *condition_col* are interchangeable. If balanc-

ing across more than 2 variables, generate interaction terms, create a column in *.obs* concatenating the two (or more) columns of interest with a semicolon (:). This way balancing can be done across as many variables as desired.

- **condition_col** (*str*, *default* = *None*) – The column in *.obs* containing the conditions to balance within each category to generate a balanced sample of the experiment. (e.g., ‘sex’, ‘treatment’). Refer to readme for further explanation on how to select category and condition columns. *category_col* and *condition_col* are interchangeable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatenating the two (or more) columns of interest with a semicolon (:). This way balancing can be done across as many variables as desired.
- **num_draws** (*int*, *default* = 100) – The number of balanced subsets to generate, by default 100.
- **save_draws** (*bool*, *default*=*False*) – Whether to save intermediate draws to *save_dir*.
- **seed** (*int*, *default* = 42) – The random seed for reproducible draws, by default 42.
- **partition_pvals** (*bool*, *default*=*True*) – Whether to compute permutation-based p-values for the *partition_label*. P-values are generated by sampling. If *sampling* = *False* and *partition_pvals* = *True*, the *sampling*=*False* will be overwritten. Calls *generate_pvals*, which can be called manually after metric generation as well.
- **block_pvals** (*bool*, *default*=*False*) – Whether to compute permutation-based p-values for the *block_label*. P-values are generated by sampling. If *sampling* = *False* and *block_pvals* = *True*, the *sampling*=*False* will be overwritten. Calls *generate_pvals*, which can be called manually after metric generation as well.
- **block_label** (*str*, *default* = *None*) – One value in the *.obs* column for *partition_label* to use for block-based permutation tests. Required if *block_pvals*=*True*.
- **n_pval_iterations** (*int*, *default*=1000) – Number of permutations to use for p-value calculation.
- **n_cpus** (*int*, *default*=1) – Number of CPU cores to use for parallel permutation testing. For this script, performance is I/O-bound and may not improve beyond 4-8 cores.’

Return type

None

Notes

- Results are saved to *save_dir* as CSV files.
- one csv file with all entropy metrics
- one csv file in a new *Psi_block_df* folder with psi block values for all blocks in a partition
- Separate file for pvals
- Separate files for each partition
- Alternate file names depending on sampling on or off.

What to expect inside ‘entropy_metrics.csv’:

- *gene_name*: All genes in *.var*
- *Psi_mean*: Psi scores averaged over n draws (between 0 and 1) corresponding to the selected partition for each gene in *.var*.

- **Psi_std**: Standard deviation of Psi scores across n draws corresponding to the selected partition for each gene in *.var*.
- **Psi_valid_counts**: Number of valid Psi scores observed across n draws. Only use genes for downstream analysis that have valid counts=num_draws. If valid counts is not close to num_draws, increase threshold for filtering genes with low reads beforehand(recommended <100 reads, increase as needed).
- **Zeta_mean**: Zeta scores averaged over n draws (between 0 and 1) corresponding to the selected partition for each gene in *.var*.
- **Zeta_std**: Standard deviation of Zeta scores across n draws corresponding to the selected partition for each gene in *.var*.
- **Zeta_valid_counts**: Number of valid Psi scores observed across n draws. Only use genes for downstream analysis that have valid counts=num_draws. If valid counts is not close to num_draws, increase threshold for filtering genes with low reads beforehand (recommended <100 reads, increase as needed).

What to expect inside ‘Psi_block_df’:

- **mean_Psi_block_df.csv** : A dataframe of mean Psi_block scores (between 0 and 1) corresponding to the selected partition for each gene in *.var*. Scores are calculated for all blocks, each column of the dataframe corresponds to one block.
- **std_Psi_block_df.csv** : A dataframe of standard deviations for Psi_block scores corresponding to the selected partition for each gene in *.var*. Scores are calculated for all blocks, each column of the dataframe corresponds to one block.

What to expect inside ‘pvals_entropy_metrics.csv’:

- **gene_name**: All genes in *.var*
- **Psi**: Psi scores averaged over n draws (between 0 and 1) generated by light_ember for each gene in *.var*.
- **Psi p-value**: Permutation based empirical p-values for observed Psi scores for each gene in *.var*.
- **Zeta**: Zeta scores averaged over n draws (between 0 and 1) generated by light_ember for each gene in *.var*.
- **Zeta p-value**: Permutation based empirical p-values for observed Zeta scores for each gene in *.var*.
- **Psi FDR**: Multiple testing corrected q-values for Psi scores.
- **Zeta FDR**: Multiple testing corrected q-values for Zeta scores. Correction performed to include all p-values generated in a single file (Psi and Zeta).

If `block_pvals = True` and a single `block_label` is given:

- **psi_block**: psi_block scores (between 0 and 1) generated by light_ember for each gene in *.var*.
- **psi_block p-value**: Permutation based empirical p-values for observed psi_block scores for each gene in *.var*.
- **psi_block FDR**: Multiple testing corrected q-values for psi_block scores. Correction performed to include all p-values generated in a single file (Psi, psi_block and Zeta).

GENERATING P-VALUES

```
ember.generate_pvals.generate_pvals(h5ad_dir, partition_label, entropy_metrics_dir, save_dir,
                                   sample_id_col, category_col, condition_col, block_label=None,
                                   seed=42, n_iterations=1000, n_cpus=1, Psi_real=None,
                                   Psi_block_df_real=None, Zeta_real=None)
```

Calculate empirical p-values for entropy metrics from permutation test results. This function can be called manually or accessed through `light_ember` with `partition_pvals = True` or `block_pvals = True`.

Manual access useful if wanting to generate p-values for multiple blocks and partitions of interest after initial investigation using entropy metrics.

Integrated access with `light_ember` is easier if investigating only a partition or a block in a partition.

Entropy metrics generated:

- **Psi** : Fraction of information explained by partition of choice
- **Psi_block** : Specificity of information to a block
- **Zeta** : Specificity to a partition/ distance of Psi_blocks distribution from uniform

Parameters

- **h5ad_dir** (*str*, *Required*) – Path to the *.h5ad* file to process. Data should be log1p and depth normalized before running ember. Remove genes with less than 100 reads before running ember.
- **partition_label** (*str*, *Required*) – Column in *.obs* used to partition cells for entropy calculations (e.g., “celltype”, “Genotype”, “Age”). Required to run process. If performing calculation on interaction term, first create a column in *.obs* concatenating the two columns of interest with a semicolon (:).
- **entropy_metrics_dir** (*str*, *Required*) – Path to csv with entropy metrics to use for generating pvals.
- **save_dir** (*str*, *Required*) – Path to directory where results will be saved.
- **sample_id_col** (*str*, *Required*) – The column in *.obs* with unique identifiers for each sample or replicate (e.g., ‘sample_id’, ‘mouse_id’).
- **category_col** (*str*, *Required*) – The column in *.obs* defining the primary group to balance across in order to generate a balanced sample of the experiment. (e.g., ‘disease_status’, ‘mouse_strain’). Refer to readme for further explanation on how to select category and condition columns. `category_col` and `condition_col` are interchangeable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatenating the two (or more) columns of interest with a semicolon (:). This way balancing can be done across as many variables as desired.

- **condition_col** (*str*, *Required*) – The column in *.obs* containing the conditions to balance within each category to generate a balanced sample of the experiment. (e.g., ‘sex’, ‘treatment’). Refer to readme for further explanation on how to select category and condition columns. category_col and condition_col are interchangeable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatenating the two (or more) columns of interest with a semicolon (:). This way balancing can be done across as many variables as desired.
- **block_label** (*str*, *default=None*) – Block in partition to calculate p-values for. Default set to None, program will continue generating p-values for only Psi and Zeta.
- **seed** (*int*, *default=42*) – The random seed for reproducible draws, by default 42.
- **n_iterations** (*int*, *default = 1000*) – Number of iterations to calculate p-values. Default set to 1000. Note that doing fewer than 1000 iterations is a good choice to get first pass p-values but for reliable p-values 1000 iterations is recommended. Larger than 1000 will give you more reliable p-values but will increase runtime significantly.
- **n_cpus** (*int*, *default=1*) – Number of cpus to use to perform p-value calculation. Default set to 1 assuming no parallel compute power on local machine. User can input -1 to use all available cpus but one.
- **Psi_real** (*pd.Series*, *default=None*) – Observed Psi values for each gene. Used by light_ember, not necessary for user use.
- **Psi_block_df_real** (*pd.DataFrame*, *default = None*) – Observed Psi_block values for all blocks in chosen partition. Used by light_ember, not necessary for user use.
- **Zeta_real** (*pd.Series*, *default=None*) – Observed Zeta values for each gene. Used by light_ember, not necessary for user use.

Return type

None

Notes**What to expect inside ‘pvals_entropy_metrics.csv’:**

- gene_name: All genes in *.var*
- Psi: Psi scores averaged over n draws (between 0 and 1) generated by light_ember for each gene in *.var*.
- Psi p-value: Permutation based empirical p-values for observed Psi scores for each gene in *.var*.
- Zeta: Zeta scores averaged over n draws (between 0 and 1) generated by light_ember for each gene in *.var*.
- Zeta p-value: Permutation based empirical p-values for observed Zeta scores for each gene in *.var*.
- Psi FDR: Multiple testing corrected q-values for Psi scores.
- Zeta FDR: Multiple testing corrected q-values for Zeta scores. Correction performed to include all p-values generated in a single file (Psi and Zeta).

if block_pvals = True and a single block_label is given:

- psi_block: psi_block scores (between 0 and 1) generated by light_ember for each gene in *.var*.
- psi_block p-value: Permutation based empirical p-values for observed psi_block scores for each gene in *.var*.
- psi_block FDR: Multiple testing corrected q-values for psi_block scores. Correction performed to include all p-values generated in a single file (Psi, psi_block and Zeta).

PLOTTING FUNCTIONS

3.1 Psi vs. Zeta scatter plots

```
ember.plots.plot_partition_specificity(partition_label, pvals_dir, save_dir, highlight_genes=None,  
                                     fontsize=18, custom_palette=None)
```

Generate a Zeta vs. Psi scatter plot to visualize partition-specific genes.

This function reads p-value data, colors genes based on their statistical significance for Psi and Zeta scores, and highlights top “marker” and “housekeeping” genes. Only interpret genes that are significant by both Psi and Zeta since those are genes that have reliable scores after permutation testing. Allows for custom highlighting of a user-provided gene list. Fontsize and color palette can be customized.

Parameters

- **partition_label** (*str*, *Required.*) – The label for the partition being plotted, used in the plot title.
- **pvals_dir** (*str*, *Required.*) – Path to the input CSV file containing p-values and scores (Psi, Zeta, FDRs). The CSV must have gene names as its index column.
- **save_dir** (*str*, *Required.*) – Path where the output plot image will be saved.
- **highlight_genes** (*list[str]*, *default=None.*) – A list of gene names to highlight and annotate on the plot, by default None.
- **fontsize** (*int*, *default=18.*) – The base font size for plot labels and text, by default 18.
- **custom_palette** (*list[str]*, *default=None.*) – A list of 7 hex color codes to customize the plot’s color scheme. If None, a default palette is used. Please provide list in this order [‘significant by psi’, ‘significant by zeta’, ‘highlight genes’, ‘significant by both’, ‘circle markers’, ‘circle housekeeping genes’, ‘significant by neither’]

Return type

None

3.2 Psi vs. psi_block scatter plots

```
ember.plots.plot_block_specificity(partition_label, block_label, pvals_dir, save_dir,  
                                  highlight_genes=None, fontsize=18, custom_palette=None)
```

Generate a psi_block vs. Psi scatter plot to visualize block-specific genes.

This function reads p-value data, colors genes based on their statistical significance for Psi and psi_block scores, and highlights the top genes significant in both metrics. Only interpret genes that are significant by both Psi and psi_block since those are genes that have reliable scores after permutation testing. Allows for custom highlighting of a user-provided gene list. Fontsize and color palette can be customized.

Parameters

- **partition_label** (*str*, *Required.*) – The label for the partition, used in the plot title.
- **block_label** (*str*, *Required.*) – The label for the block variable (e.g., a cell type or condition).
- **pvals_dir** (*str*, *Required.*) – Path to the input CSV file containing p-values and scores. The CSV must have gene names as its index column.
- **save_dir** (*str*, *Required.*) – Path where the output plot image will be saved.
- **highlight_genes** (*list[str]*, *default=None.*) – A list of gene names to highlight and annotate on the plot, by default None.
- **fontsize** (*int*, *default = 18.*) – The base font size for plot labels and text, by default 18.
- **custom_palette** (*list[str]*, *default=None.*) – A list of 6 hex color codes to customize the plot's color scheme. If None, a default palette is used. Provide list of colors in this order: ['significant by psi', 'significant by psi_block', 'highlight genes', 'significant by both', 'circle markers', 'circle housekeeping genes', 'significant by neither']

Return type

None

3.3 Descriptive bar plot of sample counts

```
ember.plots.plot_sample_counts(h5ad_dir, save_dir, sample_id_col, category_col, condition_col,  
                               fontsize=18)
```

Generate a bar plot showing the number of unique individuals per category and condition.

This function reads an AnnData object from an .h5ad file in backed mode, calculates the number of unique individuals for each combination of a given category and condition, and visualizes these counts as a grouped bar plot. Fontsize can be customized.

Parameters

- **h5ad_dir** (*str*, *Required*) – Path to the input AnnData (.h5ad) file.
- **save_dir** (*str*, *Required*) – Path to directory to save the output plot image.
- **sample_id_col** (*str*, *Required*) – The column name in adata.obs that contains unique sample IDs.
- **category_col** (*str*, *Required*) – The column name to use for the primary categories on the x-axis.
- **condition_col** (*str*, *Required*) – The column name to use for grouping the bars (hue).
- **fontsize** (*int*, *default = 18.*) – The base font size for plot labels and text, by default 18.

Return type

None

3.4 psi_blocks bar plots with error bars

`ember.plots.plot_psi_blocks(gene_name, partition_label, psi_block_df_dir, save_dir, fontsize=18)`

Generates and saves a bar plot of mean psi block values with error bars.

This function reads two CSV files from a specified directory: one for mean psi block values and one for standard deviations. It plots the mean values for a specific gene as a bar plot with corresponding standard deviation error bars. Fontsize can be customized.

Parameters

- **gene_name** (*str*, *Required*) – The name of the gene (row) to select and plot from the CSV files.
- **partition_label** (*str*, *Required*) – The partition label used to find the correct files (e.g., 'Genotype').
- **psi_block_df_dir** (*str*, *Required*) – Path to the directory containing the mean and std CSV files. Files must be named 'mean_Psi_block_df_{partition_label}.csv' and 'std_Psi_block_df_{partition_label}.csv'.
- **save_dir** (*str*, *Required*) – Path to directory to save the output plot image.
- **fontsize** (*int*, *default=18.*) – The base font size for plot labels and text, by default 18.

Return type

None

COMMAND-LINE INTERFACE (CLI)

The `ember` also has a command-line interface (CLI). This allows you to run workflows and plotting directly from the terminal.

A command-line toolkit for `ember`: Entropy Metrics for Biological ExploRation.

```
usage: ember [-h] {light_ember,generate_pvals,plot_partition_specificity,plot_block_
↪specificity,plot_sample_counts,plot_psi_blocks} ...
```

4.1 Positional Arguments

command Possible choices: `light_ember`, `generate_pvals`, `plot_partition_specificity`,
`plot_block_specificity`, `plot_sample_counts`, `plot_psi_blocks`
Available sub-commands

4.2 Sub-commands

4.2.1 `light_ember`

Runs the `ember` entropy metrics and p-value generation workflow on an `AnnData` object.

This function loads an `AnnData` `.h5ad` file, optionally performs balanced sampling across replicates, computes entropy metrics for the specified partition, and generates p-values for Psi and Zeta and optionally `Psi_block` for a block of choice.

Entropy metrics generated:

- Psi : Fraction of information explained by partition of choice
- Psi_block : Specificity of information to a block
- Zeta : Specificity to a partition / distance of Psi_blocks distribution from uniform

Notes:

- Results are saved to `save_dir` as CSV files.
- One CSV file with all entropy metrics.
- One CSV file in a new `Psi_block_df` folder with `Psi_block` values for all blocks in a partition.
- Separate file for p-values.
- Separate files for each partition.

- Alternate file names depending on sampling on or off.

```
ember light_ember [-h] [--no_sampling] [--sample_id_col SAMPLE_ID_COL] [--category_col_↵
↵CATEGORY_COL] [--condition_col CONDITION_COL]
                    [--num_draws NUM_DRAWS] [--save_draws] [--seed SEED] [--no_partition_↵
↵pvals] [--block_pvals] [--block_label BLOCK_LABEL]
                    [--n_pval_iterations N_PVAL_ITERATIONS] [--n_cpus N_CPUS]
                    h5ad_dir partition_label save_dir
```

Positional Arguments

h5ad_dir	Path to the <i>.h5ad</i> file to process. Data should be log1p and depth normalized before running ember. Remove genes with <100 reads before running ember.
partition_label	Column in <i>.obs</i> used to partition cells for entropy calculations (e.g., ‘celltype’, ‘Genotype’, ‘Age’). For interaction terms, create a new column concatenating multiple <i>.obs</i> columns with a semicolon (;).
save_dir	Path to directory where results will be saved.

Sampling Parameters

--no_sampling	Disable balanced sampling. Default: True. Note: If <i>partition_pvals</i> or <i>block_pvals</i> are enabled, sampling will be re-enabled. Default: True
--sample_id_col	Column in <i>.obs</i> with unique identifiers for each sample or replicate (e.g., ‘sample_id’, ‘mouse_id’).
--category_col	Column in <i>.obs</i> defining the primary group to balance across (e.g., ‘disease_status’, ‘mouse_strain’). Interchangeable with <i>condition_col</i> . For >2 variables, create interaction terms by concatenating columns with <i>:</i> .
--condition_col	Secondary column in <i>.obs</i> to balance sampling across (e.g., ‘sex’, ‘treatment’). Interchangeable with <i>category_col</i> . Supports interaction terms.
--num_draws	Number of balanced subsets to generate (default: 100). Default: 100
--save_draws	Save intermediate sampled draws to <i>save_dir</i> (default: False). Default: False
--seed	Random seed for reproducible draws (default: 42). Default: 42

P-value Parameters

--no_partition_pvals	Disable permutation p-value calculation for the main partition. Default: True. Default: True
--block_pvals	Enable permutation p-value calculation for a specific block. Default: False. Default: False
--block_label	Specific value in ‘partition_label’ for block p-values. Required if <i>--block_pvals</i> is set.

--n_pval_iterations Number of permutations for p-value calculation (default: 1000).
Default: 1000

Performance Parameters

--n_cpus Number of CPU cores to use for parallel processing (default: 1). Performance is I/O-bound and may not improve beyond 4–8 cores.
Default: 1

Example:

```
ember light_ember ~/ember_test/test_adata_cwc22.h5ad Genotype ~/ember_test/ --sample_id_col
Mouse_ID --category_col Genotype --condition_col Sex --num_draws 50 --no_partition_pvals
--n_cpus 4
```

4.2.2 generate_pvals

Calculate empirical p-values for entropy metrics from permutation test results.

Entropy metrics generated:

- Psi : Fraction of information explained by partition of choice
- Psi_block : Specificity of information to a block
- Zeta : Specificity to a partition / distance of Psi_blocks distribution from uniform

```
ember generate_pvals [-h] [--block_label BLOCK_LABEL] [--seed SEED] [--n_iterations N_
↪ITERATIONS] [--n_cpus N_CPUS] [--Psi_real PSI_REAL]
                        [--Psi_block_df_real PSI_BLOCK_DF_REAL] [--Zeta_real ZETA_REAL]
                        h5ad_dir partition_label entropy_metrics_dir save_dir sample_id_col_
↪category_col condition_col
```

Positional Arguments

h5ad_dir Path to the *.h5ad* file to process. Data should be log1p and depth normalized before running ember. Remove genes with <100 reads before running ember.

partition_label Column in *.obs* used to partition cells for entropy calculations (e.g., ‘celltype’, ‘Genotype’, ‘Age’). For interaction terms, create a new column concatenating multiple *.obs* columns with a semicolon (;).

entropy_metrics_dir Path to CSV with entropy metrics to use for generating p-values.

save_dir Path to directory where results will be saved.

sample_id_col Column in *.obs* with unique identifiers for each sample or replicate (e.g., ‘sample_id’, ‘mouse_id’).

category_col Column in *.obs* defining the primary group to balance across (e.g., ‘disease_status’, ‘mouse_strain’). Interchangeable with *condition_col*. For >2 variables, create interaction terms by concatenating columns with *:*.

condition_col Column in *.obs* containing the conditions to balance within each category (e.g., ‘sex’, ‘treatment’). Interchangeable with *category_col*. Supports interaction terms.

Named Arguments

--block_label Block in partition to calculate p-values for. Default: None (Psi and Zeta only).

Performance Parameters

--seed Random seed for reproducible draws (default: 42).

Default: 42

--n_iterations Number of iterations to calculate p-values (default: 1000). Use fewer for quick runs, more for reliable results.

Default: 1000

--n_cpus Number of CPUs to use for p-value calculation (default: 1). Set to -1 to use all available cores but one.

Default: 1

Internal Arguments (used by `light_ember`)

--Psi_real Observed Psi values for each gene (pd.Series). Not required for user runs.

--Psi_block_df_real Observed Psi_block values for all blocks in chosen partition (pd.DataFrame). Not required for user runs.

--Zeta_real Observed Zeta values for each gene (pd.Series). Not required for user runs.

Example:

```
ember generate_pvals test_adata_cwc22.h5ad Genotype ~/ember_test/ ~/ember_test/output
Mouse_ID Genotype Sex --block_label WSBJ --n_cpus 4
```

4.2.3 plot_partition_specificity

Generate a Zeta vs. Psi scatter plot to visualize partition-specific genes.

This function reads p-value data, colors genes based on their statistical significance for Psi and Zeta scores, and highlights top “marker” and “housekeeping” genes. Allows for custom highlighting of a user-provided gene list. Font size and color palette can be customized.

```
ember plot_partition_specificity [-h] [--highlight_genes HIGHLIGHT_GENES [HIGHLIGHT_
→GENES ...]] [--fontsize FONTSIZE]
                                [--custom_palette CUSTOM_PALETTE [CUSTOM_PALETTE ...]]
                                partition_label pvals_dir save_dir
```

Positional Arguments

partition_label Label for the partition being plotted, used in the plot title.

pvals_dir Path to input CSV containing p-values and scores (Psi, Zeta, FDRs). CSV must have gene names as its index.

save_dir Path where the output plot image will be saved.

Named Arguments

--highlight_genes List of gene names to highlight and annotate on the plot (default: None).

--fontsize Base font size for plot labels and text (default: 18).

Default: 18

--custom_palette List of 7 hex color codes to customize the color scheme. Order: ['significant by psi', 'significant by zeta', 'highlight genes', 'significant by both', 'circle markers', 'circle housekeeping genes', 'significant by neither']. Default: None (uses built-in palette).

Example:

```
ember plot_partition_specificity Genotype pvals_entropy_metrics_Genotype_WSBJ.csv output/
-highlight_genes Cwc22 -fontsize 25
```

4.2.4 plot_block_specificity

Generate a psi_block vs. Psi scatter plot to visualize block-specific genes.

This function reads p-value data, colors genes based on their statistical significance for Psi and psi_block scores, and highlights the top genes significant in both metrics. Allows for custom highlighting of a user-provided gene list. Font size and color palette can be customized.

```
ember plot_block_specificity [-h] [--highlight_genes HIGHLIGHT_GENES [HIGHLIGHT_GENES ...
→]] [--fontsize FONTSIZE]
                                [--custom_palette CUSTOM_PALETTE [CUSTOM_PALETTE ...]]
                                partition_label block_label pvals_dir save_dir
```

Positional Arguments

partition_label	Label for the partition, used in the plot title.
block_label	Label for the block variable (e.g., a cell type or condition).
pvals_dir	Path to input CSV containing p-values and scores. CSV must have gene names as its index.
save_dir	Path where the output plot image will be saved.

Named Arguments

--highlight_genes	List of gene names to highlight and annotate on the plot (default: None).
--fontsize	Base font size for plot labels and text (default: 18). Default: 18
--custom_palette	List of 6 hex color codes to customize the color scheme. Order: ['significant by psi', 'significant by psi_block', 'highlight genes', 'significant by both', 'circle markers', 'circle housekeeping genes', 'significant by neither']. Default: None (uses built-in palette).

Example:

```
ember plot_block_specificity Genotype WSBJ pvals_entropy_metrics_Genotype_WSBJ.csv output/
-highlight_genes Cwc22 -fontsize 25
```

4.2.5 plot_sample_counts

Generate a bar plot showing the number of unique individuals per category and condition.

This function reads an AnnData object from an .h5ad file in backed mode, calculates the number of unique individuals for each combination of a given category and condition, and visualizes these counts as a grouped bar plot. Font size can be customized.

```
ember plot_sample_counts [-h] [--fontsize FONTSIZE] h5ad_dir save_dir sample_id_col_
↪category_col condition_col
```

Positional Arguments

h5ad_dir	Path to the input AnnData (.h5ad) file.
save_dir	Path to directory to save the output plot image.
sample_id_col	Column name in <i>.obs</i> that contains unique sample IDs.
category_col	Column name to use for the primary categories on the x-axis.
condition_col	Column name to use for grouping the bars (hue).

Named Arguments

--fontsize	Base font size for plot labels and text (default: 18).
	Default: 18

Example:

```
ember plot_sample_counts test_adata_cwc22.h5ad ~/ember_test/output Mouse_ID Genotype Sex
--fontsize 20
```

4.2.6 plot_psi_blocks

Generates and saves a bar plot of mean psi block values with error bars.

This function reads two CSV files from a specified directory: one for mean psi block values and one for standard deviations. It plots the mean values for a specific gene as a bar plot with corresponding standard deviation error bars. Font size can be customized.

```
ember plot_psi_blocks [-h] [--fontsize FONTSIZE] gene_name partition_label psi_block_df_
↪dir save_dir
```

Positional Arguments

gene_name	Name of the gene (row) to select and plot from the CSV files.
partition_label	Partition label used to find the correct files (e.g., 'Genotype').
psi_block_df_dir	Directory containing the mean and std CSV files. Files must be named 'mean_Psi_block_df_{partition_label}.csv' and 'std_Psi_block_df_{partition_label}.csv'.
save_dir	Path to directory to save the output plot image.

Named Arguments

--fontsize	Base font size for plot labels and text (default: 18).
	Default: 18

Example:

```
ember plot_psi_blocks Cwc22 Genotype ~/ember_test/output/Psi_block_df/ ~/em-
ber_test/output/figs --fontsize 30
```

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