# ember Documentation

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CHAPTER

ONE

# 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 infromation explained by partition of choice
- Psi_block : Specificity of infromation to a block
- Zeta : Speicifcty 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* concatnating the two columns of interested 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 interchangable. If balanc-

ing across more than 2 variables, generate interaction terms, create a column in *.obs* concatnating the two (or more) columns of interested 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 categoryto 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 interchangable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatnating the two (or more) columns of interested 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_pavls, 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_pavls, 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 caluclated 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 caluclated 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 q-value: Multiple testing corrected q-values for Psi scores.
- Zeta q-value: Multiple testing corrected q-values for Zeta scores.Correction perfromed 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 q-value: Multiple testing corrected q-values for psi_block scores. Correction perfromed to include all p-values generated in a single file (Psi, psi_block and Zeta).

CHAPTER

TWO

# 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 infromation explained by partition of choice
- Psi_block : Specificity of infromation to a block
- Zeta : Speicifcty 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* concatnating the two columns of interested 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 interchangable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatnating the two (or more) columns of interested 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 interchangable. If balancing across more than 2 variables, generate interaction terms, create a column in *.obs* concatnating the two (or more) columns of interested with a semicolon (:). This way balancing can be done across as many variables as desired.
- **block_label** (*str, default=None*) – Block in partition to calucate 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 calulate p-vales. 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 relibale p-values but will increase runtime significantly.
- **n_cpus** (*int, default=1*) – Number of cpus to use to perfrom 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 q-value: Multiple testing corrected q-values for Psi scores.
- Zeta q-value: Multiple testing corrected q-values for Zeta scores.Correction perfromed 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 q-value: Multiple testing corrected q-values for psi_block scores. Correction perfromed to include all p-values generated in a single file (Psi, psi_block and Zeta).

CHAPTER

# THREE

# PLOTTING FUNCTIONS

## 3.1 Psi vs. Zeta scatter plots

ember.plots.**plot_partition_specificity**(*partition_label*, *pvals_dir*, *save_dir*, *highlight_genes=None*, *q_thresh=0.05*, *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 pallette 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, q-values). 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.
- **q_thresh** (*float, float, default = 0.05*) – Threshold for q-values. Genes are retained if both 'Psi q-value' <= q_thresh and 'Zeta q-value' <= q_thresh.
- **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', 'cirlce 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*, *q_thresh=0.05*, *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 pallette 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.
- **q_thresh** (*float, float, default = 0.05*) – Threshold for q-values. Genes are retained if both 'Psi q-value' <= q_thresh and 'psi_block q-value' <= q_thresh.
- **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 int his order: ['significant by psi', 'significant by psi_block', 'highlight genes', 'significant by both', 'cirlce 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

CHAPTER

FOUR

# EXTRACTING HIGHLY SPECIFIC AND NON-SPECIFIC GENES

## 4.1 Extract highly-specific genes by partition

ember.top_genes.**highly_specific_to_partition**(*partition_label*, *pvals_dir*, *save_dir*, *psi_thresh=0.5*, *zeta_thresh=0.5*, *q_thresh=0.05*)

Identifies significant and specific genes from a ember generated p-values/q-values CSV file based on thresholds for Psi, Zeta, and q-values.

This function reads a CSV file containing Psi and Zeta metrics (and their corresponding q-values), filters genes that meet given significance and specificity thresholds, and saves the resulting subset to a new CSV file.

**Parameters**

- **pvals_dir** (*str, Required*) – Path to the input CSV file (e.g., 'pvals_entropy_metrics_Age_E16.5.csv'). The CSV must include the following columns: 'Psi q-value', 'Zeta q-value', 'Psi', and 'Zeta'.
- **save_dir** (*str, Required*) – Directory where the filtered results CSV will be saved.
- **partition_label** (*Required*) – Name of partition used to generate entropy metrics, used to label saved csv.
- **psi_thresh** (*float, default = 0.5*) – Threshold for Psi values. Only genes with Psi > psi_thresh are kept.
- **zeta_thresh** (*float, Required, default = 0.5*) – Threshold for Zeta values. Only genes with Zeta > zeta_thresh are kept.
- **q_thresh** (*float, default = 0.05*) – Threshold for q-values. Genes are retained if both 'Psi q-value' <= q_thresh and 'Zeta q-value' <= q_thresh.

**Returns**

DataFrame containing the significant and specific genes that meet all threshold criteria. Also saved as "highly_specific_genes_to_{partition_label}.csv" in the specified save directory.

**Return type**

pd.DataFrame

## 4.2 Extract highly-specific genes by block or "Marker genes"

ember.top_genes.**highly_specific_to_block**(*partition_label*, *block_label*, *pvals_dir*, *save_dir*, *psi_thresh=0.5*, *psi_block_thresh=0.5*, *q_thresh=0.05*)

Identifies significant and specific genes from a ember generated p-values/q-values CSV file based on thresholds for Psi, psi_block, and q-values. (Potential marker genes)

This function reads a CSV file containing Psi and psi_block metrics (and their corresponding q-values), filters genes that meet given significance and specificity thresholds, and saves the resulting subset to a new CSV file.

**Parameters**

- **pvals_dir** (*str, Required*) – Path to the input CSV file (e.g., 'pvals_entropy_metrics_Age_E16.5.csv'). The CSV must include the following columns: 'Psi q-value', 'psi_block q-value', 'Psi', and 'psi_block'.
- **save_dir** (*str, Required*) – Directory where the filtered results CSV will be saved.
- **partition_label** (*Required*) – Name of partition used to generate entropy metrics, used to label saved csv.
- **block_label** (*Required*) – Name of block in partition used to generate entropy metrics, used to label saved csv.
- **psi_thresh** (*float, default = 0.5*) – Threshold for Psi values. Only genes with Psi > psi_thresh are kept.
- **psi_block_thresh** (*float, Required, default = 0.5*) – Threshold for psi_block values. Only genes with psi_block > psi_block_thresh are kept.
- **q_thresh** (*float, float, default = 0.05*) – Threshold for q-values. Genes are retained if both 'Psi q-value' <= q_thresh and 'psi_block q-value' <= q_thresh.

**Returns**

DataFrame containing the significant and specific genes that meet all threshold criteria. Also saved as "highly_specific_genes_by_{partition_label}_{block_label}.csv" in the specified save directory.

**Return type**

pd.DataFrame

## 4.3 Extract non-specific or "housekeeping" genes by partition

ember.top_genes.**non_specific_to_partition**(*partition_label*, *pvals_dir*, *save_dir*, *psi_thresh=0.5*, *zeta_thresh=0.5*, *q_thresh=0.05*)

Identifies significant and non-specific genes from a ember generated p-values/q-values CSV file based on thresholds for Psi, Zeta, and q-values. (Potential housekeeping genes)

This function reads a CSV file containing Psi and Zeta metrics (and their corresponding q-values), filters genes that meet given significance and specificity thresholds, and saves the resulting subset to a new CSV file.

**Parameters**

- **pvals_dir** (*str, Required*) – Path to the input CSV file (e.g., 'pvals_entropy_metrics_Age_E16.5.csv'). The CSV must include the following columns: 'Psi q-value', 'Zeta q-value', 'Psi', and 'Zeta'.
- **save_dir** (*str, Required*) – Directory where the filtered results CSV will be saved.
- **partition_label** (*Required*) – Name of partition used to generate entropy metrics, used to label saved csv.
- **psi_thresh** (*float, default = 0.5*) – Threshold for Psi values. Only genes with Psi > psi_thresh are kept.
- **zeta_thresh** (*float, default = 0.5*) – Threshold for Zeta values. Only genes with Zeta < zeta_thresh are kept.

- **q_thresh** (*float, default = 0.05*) – Threshold for q-values. Genes are retained if both 'Psi q-value' <= q_thresh and 'Zeta q-value' <= q_thresh.

**Returns**

DataFrame containing the significant and specific genes that meet all threshold criteria. Also saved as "non_specific_genes_to_{partition_label}.csv" in the specified save directory.

**Return type**

pd.DataFrame

CHAPTER

FIVE

# 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,highly_specific_to_partition,highly_
↪specific_to_block,non_specific_to_partition}
             ...
```

## 5.1 Positional Arguments

**command** Possible choices: light_ember, generate_pvals, plot_partition_specificity, plot_block_specificity, plot_sample_counts, plot_psi_blocks, highly_specific_to_partition, highly_specific_to_block, non_specific_to_partition

Available sub-commands

## 5.2 Sub-commands

### 5.2.1 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 *.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 (:).

**save_dir** Path to directory where results will be saved.

### Sampling Parameters

**--no_sampling** Disable balanced sampling. Default: True. Note: If partition_pvals or block_pvals are enabled, sampling will be re-enabled.

Default: `True`

**--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** Secondary column in *.obs* to balance sampling across (e.g., 'sex', 'treatment'). Interchangeable with category_col. Supports interaction terms.

**--num_draws** Number of balanced subsets to generate (default: 100).

Default: `100`

**--save_draws** Save intermediate sampled draws to save_dir (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 –block_pvals 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

### 5.2.2 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

### 5.2.3 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 ...]]
                                 [--q_thresh Q_THRESH] [--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).

**--q_thresh** Threshold for q-values ('Psi q-value' and 'Zeta q-value'). Must be <= q_thresh (default: 0.05).

Default: `0.05`

**--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

### 5.2.4 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 ...
↪]]
                             [--q_thresh Q_THRESH] [--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).

**--q_thresh** Threshold for q-values ('Psi q-value' and 'psi_block q-value'). Must be <= q_thresh (default: 0.05).

Default: `0.05`

**--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 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

### 5.2.5 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 *.obs* 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

### 5.2.6 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/ ~/ember_test/output/figs –fontsize 30

### 5.2.7 highly_specific_to_partition

Identifies significant and specific genes from an ember generated p-values/q-values CSV file based on thresholds for Psi, Zeta, and q-values. The resulting DataFrame is saved as "highly_specific_genes_to_{partition_label}.csv".

```
ember highly_specific_to_partition [-h] [--psi_thresh PSI_THRESH] [--zeta_thresh ZETA_
↪THRESH]
                                   [--q_thresh Q_THRESH]
                                   partition_label pvals_dir save_dir
```

#### Positional Arguments

**partition_label** Name of partition used to generate entropy metrics, used to label saved csv.

**pvals_dir** Path to the input CSV file (must contain 'Psi q-value', 'Zeta q-value', 'Psi', and 'Zeta').

**save_dir** Directory where the filtered results CSV will be saved.

#### Threshold Parameters

**--psi_thresh** Threshold for Psi values. Genes must have Psi > psi_thresh (default: 0.5).

Default: `0.5`

**--zeta_thresh** Threshold for Zeta values. Genes must have Zeta > zeta_thresh (default: 0.5).

Default: `0.5`

**--q_thresh** Threshold for q-values ('Psi q-value' and 'Zeta q-value'). Must be <= q_thresh (default: 0.05).

Default: `0.05`

**Example:**

ember highly_specific_to_partition Genotype pvals_entropy_metrics_Genotype.csv output/ –psi_thresh 0.6 –zeta_thresh 0.7

### 5.2.8 highly_specific_to_block

Identifies significant and specific genes from an ember generated p-values/q-values CSV file based on thresholds for Psi, psi_block, and q-values. REsultant genes are potential marker genes. The resulting DataFrame is saved as "highly_specific_genes_by_{partition_label}_{block_label}.csv".

```
ember highly_specific_to_block [-h] [--psi_thresh PSI_THRESH]
                               [--psi_block_thresh PSI_BLOCK_THRESH] [--q_thresh Q_
↪THRESH]
                               partition_label block_label pvals_dir save_dir
```

#### Positional Arguments

**partition_label** Name of partition used to generate entropy metrics.

**block_label** Name of block in partition used to generate entropy metrics.

**pvals_dir** Path to the input CSV file (must contain 'Psi q-value', 'psi_block q-value', 'Psi', and 'psi_block').

**save_dir** Directory where the filtered results CSV will be saved.

#### Threshold Parameters

**--psi_thresh** Threshold for Psi values. Genes must have Psi > psi_thresh (default: 0.5).

Default: `0.5`

**--psi_block_thresh** Threshold for psi_block values. Genes must have psi_block > psi_block_thresh (default: 0.5).

Default: `0.5`

**--q_thresh** Threshold for q-values ('Psi q-value' and 'psi_block q-value'). Must be <= q_thresh (default: 0.05).

Default: `0.05`

**Example:**

ember highly_specific_to_block Genotype WSBJ pvals_entropy_metrics_Genotype_WSBJ.csv output/ –psi_thresh 0.6 –psi_block_thresh 0.7

### 5.2.9 non_specific_to_partition

Identifies significant but non-specific genes (potential housekeeping genes) from an ember generated p-values/q-values CSV file based on thresholds for Psi, Zeta, and q-values. Note: The Zeta filter is reversed, keeping Zeta < zeta_thresh. The resulting DataFrame is saved as "non_specific_genes_to_{partition_label}.csv".

```
ember non_specific_to_partition [-h] [--psi_thresh PSI_THRESH] [--zeta_thresh ZETA_
↪THRESH]
                                [--q_thresh Q_THRESH]
                                partition_label pvals_dir save_dir
```

#### Positional Arguments

**partition_label** Name of partition used to generate entropy metrics, used to label saved csv.

**pvals_dir** Path to the input CSV file (must contain 'Psi q-value', 'Zeta q-value', 'Psi', and 'Zeta').

**save_dir** Directory where the filtered results CSV will be saved.

### Threshold Parameters

**--psi_thresh** Threshold for Psi values. Genes must have Psi > psi_thresh (default: 0.5).

Default: `0.5`

**--zeta_thresh** Threshold for Zeta values. Genes must have Zeta < zeta_thresh (default: 0.5) to be considered non-specific.

Default: `0.5`

**--q_thresh** Threshold for q-values ('Psi q-value' and 'Zeta q-value'). Must be <= q_thresh (default: 0.05).

Default: `0.05`

**Example:**

ember non_specific_to_partition Genotype pvals_entropy_metrics_Genotype.csv output/ –psi_thresh 0.6 –zeta_thresh 0.2

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