

# Package ‘TSENAT’

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**Type** Package

**Title** Tsallis Entropy Analysis Toolbox

**Version** 0.99.17

**Description** Quantifies and models isoform-usage complexity in RNA-seq data using Tsallis entropy, a scale-dependent diversity measure. By tuning the entropic index parameter ( $q$ ), TSENAT examines transcriptome heterogeneity at different scales: rare variants (low  $q$ ) or dominant isoforms (high  $q$ ). It enables computing Tsallis entropy and Tsallis divergence from transcript-level estimates, comparing measures between conditions, testing for differences, and visualizing scale-dependent complexity via  $q$ -curves.

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## Description

TSENAT is a Bioconductor package that quantifies transcriptomic complexity at the isoform level using Tsallis entropy, a generalization of Shannon entropy that captures rare-event dynamics and scale-dependent information structure. The package integrates diversity quantification, statistical resampling, linear mixed models, and information-theoretic divergence metrics into a unified analytical framework for comparative transcriptomics.

## Scientific Rationale

Classical Shannon entropy (a special case of Tsallis with  $q=1$ ) treats all probability states equally. Tsallis entropy with order parameter  $q$  weights rare and common transcripts differently:

- $q < 1$ : Emphasizes rare isoforms, capturing tail behavior
- $q = 1$ : Shannon entropy (equipartition weighting)
- $q = 2$ : Simpson entropy (dominance weighting, less sensitive to rare species)
- $q \rightarrow \infty$ : Reciprocal of the maximum transcript fraction

This multiscale perspective reveals condition-specific dependencies in splicing architecture and detects genes with asymmetric isoform distributions that traditional metrics miss.

## Workflow Overview

TSENAT implements a complete analytical pipeline:

1. **Diversity Quantification:** Compute Tsallis entropy  $H_q$  across a range of  $q$ -values (0 to  $\infty$ ) for each gene-condition group. Returns Hill numbers ( $D_q = \exp(H_q)$ ) for interpretable effective isoform counts.
2. **Jackknife Resampling:** Generate confidence intervals via leave-one-out resampling. Identifies genes with statistically significant entropy outliers and condition-biased isoform usage (isoform switching).
3. **Linear Mixed Models:** Tests gene-by-condition interaction effects on diversity landscapes using nlme, MASS, or geepack backends. Supports paired designs and random effect structures.
4. **Divergence Analysis:** Computes Tsallis divergence (generalized Kullback-Leibler) between condition groups. Detects differential transcript complexity via effect size estimation.
5. **Visualization:** Generates publication-ready plots:  $q$ -curves, volcano plots, heatmaps, violin distributions, and dimension-reduced embeddings.

## Core Functions

`TSENAT` Main orchestration function. Executes complete pipeline with configurable method backends and parallelization.

`TSENAT_config` Configure analysis parameters:  $q$ -values, condition grouping, sample pairing, and method selection.

`calculate_diversity` Compute Tsallis entropy and Hill numbers. Vectorized over genes and  $q$ -values with numerical stability safeguards.

`calculate_jeo` Resampling-based confidence intervals for diversity. Identifies outlier genes and validates  $q$ -value signal.

`calculate_sait` Statistical testing for gene-by-condition interactions. LMM, GAM, and rank-based approaches.

`calculate_divergence` Tsallis divergence between groups with effect size (Cohen's d, rank-biserial) and hypothesis testing.

`filter_analysis` Filter genes by diversity, effect size, or interaction significance (adjustable FDR, Benjamini-Hochberg).

### Statistical Methods

**Diversity Metrics** Tsallis Entropy:  $H_q = \frac{1}{q-1} (1 - \sum_i p_i^q)$  Hill Numbers:  $D_q = (\sum_i p_i^q)^{\frac{1}{1-q}}$

**Divergence** Tsallis Divergence:  $D_q(p||r) = \frac{1}{q-1} (1 - \sum_i p_i^q r_i^{1-q})$  Kullback-Leibler distance (q=1 limit):  $D_{KL}(p||r) = \sum_i p_i \log(p_i/r_i)$

**Resampling** Jackknife leave-one-out, block bootstrap (paired data), and percentile/BCA confidence intervals with accuracy enhancement for skewed distributions.

**Interaction Testing** LMM:  $H_{g,c} = \beta_0 + \beta_g(\text{gene}) + \beta_c(\text{condition}) + \beta_{gc}(\text{gene} \times \text{condition}) + \epsilon$   
With random effects for paired/nested designs.

### Key Features

- **Multiscale q-parameter sweep:** Detects biological signals at different probability scales without multiple testing correction for q-values.
- **Robust resampling:** Leave-one-out jackknife + block bootstrap handles paired designs and maintains correlation structure.
- **Multiple method backends:** nlme, MASS, geepack, or rank-based (Scheirer-Ray-Hare, sign test) for model flexibility.
- **FDR-corrected testing:** Benjamini-Hochberg adjustment across genes and q-values.
- **Production-grade visualization:** ggplot2-based plots optimized for large gene sets and publication quality.
- **Reproducibility tracking:** Automatic logging of function calls, parameters, timestamps, and package version for audit trails.
- **S4 object-oriented design:** Unified `TSENATAnalysis` container ensures metadata integrity throughout the pipeline.

### Data Input & Output

**Input** SummarizedExperiment object with gene-by-sample count matrix (raw or normalized) and sample metadata (condition, pairing, batch).

**Output** `TSENATAnalysis` S4 object containing: (1) Diversity values across q-parameter range, (2) Jackknife CIs and switching indicators, (3) LMM/interaction statistics with p-values and effect sizes, (4) Divergence metrics with hypothesis test results, (5) Cached publication-ready plots, (6) Reproducibility metadata.

### Interpreter Notes

- **q-value sweeps reveal biological complexity:** Genes showing entropy peaks at intermediate q-values (0.5-1.5) suggest balanced isoform distributions; peaks at  $q \rightarrow \infty$  indicate dominant-isoform architectures.
- **Interaction q-profiles:** q-dependent gene-by-condition interactions signal condition-specific isoform switching; flat profiles indicate constitutive splicing.
- **Divergence as biological distance:** Tsallis divergence quantifies transcript composition distance; high divergence ( $D_q > 0.5$ ) indicates distinct splicing programs.

- **Statistical interpretation:** Jackknife CIs that exclude the point estimate (valid for skewed distributions) occur with bounded entropies; not a software error.

## References

Tsallis entropy and information theory:

- Tsallis, C. (1988). Possible generalization of Boltzmann-Gibbs statistics. *Journal of Statistical Physics*, 52(1-2), 479-487.
- Furuichi, S. (2006). Information theoretical properties of Tsallis entropies. *Journal of Mathematical Physics*, 47(2), 023302.

Hill numbers and diversity indices:

- Hill, M. O. (1973). Diversity and evenness: A unifying notation and its consequences. *Ecology*, 54(2), 427-432.
- Chao, A., Chiu, C. H., & Jost, L. (2014). Unifying species diversity, phylogenetic diversity, functional diversity, and related similarities into a single framework. *Annual Review of Ecology, Evolution, and Systematics*, 45, 297-324.

Isoform switching and transcriptomics:

- Vitting-Seerup, K., et al. (2017). IsoformSwitchAnalyzeR enables robust detection of isoform switches and novel isoforms in the human transcriptome from long-read cDNA sequencing data. *Genome Biology*, 18(1), 122.

## S4 Container

[TSENATAnalysis-class](#) — Central container unifying all analysis components. Access results via slots: @diversity\_results, @sait\_results, @jackknife\_results, @divergence\_results, or accessor methods [se](#), [metadata<-](#), and [results](#).

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## See Also

[TSENAT](#) for running the complete analysis pipeline. [se](#), [metadata<-](#), and [results](#) for accessor functions. [TSENATAnalysis-class](#) for the S4 object structure. [build\\_analysis](#) for creating TSENATAnalysis objects.

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AllGenerics

*Generic Functions for TSENATAnalysis S4 Class*


---

### Description

Define generic functions for accessing components of TSENATAnalysis objects. These are the recommended way to extract results from analysis objects, following Bioconductor best practices.

### Value

These generic functions return different types depending on the specific method: - Accessor methods return data.frames or lists containing analysis results - See individual method documentation for specific return types

---

build\_analysis

*Build a Complete TSENATAnalysis Object*


---

### Description

Convenience wrapper that combines `.build_se()` and `TSENATAnalysis()` into a single function call. This creates a complete analysis object ready for Tsallis entropy computation and downstream analysis.

### Usage

```
build_analysis(
  readcounts = NULL,
  salmon_dir = NULL,
  tx2gene,
  assay_name = "counts",
  metadata = NULL,
  tpm = NULL,
  effective_length = NULL,
  config = list(),
  skip = FALSE,
  verbose = FALSE
)
```

### Arguments

readcounts	A matrix or data.frame of transcript-level read counts with transcript IDs as row names and sample names as column names. Typically output from quantification tools (SALMON, kallisto, etc. ). Optional when salmon_dir is provided (in which case readcounts are auto-loaded from quant.sf files).
salmon_dir	Optional character path to directory containing Salmon quantification output. Expected structure: salmon_dir/sample_name/quant.sf. When provided, automatically discovers and reads all quant.sf files. If both readcounts and salmon_dir are provided, salmon_dir takes precedence. Default: NULL.

tx2gene	Either: - A path to a GFF3 or GFF3.gz file containing transcript-to-gene mapping - A path to a TSV file with columns 'Transcript' and 'Gene' - A data.frame with transcript-to-gene mapping
assay_name	Character. Name for the assay (default: 'counts').
metadata	Optional data.frame with sample metadata. Should have sample names as row names and metadata columns (e.g., sample_type, condition, etc.). If NULL, will attempt to read from config\$metadata. Priority: explicit metadata argument > config\$metadata > NULL.
tpm	REQUIRED matrix of transcript-level TPM values (Transcripts Per Million). Must be provided and will be stored in the SummarizedExperiment for use during diverse filtering. Same dimensions as readcounts required (rows = transcripts, columns = samples). Typically from SALMON quantification output. If not provided to build_analysis(), subsequent filter_analysis() calls will fail with an explicit error message.
effective_length	REQUIRED numeric vector of transcript effective lengths (e.g., from SALMON EffectiveLength column). Length must match nrow(readcounts). Typically obtained as the median effective length across all samples. If not provided to build_analysis(), the data will not be stored for later use in length-normalized calculations.
config	Optional list of configuration parameters to store in the TSENATAnalysis object. Can also contain config\$metadata which will be used if the metadata argument is NULL. Following Bioconductor best practices (fail-fast principle), create configuration via TSENAT_config() FIRST, then pass to build_analysis() at object construction time. This ensures invalid parameters are caught immediately, before analysis proceeds. See examples below for recommended usage pattern.
skip	Logical. If TRUE, allow unmapped transcripts (transcripts not found in tx2gene mapping) and remove them from analysis. If FALSE (default), stop with an error when unmapped transcripts are detected. Useful for handling data with transcript IDs that don't match the annotation file provided.
verbose	Logical. If TRUE, print informative messages during execution (e.g., Salmon sample discovery, progress on data loading). Default: TRUE.

## Details

When using `salmon_dir`, the function automatically:

1. Discovers all Salmon sample folders and quant.sf files
2. Reads transcript counts (NumReads), TPM, and effective\_length
3. Extracts sample names from directory structure
4. Creates count matrix ready for analysis

The `salmon_dir` parameter provides a convenient alternative to manually constructing the readcounts matrix, especially useful in Galaxy workflows.

## Value

A TSENATAnalysis S4 object with:

@se                    The SummarizedExperiment containing transcript counts and metadata

```

@config          Analysis configuration (empty list or user-provided)
@diversity_results  Empty list (populated by calculate_diversity())
@divergence_results  Empty list (populated by calculate_divergence())
@sait_results      Empty list (populated by calculate_sait())
@jackknife_results  Empty list (populated by jackknife functions)
@plots            Empty list (populated by plotting functions)
@metadata          Metadata with package version and creation timestamp

```

### CRITICAL: TPM and effective\_length Requirements

Both `tpm` and `effective_length` MUST be provided to ensure correct filtering and normalization in downstream analysis:

- `filter_analysis()` requires TPM data (stored in metadata). If TPM is missing, the function will fail with an explicit error message that guides you to pass it to `build_analysis()`.
- `calculate_diversity()` uses `effective_length` for length-normalized entropy calculations.

Following Bioconductor best practices (fail-fast principle), these are explicit parameters, not optional. They must be passed at object construction time:

```

analysis <- build_analysis(
  readcounts = readcounts,
  metadata = metadata_df,
  tx2gene = gff3_file,
  tpm = tpm, # REQUIRED from Salmon output
  effective_length = effective_length, # REQUIRED from Salmon output
  config = config
)

```

This wrapper combines two steps into one:

1. Call `.build_se()` to create a `SummarizedExperiment` from transcript counts
2. Wrap the result in `TSENATAnalysis()` to create the analysis object

The returned object is ready for diversity analysis via `calculate_diversity()`.

If you need to inspect or filter the `SummarizedExperiment` before creating the `TSENATAnalysis` object, call `.build_se()` and `TSENATAnalysis()` separately.

### See Also

[TSENATAnalysis](#) for the S4 class structure [calculate\\_diversity](#) for computing Tsallis entropy

### Examples

```

# Create example transcript count data
set.seed(42)
n_genes <- 10
n_isoforms_per_gene <- 3
n_isoforms <- n_genes * n_isoforms_per_gene

```

```

n_samples <- 10

# Generate count matrix
counts <- matrix(rpois(n_isoforms * n_samples, lambda = 20),
                nrow = n_isoforms, ncol = n_samples)
rownames(counts) <- paste0('TX_', 1:n_isoforms)
colnames(counts) <- paste0('Sample_', 1:n_samples)

# Create tx2gene mapping
tx2gene <- data.frame(
  Transcript = rownames(counts),
  Gene = rep(paste0('GENE_', 1:n_genes), each = n_isoforms_per_gene))

# Create sample metadata
metadata <- data.frame(
  sample = colnames(counts),
  condition = rep(c('control', 'treatment'), each = 5),
  row.names = colnames(counts))

# Build analysis object - use NAMED parameters to avoid confusion
# Method 1: With explicit tx2gene data.frame (most common)
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(
  readcounts = counts,
  tx2gene = tx2gene,
  metadata = metadata,
  config = config)

# Verify the analysis object was created
analysis
print(dim(analysis))

# Method 2: From Salmon quantification folder
# Requires directory structure like:
#   salmon_output/
#   sample1/quant.sf
#   sample2/quant.sf
#   ...
#
# salmon_dir <- '/path/to/salmon/directory'
#
# First create sample metadata matching Salmon sample names
# salmon_metadata <- data.frame(
#   condition = c('control', 'control', 'treatment', 'treatment'),
#   row.names = c('sample1', 'sample2', 'sample3', 'sample4')
# )
#
# analysis_salmon <- build_analysis(
#   salmon_dir = salmon_dir,
#   tx2gene = 'annotation.gff3.gz', # Auto-parsed from GFF3
#   metadata = salmon_metadata
# )
#
# Method 3: Hybrid - Salmon counts with manual tx2gene
# analysis_hybrid <- build_analysis(
#   salmon_dir = salmon_dir,
#   tx2gene = tx2gene, # data.frame instead of file

```

```

# metadata = salmon_metadata
# )
#
# Method 4: Pass metadata via config (parameter resolution pattern)
# cfg <- TSENAT_config()
# cfg$metadata <- metadata
# analysis_with_config <- build_analysis(
#   readcounts = counts,
#   tx2gene = tx2gene,
#   config = cfg
#   # Note: metadata argument omitted - will be read from config$metadata
# )

# Advanced: Assigning metadata to assays after object creation
# When adding metadata to SummarizedExperiment assays, always use the
# S4Vectors namespace to ensure proper method dispatch:
#
# se <- getSE(analysis)
# assay_with_ci <- SummarizedExperiment::assay(se, 'log2fc_ci')
# S4Vectors::metadata(assay_with_ci)$lower <- ci_lower_bounds
# S4Vectors::metadata(assay_with_ci)$upper <- ci_upper_bounds
#
# Note: Avoid using metadata(assay) without the namespace - this can
# cause silent failures in S4 object metadata assignment.

```

---

calculate\_assumptions *Test statistical assumptions on diversity data in TSENATAnalysis*

---

## Description

Test statistical assumptions on diversity data in TSENATAnalysis

## Usage

```

calculate_assumptions(
  analysis,
  q = NULL,
  checks = "rank",
  alpha = 0.05,
  format = "text",
  ...
)

## S4 method for signature 'TSENATAnalysis'
calculate_assumptions(
  analysis,
  q = NULL,
  checks = "rank",
  alpha = 0.05,
  format = "text",
  ...
)

```

**Arguments**

analysis	TSENATAnalysis object with diversity results stored in @diversity_results.
q	numeric. Q-value(s) to extract from diversity results. If NULL, uses the first available diversity result or q=1.0.
checks	character. Which assumptions to test (default: 'rank'). Presets: - 'rank': core assumption checks (exchangeability, monotonicity, consistency) - 'all': all checks including GAM diagnostics Explicit: character vector like c('exchangeability', 'monotonicity').
alpha	numeric. Significance level for tests (default: 0.05).
format	character. Output format when used with results(). 'text' (default): formatted text output for display 'list': returns structured list for programmatic access.
...	Additional arguments (output_file, verbose for file output).

**Details**

This wrapper calls .calculate\_assumptions() on diversity data extracted from the analysis object. Evaluates data stability and consistency across dimensions. Results include:

**exchangeability** Permutation test for independence and temporal/spatial structure

**monotonicity** Spearman correlation consistency across rows

**consistency** Kendall's W concordance and ICC across samples

**\*\*Data Extraction Priority:\*\*** 1. If q specified: uses diversity result for that q-value 2. If q NULL: uses first available diversity result 3. If no diversity results: extracts from cached combined result (@metadata\$diversity\_combined)

**Value**

Modified TSENATAnalysis object with assumption test results stored in @metadata\$rankbased\_assumptions.

**Examples**

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Build analysis from vignette data and create small subset
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  q_values = seq(0, 2, by = 0.05),
  paired = FALSE
)
analysis <- build_analysis(
  readcounts = readcounts,
  metadata = metadata_df,
```

```

tx2gene = gff3_dataset,
config = config,
tpm = tpm,
effective_length = effective_length
)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes = 200)
analysis <- calculate_diversity(analysis, q = c(0.5, 1.0, 1.5))
analysis <- calculate_assumptions(analysis, q = 1.0)
# Check results using rank_test accessor
results_df <- results(analysis, type = 'rank_test')

```

---

calculate\_concordance *Compare method concordance for differential analysis results*

---

## Description

Compares statistical results from two different methods (typically SAIT/GAM for continuous data and Scheirer-Ray-Hare rank tests) to assess agreement and identify genes detected by one method but not the other.

## Usage

```
calculate_concordance(analysis_sait, analysis_rank = NULL, ...)
```

```

## S4 method for signature 'TSENATAnalysis'
calculate_concordance(
  analysis_sait,
  analysis_rank = NULL,
  verbose = FALSE,
  output_file = NULL,
  ...
)

```

## Arguments

analysis_sait	TSENATAnalysis object containing SAIT/GAM analysis results (from calculate_sait()).
analysis_rank	TSENATAnalysis object or NULL. If NULL, uses legacy single-object API with analysis_sait containing both results. If provided, compares SAIT results from analysis_sait with rank-test results from analysis_rank.
...	Additional arguments for future extensibility.
verbose	logical. Print progress messages (default: FALSE).
output_file	character or NULL. Optional file path to save results. Supported formats: .rds (for S4 objects). Default: NULL (no file output).

## Details

Compares results from two different statistical methods (typically GAM for continuous and Scheirer-Ray-Hare for rank-based analysis) on the same data. Identifies: - Genes significant in both methods (high confidence) - Genes detected by one method only (potential false positives or method-specific signal) - Spearman correlation of p-values (overall agreement trends)

**Value**

Modified TSENATAnalysis object with concordance results stored in: @metadata\$method\_concordance:

**comparison\_df** Data frame comparing results from both methods

**spearman\_rho** Spearman correlation between adjusted p-values

**high\_confidence** Genes with strong agreement

**agreement\_table** Contingency table of significant/non-significant calls

**sait\_method** Method name used for SAIT/GAM analysis

**rank\_method** Method name used for rank-based analysis

**timestamp** When concordance was computed

**Examples**

```
# Compare results from SAIT and rank-based testing
# (Requires pre-computed analysis objects from calculate_sait and calculate_srh)
# results_df <- results(calculate_concordance(analysis_sait, analysis_rank))
```

---

calculate\_divergence *Calculate divergence metrics and store in TSENATAnalysis*

---

**Description**

Wrapper around .calculate\_divergence() that manages TSENATAnalysis object. Calculates Tsallis divergence between experimental conditions for transcripts across multiple q-values to detect condition-specific transcript remodeling.

**Usage**

```
calculate_divergence(
  analysis,
  q = NULL,
  verbose = FALSE,
  nthreads = NULL,
  output_file = NULL,
  control_group = NULL,
  paired = FALSE,
  method = NULL,
  bootstrap = FALSE,
  nboot = NULL,
  progress = FALSE,
  ...
)
```

**Arguments**

analysis	TSENATAnalysis object.
q	numeric. Q-value(s) for divergence (single value or vector). If NULL, reads from analysis@config\$q. If not in config, defaults to seq(0.01, 2, by = 0.05) for full spectrum computation.
verbose	logical. Print progress messages. Default: TRUE.
nthreads	numeric or NULL. Number of CPU threads for parallel processing. If NULL, reads from @config\$nthreads (or defaults to 1).
output_file	character or NULL. Optional file path to save results. Supported formats: .rds (for S4 objects), .tsv, .csv, .txt (for tables). Default: NULL (no file output).
control_group	character or NULL. Control group identifier for divergence comparison. If NULL, reads from @config\$control_group if available.
paired	logical. Whether to use paired design. Default: FALSE. If not specified, reads from @config\$paired if available.
method	character or NULL. Statistical method for divergence calculation. If NULL, reads from @config\$method if available.
bootstrap	logical. Whether to compute bootstrap confidence intervals. Default: FALSE. If not specified, reads from @config\$bootstrap if available.
nboot	numeric or NULL. Number of bootstrap replicates. Default: NULL. If NULL, reads from @config\$nboot if available.
progress	logical. Show progress bar during computation. Default: FALSE.
...	Additional arguments passed to the base divergence function.

**Details****Key Features:**

- Multi-q analysis: Divergence computed across full q-spectrum simultaneously
- Bootstrap confidence intervals: Quantify uncertainty in divergence estimates
- Multiple testing correction: Hochberg, Benjamini-Yekutieli, or no correction
- Paired designs: Supports paired/repeated measures via subject\_col parameter
- Effect size reporting: Log-fold-change and confidence intervals per gene
- Flexible control group: Compare any condition vs. any other condition

**\*\*Mathematical Background:\*\*** Tsallis divergence  $D_q$  between two probability distributions:

$$D_q(P||Q) = (\log_2(N) - \text{entropy}_q(P) + \text{entropy}_q(Q)) / (q - 1)$$

Measures how much transcript composition changes from control to condition. Values near 0: Similar isoform composition; Large positive values: Major change.

**\*\*Example Use Case:\*\*** Control sample: All reads from dominant isoform (low entropy)

Tumor sample: Reads spread across multiple isoforms (high entropy)

Result: Large divergence indicating condition-specific isoform switching.

**\*\*Parameter Resolution:\*\*** Parameters are resolved in priority order: 1. Explicit arguments passed to function 2. Values from analysis@config (if present) 3. Function defaults

Requires diversity results from calculate\_diversity() as prerequisite.

**Value**

Modified TSENATAnalysis with divergence metrics in @divergence\_results (stored as list of data.frames or matrices).

**Examples**

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package = 'TSENAT')

# Build analysis from vignette data and create manageable subset
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)
# Use 200+ genes to ensure diversity filtering doesn't remove all genes
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes = 200)

# Compute diversity first (required for divergence)
analysis <- calculate_diversity(analysis, q = c(0.5, 1.0, 1.5))

# Calculate divergence across q-values
analysis <- calculate_divergence(analysis, q = c(0.5, 1.0, 1.5))

# Check divergence results using unified accessor
head(results(analysis, type = 'divergence'))
```

---

calculate\_diversity     *Calculate diversity and store in TSENATAnalysis*

---

**Description**

Wrapper around .calculate\_diversity() that manages TSENATAnalysis object. Calculates Tsallis entropy (diversity) across multiple q-values for each gene to quantify isoform complexity and transcript heterogeneity.

**Usage**

```
calculate_diversity(
  analysis,
  q = NULL,
```

```

norm = TRUE,
norm_method = NULL,
reference_group = NULL,
verbose = NULL,
show_messages = FALSE,
what = NULL,
nthreads = NULL,
pseudocount = NULL,
min_valid_frac = NULL,
shrinkage = NULL,
bootstrap = NULL,
nboot = NULL,
bootstrap_method = NULL,
bootstrap_ci = NULL,
bootstrap_include_diagnostics = NULL,
output_file = NULL,
...
)

```

### Arguments

analysis	TSENATAnalysis object.
q	numeric. Q-value(s) for Tsallis entropy (single value or vector). If NULL, reads from analysis@config\$q. If not in config, defaults to seq(0.01, 2, by = 0.05) for full spectrum computation.
norm	logical or character. Normalization method: TRUE, FALSE, 'none', 'range', 'zscore', 'log_odds_ratio', 'relative_reference'. If NULL, reads from @config\$norm or defaults to TRUE.
norm_method	character. Post-hoc normalization method applied after diversity computation. Options: <ul style="list-style-type: none"> <li>'default' - Simple normalization by theoretical maximum (current behavior)</li> <li>'zscore' - Z-score normalization per q-value</li> <li>'log_odds_ratio' - Log-odds ratio relative to max entropy (q and isoform-aware)</li> <li>'relative_reference' - Divide by reference group mean (requires reference_group)</li> <li>NULL - No post-hoc normalization (default)</li> </ul> If NULL, reads from @config\$norm_method if available.
reference_group	character. For norm_method = 'relative_reference', the reference group column name (e.g., from colData). If NULL, uses first group in colData. If NULL, reads from @config\$reference_group if available.
verbose	logical. Print progress messages. Default: TRUE. If not specified, reads from @config\$verbose if available.
show_messages	logical. Display verbose messages during computation. Default: FALSE.
what	character. Output type: 'S' (entropy) or 'D' (diversity). Default: 'S'. If NULL, reads from @config\$what if available.
nthreads	numeric or NULL. Number of CPU threads for parallel processing. If NULL, reads from @config\$nthreads (or defaults to 1).

pseudocount	numeric or character. Pseudocount value or 'auto'. Default: 0. If NULL, reads from @config\$pseudocount if available.
min_valid_frac	numeric. Minimum fraction of valid samples for gene filtering. Default: NULL. If NULL, reads from @config\$min_valid_frac if available.
shrinkage	character. Shrinkage method: 'none' or 'empirical_bayes'. Default: 'none'. If NULL, reads from @config\$shrinkage if available.
bootstrap	logical. Compute bootstrap confidence intervals. Default: FALSE. If not specified, reads from @config\$bootstrap if available.
nboot	numeric or NULL. Number of bootstrap replicates. Default: NULL. If NULL, reads from @config\$nboot if available.
bootstrap_method	character. Bootstrap method: 'percentile' or others. Default: 'percentile'. If NULL, reads from @config\$bootstrap_method if available.
bootstrap_ci	numeric. Bootstrap confidence interval level (0-1). Default: 0.95. If NULL, reads from @config\$bootstrap_ci if available.
bootstrap_include_diagnostics	logical. Include bootstrap diagnostic information. Default: FALSE. If NULL, reads from @config\$bootstrap_include_diagnostics if available.
output_file	character or NULL. Optional file path to save results. When provided, generates TWO files: <ol style="list-style-type: none"> <li>1. Primary output: Analysis object (.rds) or table (.tsv/.csv/.txt)</li> <li>2. Secondary output: Diversity spectrum statistics (TSV format) with suffix _spectrum.tsv</li> </ol> <p>Example: output_file = 'analysis.rds' generates:</p> <ul style="list-style-type: none"> <li>• analysis.rds - TSENATAnalysis object</li> <li>• analysis_spectrum.tsv - Spectrum statistics</li> </ul> <p>The spectrum file contains columns: q, central (median diversity), spread (IQR), count, and group (if grouping variable available). Default: NULL (no file output).</p>
...	Additional arguments passed to underlying functions for extensibility.

## Details

### Key Features:

- Multi-q analysis: Entropy computed for q = 0.01 to 2.00 (by default)
- Normalized entropy: Scale to [0, 1] using theoretical maximum log(m)
- Bootstrap confidence intervals: Quantify uncertainty in estimates
- TPM normalization: Optional SALMON TPM-based weighting
- Multiple normalization methods: Range, Z-score, log-odds-ratio, relative
- Spectrum computation: Aggregate diversity statistics across all q-values

**\*\*Mathematical Background:\*\*** Tsallis entropy  $H_q$  for q-parameter:

$$H_q(X) = (1/(q-1)) * (1 - \sum p_i^q) \quad [\text{for } q \neq 1]$$

$$H_1(X) = -\sum p_i * \log(p_i) \quad [\text{Shannon entropy, limit } q \rightarrow 1]$$

where  $p_i$  = relative abundance of isoform  $i$  for a gene. Larger  $q$  emphasizes dominant isoforms; smaller  $q$  emphasizes rare ones.

**\*\*Example Interpretation:\*\***

- Gene with 1 isoform:  $H_q = 0$  for all  $q$  (no diversity)
- Gene with 2 equal isoforms:  $H_q \sim 0.5-1.0$  depending on  $q$
- Gene with  $m$  equally abundant isoforms:  $H_q = 1.0$  (maximum diversity)

This wrapper calls `.calculate_diversity()` once per  $q$ -value, storing results as Summarized-Experiment objects. It extracts key parameters from `analysis@config` with priority resolution (`explicit > @config > default`).

**\*\*Diversity Spectrum Computation:\*\*** By default, this function computes and saves a diversity spectrum (aggregated statistics across all  $q$ -values and groups) when `output_file` is provided. The spectrum contains:

- `q`: Diversity parameter value
- `central`: Median (or mean) diversity across all genes
- `spread`: IQR (or SD) around central value
- `count`: Number of valid measurements
- `group`: Condition group (if applicable)

This provides a quick summary of how diversity changes across  $q$ -values, useful for  $q$ -curve visualization and statistical comparisons. Spectrum is saved as: `*_spectrum.tsv`

**\*\*Parameter Priority Resolution:\*\***

**q** Priority 1 (`explicit`) > Priority 2 (`@config$q`) > Priority 3 (default: `seq(0.01, 2, by=0.05)`).

Accepts single or multiple  $q$ -values (for spectrum computation).

**nthreads** Priority: `explicit > @config$nthreads > 1`

**verbose** Priority: `explicit > @config$verbose > TRUE`

**bootstrap** Priority: `explicit > @config$bootstrap > FALSE`

**pseudocount** Priority: `explicit > @config$pseudocount > 0`

**norm** Priority: `explicit > @config$norm > TRUE`

**what** Priority: `explicit > @config$what > 'S'` (Tsallis entropy)

**\*\*Audit Trail:\*\*** After execution, check:

- `analysis@config$last_diversity_run$parameters_used`: Actual parameters (not original `@config`)
- `attr(diversity(analysis, q=X), 'computed_with')`: Per- $q$ -value metadata (timestamp, bootstrap setting, `nthreads`, etc.)

For additional details on diversity spectrum calculations and normalization methods, see the package vignettes.

## Value

Modified TSENATAnalysis object with diversity results stored in `@diversity_results`, keyed by `'q_X.XX...'` format (e. g. , `'q_1.000'`). When `output_file` is provided, also generates:

- Primary file: Analysis object or table export
- Spectrum file: `*_spectrum.tsv` containing aggregated diversity statistics across  $q$ -values and groups

**Examples**

```

# Load vignette data and build analysis
data(readcounts)
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'

config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)

# Filter to manageable size (use 200+ genes to survive diversity filtering)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)

# Compute diversity and access results using unified accessor
analysis <- calculate_diversity(analysis, q = c(0.5, 1.0), verbose =
FALSE)
head(results(analysis, type = 'diversity', q = 1.0))

```

---

calculate\_effect\_sizes

*Compute Effect Sizes from Divergence Results (S4 Wrapper)*

---

**Description**

S4 wrapper for `.calculate_effect_sizes()` that extracts divergence and LM results directly from a `TSENATAnalysis` object.

**Usage**

```

calculate_effect_sizes(
  analysis,
  significance_threshold = NULL,
  enrich_per_q_pattern = NULL,
  verbose = NULL,
  output_file = NULL,
  ...
)

```

**Arguments**

`analysis` `TSENATAnalysis`. An S4 object containing divergence results (from `calculate_divergence()`) and SAIT interaction results (from `calculate_sait()`).

```

significance_threshold
    numeric. Adjusted p-value threshold for filtering significant genes (default:
    0.05).
enrich_per_q_pattern
    logical. If TRUE, enriches results with per-q divergence patterns (default:
    TRUE).
verbose
    logical. If TRUE, print diagnostic messages (default: TRUE).
output_file
    character or NULL. Optional file path to save results. Supported formats: .rds
    (for S4 objects). Default: NULL (no file output).
...
    Additional arguments passed to the base function.

```

## Details

**\*\*Workflow steps:\*\***

**Validating** Input analysis object and required results

**Extracting** Divergence SE and SAIT results from analysis slots

**Enriching** Divergence SE with gene names via tx2gene or direct mapping

**Computing** Effect sizes using base `.calculate_effect_sizes()`

**Storing** Results in metadata with function call tracking

**\*\*Parameter resolution priority\*\*** (explicit > metadata > default):

- `significance_threshold`: Uses explicit arg, else `metadata(analysis)$significance_threshold`, else 0.05
- `enrich_per_q_pattern`: Uses explicit arg, else `metadata(analysis)$enrich_per_q_pattern`, else TRUE
- `verbose`: Uses explicit arg, else `metadata(analysis)$verbose`, else TRUE

Results are accessed via: `metadata(analysis)$effect_sizes_divergence`

## Value

Modified TSENATAnalysis with effect size results stored via `metadata(analysis)$effect_sizes_divergence`. Returns the analysis object visibly to support piping and method chaining.

## See Also

[calculate\\_divergence](#) for divergence wrapper, [calculate\\_sait](#) for SAIT interaction wrapper

## Examples

```

# Setup: Create test analysis with divergence and SAIT interaction results
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

```

```

# Configure analysis parameters (best practice for reproducibility)
## Not run:
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  subject_col = 'paired_samples',
  paired = TRUE,
  control = 'normal',
  q = seq(0, 2, by = 0.5) # Multiple q-values for SAIT (5 unique values)
)
analysis <- build_analysis(readcounts = readcounts, tx2gene =
  gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)

analysis <- filter_analysis(analysis, stringency = 'severe')
analysis <- calculate_diversity(analysis)
analysis <- calculate_divergence(analysis)
analysis <- suppressWarnings(calculate_sait(analysis, method = 'gam'))

# Compute effect sizes from divergence results
analysis <- calculate_effect_sizes(analysis,
  significance_threshold = 0.05)

# Access results using unified results accessor
effect_size_results <- results(analysis, type = 'effect_sizes_divergence')

# View structure of results
str(effect_size_results, max.level = 1)

## End(Not run)

```

---

calculate\_jeo

*Jackknife resampling with confidence intervals*


---

## Description

Jackknife resampling with confidence intervals

## Usage

```

calculate_jeo(
  analysis,
  q = NULL,
  norm = NULL,
  log_base = NULL,
  top_n = NULL,
  verbose = NULL,
  nthreads = NULL,
  pseudocount = NULL,
  output_file = NULL,
  ...
)

```

**Arguments**

analysis	TSENATAnalysis object.
q	numeric. Q-value(s) for jackknife estimation. If NULL, reads from analysis@config\$q. Default: c(0, 0.5, 1, 1.5, 2) (matches calculate_jis spectrum).
norm	logical. Normalization flag. Default: NULL (uses @config\$norm or TRUE).
log_base	numeric. Logarithm base for entropy normalization. Default: NULL (uses e).
top_n	numeric. Number of top outlier samples to report. Default: 5.
verbose	logical. Print jackknife results summary. Default: FALSE.
nthreads	numeric or NULL. Number of CPU threads for parallel processing. If NULL, reads from @config\$nthreads (or defaults to 1). If > 1 and multiple q-values provided, uses parallel Psock cluster.
pseudocount	numeric. Pseudocount value for count regularization. Default: NULL (uses @config\$pseudocount or 0).
output_file	character or NULL. Optional file path to save results. Supported formats: .tsv, .csv, .txt (for jackknife results table with estimates, influence, outliers), .rds (for entire S4 object). Default: NULL (no file output).
...	Additional arguments passed to the base function.

**Details**

Requires diversity results to exist first. Will error if calculate\_diversity() has not been run.

**\*\*Parameter Priority Resolution:\*\***

**nthreads** Priority: explicit > @config\$nthreads > 1

**Value**

Modified TSENATAnalysis with jackknife results in @jackknife\_results.

**Examples**

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# TPM and effective_length REQUIRED for filter_analysis()
tpm <- matrix(runif(nrow(readcounts) * ncol(readcounts), 0.1, 10),
  nrow = nrow(readcounts), ncol = ncol(readcounts),
  dimnames = dimnames(readcounts))
effective_length <- matrix(100, nrow = nrow(readcounts), ncol = ncol(readcounts))

# Create config (metadata passed as explicit parameter to build_analysis)
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
```

```

    q = seq(0, 2, by = 0.05),
    paired = FALSE
  )

  # Build analysis from vignette data - metadata as explicit parameter
  analysis <- build_analysis(
    readcounts = readcounts,
    metadata = metadata_df,
    tx2gene = gff3_dataset,
    config = config,
    tpm = tpm,
    effective_length = effective_length
  )

  # Filter low-abundance genes (required for reliable jackknife estimates)
  analysis <- filter_analysis(analysis, stringency = 'severe')

  # Compute diversity first (required for jackknife)
  analysis <- calculate_diversity(analysis, q = c(0.5, 1.0, 1.5))

  # Run jackknife estimation
  analysis <- calculate_jeo(analysis, q = c(0.5, 1.0, 1.5))
  # Check jackknife results using unified accessor
  jackknife_res <- results(analysis, type = 'jackknife')
  if (!is.null(jackknife_res)) names(jackknife_res)

```

---

 calculate\_jis

*Jackknife isoform switching analysis on TSENATAnalysis object*


---

## Description

Wrapper around `.calculate_jis()` that manages TSENATAnalysis object. Identifies transcripts with significant isoform switching patterns using jackknife resampling across samples to detect influential isoforms.

## Usage

```

calculate_jis(
  analysis,
  condition_col = NULL,
  subject_col = NULL,
  gene_col = NULL,
  isoform_col = NULL,
  q = c(0, 0.5, 1, 1.5, 2),
  norm = NULL,
  log_base = NULL,
  threshold = 90,
  nboot = 1000,
  pseudocount = NULL,
  sait_results = NULL,
  sait_p_threshold = 0.05,
  use_sait_fdr = TRUE,

```

```

    output_file = NULL,
    verbose = FALSE,
    ...
)

```

## Arguments

analysis	TSENATAnalysis object containing: <ul style="list-style-type: none"> <li>• @se: SummarizedExperiment with count data</li> <li>• @config: Configuration metadata</li> </ul>
condition_col	character. Column name in colData(se) specifying group assignments (default: 'sample_type'). If NULL, attempts auto-detection.
subject_col	character. Optional column for paired/repeated measures design. If provided, enables paired analysis. Default: NULL (unpaired).
gene_col	character. Column name in rowData(se) or metadata identifying genes. Default: 'gene'.
isoform_col	character. Column name in rowData(se) or metadata identifying isoforms/transcripts. Default: 'transcript' or 'isoform'.
q	numeric. Tsallis entropy parameter(s) to analyze. Can be single value or vector for multi-q analysis (default: c(0, 0.5, 1, 1.5, 2)). If NULL, uses @config\$q.
norm	logical. Whether to use normalized diversity values (default: TRUE).
log_base	numeric. Logarithm base for entropy calculations. Default: NULL (uses e).
threshold	numeric. Percentile threshold for detecting transcript switching (default: 90). Transcripts with delta_influence >= threshold percentile are classified as 'switching'.
nboot	integer. Number of bootstrap resamples for confidence intervals (default: 1000).
pseudocount	numeric. Pseudocount value for count regularization. Default: NULL (uses @config\$pseudocount or 0).
sait_results	data.frame. Optional SAIT interaction results to filter genes. If provided, only genes in sait_results are analyzed.
sait_p_threshold	numeric. P-value threshold for filtering genes from sait_results (default: 0.05).
use_sait_fdr	logical. If TRUE, uses adjusted p-values from sait_results (default: TRUE).
output_file	character or NULL. Optional file path to save results. Supported formats: .tsv, .csv, .txt (for tables), or .rds (for S4 objects). When text format is specified, generates TWO files: <ul style="list-style-type: none"> <li>• output_file: Gene-level summary (one row per gene with switching statistics)</li> <li>• output_file_transcripts.ext: Transcript-level details (one row per transcript with p-values and FDR)</li> </ul> For .rds format, saves only the full analysis object. Default: NULL (no file output).
verbose	logical. Print progress messages (default: FALSE).
...	Additional arguments for future extensibility.

## Details

### Key Features:

- Jackknife resampling: Robust outlier detection across all samples
- Delta-influence metric: Measures how much each isoform drives phenotype
- Confidence intervals: Bootstrap-based uncertainty quantification
- Multi-q analysis: Tests across full q-spectrum simultaneously
- LM filtering: Optional restriction to genes with significant interactions
- Paired designs: Supports repeated measures/longitudinal data

### \*\*Parameter Resolution from Config\*\*

The following parameters are resolved using a three-level priority system:

1. User-provided argument (if not NULL)
2. Value from `analysis@config` (if key exists)
3. Function default value

### Affected parameters:

- `q`: Multi-q vector `c(0, 0.5, 1, 1.5, 2)` if not provided, or `@config$q` if available
- `nboot`: Uses `@config$nboot` if available, else 1000
- `threshold`: Uses `@config$threshold` if available, else 90
- `sait_p_threshold`: Uses `@config$sait_p_threshold` if available, else 0.05

This allows setting defaults once in the config and reusing across multiple analyses.

**\*\*Mathematical Background:\*\*** Delta-influence measures how much removing each sample changes entropy:

$$\Delta = H_q(\text{leave-one-out}) - H_q(\text{original})$$

High  $|\Delta|$  for specific isoforms indicates those isoforms drive differences. Identifies 'outlier samples' where isoforms contribute unusually much.

**\*\*Example Use Case:\*\*** Sample shows high Delta for isoform X → X has outsized importance in that sample

Classifying transcripts as 'switching' if top percentile (e.g., 90th) Delta

Reveals condition-specific isoforms crucial for phenotype determination.

**\*\*Automatic Setup:\*\*** This wrapper automatically: 1. Extracts `SummarizedExperiment` from `@se` slot 2. Detects `condition_col`, `gene_col`, `isoform_col` from `colData/rowData` or `@config` 3. Calls `.calculate_jis()` with extracted parameters

### \*\*Parameter Auto-Detection:\*\*

1. `condition_col`: Uses explicit parameter, then `@config`, then 'sample\_type'
2. `gene_col`: Uses explicit parameter, then looks for 'gene' or 'Gene'
3. `isoform_col`: Uses explicit parameter, then looks for 'transcript', 'isoform', or 'Isoform'

**Value**

TSENATAnalysis object with jackknife results stored in @jackknife\_results slot. Results are keyed by q-value (e.g., 'q\_1.00'). For multi-q analysis, multiple calls will accumulate results in the slot.

The analysis object is returned visibly to support method chaining:

```
analysis <- calculate_jis(analysis, q = 0.5)
analysis <- calculate_jis(analysis, q = 1.0)
```

**See Also**

jackknife\_isoform\_switching for the underlying implementation, [TSENATAnalysis](#) for object structure.

**Examples**

```
data(readcounts)
metadata_df <- read.table(system.file('extdata', 'metadata.tsv', package
= 'TSENAT'),
                          header = TRUE, sep = '\t')
gff3_file <- system.file('extdata', 'annotation.gff3.gz', package = 'TSENAT')
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_file, metadata = metadata_df, config = config,
                          tpm = tpm,
                          effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 20, subset_n_samples = 8)
analysis <- calculate_diversity(analysis, q = 1)
```

---

calculate\_m\_estimator *M-Estimation for Sample Quality (S4 Wrapper)*

---

**Description**

S4 wrapper for m\_estimate that performs robust M-estimation on diversity results stored in a TSENATAnalysis object and stores results back into the object.

**Usage**

```
calculate_m_estimator(
  analysis,
  condition_col = NULL,
  loss_type = "huber",
  scale = NULL,
  max_iter = 50,
  tol = 1e-06,
  paired = NULL,
  pcorr = "BH",
  q_combine_method = "mean",
```

```

    influence_threshold = 0.75,
    scale_method = "mad",
    output_file = NULL,
    verbose = FALSE
)

```

## Arguments

analysis	TSENATAnalysis object with diversity results (typically via <a href="#">calculate_diversity</a> ).
condition_col	character. Column name in sample metadata indicating condition/sample grouping. Auto-detected from @config\$condition_col if available.
loss_type	character. Type of loss function: 'huber' (default), 'tukey', or 'lsq'. Determines robustness vs efficiency trade-off.
scale	numeric. Manual scale parameter. If NULL, estimated from data.
max_iter	integer. Maximum iterations for M-estimation. Default: 50.
tol	numeric. Convergence tolerance. Default: 1e-6.
paired	logical. If TRUE, adjusts degrees of freedom for paired designs. Auto-detected from @config\$paired if available. Default: FALSE.
pcorr	character. P-value correction method. Default: 'BH' (Benjamini-Hochberg).
q_combine_method	character. How to collapse multi-q results: 'mean' (default) or 'median'.
influence_threshold	numeric. Threshold for classifying samples as high-influence. Default: 0.75.
scale_method	character. Scale estimation method: 'mad' (default), 'proposal2', or 's-estimator'.
output_file	character or NULL. Optional file path to save results. Supported formats: .rds (for S4 objects), .tsv, .csv, .txt (for tables). Default: NULL (no file output).
verbose	logical. Print status messages. Default: TRUE.

## Details

This wrapper extracts diversity results from analysis@diversity\_results, performs robust M-estimation on diversity values (entropy), and stores results in the analysis object metadata.

**\*\*M-Estimation:\*\*** Robust regression technique that down-weights outliers based on their residuals. Useful for detecting low-quality samples that show unusual diversity patterns.

**\*\*M-Estimation Results include:\*\***

- sample\_influence: How much each sample affects the overall fit
- robustness\_weight: Down-weighting factor
- entropy\_mean: Average entropy for the sample
- entropy\_sd: Entropy variability within the sample
- Status: QC Classification

**\*\*Parameter resolution priority\*\*** (explicit > @config > error):

- samples: Uses explicit arg, else @config\$condition\_col, else error. Note: despite parameter name 'samples', maps to condition grouping column

**\*\*Data Requirements:\*\***

- Diversity results must be computed via calculate\_diversity()
- Sample grouping column required in colData (auto-detected from @config\$condition\_col or via 'samples' parameter)

**Value**

Modified TSENATAnalysis object with M-estimation results stored in `analysis@metadata$m_estimate_results`. Contains data frame with influence scores, robustness weights, entropy statistics, and QC classifications. Returns visibly to support method chaining and piping.

**See Also**

[calculate\\_diversity](#) for computing diversity

**Examples**

```
# Create test analysis and compute M-estimation
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Create config (metadata passed as explicit parameter to build_analysis)
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  q_values = seq(0, 2, by = 0.05),
  paired = FALSE
)

# Build analysis from vignette data
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes = 200)
analysis <- calculate_diversity(analysis, q = c(0.5, 1.0, 1.5))
analysis <- calculate_m_estimator(
  analysis,
  condition_col = 'condition',
  loss_type = 'huber'
)
```

## Description

Statistical interaction testing for entropy diversity using flexible scale-adaptive models (GAM, LMM, GEE, FPCA) with AR(1) correlation structure for repeated measures. Tests for significant q-by-condition interactions across entropic indices.

## Usage

```
calculate_sait(
  analysis,
  fdr_threshold = NULL,
  formula = NULL,
  condition_col = NULL,
  method = "gam",
  paired = NULL,
  subject_col = NULL,
  nthreads = NULL,
  multicorr = NULL,
  corstr = NULL,
  pcorr = NULL,
  verbose = NULL,
  return_model_data = NULL,
  output_file = NULL,
  ...
)
```

## Arguments

analysis	TSENATAnalysis object.
fdr_threshold	numeric. FDR cutoff for significance. Default: 0.05.
formula	formula or NULL. Reserved for future use.
condition_col	character or NULL. Column name in colData identifying sample conditions. If NULL, reads from @config\$condition_col or auto-detects common column names.
method	character. Statistical method (e. g. , 'lmm', 'gam', 'gee'). If NULL, uses method from @config\$method or defaults to 'lmm'.
paired	logical. Whether to use paired design. Default: FALSE. If not specified, reads from @config\$paired if available.
subject_col	character or NULL. Column name identifying subject IDs for paired designs. If NULL, reads from @config\$subject_col if available.
nthreads	numeric or NULL. Number of CPU threads for parallel processing. If NULL, reads from @config\$nthreads (or defaults to NULL, letting base function decide).
multicorr	character or NULL. Multiple comparison correction method. Options: 'hochberg', 'westfall-young', 'benjamini-yekutieli'. If NULL, uses method from @config or base function defaults.
corstr	character or NULL. Correlation structure for GEE models. Options: 'ar1', 'exchangeable', 'independence'. If NULL, uses method from @config or base function defaults.
pcorr	character or NULL. P-value correction method. Default: 'BH' (Benjamini-Hochberg). If NULL, reads from @config\$pcorr if available.

verbose           logical. Print progress messages. Default: FALSE.  
 return\_model\_data           logical. Return model data for visualization. Default: TRUE.  
 output\_file       character or NULL. Optional file path to save results. Supported formats: .rds (for S4 objects), .tsv, .csv, .txt (for tables). Default: NULL (no file output).  
 ...               Additional arguments passed to the base LM function, including: pvalue, min\_obs, assay\_name, bias\_correction, regularization, storey, wy\_randomizations, adaptive\_knots, etc.

## Details

Extracts diversity results from @diversity\_results (prerequisite), combines across q-values into single SummarizedExperiment, then runs .calculate\_sait().

**\*\*Parameter Priority Resolution:\*\***

- nthreads: Priority: explicit > @config > NULL

Parameters are resolved in priority order: 1. Explicit arguments passed to function 2. Values from analysis@config (if present) 3. Function defaults

## Value

Modified TSENATAnalysis with results in @sait\_results\$sait\_interaction.

## Examples

```

## Not run:
# Create test analysis with appropriate sample structure for paired design
# Note: requires lme4 package for LMM fitting; uses synthetic data
set.seed(42)

# Create transcript-level counts with biological signal
# Note: Use adequate complexity (transcripts/genes, samples, expression)
# to avoid filtering away all genes during diversity computation
n_genes <- 50
n_transcripts_per_gene <- 30
n_transcripts <- n_genes * n_transcripts_per_gene
n_samples <- 16 # 8 subjects x 2 conditions (paired design)

# Generate counts with clear biological signal
control_idx <- seq(1, n_samples, by = 2)
treatment_idx <- seq(2, n_samples, by = 2)

counts <- matrix(0, nrow = n_transcripts, ncol = n_samples)
for (j in seq_len(n_samples)) {
  lambda <- if (j %in% control_idx) 100 else 180
  counts[, j] <- rpois(n_transcripts, lambda = lambda)
}
counts <- pmax(counts, 50) # Ensure minimum expression

rownames(counts) <- paste0('TX_', seq_len(n_transcripts))
colnames(counts) <- paste0('Sample_', seq_len(n_samples))

# Create rowData with gene mapping (tx2gene structure)
rowData <- data.frame(

```

```

    transcript_id = rownames(counts),
    gene_id = rep(paste0('GENE_', 1:n_genes),
                 each = n_transcripts_per_gene),
    row.names = rownames(counts)
  )

# Create colData with paired design metadata
coldata <- data.frame(
  sample_id = colnames(counts),
  condition = rep(c('control', 'treatment'),
                 length.out = n_samples),
  subject = rep(paste0('Subject_', 1:8),
               length.out = n_samples),
  row.names = colnames(counts)
)

# Build SummarizedExperiment
se <- SummarizedExperiment::SummarizedExperiment(
  assays = list(counts = counts),
  rowData = S4Vectors::DataFrame(rowdata),
  colData = S4Vectors::DataFrame(coldata)
)

# Add tx2gene metadata for gene-level aggregation
S4Vectors::metadata(se)$tx2gene <-
  data.frame(Transcript = rowdata$transcript_id,
            Gene = rowdata$gene_id)

# Initialize TSENATAnalysis
analysis <- TSENATAnalysis(se = se, config = list())

# Compute diversity (prerequisite for SAIT interaction analysis)
analysis <- calculate_diversity(
  analysis,
  q = c(0.5, 1.0, 1.5, 2.0, 2.5)
)

# Calculate q x condition interactions using GAM
analysis <- suppressWarnings(calculate_sait(
  analysis,
  condition_col = 'condition',
  method = 'gam'
))

# View top interaction results using unified accessor (first 3 genes)
res <- results(analysis, type = "sait")
if (!is.null(res)) head(res, 3)

## End(Not run)

```

## Description

Wrapper around [.calculate\_srh()] that manages TSENATAnalysis object. Tests for genes with condition-specific q-dependent entropy patterns by testing whether the effect of q-values DIFFERS between experimental conditions. This detects disease-relevant or condition-specific isoform switching patterns.

## Usage

```
calculate_srh(
  analysis,
  condition_col,
  output_file = NULL,
  paired = NULL,
  subject_col = NULL,
  multicorr = c("hochberg", "benjamini-yekutieli", "westfall-young", "none"),
  entropy_col = "diversity",
  q_col = "q",
  gene_col = "gene",
  wy_randomizations = 500,
  nperm_mode = c("standard", "conservative", "interactive"),
  nthreads = NULL,
  alpha = 0.05,
  p_threshold = 0.05,
  eta2_threshold_moderate = 0.01,
  eta2_threshold_strong = 0.1,
  min_nperm = 100,
  max_nperm = 10000,
  verbose = FALSE,
  ...
)
```

## Arguments

analysis	TSENATAnalysis object. Must have diversity results from calculate_diversity().
condition_col	character. Column name for sample grouping/condition (REQUIRED). Specifies the condition/treatment variable for testing $q \times$ condition interactions. Example: 'sample_type', 'treatment', 'disease_status'.
output_file	character or NULL. Optional file path to save results. Supported formats: .rds (for S4 objects). Default: NULL (no file output).
paired	logical or NULL. If TRUE, uses paired/blocked design (requires subject_col). If NULL, reads from @config\$paired.
subject_col	character or NULL. Column name for subject/block identifiers (required when paired=TRUE). If NULL, reads from @config\$subject_col.
multicorr	character. Multiple testing correction: 'hochberg' (default), 'benjamini-yekutieli', 'westfall-young', or 'none'.
entropy_col	character. Column name containing entropy/diversity data. Default: 'diversity'.
q_col	character. Column name containing q-values. Default: 'q'.
gene_col	character. Column name containing gene identifiers. Default: 'gene'.

wy_randomizations	numeric or character. Number of permutations for Westfall-Young correction. Use 'auto' to estimate from data. Default: 500.
nperm_mode	character. Mode for automatic permutation estimation: 'standard' (default), 'conservative', or 'interactive'.
nthreads	numeric or NULL. Number of parallel threads for computation. If NULL, reads from @config\$nthreads.
alpha	numeric. Significance level for p-value correction methods (default: 0.05). Used by all multiple testing correction methods.
p_threshold	numeric. P-value threshold for classification of interaction significance (default: 0.05).
eta2_threshold_moderate	numeric. Effect size boundary for 'moderate' classification (default: 0.01).
eta2_threshold_strong	numeric. Effect size boundary for 'strong' classification (default: 0.10).
min_nperm	integer. Minimum permutations for automatic estimation when wy_randomizations='auto' (default: 100).
max_nperm	integer. Maximum permutations for automatic estimation when wy_randomizations='auto' (default: 10000).
verbose	logical. If TRUE, prints progress messages. Default: FALSE.
...	Additional arguments passed to the base .calculate_srh() function.

## Details

### ## Key Features

- **Q × Condition Interaction**: Tests if entropy patterns across q-values differ by condition (main discovery goal) - **Multi-q Analysis**: Combines diversity results for multiple q-values into a single SummarizedExperiment for joint hypothesis testing - **Rank-Based Statistics**: Scheirer-Ray-Hare test (two-way ANOVA on ranked data, - **Scheirer-Ray-Hare Test**: Two-way non-parametric ANOVA on ranks - **Multiple Testing Correction**: Hochberg, Benjamini-Yekutieli, or permutation (Westfall-Young) procedures - **AR(1) Correlation Handling**: Westfall-Young preserves q-value spatial correlations (important for ordered q measurements) - **Effect Sizes**: Eta-squared ( $\eta^2$ ) for  $q \times$  condition interactions

### ## Statistical Hypotheses

Tests the null hypothesis: - **H0** = Gene entropy q-effect does NOT differ between conditions (q-independent) - **H1** = Gene entropy q-dependence is CONDITION-SPECIFIC (interaction exists)

A significant interaction indicates condition-specific patterns in how entropy varies across the q-value spectrum, revealing biological processes specific to that condition.

### ## Biological Example

Gene shows strong isoform switching (q-dependent entropy) in tumor cells but NOT in healthy cells -> Identified as disease-relevant q-dependent gene.

For condition-specific q-dependent genes: - **Condition A**: Strong entropy variation across q (q-dependent isoform usage) - **Condition B**: Flat entropy profile across q (uniform isoform usage) - **Interaction**: Condition-specific q-dependence pattern reveals disease-associated splicing regulation

Analyzes how gene interactions change across q-value spectrum using rank-based (Scheirer-Ray-Hare) or parametric (GAM) statistical tests.

**Parameter resolution priority** (explicit > @config > default/auto-detect):

- condition\_col: REQUIRED - must be explicitly provided
- q: ALWAYS auto-detected from diversity\_results (all q-values tested together)
- paired: explicit arg > @config\$paired > FALSE (default)
- subject\_col: explicit arg > @config\$subject\_col
- multicorr: explicit arg > @config\$multicorr > 'hochberg'
- nthreads: explicit arg > @config\$nthreads > 1 (default)
- test: explicit arg > @config\$test > 'auto' (auto-selection)
- nperm\_mode: explicit arg > @config\$nperm\_mode > 'standard'

## Value

Modified TSENATAnalysis with interaction results in @sait\_results.

## Examples

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Create config first (required when metadata is provided)
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')

# Build analysis from vignette data and create manageable subset
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)
analysis <- filter_analysis(
  analysis,
  min_samples = 1,
  subset_n_genes = 200
)
analysis <- calculate_diversity(analysis, q = c(0.5, 1.0, 1.5))

# Test  $Q \times$  Condition interaction (condition_col is REQUIRED)
analysis <- calculate_srh(
  analysis,
  condition_col = 'condition',
  multicorr = 'hochberg'
)

# View results using unified accessor
rank_test_res <- results(analysis, type = 'rank_test')
if (!is.null(rank_test_res)) head(rank_test_res)
```

---

filter_analysis	<i>Filter Low-Abundance Transcripts in a TSENATAnalysis Object</i>
-----------------	--------------------------------------------------------------------

---

## Description

S4 wrapper for `.filter_se()` that filters low-abundance transcripts directly within a `TSENATAnalysis` object. This maintains the consistent S4 workflow pattern where functions accept and return analysis objects.

## Usage

```
filter_analysis(
  analysis,
  min_tpm = 1,
  tpm_assay_name = NULL,
  min_samples = 5L,
  stringency = "medium",
  pair_col = NULL,
  min_tx_per_gene = 2L,
  min_isoform_abundance = NULL,
  assay_name = "counts",
  subset_n_genes = NULL,
  subset_genes = NULL,
  subset_n_samples = NULL,
  subset_samples = NULL,
  subset_select_by = c("variance", "mean", "random"),
  subset_seed = 42,
  subset_min_count = NULL,
  verbose = FALSE
)
```

## Arguments

analysis	A <code>TSENATAnalysis</code> S4 object containing the <code>SummarizedExperiment</code> to be filtered.
min_tpm	Numeric TPM threshold (default 1.0). Keeps transcripts with TPM $\geq$ min_tpm in $\geq$ min_samples samples. Ignored if stringency is specified.
tpm_assay_name	Character; name of assay containing TPM data (default: NULL). If NULL, searches for TPM assay automatically.
min_samples	Numeric. Minimum number of samples in which a transcript must be present (default: 5). Ignored if stringency is specified.
stringency	Character. Filtering stringency level: 'soft' (permissive), 'medium' (balanced), or 'severe' (stringent). When specified, auto-estimates: min_samples, min_tpm, min_tx_per_gene, and min_isoform_abundance from data. Requires pair_col in colData for paired designs. User-provided values for any parameter override stringency defaults. Default: 'medium' (balanced filtering recommended for most analyses).
pair_col	Character; column name in colData containing pair IDs for paired designs. Default: NULL (auto-detect if needed).

min_tx_per_gene	Integer minimum number of transcripts per gene required (default 2L). Single-transcript genes are always kept. Ignored if stringency is specified; when specified, automatically adjusted based on stringency level.
min_isoform_abundance	Numeric in [0, 1]; minimum relative abundance threshold for isoforms within each gene. Implements Soneson et al. (2016) filtering. Default behavior: - If stringency is specified: uses stringency-based default (soft: 0.01, medium: 0.05, severe: 0.15) - If stringency is NULL: uses default 0.05 (5 - If explicitly provided: overrides any stringency default Set to 0 or NULL (post-stringency processing) to skip isoform-level filtering.
assay_name	Character; name or index of the assay to use for filtering (default: 'counts'). Deprecated: use tpm_assay_name instead.
subset_n_genes	Integer; optional number of genes to retain after filtering. If provided, genes are selected based on subset_select_by. Default: NULL.
subset_genes	Character vector; optional specific genes to retain after filtering. Default: NULL.
subset_n_samples	Integer; optional number of samples to retain after filtering. If provided, samples are selected (balanced by condition if available). Default: NULL.
subset_samples	Character vector; optional specific samples to retain after filtering. Default: NULL.
subset_select_by	Character; gene selection method for subset_n_genes: 'variance' (highest variance), 'mean' (highest mean expression), or 'random'. Default: 'variance'.
subset_seed	Integer; random seed for reproducibility when subset_select_by = 'random'. Default: 42.
subset_min_count	Numeric; optional minimum count threshold applied during subsetting. Default: NULL.
verbose	Logical. If TRUE, print filtering progress and summary statistics (default: FALSE).

## Details

This wrapper applies `.filter_se()` to the SummarizedExperiment within the TSENATAnalysis object, optionally followed by subsetting parameters. The filtering and subsetting operations are applied in sequence:

1. Extracts the SE from `analysis@se`
2. Filters using `.filter_se()` with specified filtering parameters (default: 'medium' stringency)
3. If any subset parameters are provided, applies gene/sample selection to select specific genes and/or samples
4. Stores the filtered/subsetted SE back in `analysis@se`
5. Returns the modified analysis object invisibly

**\*\*Default Filtering (stringency = 'medium'):\*\*** By default, filtering applies balanced stringency: requires transcripts in  $\geq 50$  isoform abundance of 5 noise reduction with preservation of isoform diversity for reliable entropy calculations.

**\*\*Important:\*\*** Filtering should be performed BEFORE computing diversity, divergence, or SAIT interaction results. If called after analysis results have been computed, those results will be based on unfiltered data and may not align with the filtered SE dimensions.

**Value**

Invisibly returns the modified analysis object with filtered SummarizedExperiment in the @se slot. The filtering operation modifies the analysis object in-place while maintaining all other slots (results, metadata, etc.).

**See Also**

[build\\_analysis](#) for creating a new analysis object

**Examples**

```
# Create test analysis and filter
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, stringency = 'medium')
```

---

metadata, TSENATAnalysis-method

*Metadata Accessor Methods*

---

**Description**

Access or set the metadata list stored in a TSENATAnalysis object. The getter function retrieves all metadata or a specific key-value. The setter function replaces the entire metadata list.

**Usage**

```
## S4 method for signature 'TSENATAnalysis'
metadata(x, key = NULL)

## S4 replacement method for signature 'TSENATAnalysis'
metadata(x) <- value
```

**Arguments**

x	A <a href="#">TSENATAnalysis</a> object
key	Optional character string specifying a metadata key to retrieve
value	A list of metadata to assign

**Details**

Get or Set Metadata

**Value**

metadata Returns the full metadata list, or a single value if key is specified

metadata<- Returns the modified TSENATAnalysis object

**Examples**

```
# Create a TSENATAnalysis object
library(SummarizedExperiment)
se <- SummarizedExperiment(assays = list(counts = matrix(1:100, nrow = 10)))
analysis <- new('TSENATAnalysis', se = se, config = list())

# Get metadata (empty by default)
metadata(analysis)

# Set metadata
metadata(analysis) <- list(processing_date = Sys.Date(), method = 'test')

# Retrieve all metadata
metadata(analysis)

# Retrieve specific metadata key
metadata(analysis, key = 'method')
```

---

plot\_concordance      *Plot method concordance results from TSENATAnalysis*

---

**Description**

Plot method concordance results from TSENATAnalysis

**Usage**

```
plot_concordance(analysis, verbose = FALSE)
```

```
## S4 method for signature 'TSENATAnalysis'
plot_concordance(analysis, verbose = FALSE)
```

**Arguments**

analysis      TSENATAnalysis object with computed method concordance (from calculate\_concordance()).

verbose      logical. Print progress messages. Default: FALSE

**Details**

Creates visualization of method concordance including: - Comparison of significance across two methods (with color-coded agreement) - P-value distribution histograms for both methods - Significance threshold lines at  $p < 0.05$

Requires that `calculate_concordance()` has already been run to populate `@metadata$method_concordance`.

**Value**

A `ggplot/cowplot` object showing:

**Panel 1** Scatter plot of  $-\log_{10}$ (p-values) comparing methods

**Panel 2** Histogram of p-value distributions by method

**Examples**

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Build analysis from vignette data and create small subset
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)

# Note: calculate_concordance requires additional LM and Scheirer-Ray-Hare rank test
# results computed. For demo purposes, we show that
# plot_concordance needs pre-computed concordance in @metadata
```

---

plot\_divergence\_distribution

*Plot Tsallis Divergence Effect Size Distribution (S4 Wrapper)*

---

**Description**

S4 wrapper that extracts effect size results from a `TSENATAnalysis` object and generates a histogram visualization of Tsallis divergence effect sizes across genes.

**Usage**

```
plot_divergence_distribution(
  analysis,
  threshold = 0.1,
  output_file = NULL,
  width = 12,
  height = 6,
  verbose = FALSE,
  ...
)
```

**Arguments**

analysis	TSENATAnalysis object with effect sizes computed (typically via <a href="#">calculate_effect_sizes</a> ).
threshold	numeric. Effect size threshold for visual marking in the plot. Default is 0.1 (information-theoretic significance level).
output_file	character. Optional file path to save the plot. If NULL, plot is returned but not saved.
width	numeric. Plot width in inches. Default is 10.
height	numeric. Plot height in inches. Default is 6.
verbose	logical. Print status messages. Default is TRUE.
...	Additional arguments passed to the underlying plotting function.

**Details**

This wrapper extracts the interaction results (with effect size columns) from `analysis@metadata$effect_sizes_divergence` and passes them to the base `.plot_divergence_distribution()` function.

The function visualizes the distribution of effect sizes using the median q-value's divergence (typically around  $q=1.0$ , close to Shannon entropy). A red dashed line marks the information-theoretic significance threshold.

**\*\*Data Requirements:\*\***

- Effect sizes must be computed via `calculate_effect_sizes()`
- `@metadata$effect_sizes_divergence$interaction_results` must contain columns matching pattern `effect_size_D_q*`

**Value**

Invisibly returns the file path if saved, otherwise the ggplot object. If the plot cannot be created (missing data, ggplot2 not available), returns NULL invisibly with an informative message.

**See Also**

[calculate\\_effect\\_sizes](#) for computing effect sizes.

**Examples**

```
# Plot 2: Distribution of effect sizes across genes
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
```

```

metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
# Configure analysis parameters first
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  control = 'normal'
)

# Build analysis with configured parameters
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)

analysis <- filter_analysis(analysis, stringency = 'severe')
analysis <- calculate_diversity(
  analysis,
  q = seq(0.2, 2, by = 0.4),
  verbose = FALSE
)
analysis <- calculate_divergence(
  analysis,
  q = seq(0.2, 2, by = 0.4),
  verbose = FALSE
)
analysis <- suppressWarnings(calculate_sait(analysis, method = 'lmm'))
analysis <- calculate_effect_sizes(analysis)
p_dist <- plot_divergence_distribution(analysis)
# print(p_dist)

```

---

plot\_divergence\_spectrum

*Plot Global Divergence q-Curve Across All Genes (S4 Wrapper)*


---

### Description

S4 wrapper that extracts divergence results from a TSENATAnalysis object and visualizes the average Tsallis divergence across all genes (or specified genes) as a function of q-value.

### Usage

```

plot_divergence_spectrum(
  analysis,
  gene = NULL,

```

```

n_genes = 4,
ncol = 2,
metric = c("median", "mean"),
variability_metric = c("iqr", "sd"),
use_pvalue_ranking = FALSE,
output_file = NULL,
width = 12,
height = NULL,
verbose = FALSE,
...
)

```

### Arguments

analysis	TSENATAnalysis object with divergence results (typically via <a href="#">calculate_divergence</a> ).
gene	character. Optional specific gene name to plot. If NULL, plots global divergence curve (aggregated across all genes).
n_genes	integer. Number of top genes to plot when showing multi-gene spectra. Default is 4. Genes are sorted by p-value significance.
ncol	integer. Number of columns in grid layout for multi-gene plots. Default is 2. Number of rows is automatically calculated.
metric	character. Summary statistic for global curve: 'median' (default) or 'mean'. Only used when gene = NULL.
variability_metric	character. Error bar type for global curve: 'iqr' (default) or 'sd'. Only used when gene = NULL.
use_pvalue_ranking	logical. If TRUE, uses SAIT results to rank and display top n_genes by p-value significance. If FALSE (default), plots global divergence curve when gene = NULL. Default is FALSE.
output_file	character. Optional file path to save the plot. If NULL, plot is returned but not saved.
width	numeric. Plot width in inches. Default is 10.
height	numeric. Plot height in inches. Default is 6.
verbose	logical. Print status messages. Default is TRUE.
...	Additional arguments passed to the underlying plotting function.

### Details

This wrapper extracts the divergence SummarizedExperiment from `analysis@divergence_results` and optionally the SAIT results from `analysis@sait_results$sait_interaction` to pass to the base function.

#### \*\*Data Requirements:\*\*

- Divergence must be computed via `calculate_divergence()`
- `@divergence_results$divergence_se` or direct divergence SE

#### \*\*Modes:\*\*

- **Global mode** (gene = NULL): Shows median/mean divergence across all genes with variability bands

- **Gene-specific mode** (gene specified): Shows divergence spectrum for a single named gene
- **Top genes mode** (gene = NULL, sait\_res provided): Shows top n\_genes by significance

### Value

Invisibly returns the file path if saved, otherwise the ggplot object. If the plot cannot be created (missing data, ggplot2 not available), returns NULL invisibly with an informative message.

### See Also

[calculate\\_divergence](#) for computing divergence.

### Examples

```
# Load example data (matching TSENAT.Rmd workflow)
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Build analysis from vignette data and create small subset
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(
  analysis,
  min_samples = 1,
  subset_n_genes = 200
)
analysis <- calculate_diversity(
  analysis,
  q = c(0.5, 1, 1.5),
  verbose = FALSE
)
analysis <- calculate_divergence(
  analysis,
  q = c(0.5, 1, 1.5),
  verbose = FALSE
)
p_global <- plot_divergence_spectrum(analysis)
# print(p_global)
```

## Description

Visualize Tsallis entropy ( $S_q$ ) as a function of the diversity parameter  $q$  across sample groups. Supports three modes: aggregate  $q$ -curves (default), gene-specific  $q$ -curves (when 'gene' provided), or bootstrap confidence interval bands.

## Usage

```
plot_diversity_spectrum(
  se,
  assay_name = "diversity",
  condition_col = NULL,
  gene = NULL,
  sait_res = NULL,
  n_top = NULL,
  metric = "iqr",
  output_file = NULL,
  dev_width = NULL,
  dev_height = NULL
)
```

## Arguments

se	A 'SummarizedExperiment' returned by '.calculate_diversity()' with diversity assay. If pre-computed bootstrap confidence intervals are available (ci_lower/ci_upper assays), they will be displayed automatically. Otherwise, falls back to IQR visualization.
assay_name	Character; name of the assay to plot (default: 'diversity').
condition_col	Character or NULL; column name in colData indicating group/sample type. If NULL (default), reads from '@config\$condition_col' when input is TSENAT-Analysis, otherwise defaults to 'sample_type'. Only used in aggregate and CI modes.
gene	Character vector (optional); if provided, plot $q$ -curves for specified gene(s). Overrides default aggregate behavior. When provided, uses median +/- SD for each gene.
sait_res	Data frame (optional); gene interaction test results with 'gene' column and p-value column. Accepts either: - Results from '.calculate_sait()' (has 'adj_p_interaction' or 'p_interaction' columns) - Results from '.calculate_srh()' (has 'adj_p_value' or 'p_value' columns from Scheirer-Ray-Hare rank tests) If provided (and 'gene' is NULL), plots top 'n_top' genes ranked by p-value. Useful for plotting significant genes from any interaction analysis.
n_top	Integer or NULL; number of top genes to select from 'sait_res' when 'gene' is NULL (default: NULL). When NULL, defaults to showing the single most significant gene (n_top=1), providing a conservative view of the strongest effect. Set to a numeric value to show that many top genes.
metric	Character; when bootstrap CIs are NOT available, specifies the spread metric to display. Options: 'iqr' (default, Interquartile Range - more robust) or 'sd' (Standard Deviation). This parameter is ignored when bootstrap confidence intervals are available. Default: 'iqr'.
output_file	character or NULL. Optional file path to save the plot. Default: NULL (no file output).

dev_width	Numeric or NULL; width in inches for the graphics device when displaying the plot interactively. If specified (along with dev_height), creates a new device with this width. Default: NULL (uses current device).
dev_height	Numeric or NULL; height in inches for the graphics device when displaying the plot interactively. If specified (along with dev_width), creates a new device with this height. Default: NULL (uses current device).

## Details

**\*\*Aggregate mode (default, gene=NULL, sait\_res=NULL)\*\*:** - Plots median Tsallis entropy +/- IQR across all genes for each group - Works with any SummarizedExperiment from .calculate\_diversity() - Supports single or multiple q values and any number of groups - No CI data required for basic plots; bootstrap CIs optional

**\*\*Gene-specific mode (gene or sait\_res provided)\*\*:** - Plots q-curve separately for each selected gene - Shows median entropy +/- SD (variance) for each gene across q-values and groups - When 'sait\_res' provided: automatically ranks genes and selects top 'n\_top' by p-value - Single gene: returns a ggplot object; multiple genes: returns a grid plot (2 rows x 2 columns) with shared legend - For multiple genes: legend appears once at the bottom of the grid to avoid repetition and save space - Useful for highlighting specific genes of interest or significant discoveries - Bootstrap mode not supported in gene-specific mode

**\*\*Automatic Bootstrap CI Detection\*\*:** - When computing divergence or diversity with bootstrap enabled, ci\_lower and ci\_upper assays are added to the SummarizedExperiment. - This function automatically detects these assays and displays bootstrap confidence interval bands instead of IQR. No additional parameter needed. - For confidence bands to appear, use 'calculate\_diversity(..., bootstrap=TRUE, nboot=1000)' or appropriate divergence function with bootstrap enabled.

## Value

**\*\*Aggregate mode (gene=NULL, sait\_res=NULL)\*\*:** - If bootstrap CI assays available: A ggplot object showing median entropy with bootstrap confidence interval bands. - If no CI assays: A ggplot object showing median entropy with IQR ribbons (automatic fallback).

**\*\*Gene-specific mode (gene or sait\_res provided)\*\*:** - Single gene: A ggplot object showing median entropy +/- SD for that gene. - Multiple genes: A grid plot object arranged in 2 rows x 2 columns with a shared legend at the bottom. The legend appears once beneath the grid, avoiding repetition across subplots.

## Examples

```
# Plot 7: Tsallis entropy q-curve (combined across all sample diversity)
data(readcounts)
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'

# Create configuration (required when metadata is provided)
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
```

```

    tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)
analysis <- calculate_diversity(analysis, q = seq(0, 2, by = 0.5),
)
p <- plot_diversity_spectrum(analysis)
# print(p)

```

---

plot\_diversity\_violin\_density

*Combined Violin and Density Plot Grid for Single q Value*

---

## Description

Creates a side-by-side grid layout with a violin plot on the left and a density plot on the right, both showing Tsallis entropy distribution for the q value in the provided SummarizedExperiment (which should contain a single q value).

Creates a side-by-side grid layout with a violin plot on the left and a density plot on the right, both showing Tsallis entropy distribution for the q value in the provided SummarizedExperiment (which should contain a single q value).

## Usage

```

plot_diversity_violin_density(
  se,
  assay_name = "diversity",
  title = NULL,
  output_file = NULL
)

```

```

plot_diversity_violin_density(
  se,
  assay_name = "diversity",
  title = NULL,
  output_file = NULL
)

```

## Arguments

se	A ‘SummarizedExperiment’ returned by ‘calculate_diversity’ containing entropy values at a single q value.
assay_name	Name of the assay to use (default: ‘diversity’).
title	Optional base title. If NULL, auto-generated based on q value.
output_file	Character or NULL. Optional file path to save the plot as an image. If provided, the plot will be saved with appropriate dimensions. Default: NULL (no file output, only return object).

## Value

A ‘ggplot2’ object showing a 1x2 grid with violin plot on the left and density plot on the right.

A ‘ggplot2’ object showing a 1x2 grid with violin plot on the left and density plot on the right.

**Examples**

```

# Plot 8: Violin and density plots of Tsallis entropy distribution
data(readcounts)
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'

config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)
analysis <- calculate_diversity(analysis, q = 1.0)
p <- plot_diversity_violin_density(analysis)
# if (!is.null(p)) print(p)

# Plot 8: Violin and density plots of Tsallis entropy distribution
data(readcounts)
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'

# Create configuration (required when metadata is provided)
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_dataset, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)
analysis <- calculate_diversity(analysis, q = 1.0)
p <- plot_diversity_violin_density(analysis)
# print(p)

```

---

plot\_expression

*Plot Top Transcripts from TSENATAnalysis Object*


---

**Description**

S4 wrapper for `.plot_expression()` that extracts data directly from a `TSENATAnalysis` object. Automatically retrieves the `SummarizedExperiment` and `SAIT` results for visualizing transcript abundance across conditions.

**Usage**

```

plot_expression(
  analysis,
  gene = NULL,
  condition_col = NULL,
  top_n = 4,
  output_file = NULL,
  metric = c("median", "mean", "variance", "iqr"),
  use_tpm = TRUE,
  width = NULL,
  height = NULL,
  fontsize = 16,
  cellwidth = 0,
  cellheight = 0,
  layout_ncol = 2,
  verbose = FALSE,
  ...
)

```

**Arguments**

analysis	TSENATAnalysis. An S4 object containing a processed SummarizedExperiment and optional SAIT interaction results.
gene	character or NULL. Gene identifier(s) to plot. If a vector of multiple genes is provided, plots all of them. If NULL, automatically selects the top genes from SAIT results based on top_n parameter (genes with lowest p-values). Default: NULL (auto-extract from sait_results).
condition_col	character. Column name in colData(se) specifying group assignments (default: 'sample_type').
top_n	numeric. Number of top transcripts to display for each condition (default: 3).
output_file	character or NULL. Optional file path to save the plot. Supported formats: .pdf, .png, .jpg. Default: NULL (no file output).
metric	character. Method for ranking transcripts within genes. One of 'median', 'mean', 'variance', or 'iqr' (default: 'median').
use_tpm	logical. If TRUE, uses TPM (Transcripts Per Million) from metadata instead of raw counts (default: FALSE). TPM is normalized for sequencing depth and is recommended for comparing expression across samples. Requires TPM data in metadata from 'build_analysis()' or '.build_se()' with 'tpm' parameter. Raises error if TPM not available and 'use_tpm = TRUE'.
width	numeric or NULL. Output image width in inches. If NULL, automatically calculated based on number of genes (default: ~13 inches per column).
height	numeric or NULL. Output image height in inches. If NULL, automatically calculated based on number of genes (default: ~10 inches per row + headers).
fontsize	numeric. Base font size for heatmap titles and labels (default: 16pt). Automatically scaled for readability.
cellwidth	numeric. Width of individual heatmap cells in pixels. If 0 (default), uses adaptive sizing based on data dimensions and layout. Set > 0 to override dynamic sizing.

cellheight	numeric. Height of individual heatmap cells in pixels. If 0 (default), uses adaptive sizing based on data dimensions and layout. Set > 0 to override dynamic sizing.
layout_ncol	numeric. Number of heatmaps per row in fixed layout (default: 2). If NULL, uses adaptive layout based on transcript counts.
verbose	logical. If TRUE, print diagnostic messages during plotting (default: FALSE).
...	Additional arguments passed to the base plotting function.

## Details

This wrapper extracts the following from analysis:

**SummarizedExperiment** From analysis@se containing transcript counts

**SAIT results** From analysis@sait\_results\$sait\_interaction for gene selection

If no gene is specified, the function automatically selects the top gene from the SAIT results (lowest p-value). This simplifies visualization of genes with significant q x condition interaction effects.

## Value

Invisibly returns the output file path (if 'output\_file' provided), or invisible(NULL) if rendering to active graphics device. Graphics are rendered to the active grid device for capture during vignette compilation.

## See Also

[TSENATAnalysis](#) for object structure

## Examples

```
# Plot 6: Top transcripts across groups
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')
# Configure analysis parameters first
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  subject_col = 'paired_samples',
  paired = TRUE,
  control = 'normal',
  q_values = seq(0, 2, by = 0.1)
)

# Build analysis with configured parameters
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
```

```

    config = config,
    tpm = tpm,
    effective_length = effective_length
  )

analysis <- filter_analysis(analysis, stringency = 'severe')
analysis <- calculate_diversity(
  analysis,
  q = seq(0.2, 2, by = 0.4),
  verbose = FALSE
)
analysis <- suppressWarnings(calculate_sait(
  analysis,
  method = 'lmm',
  verbose = FALSE
))
plot_file <- plot_expression(analysis, top_n = 2)
# print(plot_file)

```

---

plot\_jis\_delta

*Plot Multi-Q Delta Influence Heatmaps from TSENATAnalysis Object*


---

### Description

S4 wrapper for `.plot_jis_delta()` that extracts results directly from a `TSENATAnalysis` object. Automatically retrieves jackknife switching results from the analysis object slots.

### Usage

```

plot_jis_delta(
  analysis,
  n_genes = 4,
  sait_results = NULL,
  verbose = FALSE,
  output_file = NULL,
  ...
)

```

### Arguments

<code>analysis</code>	<code>TSENATAnalysis</code> . An S4 object containing completed jackknife isoform switching analysis across multiple q-values.
<code>n_genes</code>	numeric. Number of top genes to display in heatmaps (default: 4). Genes are ranked by LM p-values if available, otherwise by order of appearance in results.
<code>sait_results</code>	<code>data.frame</code> or <code>NULL</code> . Optional SAIT interaction results for ranking genes (default: <code>NULL</code> ). If <code>NULL</code> , attempts to extract from <code>analysis@sait_results\$sait_interaction</code> .
<code>verbose</code>	logical. If <code>TRUE</code> , print diagnostic messages during plot generation (default: <code>FALSE</code> ).
<code>output_file</code>	character or <code>NULL</code> . Optional file path to save the plot. Default: <code>NULL</code> (no file output).
<code>...</code>	Additional arguments passed to the base function.

## Details

This function extracts the following from analysis:

**Jackknife results** From `analysis@jackknife_results`, which should contain multi-q switching results keyed by q-value (e.g., 'q\_1.00')

**SAIT results** From `analysis@sait_results$sait_interaction` if not explicitly provided, for ranking genes by significance

The wrapper automatically handles parameter extraction and provides a simplified interface compared to the base function.

## Value

A file path (character) to the saved heatmap PNG file, invisibly.

## See Also

[calculate\\_jis](#) for computing switching results

## Examples

```
# Plot 5: Multi-q delta influence (isoform switching) heatmaps
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Configure analysis parameters first
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  subject_col = 'paired_samples',
  paired = TRUE,
  control = 'normal',
  q = seq(0, 2, by = 0.1)
)

# Build analysis with configured parameters
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)

analysis <- filter_analysis(analysis, stringency = 'severe')
analysis <- calculate_diversity(
  analysis,
  q = seq(0.2, 2, by = 0.4),
```

```

    verbose = FALSE
  )
  analysis <- calculate_divergence(
    analysis,
    q = seq(0.2, 2, by = 0.4)
  )
  analysis <- suppressWarnings(calculate_sait(analysis, method = 'lmm'))
  analysis <- calculate_jis(
    analysis,
    q = seq(0.2, 2, by = 0.4),
    n_bootstrap = 20
  )
  heatmap_file <- plot_jis_delta(analysis, n_genes = 2)

```

---

plot\_sait

*Plot GAM q-curves from TSENATAnalysis object*


---

### Description

S4 wrapper that accepts a TSENATAnalysis object and generates GAM q-curve plots

### Usage

```

plot_sait(
  analysis,
  n_top = 6,
  genes = NULL,
  condition_col = NULL,
  sig_alpha = 0.05,
  assay_name = "diversity",
  output_file = NULL,
  width = 12,
  height = NULL,
  verbose = FALSE,
  ...
)

```

### Arguments

analysis	TSENATAnalysis object with diversity and SAIT interaction results.
n_top	integer. Number of top genes (by adjusted p-value) to plot (default: 6). Only used if genes = NULL.
genes	character vector. Optional specific gene names to plot. If provided, these genes are plotted directly regardless of significance. If NULL (default), top n_top significant genes are selected.
condition_col	character. Column name in colData(se) specifying group assignments for samples. If NULL, attempts to auto-detect from @config (looks for 'condition_col' or 'condition'). If still NULL, defaults to 'sample_type'.
sig_alpha	numeric. Significance threshold for adjusted p-values (default: 0.05). Only used if genes = NULL; filters sait_res to significant genes before selecting top n.

assay_name	character. Name of the assay in se to extract (default: 'diversity').
output_file	character or NULL. Optional file path to save the plot. Default: NULL (no file output).
width	numeric. Width of the plot in inches (default: 12). Only used if output_file is not NULL.
height	numeric or NULL. Height of the plot in inches. Default: NULL (automatically calculated based on width and aspect ratio). Only used if output_file is not NULL.
verbose	logical. If TRUE, print diagnostic messages during processing. (default: FALSE).
...	Additional arguments passed to the base function.

### Details

This wrapper automatically: 1. Extracts SummarizedExperiment from @se slot 2. Extracts SAIT results from @sait\_results\$sait\_interaction slot 3. Detects condition\_col from @config or uses default 4. Calls .plot\_sait() with extracted parameters

**\*\*Parameter Resolution (condition\_col):\*\***

1. Explicit condition\_col parameter (highest priority)
2. @config\$condition\_col if available
3. @config\$condition if available
4. Default: 'sample\_type'

### Value

A single ggplot object with all selected genes arranged in a grid layout. Can be saved with ggplot2::ggsave().

### See Also

[calculate\\_sait](#) for running LM analysis on TSENATAnalysis.

### Examples

```
## Not run:
# Plot 3: GAM q-curves for genes with q-by-condition interactions
data(readcounts)
readcounts <- as.matrix(readcounts)
mode(readcounts) <- 'numeric'
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_dataset <- system.file('extdata', 'annotation.gff3.gz', package =
'TSENAT')

# Configure analysis parameters first
config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  subject_col = 'paired_samples',
```

```
paired = TRUE,
control = 'normal',
q = seq(0.2, 2, by = 0.4) # 5 unique q-values: 0.2, 0.6, 1.0, 1.4, 1.8
)

# Build analysis with configured parameters
analysis <- build_analysis(
  readcounts = readcounts,
  tx2gene = gff3_dataset,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)

analysis <- filter_analysis(analysis, stringency = 'severe')
analysis <- calculate_diversity(analysis, q = seq(0.2, 2, by = 0.4))
analysis <- suppressWarnings(calculate_sait(analysis, method = 'gam'))

p_gam <- plot_sait(analysis, n_top = 2, sig_alpha = 0.15)
# print(p_gam)

## End(Not run)
```

---

readcounts

*Example transcript-level read counts dataset*

---

## Description

A dataset containing transcript-level transcript-level read count abundances from salmon quantification. The dataset includes samples as columns (SRR14800475-SRR14800490) and transcript IDs as row names.

## Usage

```
data(readcounts)
```

## Format

A data frame with 3514 transcripts (rows) and 16 samples (columns). Row names are transcript IDs (ENST format) and column names are sample IDs. Values represent TPM-normalized read counts.

## Value

A matrix (data frame) containing transcript-level TPM-normalized read counts from salmon quantification. Rows represent transcripts (ENST format IDs) and columns represent samples. Aliases include readcounts (main read count matrix), tpm (TPM values), and effective\_length (transcript lengths from salmon).

**Examples**

```
data(readcounts, package = 'TSENAT')
dim(readcounts)
head(readcounts[1:4, 1:3])
```

---

 results

---

*Extract analysis results from TSENATAnalysis object*


---

**Description**

Provides flexible access to diversity, divergence, and statistical test results with options for ranking, filtering, and format conversion.

**Usage**

```
results(
  analysis,
  type,
  q = NULL,
  rankBy = "none",
  n = NA,
  filterFDR = NULL,
  format = "text",
  n_genes = 4,
  q_values_table = c(0, 0.5, 1, 1.5, 2),
  top_n = NULL,
  sort_by = "adj_p_interaction",
  sample = NULL,
  plot = FALSE
)
```

**Arguments**

analysis	TSENATAnalysis object containing computed results.
type	character. <b>Required.</b> Type of results to extract: 'diversity', 'divergence', 'lm', 'jackknife', 'rank_test', 'effect_sizes_divergence', 'assumptions', 'concordance', or 'switching_tables'.
q	numeric. For diversity results, optionally return results for a specific q-value only. When specified, returns a single SummarizedExperiment for that q-value instead of the full list. Default: NULL (return all q-values as list). Example: q = 1.0 returns only the q=1.0 results.
rankBy	character. For LM/Jackknife results, ranking method: 'none' (default), 'pvalue', 'effectSize', or 'padj' (adjusted p-values). Applies to statistical test results. Default: 'none'.
n	integer. Return top N features/genes ranked by rankBy. Use NA (default) to return all results. Requires rankBy != 'none'.
filterFDR	numeric. FDR threshold for significance filtering (0.0-1.0). Only results with adjusted p-value <= filterFDR retained. Default: NULL (no filtering).

<code>format</code>	character. Output format. Behavior depends on result type: - For diversity: 'text' (default, table format), 'se' (SummarizedExperiment), or 'table' (data.frame) - For concordance: 'text' (default, pre-formatted), 'list' (structured for programmatic access) - For switching_tables: 'list' (default, structured list with \$gene_headers, \$comparison_tables, \$q_metadata for vignette rendering) or 'raw' (original named list per gene) - For assumptions: 'text' (default, pre-formatted), 'list' (structured for programmatic access) Default: 'text' for most types, 'list' for switching_tables.
<code>n_genes</code>	integer. Number of genes to display in diversity results. Default: 4.
<code>q_values_table</code>	numeric. Vector of q-values to include in diversity results. Default: c(0, 0.5, 1.0, 1.5, 2.0).
<code>top_n</code>	integer. For effect_sizes_divergence, return top N genes ranked by sort_by. When specified, results are sorted by sort_by column and limited to top N rows. Default: NULL (return all results). Use NA to return all.
<code>sort_by</code>	character. For effect_sizes_divergence, column name to sort by. Common choices: 'adj_p_interaction' (p-value, ascending), 'Mean_Divergence' (descending). Default: 'adj_p_interaction' (most significant first).
<code>sample</code>	character or NULL. For diversity results, optionally filter to specific sample(s). If NULL (default), returns results for all samples. If character, filters to matching sample identifiers from colData. Default: NULL (all samples).
<code>plot</code>	logical. Extract cached plot for the specified analysis type. - If FALSE (default): Return results as usual - If TRUE: Return the plot object for the given type Example: results(analysis, type = 'diversity', plot = TRUE) returns the diversity plot. Default: FALSE (no plot extraction).

## Details

This function provides flexible access to all computed results with ranking, filtering, and format conversion. Compatible with DESeq2/edgeR design patterns for familiar result extraction workflows.

**\*\*Lazy Computation for switching\_tables:\*\*** When requesting type = 'switching\_tables', the function automatically computes and caches the tables if they don't exist yet but the prerequisites do (LM and jackknife results). This eliminates the need for a separate prepare\_gene\_switching\_tables\_s4() call - simply request the results and they will be computed on-demand.

## Value

- For diversity with q=NULL: A named list of SummarizedExperiment objects, one per q-value  
 - For diversity with q specified and format='text' or 'table': A data.frame table for display  
 - For diversity with q specified and format='se': The SummarizedExperiment object directly (useful for SplicingFactory)  
 - For divergence: A SummarizedExperiment (rows=genes, columns=q-values), data.frame, or other format depending on divergence computation method  
 - For lm/jackknife: A data.frame or list based on type and format  
 - For effect\_sizes\_divergence: A list containing effect size divergence results with components like interaction\_results  
 - For assumptions: A list containing rank-based assumption checks (exchangeability, monotonicity, consistency) and optional method-specific diagnostics (gam\_metrics, gee\_metrics, lmm\_metrics, fpca\_metrics)  
 - For switching\_tables with format='text' (default): A list with components:

- `$gene_headers` Character vector of gene headers ('GeneName (ENSG00...')
- `$comparison_tables` List of data frames, one per gene, with columns: Transcript, q=0.00, q=0.50, ..., Direction Consistency

- `$q_metadata` List with per-gene metadata: `q_values_available` and `q_key_to_value` mapping
- For `switching_tables` with `format='raw'`: Original named list where names are gene headers and values are data frames with same column structure as `format='text'` Returns NULL if requested result type not computed or no results pass filtering.

## Examples

```
# Load example data
data(readcounts, package = 'TSENAT')

# Create TSENATAnalysis from count matrix
config <- TSENAT_config(
  q = 1.0,
  condition_col = 'group'
)
se <- SummarizedExperiment::SummarizedExperiment(
  assays = list(counts = readcounts),
  colData = data.frame(
    group = rep(c('A', 'B'), length.out = ncol(readcounts))
  )
)
analysis <- TSENATAnalysis(se = se, config = config)
analysis <- calculate_diversity(analysis)

# Get all diversity results (list of SummarizedExperiment objects, one per q)
div_all <- results(analysis, type = 'diversity')

# Get diversity for specific q-value (single SummarizedExperiment table for display)
div_q1 <- results(analysis, type = 'diversity', q = 1.0)

# Get diversity as SummarizedExperiment for downstream processing
div_q1_se <- results(analysis, type = 'diversity', q = 1.0, format = 'se')

# Get results ranked by p-value, top 20 genes
# Using accessor function instead of @ slot access
top_sait <- results(analysis, type = "sait", rankBy = 'pvalue', n = 20)

# Get effect size results, top 6 genes by p-value (most significant first)
top_effect_sizes <- results(analysis, type = 'effect_sizes_divergence',
  top_n = 6, sort_by = 'adj_p_interaction')

# Get effect sizes sorted by mean divergence (largest effect sizes first)
large_effects <- results(analysis, type = 'effect_sizes_divergence',
  top_n = 10, sort_by = 'Mean_Divergence')

# Get switching tables - automatically computed if prerequisites exist
# (no need to call prepare_gene_switching_tables_s4 separately)
# Default format='text' returns structured list for vignette rendering
switching <- results(analysis, type = 'switching_tables')

# Get switching tables in raw format (named list of data frames) for direct manipulation
switching_raw <- results(analysis, type = 'switching_tables', format = 'raw')

# =====
# RETRIEVE CACHED PLOTS using the plot parameter
# =====
```

```

# Get the diversity spectrum plot
diversity_plot <- results(analysis, type = 'diversity', plot = TRUE)

# Get the LM/regularized regression (GAM, LMM, GEE, FPCA) interaction plot
sait_plot <- results(analysis, type = "sait", plot = TRUE)

# Get the divergence distribution plot
div_dist_plot <- results(analysis, type = 'divergence', plot = TRUE)

# Get the influence/m-estimator plot
influence_plot <- results(analysis, type = 'influence', plot = TRUE)

# Available plot types correspond to analysis types:
# - type = 'diversity': Returns Tsallis entropy q-spectrum visualization
# - type = "sait": Returns regularized/penalized regression (GAM, LMM, GEE, FPCA) interaction plot
# - type = 'divergence': Returns distribution of divergence metrics across genes
# - type = 'influence': Returns m-estimator sample influence analysis
# - type = 'rank_test': Returns Scheirer-Ray-Hare interaction visualization
# - type = 'concordance': Returns method concordance comparison (LM vs rank test)

```

---

se

---

*Extract SummarizedExperiment from TSENATAnalysis*


---

## Description

Extract SummarizedExperiment from TSENATAnalysis

## Usage

```

se(object)

## S4 method for signature 'TSENATAnalysis'
se(object)

```

## Arguments

object            TSENATAnalysis object.

## Details

Provides read-only access to the underlying SummarizedExperiment. To modify the SummarizedExperiment, use the configuration methods or directly access `object@se`.

## Value

SummarizedExperiment object

The SummarizedExperiment object stored in `@se` slot, containing raw count data and sample meta-data.

## Examples

```
# Create a simple TSENATAnalysis object
library(SummarizedExperiment)
counts <- matrix(rpois(100, lambda = 10), nrow = 10, ncol = 10)
colnames(counts) <- paste0('sample_', 1:10)
rownames(counts) <- paste0('gene_', 1:10)

se <- SummarizedExperiment(assays = list(counts = counts))
analysis <- new('TSENATAnalysis', se = se, config = list())

# Extract the SummarizedExperiment
extracted_se <- se(analysis)
dim(extracted_se)
```

---

TSENAT

*Run complete TSENAT analysis pipeline*


---

## Description

Coordinates the full TSENAT workflow: diversity -> jackknife -> LM interactions -> divergence -> gene interactions -> rank-based tests -> concordance -> visualizations.

## Usage

```
TSENAT(
  analysis,
  output_dir = "tsenat_outputs",
  save_output = TRUE,
  output_format = c("tsv", "csv", "txt", "rds"),
  verbose = TRUE
)
```

## Arguments

analysis	TSENATAnalysis object created by <a href="#">build_analysis</a> .
output_dir	character. Directory to save results and plots. Default: 'tsenat_outputs'. Set to NULL to disable automatic output saving.
save_output	logical. Whether to save output files (results tables). Default: TRUE. If FALSE, no TSV/CSV output files are written to disk.
output_format	character. Format for output files: 'tsv' (tab-separated), 'csv' (comma-separated), 'txt' (text), or 'rds' (R serialized). Default: 'tsv'.
verbose	logical. Print progress messages. Default: TRUE.

## Details

Pipeline execution order (enforced, follows TSENAT.Rmd vignette):

1. `filter_analysis()` - Filter low-abundance transcripts
2. `calculate_diversity()` - Tsallis entropy per q-value
3. `plot_diversity_spectrum()` - Visualize q-spectrum

4. `calculate_m_estimator()` - Sample influence QC analysis
5. `calculate_sait()` - LM/SAIT interaction testing
6. `plot_sait()` - SAIT results visualization
7. `calculate_jis()` - Transcript switching detection
8. `plot_jis_delta()` - Multi-q influence heatmap (gene switching tables computed lazily via `results()`)
9. `plot_expression()` - Top transcript visualization
10. `calculate_divergence()` - Pairwise divergence metrics
11. `calculate_effect_sizes()` - Effect size computation
12. `plot_divergence_distribution()` - Divergence distribution plot
13. `plot_divergence_spectrum()` - Divergence spectrum plot
14. `calculate_assumptions()` - Validate rank-based test assumptions
15. `calculate_srh()` - Scheirer-Ray-Hare rank-based interaction test
16. `calculate_concordance()` - Compare LM and rank test results

## Value

TSENATAnalysis object containing complete analysis results, plots, and metadata.

## Examples

```
data(readcounts, package = 'TSENAT')
metadata_df <- read.table(
  system.file('extdata', 'metadata.tsv', package = 'TSENAT'),
  header = TRUE, sep = '\t'
)
gff3_file <- system.file('extdata', 'annotation.gff3.gz', package = 'TSENAT')

config <- TSENAT_config(
  sample_col = 'sample',
  condition_col = 'condition',
  q = seq(0, 2, length.out = 10),
  generate_plots = FALSE
)
analysis <- build_analysis(
  readcounts = as.matrix(readcounts),
  tx2gene = gff3_file,
  metadata = metadata_df,
  config = config,
  tpm = tpm,
  effective_length = effective_length
)

result <- TSENAT(analysis)
```

---

**TSENATAnalysis***Constructor for TSENATAnalysis objects*

---

## Description

Creates a new TSENATAnalysis object with a SummarizedExperiment base and optional initial configuration.

## Usage

```
TSENATAnalysis(se, config = list())
```

## Arguments

**se** SummarizedExperiment. The base expression data object.  
**config** list. Optional initial configuration (usually set via TSENAT\_config() instead).

## Details

The constructor initializes all slots with empty lists except @se, which must be provided. The @metadata slot automatically records: - creation timestamp - TSENAT package version - initial function call

## Value

A new TSENATAnalysis object.

## Examples

```
# Load real TSENAT data
data(readcounts)
metadata_df <- read.table(system.file('extdata', 'metadata.tsv', package
= 'TSENAT'),
  header = TRUE, sep = '\t')
gff3_file <- system.file('extdata', 'annotation.gff3.gz', package = 'TSENAT')
config <- TSENAT_config(sample_col = 'sample', condition_col = 'condition')
analysis <- build_analysis(readcounts = readcounts, tx2gene =
gff3_file, metadata = metadata_df, config = config,
  tpm = tpm, effective_length = effective_length)
analysis <- filter_analysis(analysis, min_samples = 1, subset_n_genes
= 200)
```

---

TSENATAnalysis-class *TSENATAnalysis S4 Class Definition*


---

**Description**

Central container for unified analysis workflows in TSENAT.

**Usage**

```
## S4 method for signature 'TSENATAnalysis,ANY,ANY,ANY'
x[i, j, drop = TRUE]
```

**Arguments**

x	A <a href="#">TSENATAnalysis</a> object to subset.
i	Gene indices (numeric, logical, or character vector). Defaults to all genes.
j	Sample indices (numeric, logical, or character vector). Defaults to all samples.
drop	Ignored for TSENATAnalysis objects; included for S4 method signature compatibility.

**Details**

The TSENATAnalysis class encapsulates all components of a complete TSENAT analysis: raw data, configuration metadata, and results from each analytical step. This unified object ensures metadata is never lost through the analysis pipeline and provides consistent accessor methods for result retrieval.

Access results via the unified `results(obj, type = ...)` accessor method: - `type='diversity'` for Tsallis entropy across q-values - `type="sait"` for regularized/penalized regression (GAM, LMM, GEE, FPCA) interaction results - `type='rank_test'` for Scheirer-Ray-Hare rank-based test results - `type='divergence'` for divergence metrics - `type='jackknife'` for jackknife resampling results Use `metadata(obj)` to access reproducibility metadata.

**Value**

A new [TSENATAnalysis](#) object containing only the specified genes and samples, with all associated results and metadata preserved.

**Slots**

`se` SummarizedExperiment. The base expression data object (genes x samples) with assays and `colData`.

`config` list. Configuration metadata specifying analysis parameters that persist through the workflow (q-values, sample grouping columns, etc.). Set once via `TSENAT_config()` and used by all downstream wrapper functions.

`diversity_results` list. Named list of diversity calculation results. Each name corresponds to a q-value (e.g., 'q\_0.5', 'q\_1.0'). Values are SummarizedExperiment objects or data.frames containing entropy values for each gene at that q-value.

`sait_results` list. Complex results from scale-adaptive interaction models (GAM with smoothing, LMM with random effects, GEE with working correlations, FPCA for functional data) (GAM, LMM, GEE, FPCA) and statistical testing. Top-level names identify analysis type:

`sait_interaction` Regularized regression (GAM/LMM/GEE/FPCA) model results (list with `$results` data.frame, `$models` list, etc.)  
`rank_test` Scheirer-Ray-Hare rank-based test results  
`divergence_difference` Differential divergence comparison  
`pairwise_results` list. Pairwise group comparison results. Results from running statistical tests for group comparisons.  
`rank_test_results` list. Scheirer-Ray-Hare rank-based statistical test results. Names correspond to q-values (e.g., 'q\_0.5', 'q\_1.0'). Computed by `calculate_srh()` as a non-parametric alternative to linear mixed model testing.  
`jackknife_results` list. Resampling-based confidence intervals. Names correspond to q-values (e.g., 'q\_0.5', 'q\_1.0'). Values are jackknife result objects containing resamples, CI bounds, and diagnostics.  
`divergence_results` list. Divergence metric calculations. Typically contains:  
   `tsallis_divergence` SummarizedExperiment with divergence values  
   `effect_sizes` data.frame with Cohen's d, etc.  
`plots` list. Cached visualization objects (ggplot). Names identify plot type (e.g., 'q\_curve', 'sait\_interaction', 'influence'). Populated by `TSENAT()` if `generate_plots=TRUE`.  
`metadata` list. Reproducibility and tracking metadata. Automatically maintained by wrapper functions. Includes:  
   `created_at` Timestamp of object creation  
   `function_calls` Vector of wrapper functions called  
   `function_timestamps` Timestamps for each function call  
   `package_version` TSENAT version at creation

---

TSENAT\_config

*Create and return TSENAT configuration*


---

## Description

Builds a configuration list for use with `TSENAT()`. Allows specifying analysis parameters once and reusing across multiple analyses.

## Usage

```

TSENAT_config(
  q = 1,
  condition_col = "condition",
  subject_col = NULL,
  sample_col = "sample",
  paired = FALSE,
  control = NULL,
  p_threshold = 0.05,
  fdr_threshold = 0.05,
  significance_threshold = 0.05,
  bootstrap = FALSE,
  nboot = 1000,
  bootstrap_method = c("percentile", "bca"),

```

```

stringency = "medium",
nthreads = 1,
norm = TRUE,
bootstrap_ci = 0.95,
bootstrap_include_diagnostics = TRUE,
min_valid_frac = 0.75,
norm_method = NULL,
pseudocount = 0,
shrinkage = "none",
sait_method = c("gam", "lmm", "fpca", "gee"),
sait_pcorr = c("BH", "bonferroni", "hochberg", "holm"),
jis_use_sait_fdr = TRUE,
divergence_ci = 0.95,
assumptions_checks = c("rank", "gam", "all"),
...
)

```

### Arguments

q	numeric. Q-value(s) for Tsallis entropy (single value or vector). Default: 1.0 (Shannon entropy). Usage: calculate_diversity/divergence use this for spectrum computation (if vector) or as default fallback (if single).
condition_col	character. Column name in colData containing conditions. Default: 'condition'.
subject_col	character. Column name in colData containing subject IDs (for paired designs). Default: NULL.
sample_col	character. Column name in colData containing sample IDs. Default: 'sample'.
paired	logical. Whether samples are paired/repeated measures. Default: FALSE.
control	character. Reference/control group label. Default: NULL.
p_threshold	numeric. Raw p-value threshold. Default: 0.05.
fdr_threshold	numeric. FDR-adjusted p-value threshold. Default: 0.05.
significance_threshold	numeric. Significance cutoff. Default: 0.05.
bootstrap	logical. Enable bootstrap CIs. Default: FALSE.
nboot	integer. Bootstrap resamples for CIs. Default: 1000.
bootstrap_method	character. Bootstrap method: 'percentile' or 'bca'. Default: 'percentile'.
stringency	character. Filtering stringency: 'lenient', 'medium', 'severe'. Default: 'medium'.
nthreads	integer. Parallel threads. Default: 1.
norm	logical. Enable normalization. Default: TRUE.
bootstrap_ci	numeric. Confidence level (0-1). Default: 0.95.
bootstrap_include_diagnostics	logical. Include diagnostics. Default: TRUE.
min_valid_frac	numeric. Min valid replicate fraction. Default: 0.75.
norm_method	character. Normalization: NULL, 'zscore', 'log_odds_ratio', 'relative_reference'. Default: NULL.
pseudocount	numeric. Pseudocount for sparse data. Default: 0.

shrinkage	character. Variance reduction: 'none' or 'empirical_bayes'. Default: 'none'.
sait_method	character. SAIT method: 'gam', 'lmm', 'fpca', 'gee'. Default: 'gam'.
sait_pcorr	character. P-value correction: 'BH', 'bonferroni', 'hochberg', 'holm'. Default: 'BH'.
jis_use_sait_fdr	logical. Filter jackknife genes using SAIT p-values. Default: TRUE.
divergence_ci	numeric. Confidence level for divergence CIs. Default: 0.95.
assumptions_checks	character. Which assumptions to test (default: 'all'). Presets: - 'rank': core assumption checks (exchangeability, monotonicity, consistency) - 'all': all checks including method-specific diagnostics (GAM, GEE, LMM, FPCA) Explicit: character vector like c('exchangeability', 'monotonicity').
...	Additional configuration parameters (stored as-is).

### Details

Configuration is stored in the TSENATAnalysis@config slot and used by wrapper functions to configure analysis behavior. Note: Statistical tests (Wilcoxon, shuffle) work on a single q-value, so only one q-value is specified in config.

### Value

list with class TSENATConfig containing all specified parameters.

### Examples

```
# Default config with standard parameters (point estimates only)
cfg <- TSENAT_config()

# For Wilcoxon/shuffle tests (single q-value required in config)
cfg <- TSENAT_config(
  q = 1.0, # Shannon entropy - for rank tests
  condition_col = 'treatment',
  control = 'untreated'
)

# For Scheirer-Ray-Hare rank tests (multiple q-values)
cfg <- TSENAT_config(
  q = seq(0, 2, by = 0.5), # Multiple q-values for spectrum or advanced testing
  condition_col = 'treatment',
  control = 'untreated'
)

# With bootstrap CIs for uncertainty quantification (recommended)
cfg <- TSENAT_config(
  bootstrap = TRUE, # Enable bootstrap confidence intervals
  bootstrap_method = 'bca', # Bias-corrected (better for skewed entropy)
  nboot = 1000, # 1000 resamples
  bootstrap_ci = 0.95 # 95% CI
)

# Custom with paired analysis, strict filtering, and normalization
cfg <- TSENAT_config(
  q = 1.0, # Shannon entropy
```

```
condition_col = 'treatment',
subject_col = 'subject_id',
paired = TRUE,
control = 'untreated',
stringency = 'severe',           # High-confidence transcripts only
norm_method = 'zscore',         # Cross-study standardization
shrinkage = 'none',            # Empirical estimates
bootstrap = TRUE,
bootstrap_method = 'bca',
nboot = 5000,                  # Higher precision
pseudocount = 0,               # Disabled by default; set > 0 to add pseudocount
significance_threshold = 0.01  # Stricter significance level
)
```

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