

Package ‘BreastSubtypeR’

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Type Package

Title Cohort-aware methods for intrinsic molecular subtyping of breast cancer

Description BreastSubtypeR provides an assumption-aware, multi-method framework for intrinsic molecular subtyping of breast cancer. The package harmonizes several published nearest-centroid (NC) and single-sample predictor (SSP) classifiers, supplies method-specific preprocessing and robust probe-to-gene mapping, and implements a cohort-aware AUTO mode that selectively enables classifiers compatible with the cohort composition. A local Shiny app (iBreastSubtypeR) is included for interactive analyses and to support users without programming experience.

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URL <https://doi.org/10.18129/B9.bioc.BreastSubtypeR>,<https://github.com/yqkiuo/BreastSubtypeR>,<https://github.com/JohanHartmanGroupBioteam/BreastSubtypeR>

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AIMSmodel*AIMSmodel: Model object for AIMS*

Description

Model definition for AIMS consisting of 100 pairwise rules and a Naive Bayes classifier (via **e1071**) as described by Paquet & Hallett (2015).

Usage

```
data("AIMSmodel")
```

Format

An object of class `list` of length 4.

Details

The 100 rules are of the form “EntrezID gene A < EntrezID gene B”. A subset of k rules (typically 20) is used within a Naive Bayes classifier to assign subtypes (Basal-like, HER2-enriched, LumaA, LumB, Normal-like) on a per-sample basis.

Value

<code>all.pairs</code>	Character vector of the 100 AIMS rules (EntrezID comparisons).
<code>k</code>	Integer; number of optimal rules (commonly 20).
<code>one.vs.all.tsp</code>	Naive Bayes classifier object used with the rules.
<code>selected.pairs.list</code>	Rules ranked by discriminative power per subtype.

References

Paquet ER, Hallett MT. Absolute assignment of breast cancer intrinsic molecular subtype. *J Natl Cancer Inst.* 2015;107(1):dju357. <https://doi.org/10.1093/jnci/dju357>

Examples

```
library(BreastSubtypeR)
data("AIMSmodel")
```

BreastSubtypeR

*BreastSubtypeR: A Unified R/Bioconductor Package for Intrinsic Molecular Subtyping in Breast Cancer Research***Description**

BreastSubtypeR is an R/Bioconductor package that unifies multiple published intrinsic subtyping (IS) methods for breast cancer into a single, reproducible framework. It supports both nearest-centroid (NC-based) and single-sample predictor (SSP-based) classifiers and introduces an assumption-aware **AUTO mode** that dynamically selects methods compatible with the input cohort.

By standardizing input handling, applying method-specific normalization, and providing optimised probe-to-gene mapping, BreastSubtypeR reduces inconsistencies across platforms and improves reproducibility in translational research. A companion Shiny app (**iBreastSubtypeR**) offers an intuitive GUI for non-programmers while preserving data privacy.

Workflow:

1. **Data Input:** Supply a gene expression dataset as a SummarizedExperiment. Supported inputs include raw RNA-seq counts (with gene lengths), log2(FPKM+1) RNA-seq, or log2-normalized microarray/nCounter data.
2. **Gene Mapping:** Prepare expression data with [Mapping](#), including Entrez ID-based resolution of duplicates.
3. **Subtyping:** Apply multiple classifiers simultaneously using [BS_Multi](#), or enable **AUTO mode** for cohort-aware method selection.
4. **visualization:** Summarise and interpret subtyping results with [Vis_Multi](#).

Key Features:

- **Multi-method framework:** Ten published NC- and SSP-based classifiers, harmonised under one interface.
- **AUTO mode:** Evaluates cohort composition (e.g., ER/HER2 prevalence, subtype purity, subgroup sizes) and disables classifiers with violated assumptions; improves accuracy, Cohen's kappa, and IHC concordance.
- **standardized normalization:** Upper-quartile log2-CPM for NC-based methods; FPKM for SSP-based methods.
- **Optimised gene mapping:** Entrez ID-based mapping with conflict resolution.
- **Dual accessibility:** A Bioconductor-compliant R API and a local Shiny app (iBreastSubtypeR).

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See Also

[Mapping](#), [BS_Multi](#), [Vis_Multi](#)

BreastSubtypeRobj

*BreastSubtypeRobj: Resources for NC-based methods***Description**

List of reference resources required by nearest-centroid (NC) subtyping methods: platform medians, centroids, signatures, subgroup quantiles, and metadata from the UNC232 training cohort.

Usage

```
data("BreastSubtypeRobj")
```

Format

A list with:

`medians` Matrix/data frame of platform-specific medians for **11** expression/sequencing platforms, derived as described in Picornell et al. (2019). Platform columns include: `nCounter`, `totalRNA.FFPE.20151111`, `RNAseq.Freeze.20120907`, `RNAseq.V2`, `RNAseq.V1`, `GC.4x44Kcustom`, `Agilent_244K`, `commercial_1x44k_postMeanCollapse`, `commercial_4x44k_postMeanCollapse_WashU_v2`, `htp1.5_WU_update`, `arrayTrain_postMeanCollapse`.

`centroid` PAM50 centroids used by `parker.original`.

`genes.sig50` Data frame of the 50 PAM50 genes with a proliferation flag.

`ssBC.subgroupQuantile` Subgroup-specific quantiles used by `ssBC`.

`genes.signature` Marker genes used across NC- and SSP-based methods.

`UNC232` Summary data for the UNC232 training cohort.

`platform.UNC232` Platform annotation for UNC232.

References

Parker JS, Mullins M, Cheung MCU, Leung S, Voduc D, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160–1167. <https://doi.org/10.1200/JCO.2008.18.1370>

Zhao X, Rodland EA, Tibshirani R, Plevritis S. Molecular subtyping for clinically defined breast cancer subgroups. *Breast Cancer Res.* 2015;17(1):29. <https://doi.org/10.1186/s13058-015-0520-4>

Fernandez-Martinez A, Krop IE, Hillman DW, Polley MY, Parker JS, Huebner L, et al. Survival, pathologic response, and genomics in CALGB 40601 (Alliance). *J Clin Oncol.* 2020;38(36):4184–4197. <https://doi.org/10.1200/JCO.20.01276>

Picornell AC, Echavarria I, Alvarez E, López-Tarruella S, Jerez Y, Hoadley K, et al. Breast cancer PAM50 signature: correlation and concordance between RNA-seq and digital multiplexed gene expression technologies in a TNBC series. *BMC Genomics.* 2019;20(1):452. <https://doi.org/10.1186/s12864-019-5849-0>

Examples

```
library(BreastSubtypeR)
data("BreastSubtypeRobj")
```

BS_AIMS

*AIMS Intrinsic Subtyping (BS_AIMS)***Description**

Implements the **AIMS (Absolute Assignment of Intrinsic Molecular Subtype)** method for breast cancer intrinsic subtyping. Unlike nearest-centroid (NC) approaches, AIMS is a single-sample predictor (SSP): it assigns subtypes independently for each sample using within-sample, pairwise gene expression rules. This makes it robust to cohort composition and scaling.

Usage

```
BS_AIMS(se_obj)
```

Arguments

- `se_obj` A SummarizedExperiment object containing:
- **Assay data:** A gene expression matrix with genes (Entrez IDs) as rows and samples as columns.
 - Expression values must be **positive** (e.g., FPKM or log2(FPKM+1)).
 - Values should not be gene-centered or globally scaled.

Value

A **list** with the following elements:

- `c1`: Character vector of AIMS subtype calls per sample. One of "Basal", "Her2", "LumA", "LumB", or "Normal".
- `prob`: Numeric vector of posterior probabilities corresponding to the assigned subtype in `c1` (one value per sample).
- `all.probs`: Matrix of posterior probabilities for all samples and all subtypes (rows = samples, columns = subtypes).
- `rules.matrix`: 0/1 matrix of the 100 AIMS rules used for assignment (rows = rules, columns = samples); 1 indicates the rule evaluated to TRUE.
- `data.used`: Expression values actually used to evaluate the rules (filtered/ordered subset aligned to the AIMS gene set).
- `EntrezID.used`: Character vector of Entrez IDs used by AIMS.

References

Paquet ER, Hallett MT. *Absolute assignment of breast cancer intrinsic molecular subtype*. Journal of the National Cancer Institute. 2015;107(1):dju357. <https://doi.org/10.1093/jnci/dju357>

Examples

```
## Example using SummarizedExperiment input
data("OSLO2EMIT0obj")
res <- BS_AIMS(
  se_obj = OSLO2EMIT0obj$data_input$se_SSP
)
```

BS_cIHC

Conventional IHC Intrinsic Subtyping (BS_cIHC)

Description

Implements the conventional immunohistochemistry-based (cIHC) intrinsic subtyping approach, which balances cohorts by estrogen receptor (ER) status before applying gene-expression-based subtyping. This method is useful for ER-skewed cohorts where assumptions of nearest-centroid classifiers are violated.

Usage

```
BS_cIHC(se_obj, Subtype = FALSE, hasClinical = FALSE, seed = 118)
```

Arguments

se_obj	A SummarizedExperiment object containing: • Assay data: A log2-transformed, normalized expression matrix with genes (Gene Symbols) as rows and samples as columns. • Column metadata (colData): Must include: – "PatientID": Unique sample or patient identifier. – "ER": Estrogen receptor status, coded as "ER+" or "ER-".
Subtype	Logical. If TRUE, returns only the four main subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates additional clinical variables from colData(se_obj). Required columns: • "TSIZE": Tumor size ($0 = \leq 2$ cm; $1 = > 2$ cm). • "NODE": Lymph node status ($0 =$ negative; $\geq 1 =$ positive). Must be numeric.
seed	Integer. Random seed for reproducibility of ER-balancing.

Value

A list with the following elements:

- BS.all: data.frame with PatientID, BS (five-class call) and, when Subtype = TRUE, BS.Subtype (four-class call).

- `score.ROR`: data.frame with the per-sample centroid correlations, calls, confidence, ESR1/ERBB2 expression and the ROR-S and ROR-P scores and risk groups; with `hasClinical = TRUE` also the ROR-C and ROR-PC scores and risk groups.
- `mdns`: data.frame of gene medians used for centering (reference platform medians plus the ER-balanced cohort medians).
- `outList`: list with the internal nearest-centroid results (predictions, distances, centroids, ...).

References

Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, et al. *Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer*. Cell. 2015;163(2):506–519. <https://doi.org/10.1016/j.cell.2015.09.033>

Examples

```
data("OSLO2EMIT0obj")
res <- BS_cIHC(
  se_obj = OSLO2EMIT0obj$data_input$se_NC,
  Subtype = FALSE,
  hasClinical = FALSE
)
```

BS_cIHC.itr

Iterative Conventional IHC Intrinsic Subtyping (BS_cIHC.itr)

Description

Implements an **iterative** version of the conventional IHC-based intrinsic subtyping approach. This method repeatedly balances samples by estrogen receptor (ER) status across multiple iterations, allowing refinement of subtype calls in ER-skewed cohorts. Users can customise the ER+/ER–ratio to match specific cohort assumptions (e.g., training distribution).

Usage

```
BS_cIHC.itr(
  se_obj,
  iteration = 100,
  ratio = 54/64,
  Subtype = FALSE,
  hasClinical = FALSE,
  seed = 118
)
```

Arguments

se_obj	A SummarizedExperiment object containing: • Assay data: A log2-transformed, normalized expression matrix with genes (Gene Symbols) as rows and samples as columns. • Column metadata (colData): Must include: – "PatientID": Unique sample or patient identifier. – "ER": Estrogen receptor status, coded as "ER+" or "ER-".
iteration	Integer. Number of iterations for the ER-balancing procedure. Default: 100.
ratio	Numeric. Target ratio for ER balancing. Options: • 1:1: Equal balancing. • 54:64: Default; reflects the ER+/ER- ratio in the UNC232 training cohort. The ratio is applied to the larger ER group relative to the smaller one: in each iteration $\text{ceiling}(\text{ratio} * n_{\text{smaller}})$ samples are drawn from the larger ER group and combined with all samples of the smaller group. This equals the ER+/ER- ratio when ER+ is the larger group and its inverse when ER- is the larger group. ratio must not exceed $n_{\text{larger}} / n_{\text{smaller}}$.
Subtype	Logical. If TRUE, returns only the four main subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates additional clinical variables from colData(se_obj). Required columns: • "TSIZE": Tumor size ($0 \leq 2$ cm; $1 > 2$ cm). • "NODE": Lymph node status ($0 = \text{negative}$; $\geq 1 = \text{positive}$). Must be numeric.
seed	Integer. Random seed for reproducibility.

Value

A list with the following elements:

- BS.all: data.frame with PatientID, BS (five-class consensus call across iterations) and, when Subtype = TRUE, BS.Subtype (four-class consensus call). The consensus is the most frequent call over the iterations; ties are resolved in favour of the alphabetically first subtype (Basal, Her2, LumA, LumB, Normal).
- score.ROR: data.frame with the per-sample centroid correlations (averaged over the iterations whose call equals the consensus call), calls, confidence, ESR1/ERBB2 expression and the ROR-S and ROR-P scores and risk groups computed from these averages; with hasClinical = TRUE also the ROR-C and ROR-PC scores and risk groups.
- outList: list with the consensus predictions, the averaged test data and distances, and the centroids.
- BS.itr.keep: character matrix of the five-class calls per sample (rows) and iteration (columns).
- BS.itr.keep.Subtype: the four-class analogue, present when Subtype = TRUE.

References

Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. *The genomic and transcriptomic architecture of 2,000 breast tumors reveals novel subgroups*. Nature. 2012;486(7403):346–352. <https://doi.org/10.1038/nature10983>

Examples

```
data("OSLO2EMIT0obj")
res <- BS_cIHC.itr(
  se_obj = OSLO2EMIT0obj$data_input$se_NC,
  iteration = 10, ## for final analysis, use iteration = 100
  Subtype = FALSE,
  hasClinical = FALSE
)
```

BS_Multi

Intrinsic Subtyping with Multiple Approaches (BS_Multi)

Description

Executes multiple intrinsic molecular subtyping methods in parallel. Users can either specify a set of classifiers directly, or enable the **AUTO mode**, which dynamically selects methods based on cohort composition (e.g., ER/HER2 distribution, subtype purity, subgroup size). AUTO reduces misclassification in skewed or subtype-specific cohorts by disabling methods whose assumptions are violated, but does not perform consensus voting—subtypes are still returned per method.

Usage

```
BS_Multi(data_input, methods = "AUTO", Subtype = FALSE, hasClinical = FALSE)
```

Arguments

- | | |
|------------|---|
| data_input | The output from the Mapping() function, containing processed gene expression data prepared for subtyping. |
| methods | <p>Character vector specifying the subtyping methods to run. Available options include:</p> <ul style="list-style-type: none"> • "parker.original": Original PAM50 (Parker et al., 2009). • "genefu.scale": PAM50 (scaled version; Gendoo et al., 2016). • "genefu.robust": PAM50 (robust version; Gendoo et al., 2016). • "cIHC": Conventional ER-balancing with immunohistochemistry (Ciriello et al., 2015). • "cIHC.itr": Iterative ER-balancing (Curtis et al., 2012). • "PCAPAM50": PCA-based PAM50 using ESR1 balancing (Raj-Kumar et al., 2019). • "ssBC": Subgroup-specific gene-centering (Zhao et al., 2015). |

- "ssBC.v2": Updated subgroup-specific centering (Fernandez-Martinez et al., 2020).
- "AIMS": Absolute Intrinsic Molecular Subtyping (Paquet & Hallett, 2015).
- "sspbcc": SSPBC, a large-cohort SSP trained on SCAN-B (Staaf et al., 2022).
- "AUTO": Cohort-aware selection of compatible methods (must be the only entry).

Notes:

- If "AUTO" is selected, it must be the sole value in methods.
- Otherwise, at least **two** methods must be specified.

Subtype	Logical. If TRUE, returns four subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates clinical data from <code>colData(se_obj)</code> . Required columns: • "TSIZE": Tumor size ($0 \leq 2$ cm; $1 > 2$ cm). • "NODE": Lymph node status ($0 = \text{negative}$; $\geq 1 = \text{positive}$).

Value

A list containing per-method subtype assignments for each sample.

References

- Yang Q, Hartman J, Sifakis EG. *BreastSubtypeR: A Unified R/Bioconductor Package for Intrinsic Molecular Subtyping in Breast Cancer Research*. NAR Genomics and Bioinformatics. 2025. <https://doi.org/10.1093/nargab/lqaf131>. Selected as Editor's Choice.
- Parker JS, Mullins M, Cheung MCU, Leung S, Voduc D, et al. *Supervised risk predictor of breast cancer based on intrinsic subtypes*. J Clin Oncol. 2009;27(8):1160-1167. <https://doi.org/10.1200/JCO.2008.18.1370>
- Gendoo DMA, Ratanasirigulchai N, Schröder MS, Paré L, Parker JS, Prat A, et al. *Genefu: An R/Bioconductor package for computation of gene expression-based signatures in breast cancer*. Bioinformatics. 2016;32(7):1097-1099. <https://doi.org/10.1093/bioinformatics/btv693>
- Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, et al. *Comprehensive molecular portraits of invasive lobular breast cancer*. Cell. 2015;163(2):506-519. <https://doi.org/10.1016/j.cell.2015.09.033>
- Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. *The genomic and transcriptomic architecture of 2,000 breast tumors reveals novel subgroups*. Nature. 2012;486(7403):346-352. <https://doi.org/10.1038/nature10983>
- Zhao X, Rodland EA, Tibshirani R, Plevritis S. *Molecular subtyping for clinically defined breast cancer subgroups*. Breast Cancer Res. 2015;17(1):29. <https://doi.org/10.1186/s13058-015-0520-4>
- Fernandez-Martinez A, Krop IE, Hillman DW, Polley MY, Parker JS, Huebner L, et al. *Survival, pathologic response, and genomics in CALGB 40601 (Alliance), a neoadjuvant Phase III trial of paclitaxel-trastuzumab with or without lapatinib in HER2-positive breast cancer*. J Clin Oncol. 2020;38(36):4184-4197. <https://doi.org/10.1200/JCO.20.01276>
- Paquet ER, Hallett MT. *Absolute assignment of breast cancer intrinsic molecular subtype*. J Natl Cancer Inst. 2015;107(1):dju357. <https://doi.org/10.1093/jnci/dju357>

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. *RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer*. NPJ Breast Cancer. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
## Example: run multiple methods
data("OSLO2EMIT0obj")
methods <- c("parker.original", "genefu.scale", "genefu.robust")
res.test <- BS_Multi(
  data_input = OSLO2EMIT0obj$data_input,
  methods = methods,
  Subtype = FALSE,
  hasClinical = FALSE
)
```

BS_parker

Original Parker Intrinsic Subtyping (BS_parker)

Description

Implements the original PAM50 nearest-centroid classifier as described by Parker et al. (2009), along with supported calibration strategies and variations. This function assigns intrinsic breast cancer subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like, and optionally Normal-like).

Usage

```
BS_parker(
  se_obj,
  calibration = "None",
  internal = NA,
  external = NA,
  medians = NA,
  Subtype = FALSE,
  hasClinical = FALSE
)
```

Arguments

se_obj	A SummarizedExperiment object containing: • Assay data: A log-transformed, normalized gene expression matrix with genes (Gene Symbols) as rows and samples as columns. • Column metadata (colData): Optional sample- or patient-level information.
calibration	Character. One of:

	• "None": no centering/scaling. • "Internal": center by a method derived from the current cohort (see internal). • "External": center by medians from an external cohort (see external).
internal	Internal calibration method used when calibration = "Internal". Accepts: • NA or "medianCtr" (identical): gene-wise median centering (as in Parker et al.). • "meanCtr": gene-wise z-scoring (mean 0, sd 1; as implemented in <code>genefu.scale</code>). • "qCtr": robust centering (quantile rescale with <code>mq = 0.05</code>; as in <code>genefu.robust</code>). Defaults to NA (median centering).
external	Character string specifying the external calibration source. • To use training cohort medians, provide the platform/column name. • To supply user-defined medians, set <code>external = "Given.mdns"</code> and pass values via <code>medians</code>.
medians	A matrix or data.frame of user-provided medians (required if <code>external = "Given.mdns"</code>). • First column: 50 PAM50 genes. • Second column: Corresponding median expression values.
Subtype	Logical. If TRUE, assigns only the four main intrinsic subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates clinical variables from <code>colData(se_obj)</code> . Required columns: • "TSIZE": Tumor size ($0 = \leq 2$ cm; $1 = > 2$ cm). • "NODE": Lymph node status ($0 =$ negative; $\geq 1 =$ positive). Must be numeric.

Value

A list containing PAM50 intrinsic subtype calls using the Parker classifier and selected calibration strategy.

References

- Parker JS, Mullins M, Cheung MCU, Leung S, Voduc D, et al. *Supervised risk predictor of breast cancer based on intrinsic subtypes*. Journal of Clinical Oncology. 2009;27(8). <https://doi.org/10.1200/JCO.2008.18.1370>
- Gendoo DMA, Ratanasirigulchai N, Schröder MS, Paré L, Parker JS, Prat A, et al. *Genefu: An R/Bioconductor package for computation of gene expression-based signatures in breast cancer*. Bioinformatics. 2016;32(7). <https://doi.org/10.1093/bioinformatics/btv693>

Examples

```
data("OSL02EMIT0obj")
res <- BS_parker(
  se_obj = OSL02EMIT0obj$data_input$se_NC,
  calibration = "Internal",
```

```

    internal = NA, # NA is equal to "medianCtr"
    Subtype = FALSE,
    hasClinical = FALSE
  )

```

BS_PCAPAM50

PCA-PAM50 Intrinsic Subtyping (BS_PCAPAM50)

Description

Implements the PCA-PAM50 method, which integrates **Principal Component Analysis (PCA)** of ESR1 expression to adjust for estrogen receptor (ER) imbalance prior to applying the PAM50 nearest-centroid classifier. This approach improves subtype consistency, particularly in ER-skewed cohorts.

Usage

```
BS_PCAPAM50(se_obj, Subtype = FALSE, hasClinical = FALSE, seed = 118)
```

Arguments

se_obj	A SummarizedExperiment object containing: • Assay data: A log2-transformed, normalized expression matrix with genes (Gene Symbols) as rows and samples as columns. • Column metadata (colData): Must include: – "PatientID": Unique sample or patient identifier. – "ER": Estrogen receptor status, coded as "ER+" or "ER-".
Subtype	Logical. If TRUE, returns only the four main subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates additional clinical variables from colData(se_obj). Required columns: • "TSIZE": Tumor size ($0 = \leq 2$ cm; $1 = > 2$ cm). • "NODE": Lymph node status ($0 =$ negative; $\geq 1 =$ positive). Must be numeric.
seed	Integer. Random seed for reproducibility.

Value

A list with the following elements:

- `BS.all`: data.frame with PatientID, BS (five-class call) and, when Subtype = TRUE, BS.Subtype (four-class call).
- `score.ROR`: data.frame with the per-sample centroid correlations, calls, confidence, ESR1/ERBB2 expression and the ROR-S and ROR-P scores and risk groups; with hasClinical = TRUE also the ROR-C and ROR-PC scores and risk groups.

- `mdns.fl`: data.frame of gene medians used for centering (reference platform medians plus the PCA-PAM50 refined cohort medians).
- `outList`: list with the internal nearest-centroid results (predictions, distances, centroids, ...).

References

Raj-Kumar PK, Liu J, Hooke JA, Kovatich AJ, Kvecher L, Shriver CD, et al. *PCA-PAM50 improves consistency between breast cancer intrinsic and clinical subtyping, reclassifying a subset of luminal A tumors as luminal B*. Scientific Reports. 2019;9(1):1–12. <https://doi.org/10.1038/s41598-019-44339-4>

Examples

```
data("OSLO2EMIT0obj")
res <- BS_PCAPAM50(
  se_obj = OSLO2EMIT0obj$data_input$se_NC,
  Subtype = FALSE,
  hasClinical = FALSE
)
```

BS_ssBC

Subgroup-Specific Gene-Centering Intrinsic Subtyping (BS_ssBC)

Description

Implements the **subgroup-specific gene-centering (ssBC)** method for breast cancer intrinsic subtyping. The ssBC approach applies precomputed, subgroup-specific centering values to adjust PAM50 nearest-centroid classification when the study cohort is skewed relative to the original training cohort (e.g., ER-selected, HER2-enriched, or triple-negative cohorts).

Usage

```
BS_ssBC(se_obj, s, Subtype = FALSE, hasClinical = FALSE)
```

Arguments

- `se_obj` A SummarizedExperiment object containing:
- **Assay data**: A log2-transformed, normalized expression matrix with genes (Gene Symbols) as rows and samples as columns.
 - **Column metadata** (`colData`): If `hasClinical = TRUE`, must include:
 - "PatientID": Unique patient/sample identifier.
 - Depending on the chosen `s` parameter:
 - * "ER": Estrogen receptor status ("ER+" or "ER-") if `s = "ER"`.
 - * "HER2": HER2 status ("HER2+" or "HER2-") if `s = "ER.v2"`.
 - * "TN": Triple-negative status ("TN" or "nonTN") if `s = "TN" or "TN.v2"`.

s	Character. Specifies which subgroup-specific quantiles to use: • "ER", "TN": Original subgroup-specific quantiles (<i>Breast Cancer Research</i>, 2015). • "ER.v2", "TN.v2": Updated subgroup-specific quantiles (<i>Journal of Clinical Oncology</i>, 2020).
Subtype	Logical. If TRUE, returns only the four main subtypes (Luminal A, Luminal B, HER2-enriched, Basal-like), excluding Normal-like.
hasClinical	Logical. If TRUE, incorporates additional clinical variables from colData(se_obj). Required columns: • "TSIZE": Tumor size ($0 = \leq 2$ cm; $1 = > 2$ cm). • "NODE": Lymph node status ($0 =$ negative; $\geq 1 =$ positive). Must be numeric.

Value

A list with the following elements:

- BS.all: data.frame with PatientID, BS (five-class call) and, when Subtype = TRUE, BS.Subtype (four-class call). Samples whose subgroup (s) status is missing receive NA.
- score.ROR: data.frame with the per-sample centroid correlations, calls, confidence, ESR1/ERBB2 expression and the ROR-S and ROR-P scores and risk groups; with hasClinical = TRUE also the ROR-C and ROR-PC scores and risk groups.
- mdns: data.frame of the precomputed subgroup-specific quantiles used for gene centering.
- outList: list with the internal nearest-centroid results (predictions, distances, centroids, ...).

References

Zhao X, Rodland EA, Tibshirani R, Plevritis S. *Molecular subtyping for clinically defined breast cancer subgroups*. Breast Cancer Research. 2015;17(1):29. <https://doi.org/10.1186/s13058-015-0520-4>

Fernandez-Martinez A, Krop IE, Hillman DW, Polley MY, Parker JS, Huebner L, et al. *Survival, pathologic response, and genomics in CALGB 40601 (Alliance), a neoadjuvant Phase III trial of paclitaxel–trastuzumab with or without lapatinib in HER2-positive breast cancer*. Journal of Clinical Oncology. 2020;38(36):4184–4197. <https://doi.org/10.1200/JCO.20.01276>

Examples

```
## Example: Updated subgroup-specific quantiles (ER.v2)
data("OSL02EMIT0obj")
res <- BS_ssBC(
  se_obj = OSL02EMIT0obj$data_input$se_NC,
  s = "ER.v2",
  Subtype = FALSE,
  hasClinical = FALSE
)
```

BS_sspbc

Intrinsic Subtyping using SSPBC (BS_sspbc)

Description

Implements **SSPBC (Single Sample Predictor for Breast Cancer)**, a refinement of the original AIMS methodology trained on the large, population-based SCAN-B RNA-seq cohort. SSPBC provides robust single-sample predictions, independent of cohort composition, and supports multiple model variants for different applications.

Usage

```
BS_sspbc(se_obj, ssp.name = "ssp.pam50")
```

Arguments

- | | |
|----------|---|
| se_obj | <p>A SummarizedExperiment object containing:</p> <ul style="list-style-type: none"> • Assay data: A gene expression matrix with genes (Entrez IDs) as rows and samples as columns. <ul style="list-style-type: none"> – Expression values must be positive (e.g., FPKM or log2(FPKM+1)). – Values should not be gene-centered or globally scaled. |
| ssp.name | <p>Character. Specifies the SSPBC model to use:</p> <ul style="list-style-type: none"> • "ssp.pam50": Predicts PAM50-based intrinsic subtypes. • "ssp.subtype": Predicts Prosigna-like subtypes (four subtypes, excluding Normal-like). |

Value

A **list** with the following elements:

- c1: Molecular class identified by the sspbc models for each sample (one of "Basal", "Her2", "LumA", "LumB", with or without "Normal").
- prob: Numeric vector of posterior probabilities corresponding to the assigned subtype in c1 (one value per sample).
- all.probs: Matrix of posterior probability values for all samples and all subtypes (rows = samples, columns = subtypes).
- rules.matrix: Binary (0/1) matrix of the pairwise gene-expression rules (*gene A* < *gene B*) used for assignment (rows = rules, columns = samples); 1 indicates the rule evaluated to TRUE for that sample.
- data.used: Expression values actually used to evaluate the simple rules.
- EntrezID.used: Character vector of EntrezGene IDs used for rule evaluation.

References

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. *RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer*. *NPJ Breast Cancer*. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
## Example using SSPBC with the PAM50 model
data("OSL02EMIT0obj")
res <- BS_sspbc(
  se_obj = OSL02EMIT0obj$data_input$se_SSP,
  ssp.name = "ssp.pam50"
)
```

Gene.ID.ann

Gene.ID.ann: Gene annotation table

Description

Annotation table for GENCODE Human Release 27 genes (Gene.ID) used by StringTie summarisation. Includes HGNC, EntrezGene, and RefSeq identifiers derived from GENCODE v27 metadata.

Usage

```
data("Gene.ID.ann")
```

Format

An object of class `data.frame` with 19675 rows and 6 columns.

Details

Used by internal SSP application functions to translate identifiers prior to classification with SSP models.

Value

Gene.ID.ann Data frame of annotations for GENCODE v27 genes.

References

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. *RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer*. *NPJ Breast Cancer*. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
library(BreastSubtypeR)
data("Gene.ID.ann")
```

iBreastSubtypeR	<i>Launch the iBreastSubtypeR Shiny app</i>
-----------------	---

Description

Starts the Shiny UI bundled with the BreastSubtypeR package. The launcher can (optionally) attach Shiny/Bslib so UI/server can use unqualified functions like tags, icon, fileInput, etc.

Usage

```
iBreastSubtypeR(
  attach = c("shiny", "bslib"),
  attach_tidyverse = FALSE,
  max_upload_mb = 1000
)
```

Arguments

attach	Character vector of packages to attach before launch. Defaults to c("shiny","bslib"). Set to character(0) to skip attaching.
attach_tidyverse	Logical; if TRUE and tidyverse is installed, it will be attached quietly for the session (purely optional).
max_upload_mb	Numeric; Shiny upload size limit (in MB). Default 1000.

Value

The value returned by shiny::runApp() (usually invisible(NULL)).

Examples

```
if (interactive()) {
  iBreastSubtypeR()
  iBreastSubtypeR(attach = character(0))
}
```

Mapping

*Gene ID Mapping***Description**

Preprocesses and maps gene expression input to prepare for intrinsic subtyping workflows (NC- and SSP-based).

Usage

```
Mapping(
  se_obj,
  RawCounts = FALSE,
  method = c("max", "mean", "median", "iqr", "stdev"),
  impute = TRUE,
  verbose = TRUE
)
```

Arguments

se_obj

A SummarizedExperiment object containing:

- **Assay data:**
 - If RawCounts = FALSE: assay() must contain log2-normalized expression (e.g., pre-normalized microarray/nCounter, or log2(FPKM+1) RNAseq).
 - If RawCounts = TRUE: assay() contains raw RNA-seq counts (see RawCounts).
- **Row metadata** (required):
 - "probe": feature identifiers (e.g., gene symbols or probe IDs)
 - "ENTREZID": corresponding Entrez Gene IDs.
 - If row names are gene symbols, provide an additional SYMBOL column, renamed as probe.
- **Column metadata:** sample-level metadata in colData(). Specifically:
 - PatientID (**required**).
 - **Receptor fields required by specific methods** (and by BS_Multi() / AUTO):
 - * ER - required for ssBC, cIHC / cIHC.itr, PCAPAM50, and AUTO.
 - * HER2 - required for ssBC with s="ER.v2" and for AUTO.
 - * TN - per-sample annotation required for ssBC with s="TN" / "TN.v2"; AUTO uses TNBC-specific cohort handling only when all evaluable TN annotations indicate TN.
 - **Clinical covariates for ROR (NC-based methods only):**
 - * TSIZE: Tumor size (0 = $\leq$ 2 cm; 1 = $>$ 2 cm). Must be numeric (0/1).
 - * NODE: Lymph node status (0 = negative; $\geq$ 1 = positive). Must be numeric (0/1).

	– Preferred canonical receptor coding: * ER: ER+, ER– * HER2: HER2+, HER2– (note: HER2 "2+" is not coerced) * TN: TN, nonTN – Automatic normalization: Mapping() maps common variants to canonical forms (e.g., Pos/Neg, Positive/Negative, 1/0, True/False). Ambiguous HER2="2+" is not remapped. – These receptor fields are not required for purely SSP methods (AIMS, sspsc) or for PAM50 when used without AUTO/ROR; they are recommended so AUTO can select suitable methods.
RawCounts	Logical. If TRUE, indicates that assay() holds raw RNA-seq counts. In this case, rowData() must also provide gene lengths (column "Length", in base pairs), used for: • NC-based methods: log2-CPM (upper-quartile normalization). • SSP-based methods: linear FPKM (not log-transformed).
method	Strategy for resolving duplicate probes/genes, i.e. several rows mapping to the same Entrez ID. "mean", "median", "iqr" and "stdev" follow collapseIDs() of the original PAM50 bioclassifier code (Parker et al., 2009); "max" is an addition of this package. Options: • "mean": per-sample mean across the duplicate probes (collapses the probes into one row; long-oligo arrays, e.g., Agilent/Illumina). • "median": per-sample median across the duplicate probes (collapses the probes into one row). • "iqr": keeps the single probe with the highest interquartile range (short-oligo arrays, e.g., Affymetrix). • "stdev": keeps the single probe with the highest standard deviation. • "max": keeps the single probe with the largest row sum across samples (often used for RNA-seq).
impute	Logical. If TRUE, applies KNN-based imputation to missing values.
verbose	Logical. If TRUE, prints progress messages during execution.

Details

Mapping() supports multiple input types:

- **Raw RNA-seq counts** (with gene lengths): normalized to CPM (NC) or FPKM (SSP).
- **Precomputed log2(FPKM+1)**: used directly for NC; back-transformed for SSP.
- **log2-normalized microarray/nCounter data**: used directly for NC; back-transformed for SSP.

This design allows users to supply a single expression format, while BreastSubtypeR automatically applies method-specific preprocessing.

Phenodata normalization. #' If receptor fields are present, they are coerced to canonical encodings (ER -> {ER+, ER–}, HER2 -> {HER2+, HER2–}, TN -> {TN, nonTN}). Ambiguous values (e.g., HER2 == "2+") are left unchanged and emit a warning.

Value

A named list with:

se_NC SummarizedExperiment holding log2-transformed data prepared for NC-based methods (assay name: counts).

se_SSP SummarizedExperiment holding linear-scale data prepared for SSP-based methods (assay name: counts).

References

Yang Q, Hartman J, Sifakis EG. *BreastSubtypeR: A Unified R/Bioconductor Package for Intrinsic Molecular Subtyping in Breast Cancer Research*. NAR Genomics and Bioinformatics. 2025. <https://doi.org/10.1093/nargab/lqaf131>. Selected as Editor’s Choice.

Examples

```
if (requireNamespace("SummarizedExperiment", quietly = TRUE)) {
  # Using example raw RNA-seq counts (with gene lengths)
  data("TCGABRCAobj")
  se_obj_counts <- TCGABRCAobj$se_obj[, 1:3] # tiny subset to keep checks fast
  res <- Mapping(se_obj_counts, RawCounts = TRUE)

  # Using example pre-normalized log2(FPKM+0.1)
  data("OSLO2EMIT0obj")
  se_obj_fpkm <- OSLO2EMIT0obj$se_obj[, 1:3] # tiny subset to keep checks fast
  res <- Mapping(se_obj_fpkm, RawCounts = FALSE)
}
```

OSLO2EMIT0obj	<i>OSLO2EMIT0obj: Example dataset (OSLO2-EMIT0 cohort subset)</i>
---------------	---

Description

Example object derived from the OSLO2-EMIT0 cohort (Staaf et al., 2022). Includes a subset of normalized expression data, clinical metadata, feature annotations, and example outputs from Mapping() and BS_Multi().

Usage

```
data("OSLO2EMIT0obj")
```

Format

A list with:

se_obj A SummarizedExperiment containing a subset of the log2-transformed, normalized expression matrix (log2(FPKM+0.1)) with colData clinical metadata and row-level feature annotations.

`data_input` Example output structure produced by `Mapping()`.

`res` Example results from `BS_Multi()` run in *AUTO* mode.

References

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer. *NPJ Breast Cancer*. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
library(BreastSubtypeR)
data("OSL02EMIT0obj")
```

`ssbbc.models`

ssbbc.models: Short names for 11 SSPBC predictors

Description

List of 11 single-sample predictor (SSP) models from Staaf et al. (2022), indexed by short names used by `ssbbc`.

Usage

```
data("ssbbc.models")
```

Format

An object of class `list` of length 11.

Details

Names correspond to short model identifiers. The contents are identical to `ssbbc.models.fullname`, which uses full model names.

Value

`ssbbc.models` Named list of 11 SSP models used by `ssbbc`.

References

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer. *NPJ Breast Cancer*. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
library(BreastSubtypeR)
data("sspbcr.models")
```

sspbcr.models.fullname *sspbcr.models.fullname: Full names for 11 SSPBC predictors*

Description

List of the same 11 SSP models (Staaf et al., 2022) indexed by full model names.

Usage

```
data("sspbcr.models.fullname")
```

Format

An object of class `list` of length 11.

Details

Identical content to `sspbcr.models` but with full model names as list keys.

Value

```
sspbcr.models.fullname
      Named list of 11 SSP models used by sspbc.
```

References

Staaf J, Häkkinen J, Hegardt C, Saal LH, Kimbung S, Hedenfalk I, et al. RNA sequencing-based single sample predictors of molecular subtype and risk of recurrence for clinical assessment of early-stage breast cancer. *NPJ Breast Cancer*. 2022;8(1):27. <https://doi.org/10.1038/s41523-022-00465-3>

Examples

```
library(BreastSubtypeR)
data("sspbcr.models.fullname")
```

TCGABRCAobj

*TCGABRCAobj: Example dataset (TCGA-BRCA subset)***Description**

Example object derived from TCGA-BRCA. Includes a subset of normalized metadata

- raw counts (as a SummarizedExperiment), and example outputs from Mapping() and BS_Multi() to facilitate runnable examples.

Usage

```
data("TCGABRCAobj")
```

Format

A list with:

se_obj A SummarizedExperiment containing the integer raw-count matrix (top 5,000 variable genes), rowData with probe, SYMBOL, ENTREZID, Length, and colData with PatientID, ER, PR, HER2.

data_input Example Mapping() output created from se_obj.

res Example BS_Multi() results (e.g., run in *AUTO* mode).

Source

The Cancer Genome Atlas (TCGA) BRCA via GDC; counts summarized with recount3; clinical data retrieved with TCGAbiolinks.

References

The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumors. *Nature*. 2012;490(7418):61–70. <https://doi.org/10.1038/nature11412>

Colaprico A, Silva TC, Olsen C, Garofano L, Cava C, Garolini D, et al. TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res*. 2016;44(8):e71. <https://doi.org/10.1093/nar/gkv1507>

Collado-Torres L, Nellore A, Kammers K, Ellis SE, Taub MA, Hansen KD, et al. Reproducible RNA-seq analysis using recount2. *Nat Biotechnol*. 2017;35(4):319–321. <https://doi.org/10.1038/nbt.3838>

Examples

```
library(BreastSubtypeR)
data("TCGABRCAobj")
names(TCGABRCAobj)
# str(TCGABRCAobj$se_obj); head(colData(TCGABRCAobj$se_obj))
```

Vis_boxplot

Boxplot of Correlation per Subtype

Description

This function generates a boxplot to visualize the correlation distribution between different subtypes of breast cancer, based on the provided correlation table and subtype information.

Usage

```
Vis_boxplot(out, correlations)
```

Arguments

out	A data frame containing the columns "PatientID" and "Subtype". The "PatientID" column should have unique identifiers for each patient, and the "Subtype" column should specify the assigned subtype for each patient.
correlations	A data frame or matrix containing the correlation values computed from NC-based methods.

Value

A ggplot object representing the boxplot visualization of the correlation distributions across the different subtypes.

Examples

```
data("OSL02EMIT0obj")
res <- OSL02EMIT0obj$res

# Prepare data: Subtype information and correlation matrix
out <- data.frame(
  PatientID = res$results$genefu.robust$BS.all$PatientID,
  Subtype = res$results$genefu.robust$BS.all$BS
)

correlations <- res$results$genefu.robust$outList$distances

# Generate the boxplot
p <- Vis_boxplot(out, correlations)
plot(p)
```

Description

This function generates a heatmap to visualize gene expression patterns across breast cancer subtypes, based on the provided gene expression matrix and subtype information.

Usage

```
Vis_heatmap(x, out)
```

Arguments

<code>x</code>	A gene expression matrix, where genes are rows and samples are columns. The data should be log2 transformed.
<code>out</code>	A data frame containing two columns: "PatientID" and "Subtype". The "PatientID" column should contain unique patient identifiers, and the "Subtype" column should specify the assigned subtype for each patient.

Value

A ggplot or heatmap object (depending on implementation) representing the heatmap of gene expression across different subtypes.

Examples

```
library(SummarizedExperiment)
data("OSL02EMIT0obj")
res <- OSL02EMIT0obj$res

# Prepare data: Gene expression matrix and subtype information
x <- assay(OSL02EMIT0obj$data_input$se_NC)
out <- data.frame(
  PatientID = res$results$genefu.robust$BS.all$PatientID,
  Subtype = res$results$genefu.robust$BS.all$BS
)

# Generate the heatmap
p <- Vis_heatmap(x, out)
plot(p)
```

Vis_Multi	<i>Multi-Method Subtype Heatmap Visualization</i>
-----------	---

Description

This function generates a heatmap to visualize breast cancer subtypes classified by multiple subtyping methods. It helps users compare how different methods assign subtypes to the same set of samples.

Usage

```
Vis_Multi(data)
```

Arguments

data Output of the [BS_Multi](#) function.

Value

Returns a heatmap visualizing the subtype classifications across multiple methods.

Examples

```
data("OSLO2EMIT0obj")

# Assuming `OSLO2EMIT0obj$res$res_subtypes` contains multi-method subtype results
p <- Vis_Multi(OSLO2EMIT0obj$res$res_subtypes)
plot(p)
```

Vis_PCA	<i>PCA Plot Visualization of Gene Expression by Subtype</i>
---------	---

Description

This function generates a PCA plot to visualize the principal components of gene expression data, colored by the assigned subtypes. Optionally, it can display a scree plot of eigenvalues to evaluate the explained variance.

Usage

```
Vis_PCA(x, out, Eigen = FALSE)
```

Arguments

x	A gene expression matrix, where genes are rows and samples are columns. The data should be log2 transformed.
out	A data frame containing two columns: "PatientID" and "Subtype". The "PatientID" column should contain unique patient identifiers, and the "Subtype" column should specify the assigned subtype for each patient.
Eigen	Logical. If TRUE, the function will display a scree plot showing the eigenvalues of the principal components.

Value

A ggplot object representing the PCA plot, colored by subtype. If Eigen is set to TRUE, a scree plot of the eigenvalues is also included.

Examples

```
library(SummarizedExperiment)
data("OSL02EMIT0obj")
res <- OSL02EMIT0obj$res

# Prepare data: Gene expression matrix and subtype information
x <- assay(OSL02EMIT0obj$data_input$se_NC)
out <- data.frame(
  PatientID = res$results$genefu.robust$BS.all$PatientID,
  Subtype = res$results$genefu.robust$BS.all$BS
)

# Generate the PCA plot
p <- Vis_PCA(x = x, out = out)
plot(p)

# Generate PCA plot with scree plot of eigenvalues
p_with_eigen <- Vis_PCA(x = x, out = out, Eigen = TRUE)
plot(p_with_eigen)
```

Vis_pie

*Pie Chart Visualization of Subtype Distribution***Description**

This function generates a pie chart to visualize the distribution of breast cancer subtypes in a cohort, based on the provided Subtype data.

Usage

```
Vis_pie(out)
```

Arguments

out A data frame containing two columns: "PatientID" and "Subtype". The "PatientID" column should contain unique patient identifiers, and the "Subtype" column should specify the assigned subtype for each patient.

Value

A ggplot object representing a pie chart showing the proportion of each subtype in the dataset.

Examples

```
data("OSL02EMIT0obj")
res <- OSL02EMIT0obj$res

# Prepare data: Subtype information
out <- data.frame(
  PatientID = res$results$genefu.robust$BS.all$PatientID,
  Subtype = res$results$genefu.robust$BS.all$BS
)

# Generate the pie chart
p <- Vis_pie(out = out)
plot(p)
```

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