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DS_Type: A framework to detect phenotype-specific co-expression shifts in small-n RNA-seq

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Protocol status: In development

We are still developing and optimizing this protocol

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Keywords: gene co-expression analysis, differential network analysis, small-n RNA-seq, classifies gene pairs into ds1, specific changes in gene, classifies gene pair, ds-type, rna, ds1, gene, ds3, ds2, phenotype, seq this protocol, seq design, seq

Abstract

This protocol provides an executable workflow to detect phenotype-specific changes in gene–gene co-expression from small-N RNA-seq designs. The procedure reconstructs per-condition correlations, estimates empirical *P*-values by permutation, and classifies gene pairs into DS1 (gain), DS2 (loss), or DS3 (changed magnitude) relative to paired before/after states (e.g., DMSO → GL24) for each cell line.

Before start

- Operating system: Linux/macOS/Windows with Rscript available.
- R (≥ 4.2) with packages: data.table, stringr (install commands below).
- Source code and toy example: GitHub release/DS_type (https://github.com/telunmai/DS_type)

Section 1 — Environment setup

1 Install R packages

```
install.packages(data.table)
install.packages(stringr)
```

2 Load R packages

```
library(data.table)
library(stringr)
```

Section 2 — Step 1: Per-condition correlations with empirical P

3 Run Step 1 (default settings)

Script 1 builds per-group correlation statistics and permutation empirical P -values.

```
Rscript Step1.Correlation_for_DS.R
```

Input file: TPM_matrix_sample.txt (genes $\times$ samples, with one **gene ID column**.)

Output file: DS_pairwise_cor_stats.tsv (Build per-group stats (V_, Cor_, P_, empP_))

Notes.

- A gene pair is evaluable within a group if ≥ 3 valid paired observations.
- The script sets an internal random seed per permutation replicate to support reproducibility.
- The number of permutations (B_perm) controls stability of empirical P ; increase for larger datasets.

Section 3 — Step 2: DS classification

4 Run Step 2 (default settings)

Script 2 calls **S/NS/NA** per condition, derives per-line transitions, and classifies **DS1/DS2/DS3**.

```
Rscript Step2.Identify_DS_types.R
```

Input file: DS_pairwise_cor_stats.tsv

Output file: DS_types.tsv (Classify DS1/DS2/DS3 from the stats table)

Notes.

- Significant (S): $P < 0.005$ and $\text{emp}P < 0.005$.
- Non-significant (NS): $P > 0.05$ (irrespective of $\text{emp}P$)
- NA: any other case

5 Results: DS classification

For each line's before → after switch:

- **DS1 (gain):** all evaluable "effective" lines switch from NS/NA → S; reference lines remain unchanged (if required).
- **DS2 (loss):** all evaluable "effective" lines switch from S → NS/NA; reference lines unchanged (if required).
- **DS3 (changed magnitude):** evaluable "effective" lines remain S→S, **and** the absolute change in correlation magnitude satisfies (default delta_r_thresh is 0.10):

$$\Delta r = ||r_{\text{after}}| - |r_{\text{before}}|| \geq \text{delta_r_thresh}$$