

# PECA: Probe-level Expression Change Averaging

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January 30, 2026

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## Contents

<b>1</b>	<b>Introduction</b>	<b>2</b>
<b>2</b>	<b>Proteomics data</b>	<b>3</b>
<b>3</b>	<b>Affymetrix microarray data</b>	<b>4</b>

# 1 Introduction

PECA determines differential gene/protein expression using directly the probe/peptide-level measurements from Affymetrix gene expression microarrays or proteomic datasets, instead of the common practice of using pre-calculated gene/protein-level values. An expression change between two groups of samples is first calculated for each measured probe/peptide. The gene/protein-level expression changes are then defined as medians over the probe/peptide-level changes. This is illustrated in fig 1. For more details about the probe-level expression change averaging (PECA) procedure, see Elo et al. (2005), Laajala et al. (2009) and Suomi et al. (2015).



Figure 1: Probe-level expression change averaging.

PECA calculates the probe/peptide-level expression changes using the ordinary or modified t-statistic. The ordinary t-statistic is calculated using the function `rowttests` in the Bioconductor `genefilter` package. The modified t-statistic is calculated using the linear modeling approach in the Bioconductor `limma` package. Both paired and unpaired tests are supported.

The significance of an expression change is determined based on the analytical p-value of the gene-level test statistic. Unadjusted p-values are reported along with the corresponding p-values looked up from beta distribution taking into account the number of probes/peptide per gene/protein. The quality control and filtering of the data (e.g. based on low intensity or probe specificity) is left to the user.

## 2 Proteomics data

This is an example of tab-separated text file being used as an input. First row of the file contains the column names, which can be later used to select the sample groups for comparison (here from A1 to B3). First column should always contain the id (e.g. UniProt Accession) for which the results are summarized for. Also the column names should not start with a number or contain any special letters.

```
> filePath <- system.file("extdata", "peptides.tsv", package="PECA")
> data <- read.csv(file=filePath, sep="\t")
> head(data)
```

	Accession	Sequence	A1	A2	A3	B1
1	P00890	AIGVLPQLIIDR	473957.7045	797849.8423	889740.345	814284.358
2	P00890	AIGVLPQLIIDR	2938.4231	2624.9122	4251.228	10801.785
3	P00890	AIGVLPQLIIDR	523.6715	267.5509	1114.278	5234.959
4	P00890	ALSADLAAR	1088987.8817	752050.9742	619136.168	934525.787
5	P00890	ANQEVLEWLFK	105059.5950	66372.1954	158287.020	207838.534
6	P00890	ANQEVLEWLFK	2106.2001	5952.6551	4077.048	16127.419
		B2	B3			
1	898365.633	789901.918				
2	18419.565	15483.544				
3	4311.213	2235.556				
4	750771.408	832069.894				
5	151461.468	300008.469				
6	9989.237	6234.200				

PECA can be run by loading the package and setting the sample names of groups to be compared. These should match the column names in the input text file. After this the PECA\_tsv function can be called using the input file path and groups as parameters. Note that the sample names provided by user are used to subset the data so that the input file may contain more columns (e.g. samples or other information) than those used for comparison.

```
> library(PECA)
> group1 <- c("A1", "A2", "A3")
> group2 <- c("B1", "B2", "B3")
> results <- PECA_tsv(filePath, group1, group2)
```

Results can then be viewed, stored to disk, or processed further using R.

```
> head(results)
```

	slr	t	score	n	p	p.fdr
P00890	-0.4740294	-1.3423222	0.2506136	34	0.0008385463	0.01048183

```
P00899 -0.3112597 -0.8144448 0.4610984 6 0.4211490342 0.76510811
P00924 0.1707304 0.5772173 0.5947238 4 0.6579379772 0.96755585
P00925 -0.1036027 -0.5590090 0.6059719 14 0.7929340751 1.00000000
P00927 -0.3864132 -0.6227038 0.5672214 5 0.6245295607 0.96755585
P00931 -0.1835956 -0.8090350 0.4638697 9 0.4117016088 0.76510811
```

### 3 Affymetrix microarray data

First we load the example library `SpikeIn` containing the `SpikeIn133` dataset that we can use for input. This package needs to be installed separately from Bioconductor.

```
> library(SpikeIn)
> data(SpikeIn133)
```

We subset the original dataset for our purposes, which are two groups with three replicates. First group contains indexes 1, 15, and 29. Second group contains indexes 2, 16, and 30. This subset is then used as input for PECA.

```
> data <- SpikeIn133[,c(1,15,29,2,16,30)]
> results <- PECA_AffyBatch(normalize="true", affy=data)
```

Results can then be viewed, stored to disk, or processed further using R.

```
> head(results)
```

	slr	t	score	n	p	p.fdr
1007_s_at	-0.062497118	-0.7880544	0.4747409	16	0.4186358	1
1053_at	-0.033076247	-0.2606295	0.8072457	16	0.9974403	1
117_at	0.016630316	0.1743281	0.8700752	16	0.9998545	1
121_at	-0.001890579	-0.0300819	0.9774428	16	1.0000000	1
1255_g_at	-0.018923755	-0.1613340	0.8796512	16	0.9999185	1
1294_at	-0.024047196	-0.4637408	0.6669455	16	0.9188036	1