

HowTo plw

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1 Introduction

This vignette describes how to use *plw*, an R implementation of the Probe level Locally moderated Weighted median-t (PLW) method (Åstrand et al., 2007a) for finding differentially expressed genes. PLW uses an empirical Bayes model taking into account the dependency between variability and intensity-level. A global covariance matrix is also used allowing for differing variances between arrays as well as array-to-array correlations, and thus PLW performs weighted analysis. PLW is specially designed for Affymetrix type arrays (or other multiple-probe arrays). Instead of making inference on probe-set summaries, comparisons are made separately for each perfect-match probe and are then summarized into one score for the probe-set. The Locally Moderated Weighted-t (LMW) method, applying the model of PLW on probe-set summaries or data from single probe arrays, is also implemented in the *plw* package. See Åstrand et al. (2007a) for details on PLW and LMW, and Kristiansson et al. (2005, 2006), Sjögren et al. (2007), and Åstrand et al. (2007b) for details on weighted analysis for microarrays. PLW is demonstrated in Sections 2 to 4, and LMW in Section 5.

2 Data

The R-package *plw* depends on the *affy* package, available from the Bioconductor¹ project, which is loaded automatically when loading *plw*:

```
> require(plw)
```

The *affy* package contains functions for reading CEL-file data into an **AffyBatch** object using the function **ReadAffy**. It also contains functions for doing low-level analysis, such as background correction, normalization, and calculating expression indexes. For example, the **rma** function performs all three steps in one call and returns an **ExpressionSet** object holding RMA expression indexes. For further details on how to read CEL-file data into R use

```
> help(ReadAffy)
```

In this vignette the PLW method is demonstrated using the **AffySpikeU95Subset** data set of 6 arrays and 1016 probe-sets. The data set was loaded using the **ReadAffy** function and is included in the *plw* package. **AffySpikeU95Subset** is a sub-set of the Affymetrix U95 Latin-Square spike-in data set of 59 arrays and 12626 probe-sets. For these data there are 16 known differentially expressed genes/probe-sets Cope et al. (2004), of which all 16 are included in **AffySpikeU95Subset**. The data set is loaded using

```
> data(AffySpikeU95Subset)
```

```
> AffySpikeU95Subset
```

¹<http://bioconductor.org/>

```

AffyBatch object
size of arrays=182x182 features (14 kb)
cdf=HGU95subset1016 (1016 affyids)
number of samples=6
number of genes=1016
annotation=hgu951016
notes=

```

3 Running PLW

The `AffySpikeU95Subset` data set use data from groups a and b of the Affymetrix U95 Latin-Square spike-in data set. Here we show how to do a comparison of these two groups. The fifth letter of the CEL-file names holds the group assignment of each array which we can inspect using the `pData` function

```

> pData(AffySpikeU95Subset)

              sample
1521a99hpp_av06.CEL      1
1532a99hpp_av04.CEL      2
2353a99hpp_av08.CEL      3
1521b99hpp_av06.CEL      4
1532b99hpp_av04.CEL      5
2353b99hpp_av08r.CEL     6

```

We define a design using the function `model.matrix`, and a contrast matrix for comparing groups a and b.

```

> group <- factor(rep(letters[1:2], each = 3))
> design <- model.matrix(~group - 1)
> contrast <- matrix(c(1, -1), 1, 2)

```

```

> design

  groupa groupb
1      1      0
2      1      0
3      1      0
4      0      1
5      0      1
6      0      1
attr(,"assign")
[1] 1 1
attr(,"contrasts")
attr(,"contrasts")$group
[1] "contr.treatment"

> contrast

      [,1] [,2]
[1,]    1  -1

```

Now we are ready to use the `plw` function.

```

> plwFit <- plw(AffySpikeU95Subset, design = design, contrast = contrast,
+   epsilon = 1e-05)

```

```
> plwFit
```

```
Call:
```

```
plw(x = AffySpikeU95Subset, design = design, contrast = contrast,      epsilon = 1e-05)
```

```
Number of arrays      : 6
Number of probe-sets  : 1016
Number of PM probes   : 16256
Number of knots for v: 6
m parameter           : 9.328
Df for probe t-stat.  : 13.3
Convergence status    : TRUE
Number of iterations  : 51 12
```

From the output we can see that steps 1 and 2 of the procedure used in `plw` required 51 and 12 iterations, respectively (see Åstrand et al. (2007a) for details of the procedure). The estimated value for the m -parameter is 9.328 and the degrees of freedom for the moderated t-statistics is 13.3.

4 Analysing PLW output

There are three functions for displaying the ranking of probe-sets with respect to differential expression, `topRankSummary`, `plotSummaryT`, and `plotSummaryLog2FC`. All three show results for a given number of top ranking probe-sets (e.g. probe-set ranked 1-20), for a specific list of ranks (e.g. probe-set ranked 1,5, and 7), or for a specific list of probe-sets. For example we can display the result for the 16 spiked-in probsets.

```
> topRankSummary(plwFit, genes = spikedProbesU95)
```

	Rank	Median t	Q1-t	Q3-t	Med. log2FC
37777_at	16	-1.032	-2.11	-0.5477	-0.323
684_at	61	-0.702	-1.64	-0.0697	-0.145
1597_at	54	-0.709	-1.56	-0.0339	-0.138
38734_at	8	-3.948	-4.94	-1.7735	-0.666
39058_at	10	-3.148	-4.32	-2.4664	-0.562
36311_at	4	-5.612	-6.89	-3.7220	-0.794
36889_at	9	-3.657	-4.82	-1.4953	-0.650
1024_at	3	-5.719	-6.84	-5.1256	-0.895
36202_at	2	-6.059	-7.06	-5.3868	-0.827
36085_at	5	-5.394	-6.08	-4.3263	-0.569
40322_at	11	-2.627	-3.25	-1.9677	-0.250
407_at	13	-1.203	-2.51	-0.1519	-0.353
1091_at	12	-1.703	-3.50	-0.7309	-0.165
1708_at	1	37.206	31.92	45.3687	7.049
33818_at	7	-4.718	-4.86	-3.3829	-0.512
546_at	6	-4.759	-5.90	-2.3678	-0.695

We can also display results for probe-sets ranked 11 to 20,

```
> topRankSummary(plwFit, genesOfRank = 11:20)
```

	Rank	Median t	Q1-t	Q3-t	Med. log2FC
40322_at	11	-2.627	-3.251	-1.968	-0.250
1091_at	12	-1.703	-3.500	-0.731	-0.165
407_at	13	-1.203	-2.513	-0.152	-0.353

36400_at	14	1.126	0.530	1.565	0.273
33040_at	15	1.053	0.156	2.100	0.311
37777_at	16	-1.032	-2.107	-0.548	-0.323
31642_at	17	1.026	0.700	1.972	0.342
39311_at	18	1.008	0.168	1.324	0.165
39045_at	19	-0.996	-1.400	0.126	-0.133
33527_at	20	0.967	0.306	1.286	0.348

Alternatively, we can display the result for the 20 top ranking probe-sets,

```
> topRankSummary(plwFit, nGenes = 20)
```

	Rank	Median	t	Q1-t	Q3-t	Med. log2FC
1708_at	1	37.206	31.924	45.369	7.049	
36202_at	2	-6.059	-7.058	-5.387	-0.827	
1024_at	3	-5.719	-6.845	-5.126	-0.895	
36311_at	4	-5.612	-6.886	-3.722	-0.794	
36085_at	5	-5.394	-6.085	-4.326	-0.569	
546_at	6	-4.759	-5.895	-2.368	-0.695	
33818_at	7	-4.718	-4.856	-3.383	-0.512	
38734_at	8	-3.948	-4.941	-1.774	-0.666	
36889_at	9	-3.657	-4.818	-1.495	-0.650	
39058_at	10	-3.148	-4.317	-2.466	-0.562	
40322_at	11	-2.627	-3.251	-1.968	-0.250	
1091_at	12	-1.703	-3.500	-0.731	-0.165	
407_at	13	-1.203	-2.513	-0.152	-0.353	
36400_at	14	1.126	0.530	1.565	0.273	
33040_at	15	1.053	0.156	2.100	0.311	
37777_at	16	-1.032	-2.107	-0.548	-0.323	
31642_at	17	1.026	0.700	1.972	0.342	
39311_at	18	1.008	0.168	1.324	0.165	
39045_at	19	-0.996	-1.400	0.126	-0.133	
33527_at	20	0.967	0.306	1.286	0.348	

The other two functions plot individual values for each perfect-match probe together with the median value. The `plotSummaryT` plots t-statistics, whereas `plotSummaryLog2FC` plots logged fold-change values, as shown in Figures 1 and 2, respectively.

```
> plotSummaryT(plwFit, genes = spikedProbesU95)
```

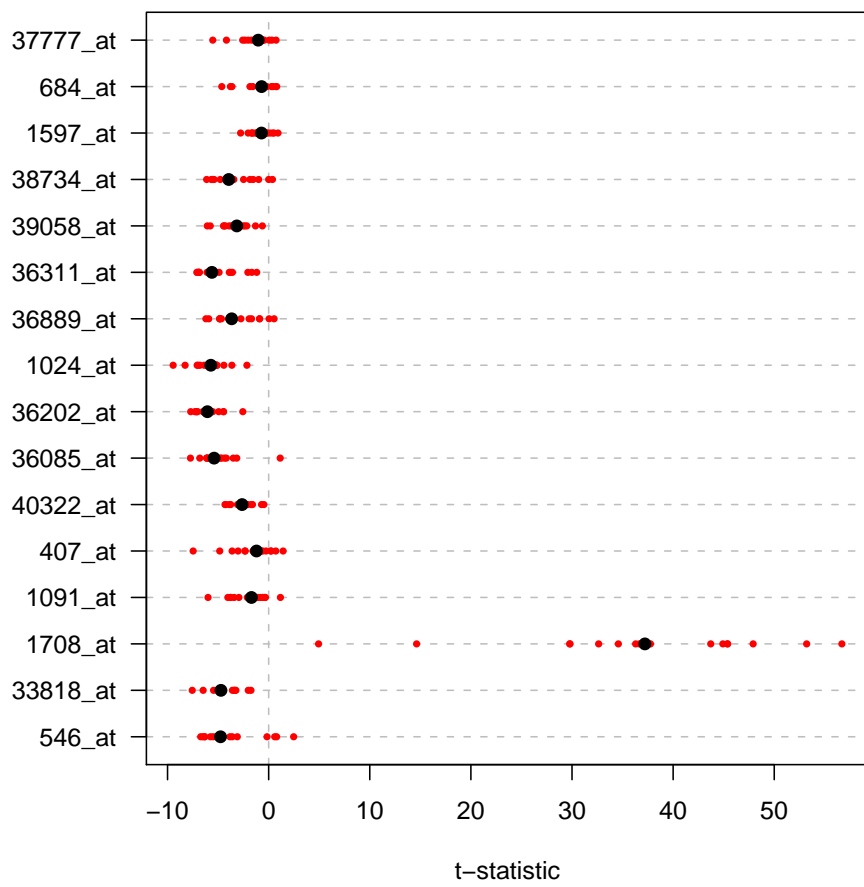


Figure 1: T-statistics for spiked-in probsets.

```
> plotSummaryLog2FC(plwFit, nGenes = 15)
```

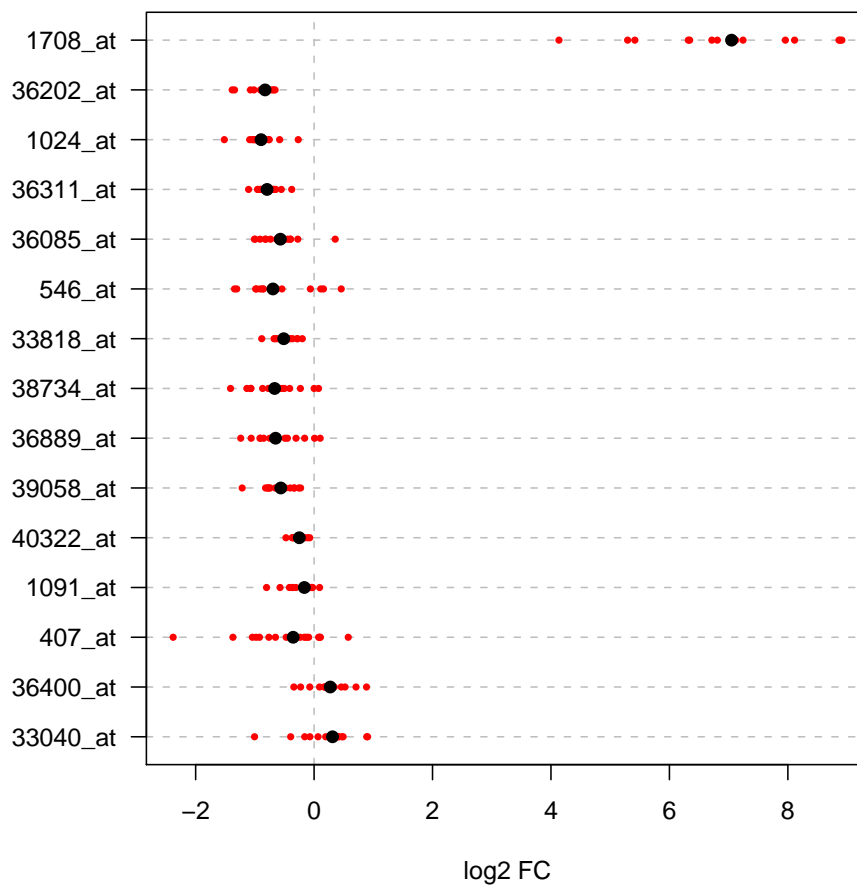


Figure 2: Logged fold-change values for the 15 top ranking probe-sets.

The `plw` function uses an empirical bayes model with an inverse-gamma prior for the unknown variances, where the scale parameter of the inverse-gamma prior is modeled as a function of mean intensity. With the `varHistPlot` function we can compare the fitted distribution for $\log(s^2)$ with the observed data, and with the `scaleParameterPlot` function we can look at the fitted curve for the scale parameter ν of the inverse-gamma prior. See Figures 3 and 4, respectively.

```
> varHistPlot(plwFit)
```

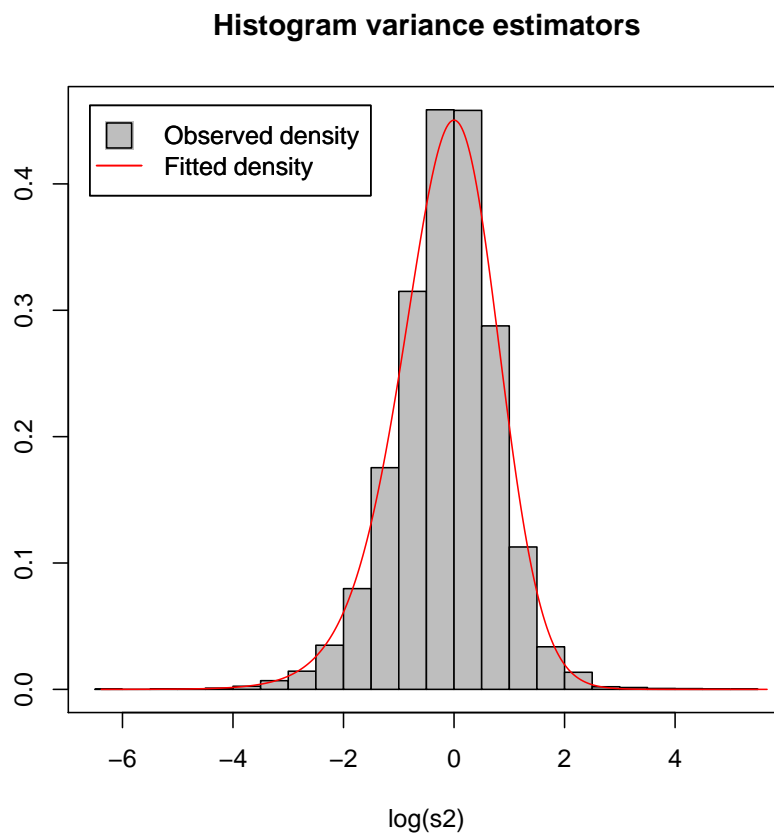


Figure 3: Comparing the fitted distribution for $\log(s^2)$ with the observed data.

```
> scaleParameterPlot(plwFit)
```

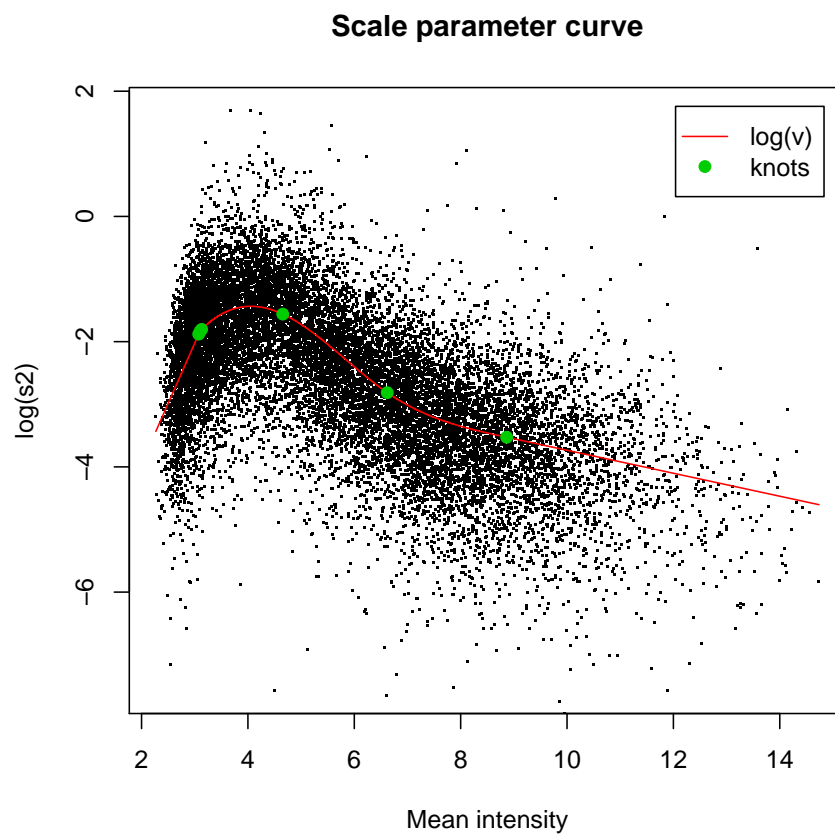


Figure 4: Fitted curve for the scale parameter ν of the inverse-gamma prior.

5 LMW on two-color microarray data

In Åstrand et al. (2007a) the LMW method is used on RMA expression indexes, and `example(lmw)` shows how to use LMW on Affymetrix or other one-color array data. This section demonstrates how to use LMW on the ApoAI data-set (Callow et al., 2000), comparing 8 ApoAI knockout mice with 8 normal mice using a set of $n = 16$ two-color cDNA-arrays. Data was pre-processed as described in (Callow et al., 2000) and the analysis presented here is based on the 6068 genes (out of 6226) having no missing values.

```
> source("http://www.math.chalmers.se/~astrandm/plw/GetApoAIdata.R")
> RG <- GetApoAIdata()
> require(limma)
> MA <- normalizeWithinArrays(RG)
> rownames(MA$M) <- MA$genes$Name
> ii <- apply(is.na(MA$M), 1, any)
> MA$A <- MA$A[!ii, ]
> MA$M <- MA$M[!ii, ]
```

Arrays 1 to 8 is the control group with mRNA from normal mice, whereas arrays 9 to 16 are from the knockout group. Thus, we specify a design and contrast matrix for the comparison of knock out mice with the control group of normal mice.

```
> design <- cbind("Control-Ref" = 1, "KO-Control" = MA$targets$Cy5 ==
+ "ApoAI KO")
> contrast <- matrix(0:1, ncol = 2)
```

```
> design
```

	Control-Ref	KO-Control
[1,]	1	0
[2,]	1	0
[3,]	1	0
[4,]	1	0
[5,]	1	0
[6,]	1	0
[7,]	1	0
[8,]	1	0
[9,]	1	1
[10,]	1	1
[11,]	1	1
[12,]	1	1
[13,]	1	1
[14,]	1	1
[15,]	1	1
[16,]	1	1

```
> contrast
```

	[,1]	[,2]
[1,]	0	1

The analysis using LMW is done using the mean intensity of the sum of logged green and red signal, respectively, to model the scale parameter of the inverse-gamma prior for error variances. Also, the spline-knots for the scale-parameter function are set using a set of sample quantiles (10, 30, 50, 70, and the 90% quantile) of the mean intensity instead of the default knots computing using an internal function.

```

> meanX <- apply(MA$A, 1, mean)
> knots <- quantile(meanX, seq(0.1, 0.9, by = 0.2))
> lmwFit <- lmw(MA$M, design = design, contrast = contrast, meanX = meanX,
+   knots = knots)

> lmwFit

```

Call:

```
lmw(x = MA$M, design = design, contrast = contrast, meanX = meanX,   knots = knots)
```

```

Number of arrays      : 16
Number of probe-sets : 6226
Number of knots for v: 5
m parameter           : 6.051
Df for probe t-stat. : 20.1
Convergence status    : TRUE
Number of iterations  : 21 37

```

From the fitted model we can select the top 10 ranked genes from the analysis,

```
> topRankSummary(lmwFit, nGenes = 10)
```

	Rank	t-statistic	Estimate
ApoAI,lipid-Img	1	-24.781448	-3.1850746
EST,HighlysimilartoA	2	-13.083237	-2.9606061
CATECHOLO-METHYLTRAN	3	-11.818502	-1.7719091
EST,WeaklysimilartoC	4	-11.251879	-0.9573663
ApoCIII,lipid-Img	5	-10.569031	-0.8991223
ESTs,Highlysimilarto	6	-10.546552	-1.0038929
est	7	-9.275085	-0.9190465
similartoyeaststerol	8	-8.518292	-0.9360310
5'similartoPIR:S5501	9	-4.625750	-0.5658059
EST,WeaklysimilartoF	10	-4.273222	-0.4782200

and inspect the model fit for the inverse-gamma prior together with the estimated scale-parameter curve, see Figures 5 and 6, respectively.

```
> varHistPlot(lmwFit)
```

Histogram variance estimators

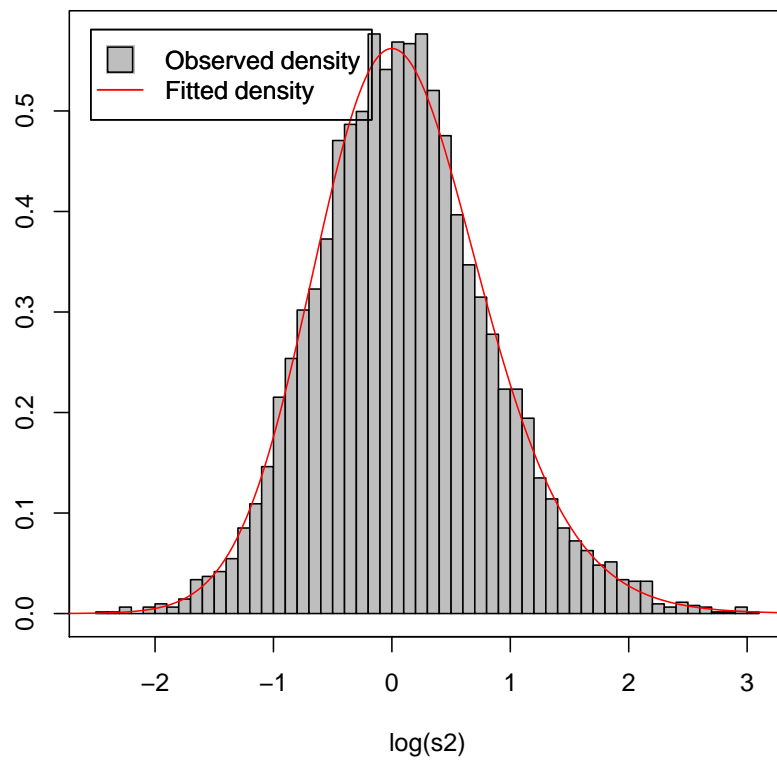


Figure 5: Comparing the fitted distribution for $\log(s^2)$ with the observed data from the ApoAI knockout experiment.

```
> scaleParameterPlot(lmwFit)
```

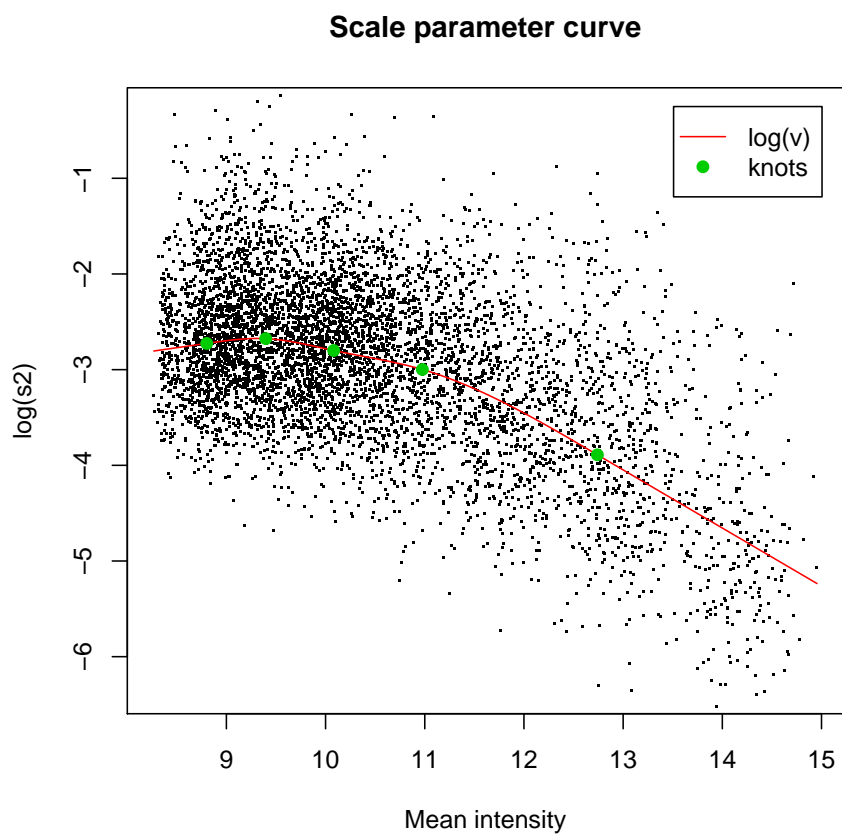


Figure 6: Fitted curve for the scale parameter ν of the inverse-gamma prior from the analysis of the ApoAI data-set.

References

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- Anders Sjögren, Erik Kristiansson, Mats Rudemo, and Olle Nerman. Weighted analysis of general microarray experiments. *BMC Bioinformatics*, 8(1):article 387, 2007.