A Bioconductor workflow for the Bayesian Analysis of Spatial proteomics

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1 Versions

- **R**: R version 3.5.1 Patched (2018-11-02 r75540)
- **Bioconductor**: 3.8
- **pRoloc** 1.23.2

2 Introduction

Quantifying uncertainty in the spatial subcellular distribution of proteins allows for novel insight into protein function (Crook et al. 2018). Many proteins live in a single location within the cell, however there are those that reside in mutiple locations and those that dynamically relocalise (Thul et al. 2017). Functional comparmentalisation of proteins allows the cell to control biomolecular pathways and biochemical process within the cell. Therefore, proteins with multiple localisation may have mutiple functional roles (Jeffery 2009). Machine learning algorithms that fail to quantify uncertainty are unable to draw deeper insight into understanding cell biology from mass-spectrometry (MS) based spatial proteomics experiments.

Bayesian approaches to machine learning and statistical analysis can provide more insight into the data, since uncertainty quantification arises as a consequence of a generative model for the data (Gelman et al. 1995). In a Bayesian framework, a model with paramters for the data is proposed, along with a statement about our prior beliefs of the model paramters. Bayes' theorem tells us how to update the prior distribution of the parameters to obtain the posterior distribution of the parameters after observing the data. It is the posterior distribution which quantifies the uncertainty in the parameters and quantities of interest derived from the data. This contrasts from a maximum-likelihood approach where we obtain only a point estimate of the parameters.

Adopting a Bayesian framework for data analysis, though of much interest to experimentalists, can be challenging. Once we have obtained a probabilistic model, complex algorithms are used to obtain the posterior distribution upon observation of the data. These algorithms can have tuning parameters and many settings, hindering their practical use for those not versed in Bayesian methodology. Even once the algorithms have been correctly set-up, assessments of convergence and guidance on how to intepret the results are often sparse. This workflow presents a Bayesian analysis of spatial proteomics to elucidate the process to any practioners. We hope that it goes beyond simply the methods, data structures and biology presented here, but provides a template for others to design tools using Bayesian methodology for the biological community.

Our model for the data is the t-augmented Gaussian mixture (TAGM) model proposed in (Crook et al. 2018). Crook et al. (2018) provide a detailed description of the model, rigorous comparisons and testing on many spatial proteomics datasets and a case study on a hyperLOPIT experiment on mouse pluripotent stem cells (Christoforou et al. 2016; Mulvey et al. 2017). Revisiting these details is not the purpose of this computational protocol, rather we present how to correctly use the software and provide step by step guidance for interpreting the results.

In brief, the TAGM model posits that each annotated sub-cellular niche can be described by a Gaussian distribution. Thus the full complement of proteins within the cell is captured as a mixture of Gaussians. The highly dynamic nature of the cell means that many proteins are not well captured by any of these multivariate Gaussian distributions, and thus the model also includes an outlier component, mathematically described as multivariate student's t distribution. The heavy tails of the t distribution allow it to better capture dispersed proteins.

To perform inference in the TAGM model there are two approaches. The first, which we refer to as TAGM MAP, allows us to obtain *maximum a posteriori* estimates of posterior localisation probabilities; that is, the modal posterior probability that a protein localises to that class. This approach uses the expectation-maximisation (EM) algorithm to perform inference (Dempster, Laird, and Rubin 1977). Whilst this is a interpretable summary of the TAGM model, it only provides point estimates. For a richer analysis, we present a Markov-chain Monte-Carlo (MCMC) method to perform fully Bayesian inference in our model, allowing us to obtain full posterior localisation distributions. This method is refered to as TAGM MCMC throughout the text.

This workflow begins with a brief review of some of the basic features of mass spectrometry-based spatial proteomics data, including the state-of-the-art computational infrastructure and bespoke software suite. We then present each method in turn, detailing how to obtain high quality results. We provide an extended dicussion of the TAGM MCMC method to highlight some of the challenges when apply this method. This includes how to assess convergence of MCMC methods, as well as methods for manipulating the output. We then take the processed output and explain how to intepret the results, as well as providing some tools for visualisation. We conclude with some remarks and directions for the future.

3 Getting started and infrastructure

In this workflow, we are currently using version 1.23.2 of `pRoloc` (Gatto et al. 2014). The pacakge `pRoloc` contains algorithms and methods for analysing spatial proteomics data, building on the `MSnSet` structure provided in `MSnbase`. The `pRolocdata` package provides many annotated datasets from a variety of species and experimental procedures. The following code chunks install and load the suite of packages require for the analysis.

```r
if (!require("BiocManager"))
    install.package("BiocManager")
BiocManager::install(c("pRoloc", "pRolocdata"))
```

```r
library("pRoloc")
library("pRolocdata")
```

We assume that we have a MS-based spatial proteomics dataset contained in a `MSnSet` structure. For information on how to import data, perform basic data processing, quality control, supervised machine learning and transfer learning see following workflow (Breckels et al. 2016). We use a spatial proteomics dataset on mouse E14TG2a embryonic stem cells (Breckels et al. 2013). The LOPIT protocol (Dunkley et al. 2004, 2006) was used and normalised intensity of proteins from eight iTRAQ 8-plex labelled fraction are provided. The methods provided here are independent of labelling procedure, fractionation process or workflow. Examples of valid experimental protocols are LOPIT (Dunkley et al. 2004), hyperLOPIT (Christoforou et al. 2016; Mulvey et al. 2017), label-free methods such as PCP (Foster et al. 2006), and when fractionation is perform by differential centrifugation (Itzhak et al. 2016, @DC:2018).

In the code chunk below, we load the aforementioned dataset. The printout demonstrates that this experiment quantified 2031 proteins over 8 fractions.

```r
data("E14TG2aR") # load experimental data
E14TG2aR
```

```
## MSnSet (storageMode: lockedEnvironment)
## assayData: 2031 features, 8 samples
##   element names: exprs
## protocolData: none
## phenoData
##   sampleNames: n113 n114 ... n121 (8 total)
##   varLabels: Fraction.information
##   varMetadata: labelDescription
```
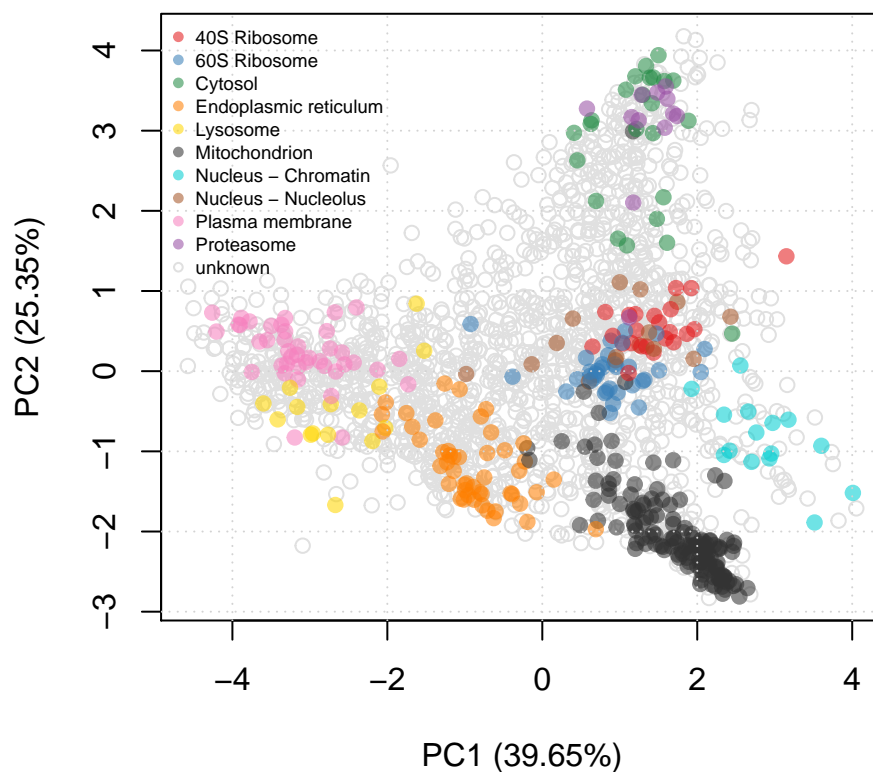
Figure 1: First two principal components of mouse stem cell data.

```
## featureData
##   featureNames: Q62261 Q9JHU4 ... Q9EQ93 (2031 total)
##   fvarLabels: Uniprot.ID UniprotName ... markers (8 total)
##   fvarMetadata: labelDescription
## experimentData: use 'experimentData(object)'
## Annotation:
## - - - Processing information - - -
## Loaded on Thu Jul 16 15:02:29 2015.
## Normalised to sum of intensities.
## Added markers from  'mrk' marker vector. Thu Jul 16 15:02:29 2015
##   MSnbase version: 1.17.12
```

On figure 1 (left), we can visualise the mouse stem cell dataset use the `plot2D` function. We observe that some of the organelle classes overlap and this is a typical feature of biological datasets. Thus, it is vital to perform uncertainty quantification when analysising biological data.

```
plot2D(E14TG2aR)
addLegend(E14TG2aR, where = "topleft", cex = 0.6)
```

4 Methods: *TAGM MAP*

4.1 Introduction to TAGM MAP

We can perform *maximum a posteriori* (MAP) estimation to perform Bayesian inference in our model. The *maximum a posteriori* estimate equals the mode of the posterior distribution and can be used to provide a point estimate summary of the posterior localisation probabilities. It does not provide samples from the posterior distribution, however an extended version of the expectation-maximisation (EM) algorithm can be used in our case, allowing fast inference. The EM algorithm is an algorithm that iterates between an expectation step and a maximisation step. This allows us to find parameters which maximise the logarithm of the posterior, in the presence of latent (unobserved) variables. The EM algorithm is guaranteed to converge to a local mode. The code chunk below excutes the `tagmMapTrain` function for a default of 100 iterations. We use the default priors for simplicity and convenience, however they can be changed, which we explain in a later section. The output is an object of class `MAPParams`, that captures the details of the TAGM MAP model.

```
set.seed(2)
mappars <- tagmMapTrain(E14TG2aR)

## co-linearity detected; a small multiple of
##              the identity was added to the covariance
mappars

## Object of class "MAPParams"
##  Method: MAP
```

TODO Add a note on co-linearity of data.

The results of the modelling can be visualised with the `plotEllipse` function on figure 2. The outer ellipse contains 99% of the total probability whilst the middle and inner ellipses contain 95% and 90% of the probability respectively. The centres of the clusters are represented by black circumpunct (circled dot). We can also plot the model in other principal components. The code chunk below plots the probability ellipses along the first and second, as well as the fourth prinipal component. The user can change the components visualised by altering the `dims` argument.

```
par(mfrow = c(1, 2))
plotEllipse(E14TG2aR, mappars)
plotEllipse(E14TG2aR, mappars, dims = c(1, 4))
```

4.2 The expectation-maximisation algorithm

The EM algorithm is iterative; that is, the algorithm iterates between an expectation step and a maximisation step until the value of the log-posterior does not change (Dempster, Laird, and Rubin 1977). This fact can be used to assess the convergence of the EM algoritm. The value of the log-posterior at each iteration can be accessed with the `logPosteriors` function on the `MAPParams` object. The code chuck below plots the log posterior at each iteration and we see on figure 3 the algorithm rapidly plateaus and so we have acheived convergence. If convergence has not been reached during this time, increase the number of iteration by changing the parameter `numIter` in the `tagmMapTrain` method. In practice, it is not unexpected to observe small fluctations due to numerical errors and this should not concern users.

```
plot(logPosteriors(mappars), type = "b", col = "blue",
     cex = 0.3, ylab = "log-posterior", xlab = "iteration")
```

The code chuck below uses the `MAPParams` object to classify the proteins of unknown localisation using `tagmPredict` function. This method appends new columns to the `fData` columns of the `MSnSet`.

```
E14TG2aR <- tagmPredict(E14TG2aR, mappars) # Predict protein localisation
```
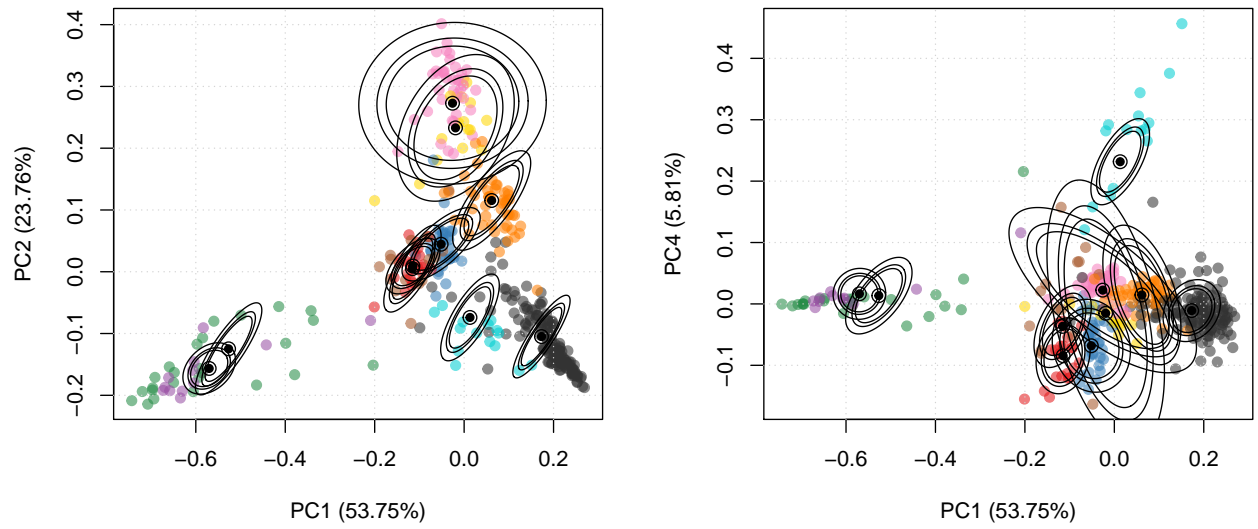
Figure 2: PCA plot with probability ellipses along PC 1 and 2 (left) and PC 1 and 4 (right)
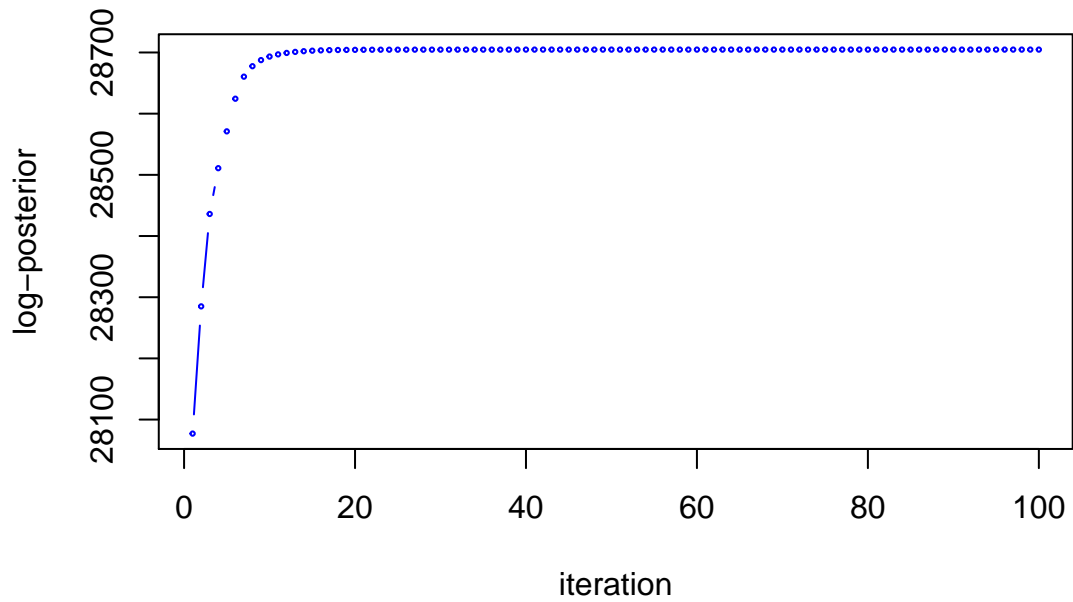

Figure 3: Log-posterior at each iteration of the EM algorithm demonstrating convergence.

The new feature variables that are generated are:

- `tagm.map.allocation`: the TAGM MAP predictions for the most probable protein sub-cellular allocation.

```
table(fData(E14TG2aR)$tagm.map.allocation)
```

```
##
##          40S Ribosome          60S Ribosome               Cytosol
##                    34                    85                   328
## Endoplasmic reticulum              Lysosome         Mitochondrion
##                   284                   147                   341
##   Nucleus - Chromatin   Nucleus - Nucleolus       Plasma membrane
##                   143                   322                   326
##            Proteasome
##                    21
```

- `tagm.map.probability`: the posterior probability for the protein sub-cellular allocations.

```
summary(fData(E14TG2aR)$tagm.map.probability)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.3121  0.9693  0.9995  0.9465  1.0000  1.0000
```

- `tagm.map.outlier`: the posterior probability for that protein to belong to the outlier component rather than any annotated component.

```
summary(fData(E14TG2aR)$tagm.map.outlier)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##       0       0       0       0       0       0
```

We can visualise the results by scaling the pointer according the posterior localisation probabilities. To do this we extract the MAP localisation probabilities from the feature columns of the the `MSnSet` and pass these to the `plot2D` function (figure 4).

```
ptsze <- fData(E14TG2aR)$tagm.map.probability # Scale pointer size
plot2D(E14TG2aR, fcol = "tagm.map.allocation", cex = ptsze)
addLegend(E14TG2aR, where = "topleft", cex = 0.6, fcol = "tagm.map.allocation")
```

The TAGM MAP method is easy to use and it is simple to check convergence, however it is limited in that it can only provide point estimates of the posterior localisation distributions. To obtain the full posterior distributions and therefore a rich analysis of the data, we resort to using Markov-Chain Monte-Carlo methods. In our particular case, we use a so-called collapsed Gibbs sampler (Smith and Roberts 1993).

5   Methods: *TAGM MCMC* a brief overview

The TAGM MCMC method allows a fully Bayesian analysis of spatial proteomics datasets. It employs a collapsed Gibbs sampler to obtain samples from the posterior distribution of localisation probablities, providing a rich analysis of the data. This section demonstrates the advantage of taking a Bayesian approach and the biological information that can be extracted from this analysis.

Since our audience is unlikely to be versed in Bayesian methodology, we explain some of the key ideas for a more complete understanding. Firstly, MCMC based inference constrasts with MAP based inference in that in produces *samples* from the posterior distribution of localisation probabilities. Hence, we do not just have a single estimate for each quantity but a distribution of estimates. MCMC methods are a large class of algorithms used to sample from a probability distribution, in our case the posterior distribution of the parameters (Gilks, Richardson, and Spiegelhalter 1995). They design a Markov-chain; that is, a random sequence of events where the probability of the next event only depends on the current state, which, after
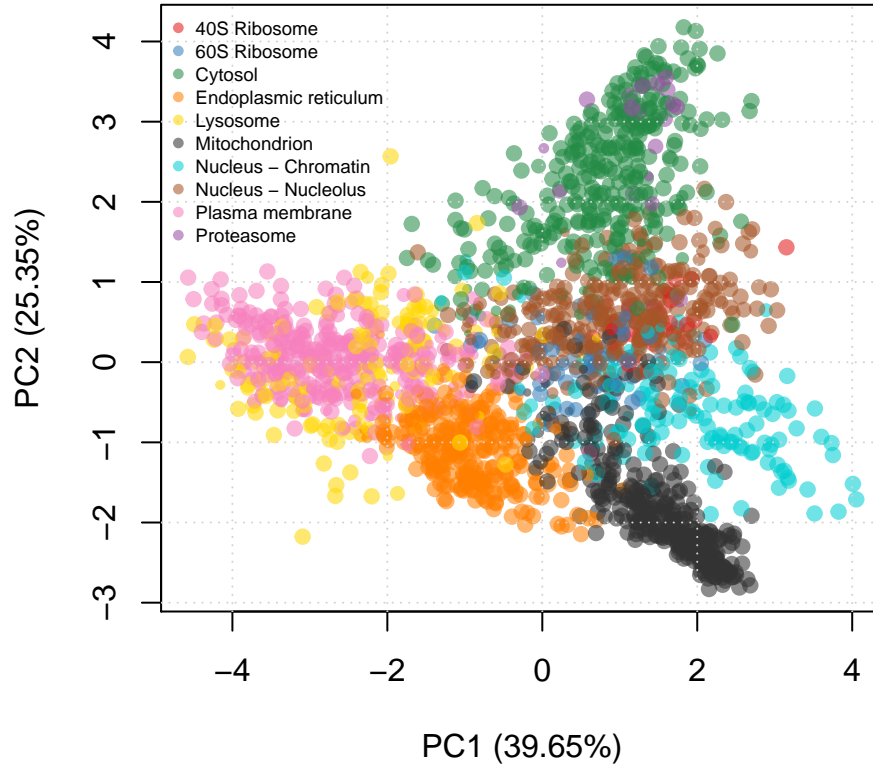
Figure 4: TAGM MAP allocations

convergence, obtains samples form the posterior distribution. A specific example of an MCMC algorithm is the Gibbs sampler, which can be applied when the parameters are conditionally conjugate. Often one can perform Rao-Blackwellisation , a method to reduce posterior variance, to obtain a collapsed Gibbs sampler (Casella and Robert 1996). Once one has obtained samples from the posterior distribution, we can estimate the true mean of the posterior distribution by simply taking the mean of the samples. In a similar fashion, we can obtain other summaries of the posterior distribution.

The TAGM MCMC method is computationally intensive and requires at least modest processing power. Leaving the MCMC algorithm to run overnight on a modern desktop is usually sufficient, however this, of course, depends on the exact system properties. Do note expect the analysis to finish in a couple of hours on a medium specification laptop, for example.

To demonstrate the class structure and expected outputs of the TAGM MCMC method, we run a brief analysis on the a subsest (400 randomly chosen proteins) of the `tan2009r1` dataset from the `pRolocdata` purely for illustration. This is to provide a bare bones analysis of these data without being held back by computational requirements. We perform a complete demonstration and provide precise details of the analysis of the stem cell dataset considered above in the next section.

```
set.seed(1)
data(tan2009r1)
tan2009r1 <- tan2009r1[sample(nrow(tan2009r1), 400), ]
```

The first step is to run a few MCMC chains (below we use only 2) for a few iterations (three below, but typically in the order of ten thousands) of the algorithm using the `tagmMcmcTrain` function. This function will generate a object of class `MCMCParams`.

```
p <- tagmMcmcTrain(object = tan2009r1, numIter = 3,
                   burnin = 1, thin = 1, numChains = 2)
```

```
p
```

```
## Object of class "MCMCParams"
## Method: TAGM.MCMC
## Number of chains: 2
```

Information for each MCMC chain is contained within the chains slot. If needed, this information can be accessed manually. The function `MCMCProcess` processed the `MCMCParams` object and populates the summary slot.

```
p <- tagmMcmcProcess(p)
p
```

```
## Object of class "MCMCParams"
## Method: TAGM.MCMC
## Number of chains: 2
## Summary available
```

The summary slot has now been populated to include basic summaries of the MCMC chains, such as organelle allocations and localisation probabilities. Protein information can be appended to the feature columns of the `MSnSet` by using the `tagmPredict` function, which extracts the required information from the summary slot of the `MCMCParams` object.

```
res <- tagmPredict(object = tan2009r1, params = p)
```

One can now access new features variables:

- `tagm.mcmc.allocation`: the TAGM MCMC prediction for the most likely protein sub-cellular annotation.

```
table(fData(res)$tagm.mcmc.allocation)
```

```
##
## Cytoskeleton              ER         Golgi       Lysosome mitochondrion
##           11              97            23              9            40
##      Nucleus      Peroxisome            PM     Proteasome  Ribosome 40S
##           25               3           101             31            31
## Ribosome 60S
##           29
```

- `tagm.mcmc.probability`: the mean posterior probability for the protein sub-cellular allocations.

```
summary(fData(res)$tagm.mcmc.probability)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##  0.3599  0.8965  0.9880  0.9091  1.0000  1.0000
```

As well as other useful summaries of the MCMC methods:

- `tagm.mcmc.outlier` the posterior probability for the protein to belong to the outlier component.

- `tagm.mcmc.probability.lowerquantile` and `tagm.mcmc.probability.upperquantile` are the lower and upper boundaries to the equi-tailed 95% credible interval of `tagm.mcmc.probability`.

- `tagm.mcmc.mean.shannon` a Monte-Carlo averaged shannon entropy, which is a measure of uncertainty in the allocations.

6 Methods: *TAGM MCMC* the details

In the code chunk below, we load the pre-computed TAGM MCMC model of class `MCMCParams` that is available at https://drive.google.com/open?id=1zozntDhE6YZ-q8wjtQ-lxZ66EEszOGYi.

```
load("e14Tagm.rda")
```

This section explains how to manually manipulate the MCMC output of the TAGM model. The data file 'tagmE14.rda' is available online and is not directly loaded into this package for size. The file itself if around 500mb, which is too large to directly load into a package. The following code, which is not evaluated, was used to produce the `tagmE14` MCMCParams object. We run the MCMC algorithm for 20000 iterations with 10000 iterations discarded for burnin. We then thin the chain by 20. We ran 6 chains in parallel and so we obtain 500 samples for each of the 6 chains, totalling 3000 samples. The resulting file is assumed to be in our working directory.

```
tagmE14 <- tagmMcmcTrain(E14TG2aR2,
                         numIter = 20000,
                         burnin = 10000,
                         thin = 20,
                         numChains = 6)
```

Manually inspecting the object we see that it is a `MCMCParams` object with 6 chains.

```
tagmE14
```

```
## Object of class "MCMCParams"
## Method: TAGM.MCMC
## Number of chains: 6
```

6.1 Data exploration and convergence diagnostics

Assessing whether or not an MCMC algorithm has converged is challenging. Assessing and diagnosing convergence is an active area of research and throughout the 1990s many approaches were proposed [Geweke:1992, Gelman:1992; Roberts:1994; Brooks:1998]. A converged MCMC algorithm should be oscillating rapidly around a single value with no monotonicity. We provide a more detailed exploration of this issue, but the readers should bare in mind that the methods provided below are diagnostics and cannot guarantee success. We direct readers to several important works in the literature discussing the assesment of convergence. Users that do not assess convergence and base their downstream analysis on unconverged chains are likely to obtain poor quality results.

We first assess convergence using a parallel chains approach. We find producing multiple chains is benifical not only for computational advantages but also for analysis of convergence of our chains.

```
## Get number of chains
nChains <- length(tagmE14)
nChains
```

```
## [1] 6
```

The following code chunks sets up a manual convegence diagnostic check. We make use of objects and methods in the package *coda* to peform this analysis (Plummer et al. 2006). Our function below automatically coerces our objects into *coda* for ease of analysis. We calculate the total number of outliers at each iteration of each chain and if the algorithm has converged this number should be the same (or very similar) across all 6 chains. We can observe this from the trace plots and histrograms for each MCMC chain. Unconverged chains are discharded from downstream analysis.

```
## Convergence diagnostic to see if more we need to discard any
## iterations or entire chains: compute the number of outliers for
## each iteration for each chain
```
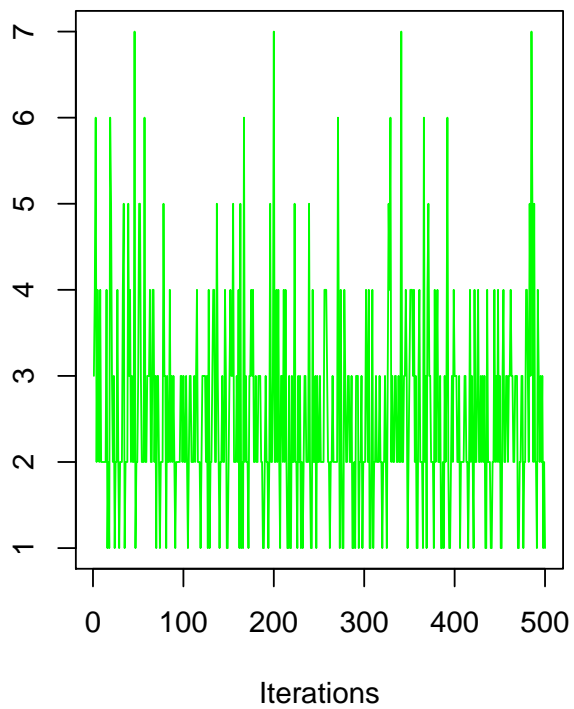
```
out <- mcmc_get_outliers(tagmE14)

## Using coda S3 objects to produce trace plots and histograms
plot(out[[1]], col = "blue",    main = "Chain 1")
```
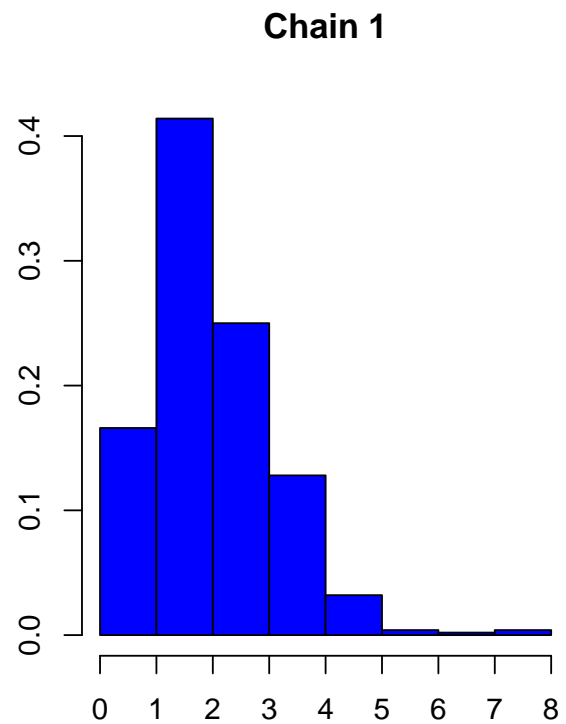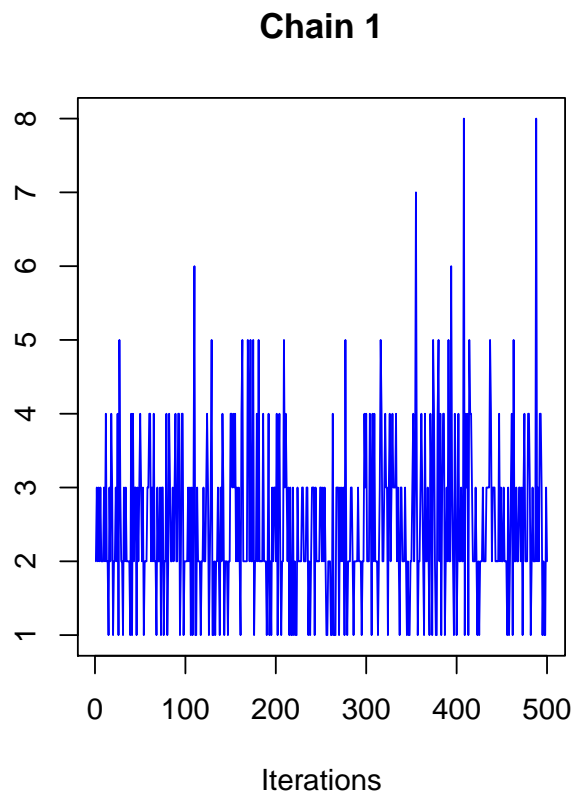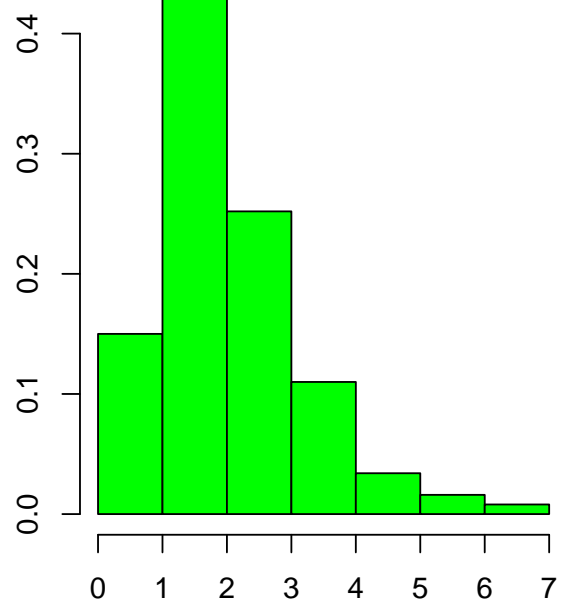


```
plot(out[[2]], col = "green",   main = "Chain 2")
```
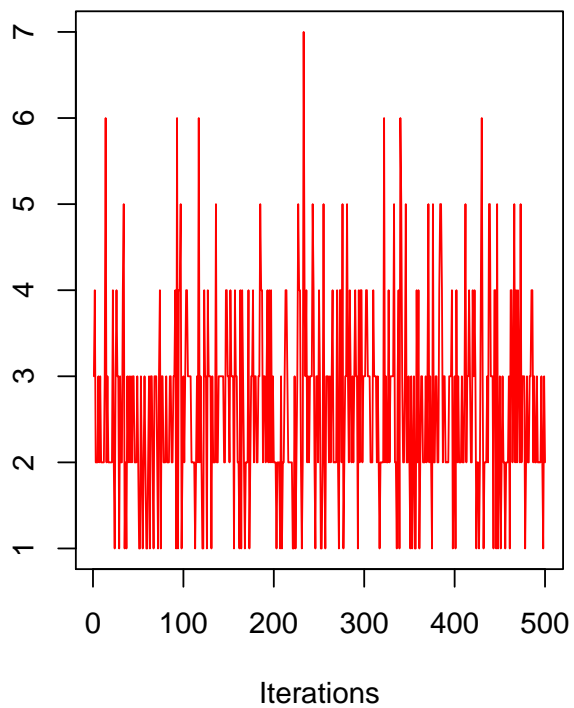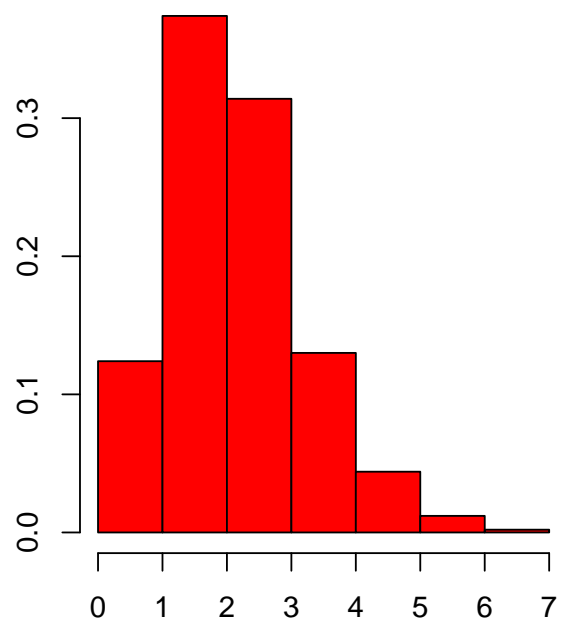
Chain 2

Chain 2

```r
plot(out[[3]], col = "red",    main = "Chain 3")
```

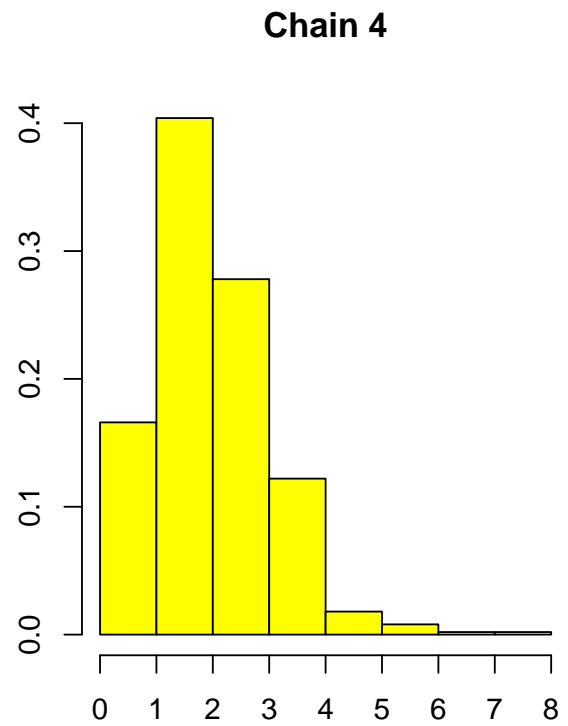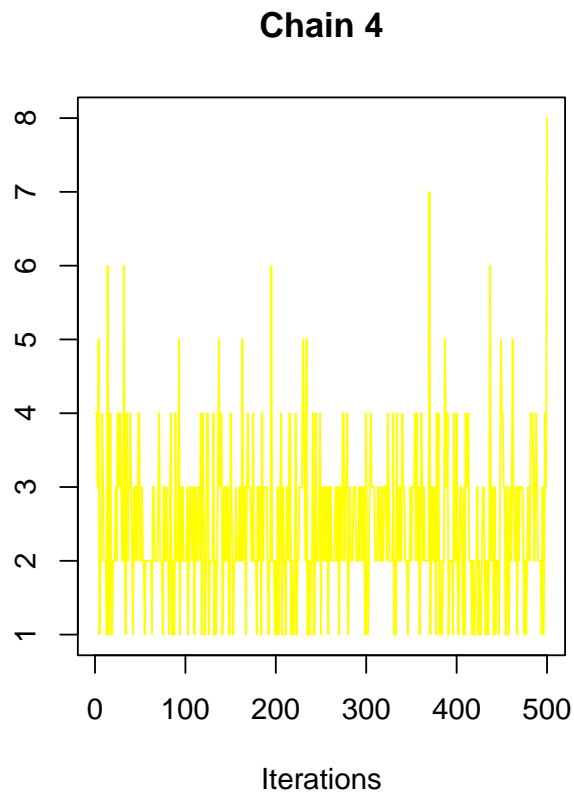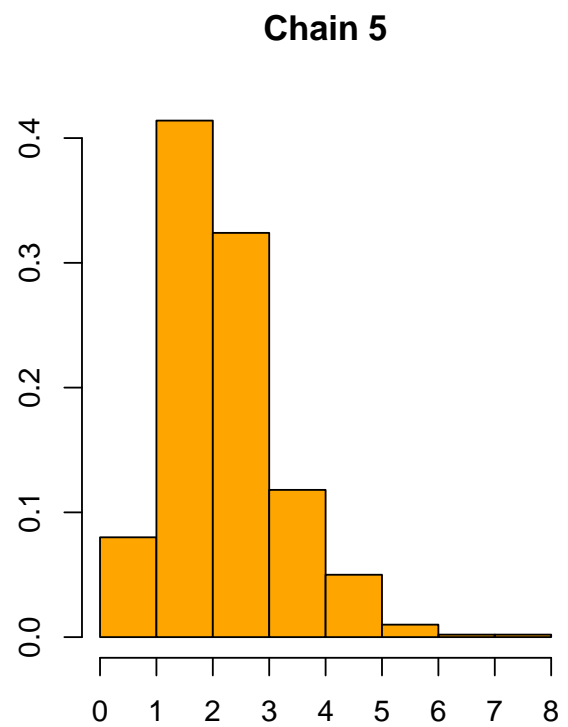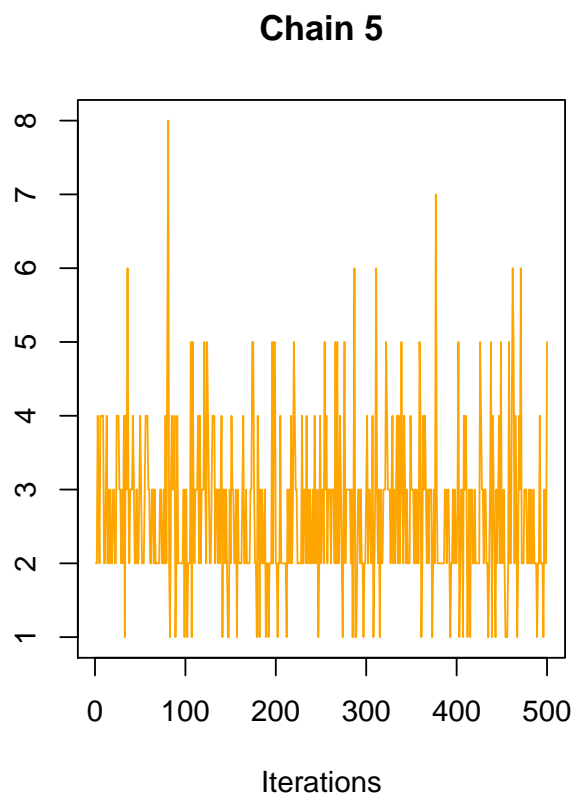## Chain 3

Chain 3

```r
plot(out[[4]], col = "yellow", main = "Chain 4")
```

Chain 4

Chain 4

Iterations

```r
plot(out[[5]], col = "orange", main = "Chain 5")
```

Chain 5

Chain 5

Iterations

```r
plot(out[[6]], col = "purple", main = "Chain 6")
```

Chain 6

Chain 6

Iterations
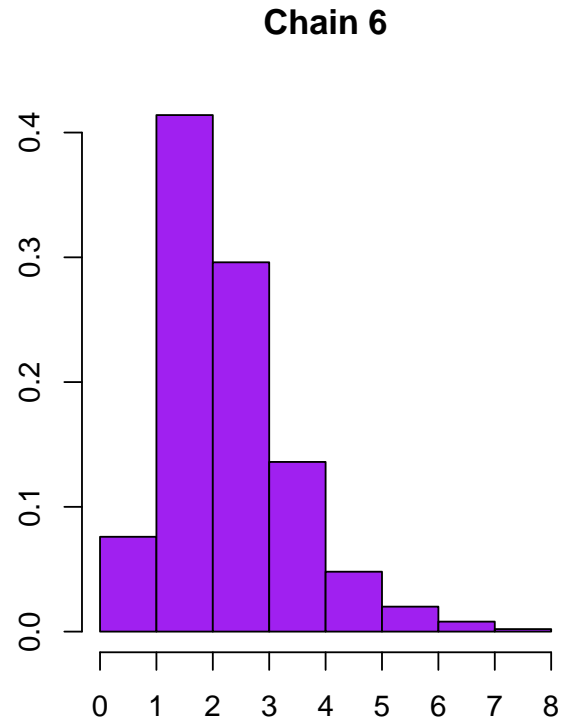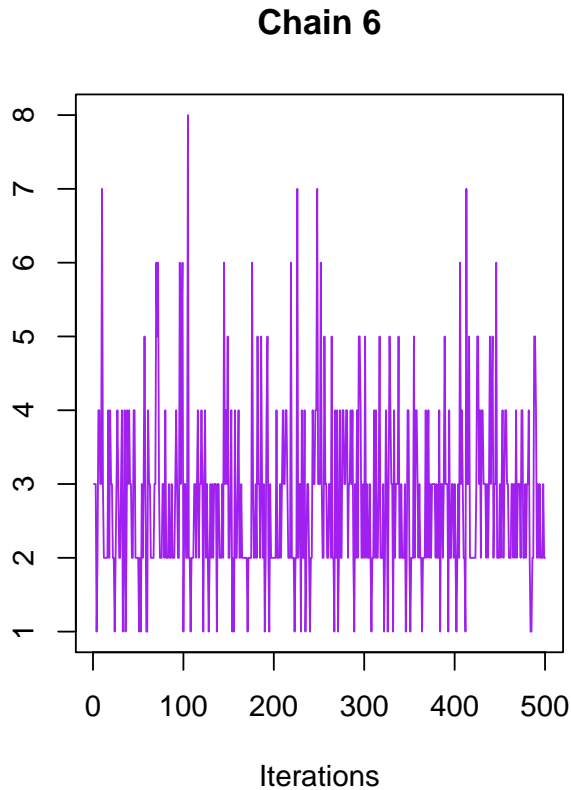
All of the chains are are oscillating around 2.5 and demonstrate similar structure. This is indicative of convergence. We can use the *coda* package to produce summaries of our chains. Here is the `coda` summary for the first chain.

```r
## all chains average around 2.5 outliers
summary(out[[1]])
```

```
##
## Iterations = 1:500
## Thinning interval = 1
## Number of chains = 1
## Sample size per chain = 500
##
## 1. Empirical mean and standard deviation for each variable,
##    plus standard error of the mean:
##
##           Mean            SD      Naive SE Time-series SE
##        2.48600       1.11368       0.04981        0.04981
##
## 2. Quantiles for each variable:
##
##   2.5%    25%    50%    75% 97.5%
##      1      2      2      3      5
```

6.1.1 Applying the Gelman diagnostic

Thus far, our analysis appears promising. Each chain oscillates around an average of 2.5 outliers and there is no observed monotonicity in our output. However, for a more rigorous and unbiased analysis of convergence

we can calculate the Gelman diagnostic using the *coda* package (Gelman and Rubin 1992; Brooks and Gelman 1998). This statistics is often refered to as $\hat{R}$ or the potential scale reduction factor. The idea of the Gelman diagnostics is to compare the inter and intra chain variances. The ratio of these quantities should be close to one. The actual statistics computed is more complicated, but we do not go deeper here and a more detailed and in depth discussion can be found in the references. The *coda* package also reports the 95% upper confidence interval of the $\hat{R}$ statistic. In this case ,our samples are not normally distributed. The *coda* package allows for transformations to improve normality of the data, in our case a log tranform is performed. Gelman and Rubin (1992) suggests that chains with $\hat{R}$ value of less than 1.2 are likely to have converged.

```
## We can check Gelman diagnostic for convergence
## (values less than <1.2  are good for convergence)
gelman.diag(out, transform = TRUE) # the Upper C.I. is 1.03 so mcmc has likely converged
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]       1.01       1.03
```

We can also look at the Gelman diagnostics statistics for groups or pairs of chains. The first line below compute the Gelman diagnostic across the first three chains, whereas the second calculates between chain 2 and chain 5.

```
## We can also check individual pairs of chains for convergence
gelman.diag(out[1:3], transform = TRUE) # the upper C.I is 1.02
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]       1.01       1.02
```

```
gelman.diag(out[c(2,5)], transform = TRUE) # the upper C.I is 1.08
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]       1.02       1.08
```

To assess another summary statistics, we can look at the mean component allocation at each iteration of the MCMC algorithm and as before we produce trace plots of this quantity.
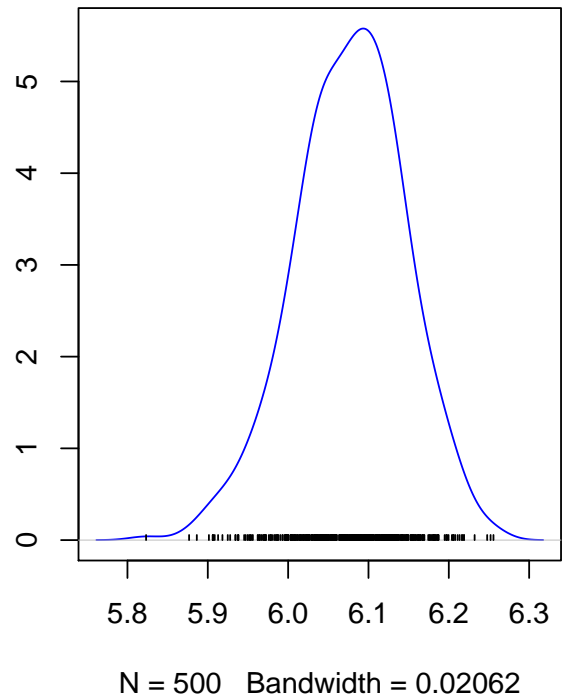
```
# Compute the mean component allocation at each mcmc iterations
meanAlloc <- mcmc_get_meanComponent(tagmE14)
```

```
plot(meanAlloc[[1]], col = "blue",   main = "Chain 1")
```

Chain 1
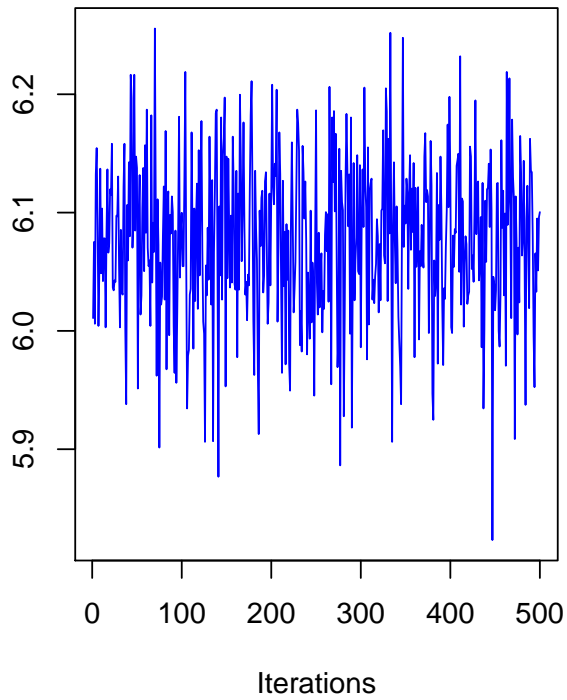
Chain 1

```r
plot(meanAlloc[[2]], col = "green",  main = "Chain 2")
```
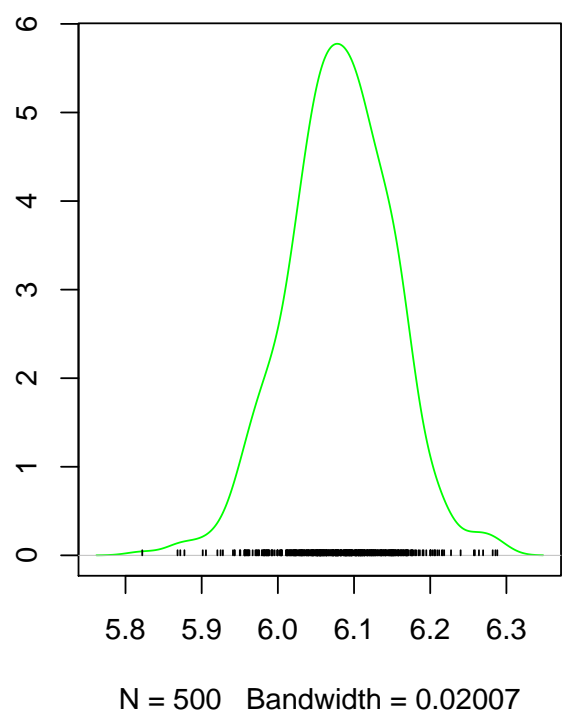
Chain 2

Chain 2

```
plot(meanAlloc[[3]], col = "red",    main = "Chain 3")
```

Chain 3

Iterations

Chain 3

N = 500   Bandwidth = 0.01654
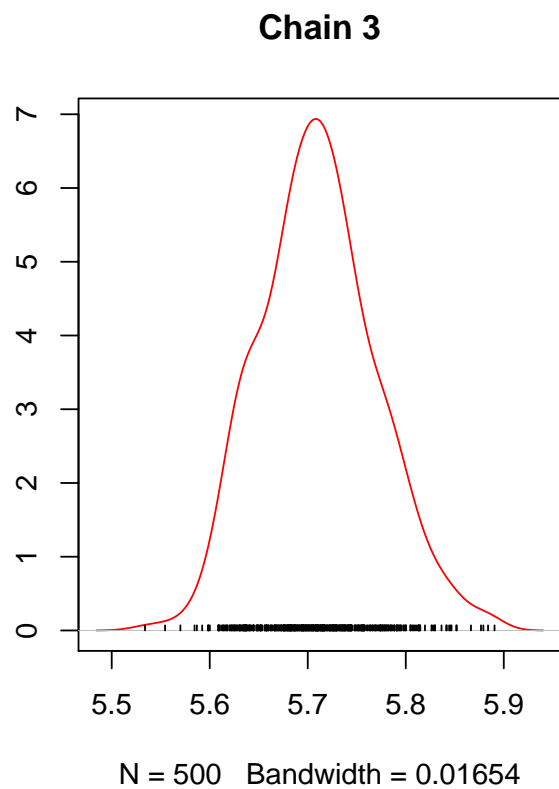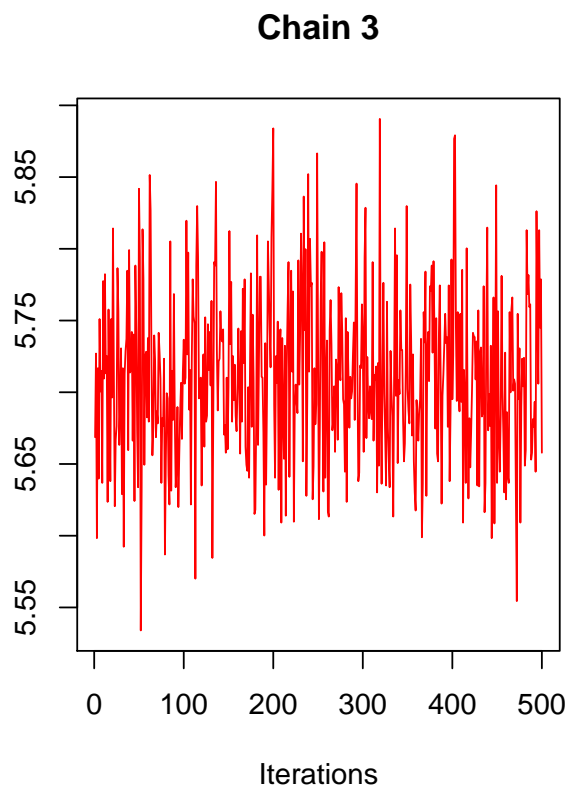
```
plot(meanAlloc[[4]], col = "yellow", main = "Chain 4")
```

Chain 4

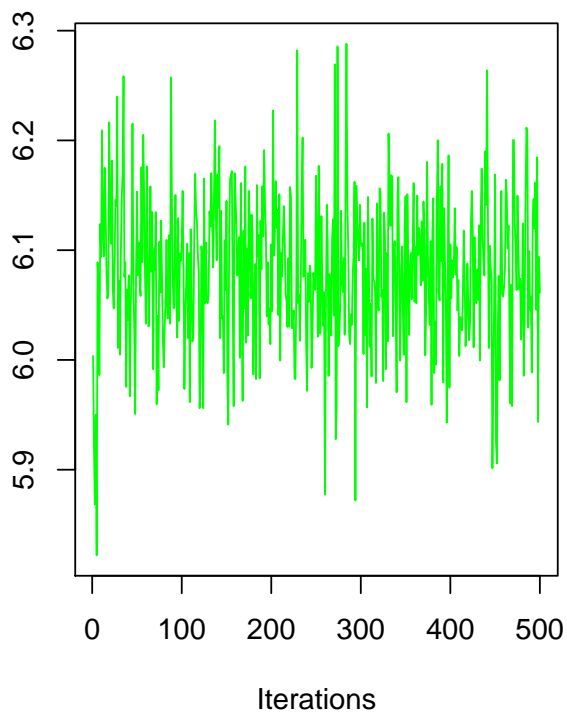

Iterations

Chain 4

N = 500   Bandwidth = 0.02076

16
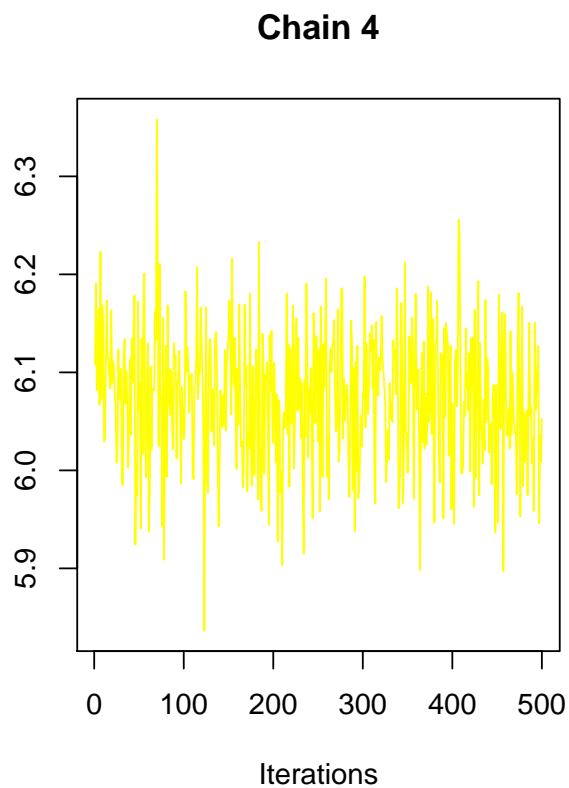
```
plot(meanAlloc[[5]], col = "orange", main = "Chain 5")
```
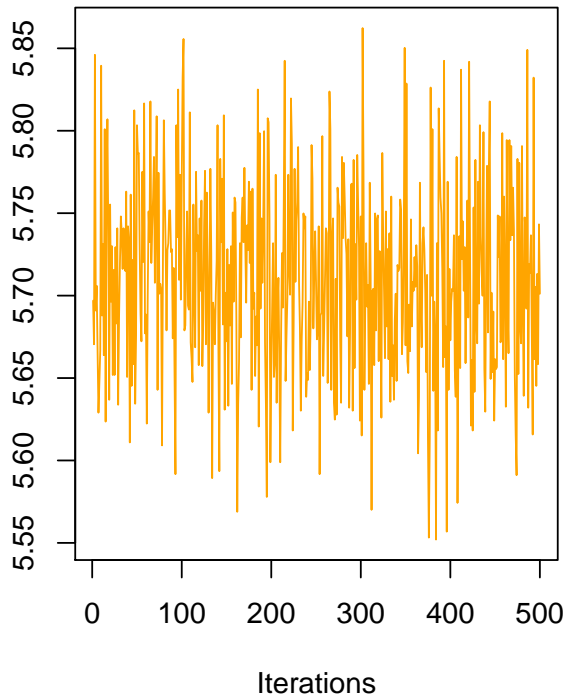

Chain 5

Chain 5

```
plot(meanAlloc[[6]], col = "purple", main = "Chain 6")
```
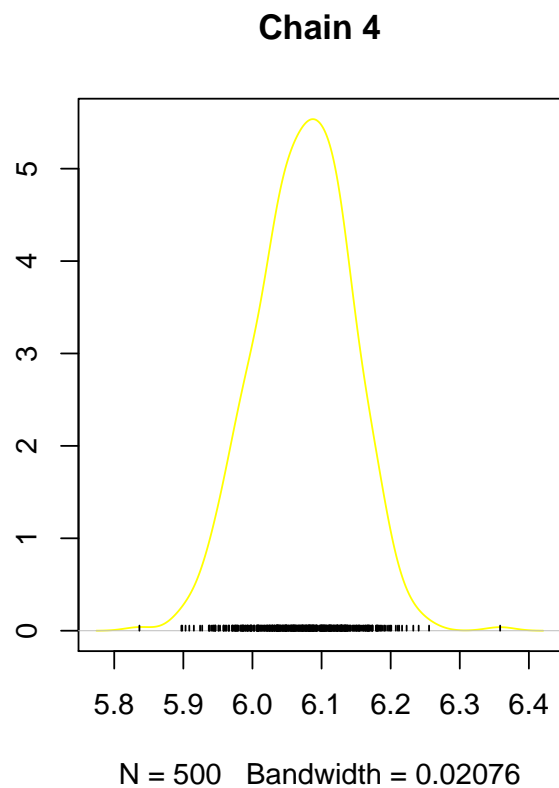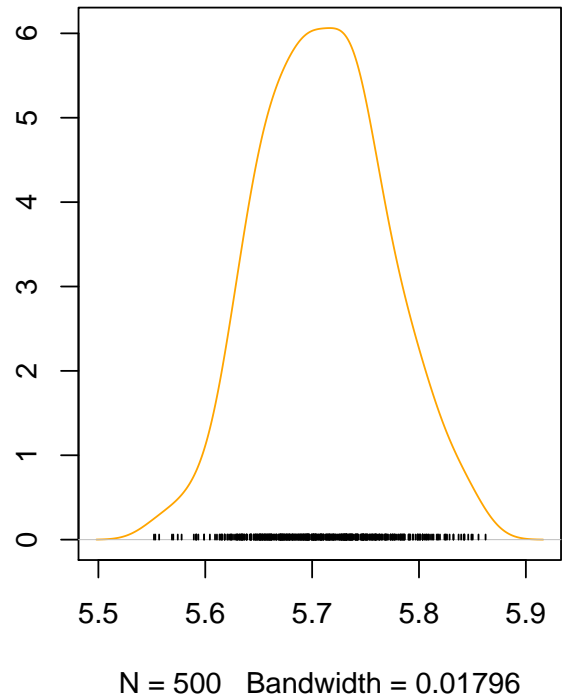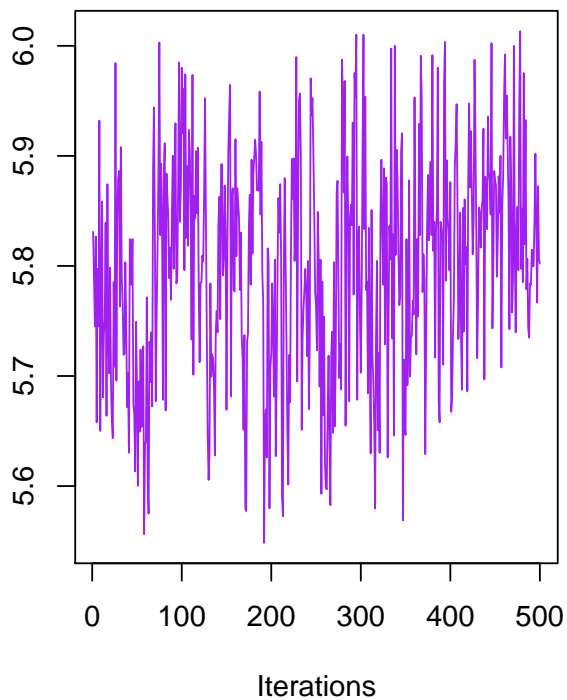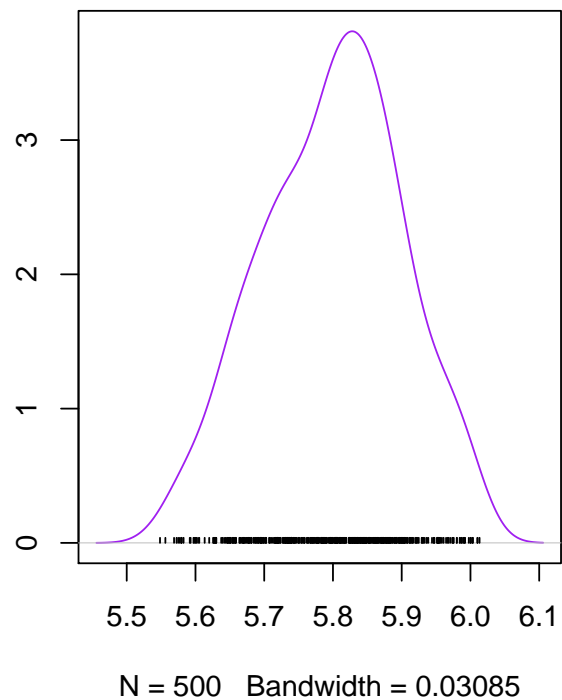

Chain 6

Chain 6

As before we can produce summaries of the data.

```
summary(meanAlloc[[1]])
```

```
##
## Iterations = 1:500
## Thinning interval = 1
## Number of chains = 1
## Sample size per chain = 500
##
## 1. Empirical mean and standard deviation for each variable,
##    plus standard error of the mean:
##
##          Mean             SD      Naive SE Time-series SE
##      6.076683       0.068538      0.003065       0.003271
##
## 2. Quantiles for each variable:
##
##   2.5%   25%   50%   75% 97.5%
## 5.934 6.033 6.081 6.123 6.205
```

We can already observe that there are difference between these chains and they oscillate around slightly different values, this raises suspicion that some of the chains may not have converged. For example chains $1, 2$ and 4 appear to oscillate around a mean value of 6.1. Chains 3 and 5 appear to increase and decrease rather than rapidly osclate. Chain 5 seems to be oscillating around 5.7, which is not in consensus with the other chains. For a more quantitaive analysis, we again apply the Gelman diagnostics to these summaries.

```
gelman.diag(meanAlloc)
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]       3.38       5.46
```

The above values are quite distant from 1 and clearly greater than 1.2, therefore we should believe these chains have not converged. As observed previously, chains $3, 5, 6$ look quite different from the other chains and so we recalculate the diagnostic excluding these chains. The computed Gelman diagnostic below suggest that chains $1, 2$ and 4 have converged and that we should discard chains $3, 4$ and 6 from further analysis.

```
gelman.diag(meanAlloc[c(1,2,4)])
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]          1       1.01
```

For a further check, we can look at the mean outlier probability at each iteration of the MCMC algorithm and again computing the Gelman diagnostics between chains $1, 2$ and 4. An $\hat{R}$ statistics of 1 is indicative of convergence, since it is less than the recommend value of 1.2.

```
meanoutProb <- mcmc_get_meanoutliersProb(tagmE14)
gelman.diag(meanoutProb[c(1,2,4)])
```

```
## Potential scale reduction factors:
##
##      Point est. Upper C.I.
## [1,]          1          1
```

6.1.2 Applying the Geweke diagnostic

Along with the Gelman diagnostics, which uses parallel chains, we can also apply a single chain analysis using the Geweke diagnostic. The Geweke diagnostic tests to see whether the mean calculate from the first 10% of iterations is significantly different from the the mean calculated from the last 50% of iterations. If they are significantly different, at say a level 0.01, then this is evidence that particular chains has not converged. The following code chunk calculates the Geweke diagnostic for each chain on the summarising quantities we have previously computed.

```
geweke_test(out)
```

```
##            chain 1   chain 2     chain 3    chain 4   chain 5    chain 6
## z.value -0.9201585 1.2916455 -2.43855877 1.69642764 1.6253508 -0.6003274
## p.value  0.3574900 0.1964799  0.01474596 0.08980492 0.1040878  0.5482881
```

```
geweke_test(meanAlloc)
```

```
##            chain 1     chain 2   chain 3    chain 4   chain 5      chain 6
## z.value 0.4307900 -0.08817564 0.1194190 1.82904303 0.7542722 -3.197185476
## p.value 0.6666211  0.92973708 0.9049434 0.06739316 0.4506858  0.001387757
```

```
geweke_test(meanoutProb)
```

```
##            chain 1   chain 2    chain 3   chain 4   chain 5     chain 6
## z.value -0.8845583 1.6385473 -0.8954649 1.0480938 0.6630499 -1.0246155
## p.value  0.3763949 0.1013076  0.3705386 0.2945954 0.5072986  0.3055447
```

The first test suggest chain 3 has not converged, since the p-value is roughly 0.01 suggesting that the mean in the first 10% of iterations is significantly different from those in the final 50%. Moreover, the second test suggests that chain 6 has not converged, as can be seen from a p-value close to 0.001, supporting our earlier beliefs that these chains have not conveged. These convergence diagnostics are not limited to the quantities we have computed here and further diagnostics can be perform on any summary of the data.

An important question to consider is whether removing an early portion of the chain might lead to improvment of the convergence diagonistics. This might be particularly relevant if a chain converges some iterations after our orginally specified `burin`. For example let us take the first Geweke test above, which suggested chain 3 had not converged and see if discarding the initial 10% of the chain improves the statistic. The function below removes 50 samples , informally known as burning, from the beginning of each chain and the output shows that we now have 450 samples in each chain.

```
burntagmE14 <- mcmc_burn_chains(tagmE14, 50)
chains(burntagmE14)
```

```
## Object of class "MCMCChains"
##  Number of chains: 6
```

```
chains(burntagmE14)[[1]]
```

```
## Object of class "MCMCChain"
##  Number of components: 10
##  Number of proteins: 1663
##  Number of iterations: 450
```

The following function recomputes the number of outliers in each chain at each iteration of each Markov-chain.

```
newout <- mcmc_get_outliers(burntagmE14)
```

The code chuck below compute the Geweke diagonstic for this new truncated chain and demonstrates that chain 3 has an improved Geweke diagnostic. Thus, in practice, it maybe useful to remove iterations from the

beginning of the chain. However, as chain 3 did not pass the Gelman diagnostics we still discard it from downstream analysis.

```
geweke_test(newout)
```

```
##           chain 1   chain 2   chain 3    chain 4   chain 5   chain 6
## z.value -0.4375964 0.7167981 -1.8993795 -1.77905509 0.4345828 -0.2522779
## p.value  0.6616789 0.4734987  0.0575146  0.07523073 0.6638653  0.8008262
```

6.2   Processing converged chains

Having made an assessment of convergence, we decide to discard chains $3, 5$ and 6 from any further analysis. The code chunk below remove these chains and creates and new object to store the converged chains.

```
removeChain <- c(3, 5, 6) # The chains to be removed
tagmE14_converged <- tagmE14[seq_len(nChains)[-removeChain]] # Create new object
```

The `MCMCParams` object can be large and therefore if we have a large number of samples we may want to subsample our chain, informally known as thinning, to reduce the number of samples. Thinning also has another purpose. We may desire indepedent samples from our posterior distribution but the MCMC algorithm produces autocorrelated samples. Thinning can be applied to reduce the autocorrelation between samples. The code chuck below, which is not evaluate demonstrates retaining every 5th iteration. Recall that we we thinned by 20 when we first ran the MCMC algorithm.

```
tagmE14_converged_thinned <- mcmc_thin_chains(tagmE14_converged, freq = 5)
```

We initially ran 6 chains and after having made an assesssment of convergence we decided to discard 3 of the chains. We desire to make inference using sample from all 3 chains, since this leads to better posterior estimates. In their current class strucutre all the chains are stored separately, so the following function pools all sample for all chains together to make a single longer chain with all samplers. Pooling a mixture of converged and unconverged chains in likely to lead to poor quality results.

```
tagmE14_converged_pooled <- mcmc_pool_chains(tagmE14_converged)
tagmE14_converged_pooled
```

```
## Object of class "MCMCParams"
## Method: TAGM.MCMC
## Number of chains: 1
```

```
tagmE14_converged_pooled[[1]]
```

```
## Object of class "MCMCChain"
##  Number of components: 10
##  Number of proteins: 1663
##  Number of iterations: 1500
```

To populate the summary slot of the converged and pooled chain, we can use the `tagmMcmcProcess` function. As we can see from the object below a summary is now available. In information now available in the summary slot was detailed in section the previous section. We note that if there is more than 1 chain in the `MCMCParams` object then the chains are automatically pooled to compute the summaries.

```
tagmE14_converged_pooled <- tagmMcmcProcess(tagmE14_converged_pooled)
tagmE14_converged_pooled
```

```
## Object of class "MCMCParams"
## Method: TAGM.MCMC
## Number of chains: 1
## Summary available
```

To create new feature columns in the `MSnSet` and appened the summary information, we apply the `tagmPredict` function. The `probJoint` argument indicates whether or not to add probablistic information for all organelles for all proteins, rather than just the information for the most probable organelle. The outlier probabilities are also return by default, but users can change this using the `probOutlier` argument.

```
E14TG2aR <- tagmPredict(object = E14TG2aR,
                        params = tagmE14_converged_pooled,
                        probJoint = TRUE)
head(fData(E14TG2aR))
```

```
##         Uniprot.ID UniprotName
## Q62261     Q62261 SPTB2_MOUSE
## Q9JHU4     Q9JHU4 DYHC1_MOUSE
## Q9QXS1     Q9QXS1  PLEC_MOUSE
##                                     Protein.Description Peptides PSMs
## Q62261 Spectrin beta chain, brain 1 (multiple isoforms)       42   42
## Q9JHU4               Cytoplasmic dynein 1 heavy chain 1       33   33
## Q9QXS1                         Isoform PLEC-1I of Plectin       33   33
##         GOannotation markers.orig markers    tagm.map.allocation
## Q62261     PLM-SKE       unknown unknown Endoplasmic reticulum
## Q9JHU4         SKE       unknown unknown    Nucleus - Chromatin
## Q9QXS1     unknown       unknown unknown        Plasma membrane
##         tagm.map.probability tagm.map.outlier tagm.mcmc.allocation
## Q62261            0.5710958                0         60S Ribosome
## Q9JHU4            1.0000000                0  Nucleus - Chromatin
## Q9QXS1            0.9999997                0  Nucleus - Nucleolus
##         tagm.mcmc.probability tagm.mcmc.probability.lowerquantile
## Q62261            0.9861265                          0.9464695
## Q9JHU4            0.9998994                          0.9997662
## Q9QXS1            0.9834691                          0.9446681
##         tagm.mcmc.probability.upperquantile tagm.mcmc.mean.shannon
## Q62261                          0.9993088          0.065074712
## Q9JHU4                          0.9999656          0.001051751
## Q9QXS1                          0.9946974          0.079897555
##         tagm.mcmc.outlier tagm.mcmc.joint.40S Ribosome
## Q62261      7.470143e-04               1.050019e-20
## Q9JHU4      2.285514e-07               6.396876e-09
## Q9QXS1      6.114878e-05               3.009322e-09
##         tagm.mcmc.joint.60S Ribosome tagm.mcmc.joint.Cytosol
## Q62261               9.861265e-01            6.446086e-24
## Q9JHU4               8.306954e-05            5.115482e-23
## Q9QXS1               1.650051e-02            5.714153e-21
##         tagm.mcmc.joint.Endoplasmic reticulum tagm.mcmc.joint.Lysosome
## Q62261                          1.233029e-05            2.165666e-09
## Q9JHU4                          5.093705e-44            1.834610e-27
## Q9QXS1                          6.035255e-13            3.767776e-07
##         tagm.mcmc.joint.Mitochondrion tagm.mcmc.joint.Nucleus - Chromatin
## Q62261                  1.300557e-06                        9.285534e-04
## Q9JHU4                  2.054354e-34                        9.998994e-01
## Q9QXS1                  1.560553e-14                        2.954412e-05
##         tagm.mcmc.joint.Nucleus - Nucleolus tagm.mcmc.joint.Plasma membrane
## Q62261                          1.293132e-02                        2.879137e-23
## Q9JHU4                          1.751793e-05                        1.221535e-68
## Q9QXS1                          9.834691e-01                        4.552436e-07
##         tagm.mcmc.joint.Proteasome
```

```
## Q62261             5.600742e-45
## Q9JHU4             6.762373e-46
## Q9QXS1             1.198713e-36
##  [ reached getOption("max.print") -- omitted 2 rows ]
##  [ reached 'max' / getOption("max.print") -- omitted 1 rows ]
```

6.2.1   Aside: *Priors*

Bayesian analysis requires users to specify prior information about the parameters. This potentially appears a challenging task; however, good default options are often possible. Should expert information be available for any of these priors then the users should provide this, else we have found that the default choices work well in practice. The priors also provide regularisation and shrinkage to avoid overfitting. Given enough data the likelihood overhelms the prior and little inference is based on the prior. There is little guidance in the literature on how to choose priors or even what they mean for the data analysis.

We place a normal inverse-Wishart prior on the normally distributed mixture components. The normal inverse-Wishart prior has 4 hyperparameters to be chosen to specify the prior beliefs in the mean and covariance of these mixtures. These are the prior mean `mu0` expressing the prior location of each organelle; a prior shrinkage `lambda0` a scaler expressing uncertainty in the prior mean; the prior degress of freedom `nu0` and a scale prior `S0` on the covariance. Together `nu0` and `S0` specify the prior variability on organelle covariances. All components share the same prior information.

The default options for these hold little information and are based on choice recommended by (Fraley and Raftery 2005). The prior mean `mu0` is set to mean of the data. `lambda0` is set to be 0.01 meaning some uncertainty in the covariance is propogated to the mean, increasing `lambda0` increase shrinkage towards the prior. `nu0` is set to the number of feature variables plus 2, which is the smallest integer value that ensures a finite covariance matrix. The prior scale matrix $S0$ is set to

$$S_0 = \frac{\text{diag}(\frac{1}{n} \sum (X - \bar{X})^2)}{K^{1/D}}, \tag{1}$$

and represents a diffuse prior on the covariance. Another good choice which is often used is a constant multiple of the identity matrix. The prior for the Dirichlet distribution concentration paramters `beta0` is set to 1 for each organelle. This represents a symmetric belief about the number of proteins allocated to each organelle. Another reasonable choice would be the non-informative Jeffery's prior for the Dirichlet hyperparameter, which sets `beta0` is 0.5 for each organelle. The prior weight for the outlier detection class is a $\mathcal{B}(u, v)$ distribution. The default for $u = 2$ and the default for $v = 10$. This represent the reasonable belief that $\frac{u}{u+v} = \frac{1}{6}$ proteins *a prior* might be an outlier and we believe is unlikely that more than 50% of proteins are outliers. Decreasing the value of v, represent more uncertainty about the number of protein that are outliers.

6.3   Analysis, visualisation and interpretation of results

Now that we have single pooled chain of samples from a converged MCMC algorithm, we can begin to analyse the results. Preliminary analysis includes visualising the allocated organelle and localisation probabilty of each protein to its most probable organelle.

```r
ptsze <- fData(E14TG2aR)$tagm.mcmc.probability

plot2D(E14TG2aR, fcol = "tagm.mcmc.allocation", cex = ptsze,
       main = "TAGM MCMC allocations")
addLegend(E14TG2aR, fcol = "markers",
          where = "topleft", ncol = 2, cex = 0.6)
```

TAGM MCMC allocations
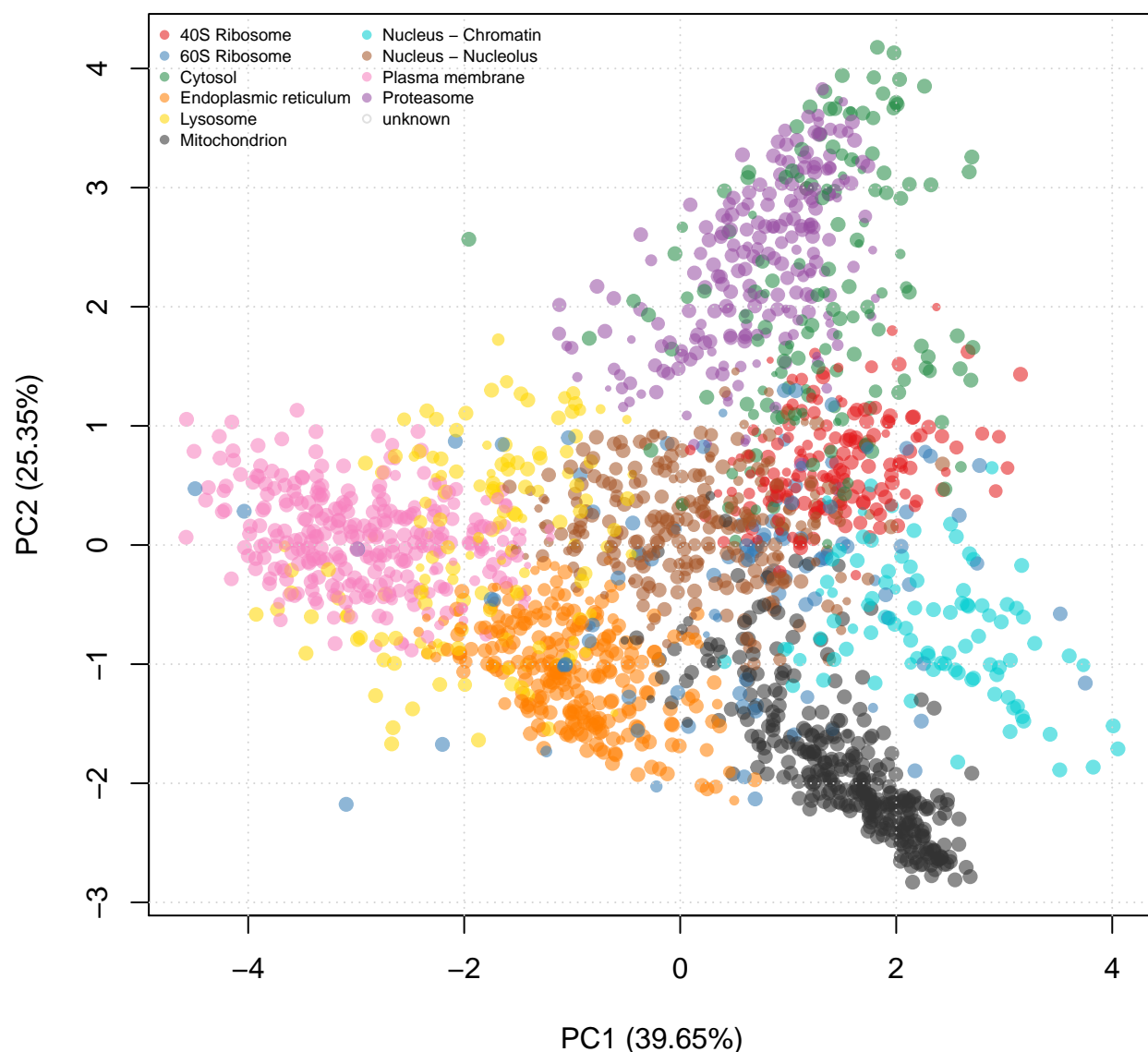
We can visualise other summaries of the data including a Monte-Carlo averaged Shannon entropy. This is a measure of uncertainty and poteins with greater shannon entropy have more uncertainty in their localisation. We observe global patterns of uncertainty, particulary in areas where organelle boundaries overlap. There are also regions of low uncertainty indicating little doubt about the localisation of these proteins.

```
ptsze2 <- fData(E14TG2aR)$tagm.mcmc.mean.shannon

plot2D(E14TG2aR, fcol = "tagm.mcmc.allocation",
       cex = ptsze2,
       main = "Visualising global uncertainty")
addLegend(E14TG2aR, fcol = "markers",
          where = "topleft", ncol = 2, cex = 0.6)
```

Visualising global uncertainty
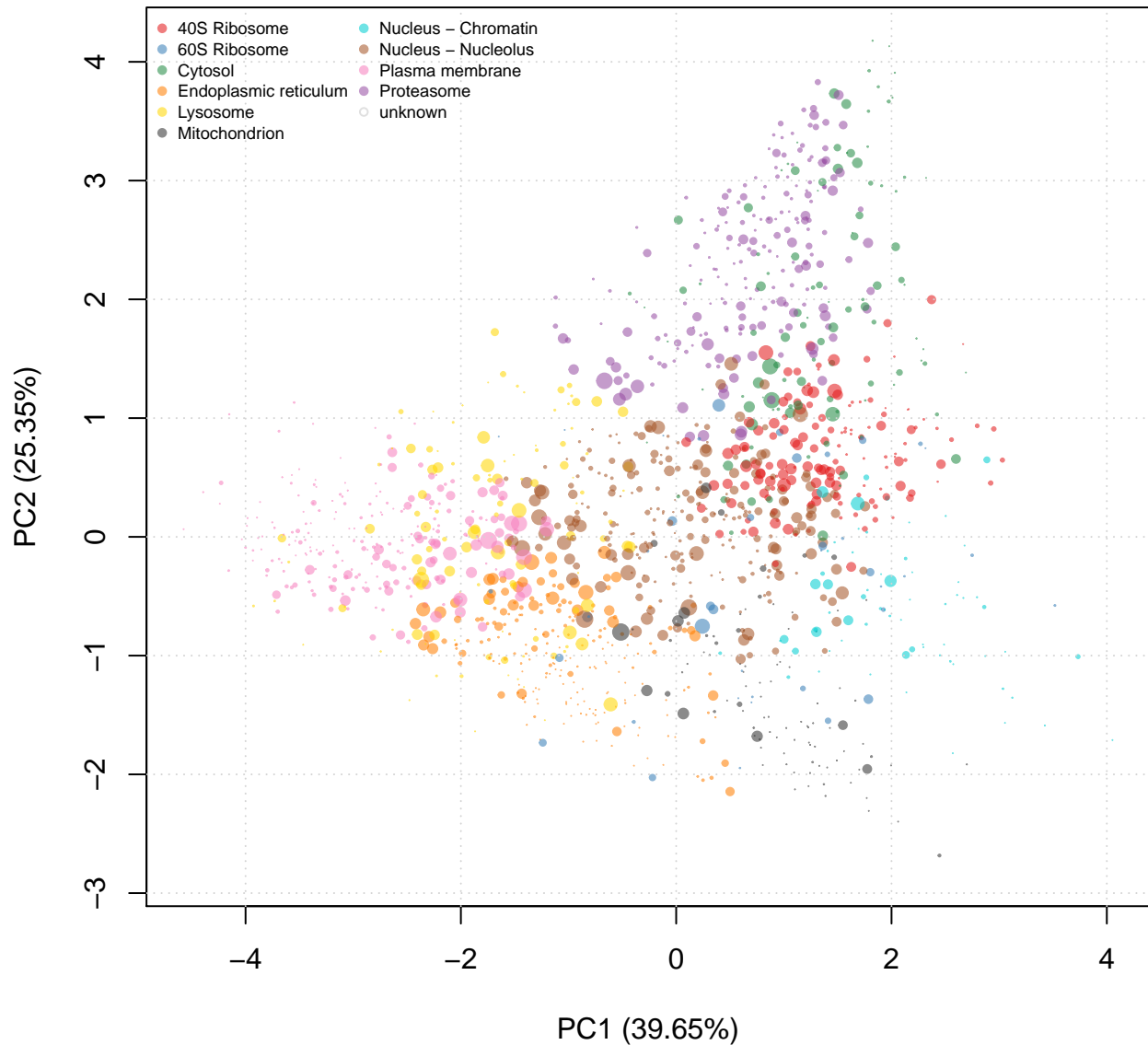
We are also interested in the relatonship between localisation probability to the most probable class and the Shannon entropy. Eventhough the two quantities are evidently correlated there is still consderable spread. Thus it is important to based inference not only on localisation probability but also a measure of uncertainty for example the Shannon entropy.

```
myColours <- getStockcol()[as.factor(fData(E14TG2aR)$tagm.mcmc.allocation)]
plot(ptsze, ptsze2, col = myColours, pch = 19,
     xlab = "Localisation probability",
     ylab = "Shannon entropy",
     main = "Shannon entropy and localisation probability")
addLegend(E14TG2aR, fcol = "markers",
          where = "topright", ncol = 2, cex = 0.6)
```

Shannon entropy and localisation probability
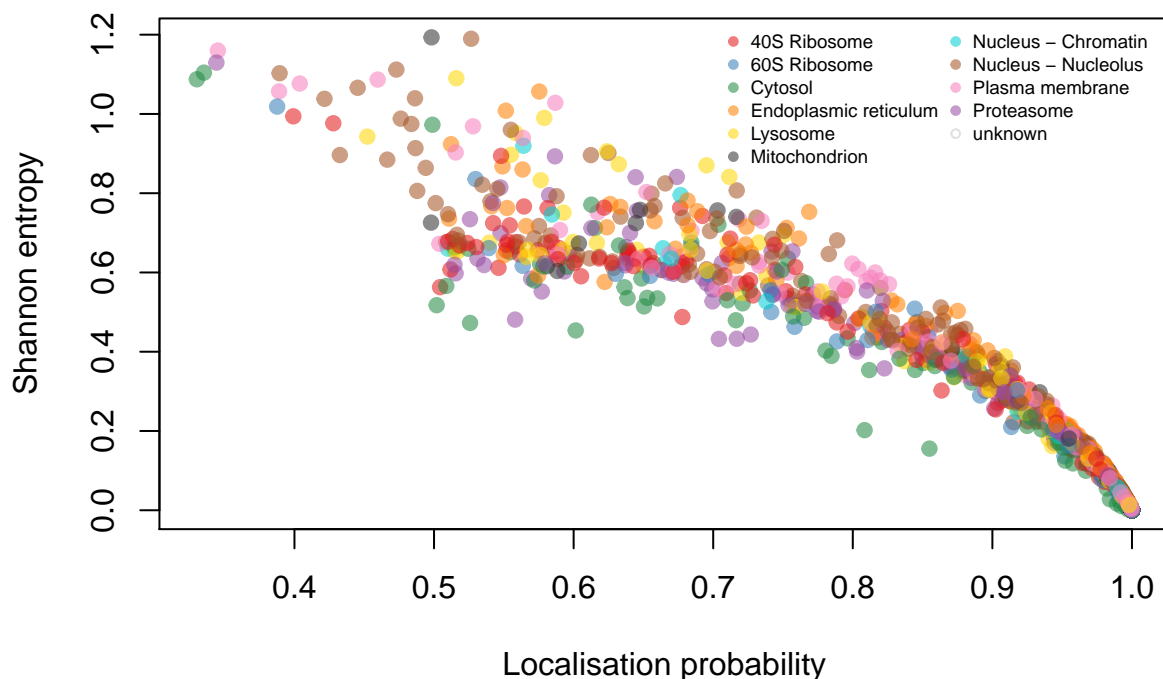
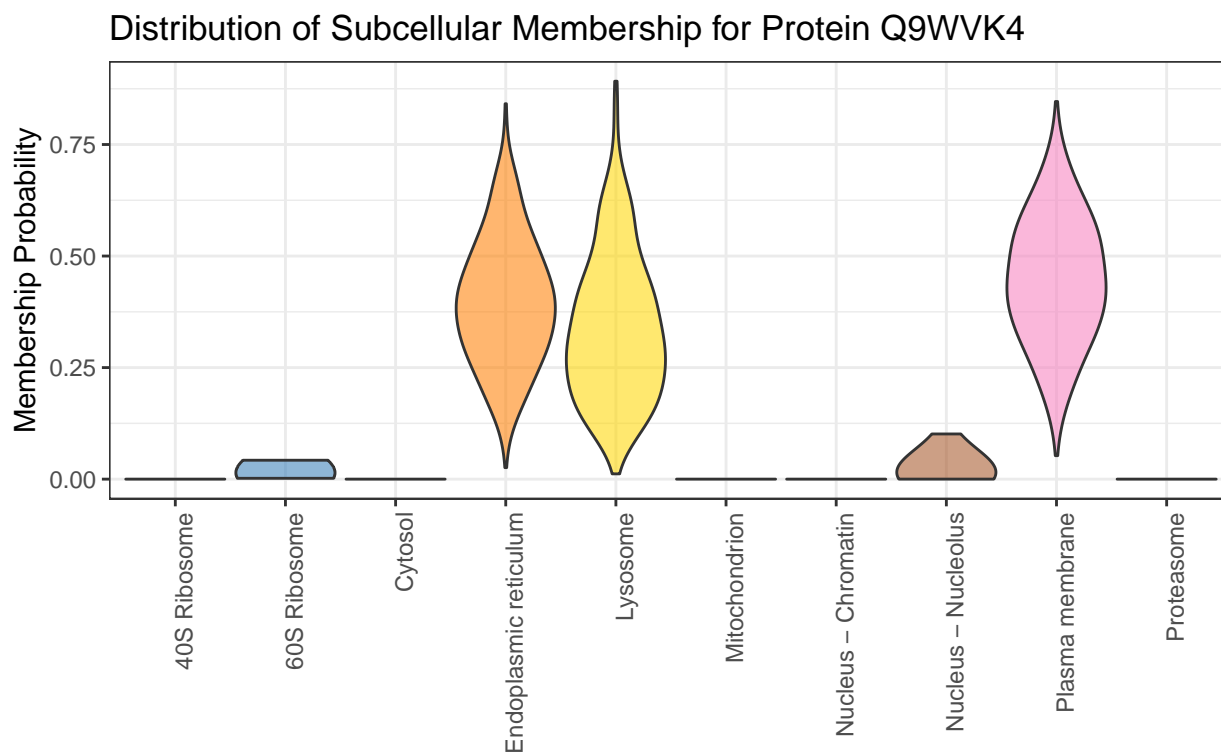Aside from global visualisation of the data, we can also interogate each individual protein. We can obtain the full posterior distribution of localisation probabilities for each protein from the `tagmE14_converged_pooled` object. We can use the `plot` generic on the `MCMCParams` object to obtain a violin plot of the localisation distribution. Simply providing the name of the protein in the second argument produce the plot for that protein. The transcription factor Q62318, also refered to as KAP1, is most probably localised to nucleus-Chomatin but also shows some uncertainty, potentially also localising to other comparments. The first violin plot visualises this uncertainty. The nucleur localisation sequence targets Q62318 to the nucleus to regulate cellular differentiation and proliferation. Thus, quantifying uncertainty recovers important bioligcal processes. Q9WVK4 is a membrane binding protein, as well as being involved in intracellular protein transport it regulates localisation to tubular recycling endosome membranes and so it unsurpising that it localisation is uncertain, because of its dynamic sub-cellular role.

```r
par(mfrow = c(1,2)) # Create a two figure panel
plot(tagmE14_converged_pooled, "Q62318")
```

Distribution of Subcellular Membership for Protein Q62318


```
plot(tagmE14_converged_pooled, "Q9WVK4")
```
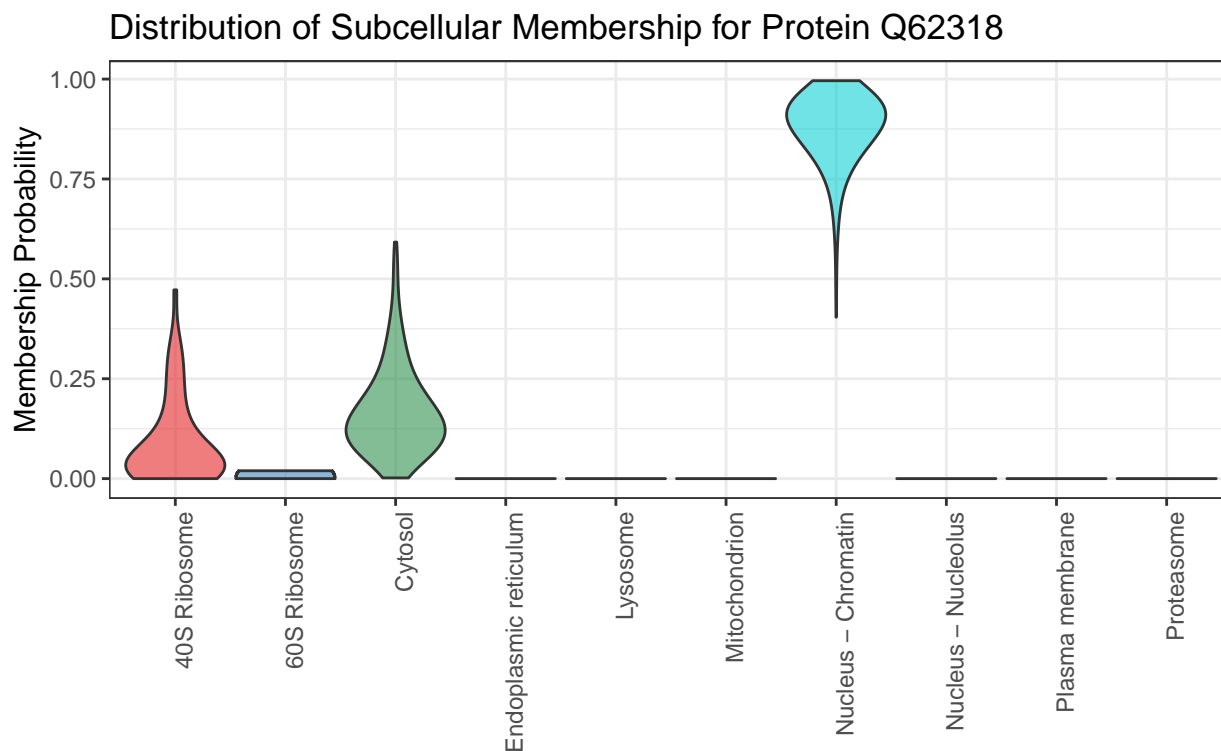
Distribution of Subcellular Membership for Protein Q9WVK4

6.4   Discussion

We have provided a workflow for the Bayesian analysis of spatial proteomics using the `pRoloc` and `MSnbase` software. We have demonstrated, in a step by step fashion, the challenges and advantages to taking a Bayesian

approach to data analysis. We hope this workflow inspires others to create documentation for the Bayesian analysis of biolgical data.

7   Session information

Below, we provide a summary of all packages and versions used to generate this document.

sessionInfo()

```
## R version 3.5.1 Patched (2018-11-02 r75540)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Manjaro Linux
##
## Matrix products: default
## BLAS: /usr/lib/libblas.so.3.8.0
## LAPACK: /usr/lib/liblapack.so.3.8.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] pRolocdata_1.20.0   pRoloc_1.23.2       coda_0.19-2
##  [4] BiocParallel_1.16.5 MLInterfaces_1.62.0 cluster_2.0.7-1
##  [7] annotate_1.60.0     XML_3.98-1.16       AnnotationDbi_1.44.0
## [10] IRanges_2.16.0      MSnbase_2.9.3       ProtGenerics_1.14.0
## [13] S4Vectors_0.20.1    mzR_2.16.1          Rcpp_1.0.0
## [16] Biobase_2.42.0      BiocGenerics_0.28.0
##
## loaded via a namespace (and not attached):
##   [1] plyr_1.8.4             igraph_1.2.2           lazyeval_0.2.1
##   [4] splines_3.5.1          ggvis_0.4.4            crosstalk_1.0.0
##   [7] ggplot2_3.1.0          digest_0.6.18          foreach_1.4.4
##  [10] htmltools_0.3.6        viridis_0.5.1          gdata_2.18.0
##  [13] magrittr_1.5           memoise_1.1.0          doParallel_1.0.14
##  [16] mixtools_1.1.0         sfsmisc_1.1-3          limma_3.38.3
##  [19] recipes_0.1.4          gower_0.1.2            rda_1.0.2-2.1
##  [22] lpSolve_5.6.13         prettyunits_1.0.2      colorspace_1.4-0
##  [25] blob_1.1.1             xfun_0.4               dplyr_0.7.8
##  [28] crayon_1.3.4           RCurl_1.95-4.11        hexbin_1.27.2
##  [31] genefilter_1.64.0      bindr_0.1.1            impute_1.56.0
##  [34] survival_2.43-1        iterators_1.0.10       glue_1.3.0
##  [37] gtable_0.2.0           ipred_0.9-8            zlibbioc_1.28.0
##  [40] kernlab_0.9-27         prabclus_2.2-6         DEoptimR_1.0-8
##  [43] scales_1.0.0           vsn_3.50.0             mvtnorm_1.0-8
##  [46] DBI_1.0.0              viridisLite_0.3.0      xtable_1.8-3
```

```
##  [49] progress_1.2.0        bit_1.1-14            proxy_0.4-22
##  [52] mclust_5.4.2          preprocessCore_1.44.0 lava_1.6.4
##  [55] prodlim_2018.04.18    sampling_2.8          htmlwidgets_1.3
##  [58] httr_1.4.0            threejs_0.3.1         FNN_1.1.2.2
##  [61] RColorBrewer_1.1-2    fpc_2.1-11.1          modeltools_0.2-22
##  [64] pkgconfig_2.0.2       flexmix_2.3-14        nnet_7.3-12
##  [67] caret_6.0-81          labeling_0.3          tidyselect_0.2.5
##  [70] rlang_0.3.1           reshape2_1.4.3        later_0.7.5
##  [73] munsell_0.5.0         mlbench_2.1-1         tools_3.5.1
##  [76] LaplacesDemon_16.1.1  generics_0.0.2        RSQLite_2.1.1
##  [79] pls_2.7-0             evaluate_0.12         stringr_1.3.1
##  [82] mzID_1.20.1           yaml_2.2.0            ModelMetrics_1.2.2
##  [85] knitr_1.21            bit64_0.9-7           robustbase_0.93-3
##  [88] randomForest_4.6-14   purrr_0.2.5           dendextend_1.9.0
##  [91] bindrcpp_0.2.2        ncdf4_1.16            nlme_3.1-137
##  [94] whisker_0.3-2         mime_0.6              biomaRt_2.38.0
##  [97] compiler_3.5.1        e1071_1.7-0           affyio_1.52.0
## [100] tibble_2.0.1
##  [ reached getOption("max.print") -- omitted 36 entries ]
```

The source of this document, including the code necessary to reproduce the analyses and figures is available in a public manuscript repository on GitHub~[**?**].

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