

FACETS: A Package for Analyzing Allele-specific Copy Number and Clonal Heterogeneity from High-throughput Sequencing

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1 Overview

This document presents an overview of the FACETS package which implements the methods described in (Shen and Seshan, 2016). This package is for analyzing allele-specific DNA copy number and clonal heterogeneity from high-throughput sequencing including whole-genome, whole-exome, and some targeted cancer gene panels. The method implements a bivariate genome segmentation, followed by allele-specific copy number calls. Tumor purity, ploidy, and cellular fractions are estimated and reported from the output.

2 Data

The file `stomach.csv.gz` in `extdata` directory contains data from a TCGA stomach cancer sample. To generate these data, tumor and normal bam files were downloaded from CGHub <https://cghub.ucsc.edu/>. Reference and variant allele read counts were extracted from the bam files for germline polymorphic sites catalogued in the dbSNP and 1000genome

database (~ 1.9 million polymorphic positions fall in regions targeted by whole exome sequencing). C++ code (`snp-pileup`) to generate such read count files and instructions for using it are available in `extcode` directory. This program generates csv file in which the first 4 columns are: Chromosome, Position, Ref and Alt. Then for each bam file 4 columns with names File#R, File#A, File#E and File#D giving the counts of number of reads with the ref allele, alt allele, errors (neither ref nor alt) and deletions in that position. The hashmark (#) is the number in position of the file in the sequence of bam files given to the pileup code. In the included data the ref and alt alleles were replaced with “N” and random noise added to the SNP positions to deidentify the case.

```
> datafile = system.file("extdata", "stomach.csv.gz", package="facets")
> head(read.csv(datafile)[,c(1:2,5:12)])
```

	Chromosome	Position	File1R	File1A	File1E	File1D	File2R	File2A	File2E	File2D
1	1	69424	170	117	0	0	158	103	0	0
2	1	69515	0	76	0	0	0	77	0	0
3	1	69536	103	0	0	0	99	0	0	0
4	1	808866	96	0	0	0	133	0	0	0
5	1	809120	66	0	0	0	105	0	0	0
6	1	809176	79	0	0	0	126	0	0	0

NOTE: Since the read counts need to be in normal-tumor order we invoke the command as `snp-pileup -g -q15 -Q20 -P100 -r25,0 vcffile outputfile normalbam tumorbam`. Meaning of the flags and instructions for using `snp-pileup` to generate the read count matrix are given in the included `README.txt` file.

3 An Example

Here we perform an analysis on the stomach cancer whole-exome described above.

```
> library(facets)
```

We first perform various pre-processing steps to prepare the data for segmenation analysis. Positions with total read count below a lower depth threshold (default 35, use `ndepth` to change the default value) or exceed an upper threshold (> 1000) (excessive coverage) in the matched normal sample were removed. We scan all positions by 150-250 bp interval to space out SNP-dense regions to reduce local patterns of serial dependencies that can result in hyper-segmentation in the downstream steps. Read depth ratio between tumor and normal gives information on total copy number. The variant (non-reference) allele frequency at heterozygous loci (germline variant allele frequency greater than 0.25 or less than 0.75) contain information on allelic imbalance. This pre-processing procedure on average yields 250K SNP loci from TCGA whole-exomes that pass these quality filters, and 10% of which are heterozygous. At each position, `logR` is defined by the log-ratio of total read depth in the tumor versus that in the normal and `logOR` is defined by the log-odds ratio of the variant allele count in the tumor versus in the normal. A normalizing constant is calculated for

each tumor/normal pair to corrected for total library size, and GC-bias is corrected using a loess regression of logR over GC content along 1kb windows along the genome. Since `preProcSample` samples the snps to be used the random number generator seed should be set/saved for reproducible results.

```
> set.seed(1234)
> datafile = system.file("extdata", "stomach.csv.gz", package="facets")
> rcmat = readSnpMatrix(datafile)
> xx = preProcSample(rcmat)
```

A bivariate genome segmentation is performed on logR and logOR by extending the CBS algorithm (Olshen et al., 2004; Venkatraman and Olshen, 2007) to the bivariate scenario using a T^2 statistic for identifying change points. If the maximal statistic is greater than a pre-determined critical value (cval), we declare a change exists and the change points that maximize this statistic. Lower cval lead to higher sensitivity for small changes. After segmentation, a clustering process is applied to group the segments into clusters of the same underlying genotype.

```
> oo=procSample(xx, cval=150)
```

We note that logR estimates are proportional to the absolute total copy number up to a location constant. In diploid genome, $\log R = 0$ is the normal 2-copy state. However, aneuploidy can lead to systematic shift of the normal diploid state. In order to obtain correct genotype calls for copy number, we need to identify the location of the normal diploid state. We use the logOR summary measure estimates to identify the segment clusters in allelic balances, and use these segments to determine the 2-copy state. We call the logR for the 2-copy state $\log R_0$ which is output below.

```
> oo$dipLogR
[1] -0.04444348
```

Call allele-specific copy number and associated cellular fraction, estimate tumor purity and ploidy.

```
> fit=emcncf(oo)
```

Once the logR value for the diploid state is obtained we calculate the observed copy number for each cluster as $\exp(\log R_c - \log R_0)$ where $\log R_c$ is the logR summary for the cluster and $\log R_0$ is the diploid state level. Once the observed total number is obtained we obtain the allele specific copy numbers m and p and the cellular fraction ϕ using the logOR data. The cellular fraction is associated with the aberrant genotype. For clonal copy number alterations, ϕ equals tumor purity. For subclonal events, ϕ will be lower than the overall sample purity.

To further refine these initial estimates and obtain a genome-wide optimization, we apply a genotype mixture model and maximize a joint likelihood that summarizes over all SNP loci and segment clusters across the genome. An expectation-maximization (EM) algorithm

is used for the estimation procedure. It can be viewed as an estimation problem with the latent copy number states as “missing” data. In the E-step of the EM procedure, Bayes theorem is used to compute the posterior probability of a segment cluster being assigned copy number state g given the parameter estimates at the k th iteration. In the M-step, given the imputed genotype, we update the model parameters by maximizing the complete-data likelihood. This procedure is iterated until convergence. For a segment, if the number of heterozygous SNPs is less than 15, only the total copy number is given. For male gender, the number of copy for chromosome X is 1 in the normal. The X chromosome copy number in the tumor is adjusted accordingly.

The segmentation result and the EM fit output looks like this

```
> head(fit$cncf)
```

	chrom	seg	num.mark	nhet	cnlr.median	mafR	segclust	cnlr.median.clust
1	1	1	2631	158	0.50028101	0.305665373	12	0.49145998
2	1	2	4598	249	-0.03699913	0.021520755	7	-0.04444348
3	1	3	6342	408	0.49218666	0.404039235	12	0.49145998
4	2	4	7006	375	-0.03654935	0.019844538	7	-0.04444348
5	2	5	14	0	-0.85252562	0.000000000	6	-0.88771396
6	2	6	2299	96	-0.05328541	0.001226293	7	-0.04444348

	mafR.clust	start	end	cf.em	tcn.em	lcn.em
1	0.38807104	69424	29651873	0.8767632	3	1
2	0.01655667	31188034	144922109	1.0000000	2	1
3	0.38807104	144922463	249212378	0.8767632	3	1
4	0.01655667	41507	197673978	1.0000000	2	1
5	NA	197706054	197873655	0.8919360	1	0
6	0.01655667	197947995	243061179	1.0000000	2	1

In the output, cf, tcn, lcn are the initial estimates of cellular fraction, total and minor copy number estimates, and cf.em, tcn.em, lcn.em are the estimates by the mixture model optimized using the EM-algorithm. cf is used as initial values for the EM algorithm. For diploid normal segments (total copy=2, minor copy=1), we report cellular fraction as 1 (100% normal). The logOR data for a segment are summarized using the square of expected log-odds-ratio (mafR column).

Estimated tumor sample purity and ploidy are reported:

```
> fit$purity
```

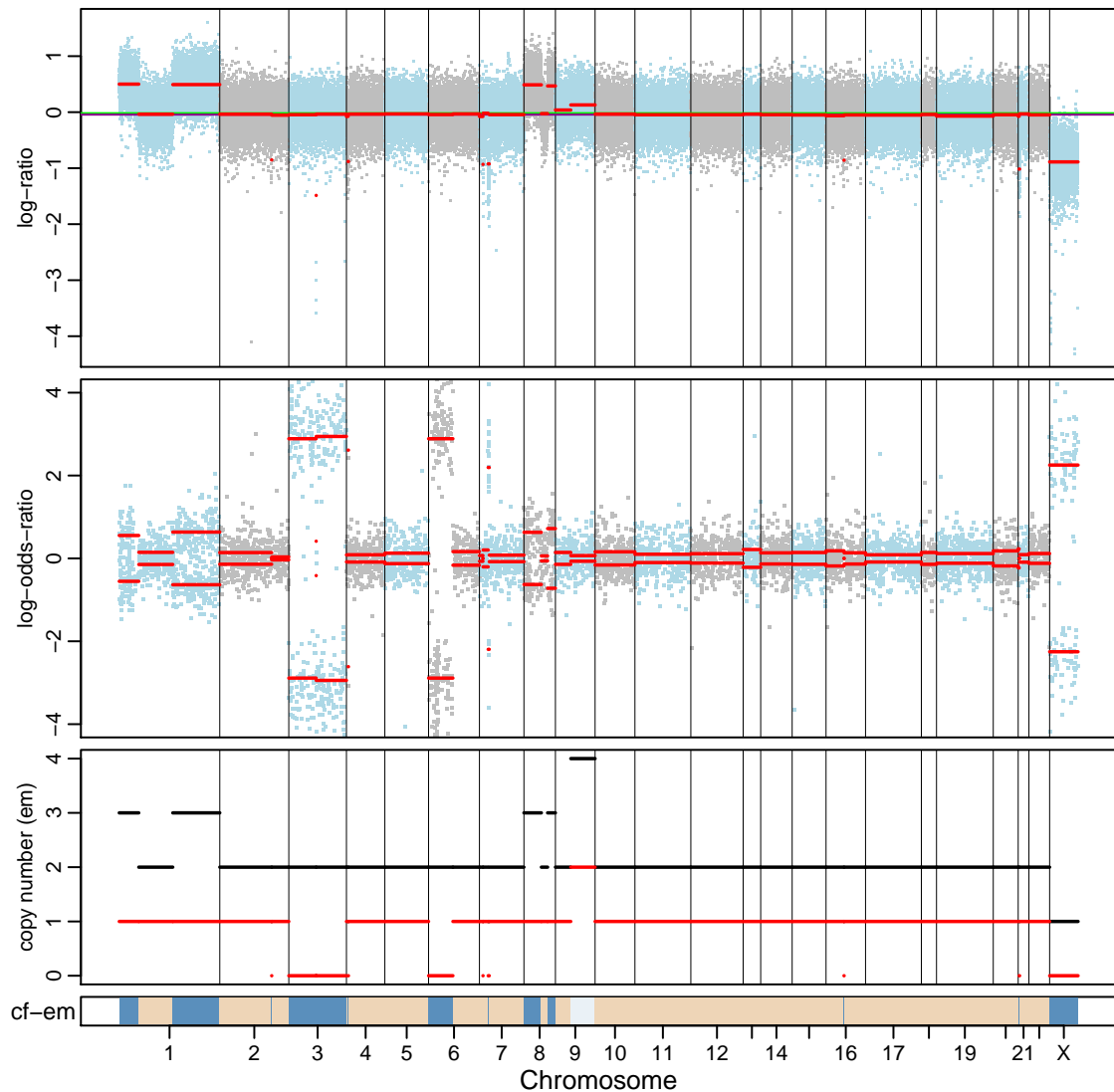
```
[1] 0.891936
```

```
> fit$ploidy
```

```
[1] 2.070151
```

We provide a plot function to visualize the genome-wide profile and FACETS output.

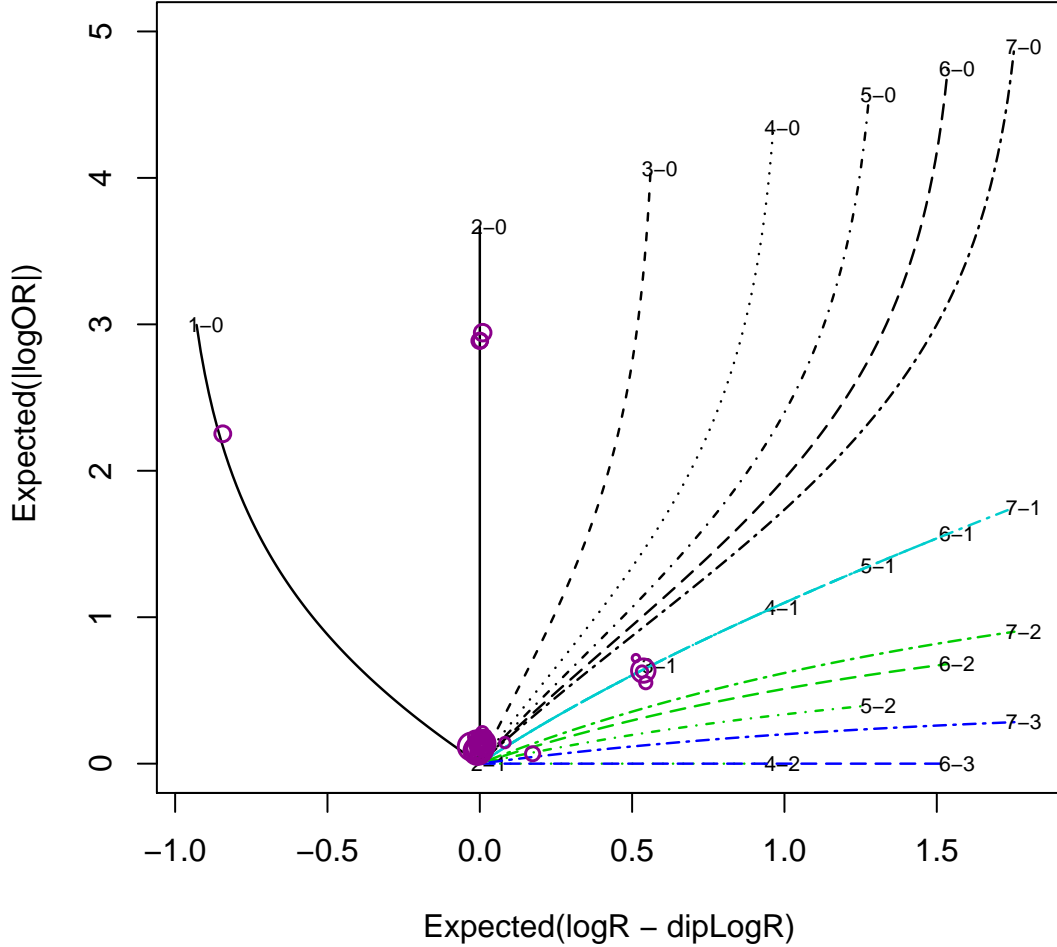
```
> plotSample(x=oo,emfit=fit)
```



The top panel of the figure displays logR with chromosomes alternating in blue and gray. The green line indicates the median logR in the sample. The purple line indicates the logR of the diploid state. The second panel displays logOR. Segment means are plotted in red lines. The third panel plots the total (black) and minor (red) copy number for each segment. The bottom bar shows the associated cellular fraction (cf). Dark blue indicates high cf. Light blue indicates low cf. Beige indicates a normal segment (total=2,minor=1).

A diagnostic plot for the fit can be generated using the function

```
> logRlogORspider(oo$out, oo$dipLogR)
```



The segment summaries are plotted as circles where the size of the circle increases with the number of loci in the segment. The expected value for various integer copy number states are drawn as curves for purity ranging from 0 to 0.95. For a good fit, the segment summaries should be close to one of the lines.

The emcnf algorithm allows a separate cellular fraction estimate for each segment cluster. In order to impose a clonally constrained structure on ϕ , we included a sequential approach where the modified algorithm starts with a single clonal cluster ($k=1$) with cellular fraction parameter ϕ_1 . We then identify segment clusters for which segment cluster-specific estimates is non-trivially lower (at least by 0.05 although this can be changed by setting desired value for difcf) from the clonally constrained estimates that result in a suboptimal fit under $k=1$. These segment clusters with discordant cellular fraction estimates then form a candidate subclonal cluster of events at a lower cellular fraction ϕ_2 , and a model is fitted with the joint likelihood optimized under $k=2$. This procedure is iterated until no additional

discordance in cellular fraction estimates are found, or a specified maximum k is reached. In the default parameter setting, a maximum $k=5$ is allowed although user can change it to a higher number if greater intratumor heterogeneity is expected. In the output, the estimated ϕ_1 is the cellular fraction for the clonal events and also the tumor purity by definition, and the estimated $\phi_k, k > 1$ for any subclonal clusters identified in the tumor sample.

```
> fit2=emcncf2(oo)
```

```
fitting 1 clonal cluster ...
```

output file

```
> head(fit2$cncf)
```

	chrom	seg	num.mark	nhet	cnlr.median	mafR	segclust	cnlr.median.clust
1	1	1	2631	158	0.50028101	0.305665373	12	0.49145998
2	1	2	4598	249	-0.03699913	0.021520755	7	-0.04444348
3	1	3	6342	408	0.49218666	0.404039235	12	0.49145998
4	2	4	7006	375	-0.03654935	0.019844538	7	-0.04444348
5	2	5	14	0	-0.85252562	0.000000000	6	-0.88771396
6	2	6	2299	96	-0.05328541	0.001226293	7	-0.04444348

	mafR.clust	start	end	cf.em	tcn.em	lcn.em	clonal.cluster
1	0.38807104	69424	29651873	0.891936	3	1	1
2	0.01655667	31188034	144922109	1.000000	2	1	1
3	0.38807104	144922463	249212378	0.891936	3	1	1
4	0.01655667	41507	197673978	1.000000	2	1	1
5	NA	197706054	197873655	0.891936	1	0	1
6	0.01655667	197947995	243061179	1.000000	2	1	1

References

- Olshen, A. B., Venkatraman, E. S., Lucito, R., and Wigler, M. (2004). Circular binary segmentation for the analysis of array-based dna copy number data. *Biostatistics*, 5:557–72.
- Shen, R. and Seshan, V. E. (2016). Facets: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput dna sequencing. *Nucleic acids research*, 44(16):e131–e131.
- Venkatraman, E. S. and Olshen, A. B. (2007). A faster circular binary segmentation algorithm for the analysis of array cgh data. *Bioinformatics*, 23:657–63.