

PopGenome Session

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1 Reading data

Loading the PopGenome package

```
> library(PopGenome)
```

Reading three alignments in FASTA-format stored in the folder "FASTA".

```
> GENOME.class <- readData("FASTA")
```

```
|          :          |          :          | 100 %  
|=====
```

GENOME.class is an object of class GENOME.

```
> GENOME.class
```

Modules:

	Calculation	Description	Get.the.Result
1	readData	Reading data	get.sum.data
2	neutrality.stats	Neutrality tests	get.neutrality
3	linkage.stats	Linkage disequilibrium	get.linkage
4	recomb.stats	Recombination	get.recomb
5	F_ST.stats	Fixation index	get.F_ST, get.diversity
6	MKT	McDonald-Kreitman test	get.MKT
7	detail.stats	Mixed statistics	get.detail
8	MS	Coalescent simulation	@
9	-----	-----	-----
10	set.populations	Defines the populations	
11	sliding.window.transform	Sliding window	
12	splitting.data	Splits the data	
13	show.slots	?provided slots?	
14	get.status	Status of calculations	

The class GENOME contains all observed data and statistic values which are presentable in a multi-locus-scale. Use the function `show.slots(GENOME.class)` to get an overview or check out the manual. To access those values we use the @-operator.

How many sites were analyzed in each alignment ?

```
> GENOME.class@n.sites
```

```
4CL1tl.fas  C4Htl.fas  CADtl.fas
      2979      2620      2930
```

```
> GENOME.class@region.names
```

```
[1] "4CL1tl.fas" "C4Htl.fas" "CADtl.fas"
```

To get some summary information from the alignments use the `get.sum.data` function. This function extracts the values from the class `GENOME` and puts them into a matrix. You can also look at those values separately with the `@`-operator (`GENOME.class@n.biallelic.sites`).

```
> get.sum.data(GENOME.class)
```

	n.sites	n.biallelic.sites	n.gaps	n.unknowns	n.valid.sites
4CL1tl.fas	2979	176	617	0	2362
C4Htl.fas	2620	84	1454	0	1161
CADtl.fas	2930	197	740	0	2189

	n.polyallelic.sites	trans.transv.ratio
4CL1tl.fas	0	1.120482
C4Htl.fas	5	1.470588
CADtl.fas	1	0.970000

The Slot `region.data` contains some detail (site specific) informations, which are not presentable in a multi-locus-scale. `region.data` is another class and its slots are accessible with the `@` operator.

```
> GENOME.class@region.data
```

```
-----
```

```
SLOTS:
```

```
-----
```

	Slots	Description
1	populations	Samples of each population (rows)
2	populations2	Samples of each population (names)
3	outgroup	Samples of outgroup
4	transitions	Biallelic site transitions
5	biallelic.matrix	Biallelic matrix
6	n.singletons	Number of singletons
7	biallelic.sites	Position of biallelic sites
8	reference	SNP reference
9	n.nucleotides	Number of nucleotides per sequence
10	biallelic.compositions	Nucleotides per sequence (biallelic)
11	synonymous	Synonymous biallelic sites
12	biallelic.substitutions	Biallelic substitutions
13	polyallelic.sites	Sites with >2 nucleotides
14	sites.with.gaps	Sites with gap positions
15	sites.with.unknowns	Sites with unknown positions
16	minor.alleles	Minor alleles
17	codons	Codons of biallelic substitutions

18	IntronSNPS	SNPs in intron region
19	UTRSNPS	SNPs in UTR region
20	CodingSNPS	SNPs in coding region
21	ExonSNPS	SNPs in exon region
22	GeneSNPS	SNPs in gene region

These are the Slots (class region.data)

The first 10 biallelic positions of the first alignment:

```
> GENOME.class@region.data@biallelic.sites[[1]][1:10]

[1] 12 13 31 44 59 101 121 154 165 202
```

Which of those biallelic sites are transitions ?

```
> GENOME.class@region.data@transitions[[1]][1:10]

[1] TRUE TRUE TRUE TRUE TRUE FALSE TRUE FALSE FALSE FALSE
```

2 Reading data with GFF/GTF information

The GFF folder contains GFF-files for each alignment. The GFF-files have the same names as the corresponding alignments

```
> GENOME.class <- readData("FASTA",gffpath="GFF")

|           :           |           :           | 100 %
|=====|
```

Which of the first 10 SNPs of the second [[2]] alignment are part of an synonymous mutation ?

```
> GENOME.class@region.data@synonymous[[2]][1:10]

[1] TRUE TRUE TRUE TRUE TRUE TRUE  NA  NA  NA  NA
```

NA values indicate that the sites are not in a coding region

```
> GENOME.class@region.data@CodingSNPS[[2]][1:10]

[1] 1413 1428 1446 1455 1482 1488 1744 1756 1798 1802
```

2.1 Splitting the data in subsites

If the number of individuals are identical, you can use the `splitting.data` function to split the data in subsites. In this example we are splitting into coding (CDS) regions. The returned value is again an object of class `GENOME`.

```
> GENOME.class.split <- splitting.data(GENOME.class, subsites="coding")

|           :           |           :           | 100 %
|=====|
|=====|
```

Each region contains now the SNP-informations of each coding region defined in the gff-files. In case of whole-genome SNP data this mechanism can be very useful. (manual:readSNP,readVCF)

```
> GENOME.class.split@n.sites

[1] 1056  413  103   96  785  132  595   92  112  226  438  220

> GENOME.class.split <- neutrality.stats(GENOME.class.split)
```

Apply the neutrality module to all synonymous SNPs in the coding regions.

```
> GENOME.class.split <- neutrality.stats(GENOME.class.split, subsites="syn")
> GENOME.class.split@Tajima.D
```

3 Define populations

Define two populations as a list.

```
> GENOME.class <- set.populations(GENOME.class,list(
+   c("CON","KAS-1","RUB-1","PER-1","RI-0","MR-0","TUL-0"),
+   c("MH-0","YO-0","ITA-0","CVI-0","COL-2","LA-0","NC-1")
+ ))

|           :           |           :           | 100 %
|=====
```

4 Statistics

4.1 Neutrality statistics

```
> GENOME.class <- neutrality.stats(GENOME.class)

|           :           |           :           | 100 %
|=====
```

Getting the result from the object of class GENOME.

```
> get.neutrality(GENOME.class)

      neutrality stats
pop 1 Numeric,27
pop 2 Numeric,27
```

Lets look at the first population [[1]].

```
> get.neutrality(GENOME.class)[[1]]
```

	Tajima.D	n.segregating.sites	Rozas.R_2	Fu.Li.F	Fu.Li.D
4CL1tl.fas	-1.1791799	16	NA	-0.9247377	-1.1331823
C4Htl.fas	0.6987394	17	NA	0.6742517	0.4167836
CADtl.fas	0.5503743	14	NA	0.4458431	0.1590690

	Fu.F_S	Fay.Wu.H	Zeng.E	Strobeck.S
4CL1tl.fas	NA	NaN	NaN	NA
C4Htl.fas	NA	NaN	NaN	NA
CADtl.fas	NA	NaN	NaN	NA

The NA values indicates that the statistics could not be calculated. This can have several reasons.

- the statistic needs an outgroup
- the statistic was not switched on
- there are no SNPs in the entire region

In each module you can switch on/off statistics and define an outgroup. (check the manual !). PopGenome also provides a population specific view of each statistic value.

```
> GENOME.class@Tajima.D
```

	pop 1	pop 2
4CL1tl.fas	-1.1791799	-0.0702101
C4Htl.fas	0.6987394	1.1819777
CADtl.fas	0.5503743	0.2682897

If there there was a GFF/GTF file specified, you can also analyse subsites like SNPs exon, coding, utr or intron regions.

```
> GENOME.class <- neutrality.stats(GENOME.class, subsites="coding")
```

	:		:		100 %
=====					

```
> GENOME.class@Tajima.D
```

	pop 1	pop 2
4CL1tl.fas	-1.023785	0.2626617
C4Htl.fas	1.013372	1.9121846
CADtl.fas	1.981520	1.5191652

Or each subsite-region separately by splitting the data as described in section 2.1.

```
> GENOME.class.split <- splitting.data(GENOME.class, subsites="coding")
```

	:		:		100 %
=====					
=====					

```
> GENOME.class.split <- neutrality.stats(GENOME.class.split)
```

```
|          :          |          :          | 100 %
|=====
```

```
> GENOME.class.split@Tajima.D
```

	pop 1	pop 2
240 - 1295	-0.2749244	-0.3186974
1890 - 2302	-1.0062306	0.7546749
2679 - 2781	-1.0062306	0.5590170
2884 - 2979	-1.0062306	NaN
3465 - 4249	NA	NA
4337 - 4468	NaN	NaN
4696 - 5290	-1.6097384	2.1259529
6181 - 6272	NaN	NaN
6412 - 6523	NaN	NaN
7320 - 7545	0.2390231	1.8112198
7643 - 8080	-0.3018700	1.1684289
8176 - 8395	NaN	NaN

The PopGenome framework provides several modules to calculate statistics. All methods will work as the `neutrality.stats()` function described above. Please read the user manual.

4.2 The slot `region.stats`

The slot `region.stats` includes some site-specific statistics or values that can not be shown in a multi-locus-scale.

```
> GENOME.class@region.stats
```

```
-----
SLOTS:
-----
```

	Slots	Description	Module
1	nucleotide.diversity	Nucleotide diversity	FST
2	haplotype.diversity	Haplotype diversity	FST
3	haplotype.counts	Haplotype distribution	FST
4	minor.allele.freqs	Minor allele frequencies	Detail
5	linkage.disequilibrium	Linkage disequilibrium	Linkage
6	biallelic.structure	Shared and fixed polymorphisms	Detail

```
-----
These are the Slots (class region.data)
```

```
> GENOME.class <- F_ST.stats(GENOME.class)
```

```
|          :          |          :          | 100 %
|=====
```

```
> GENOME.class@region.stats@nucleotide.diversity
```

```
[[1]]
      pop 1      pop 2
pop 1 5.142857      NA
pop 2 6.163265 5.238095
```

```
[[2]]
      pop 1 pop 2
pop 1 7.809524      NA
pop 2 8.816327      4
```

```
[[3]]
      pop 1      pop 2
pop 1 6.285714      NA
pop 2 5.836735 4.285714
```

5 Sliding Window Analysis

The `sliding.window.transform()` transforms an object of class `GENOME` in another object of class `GENOME`. This mechanism enables the user to apply all methods existing in the PopGenome framework.

PopGenome tries to concatenate the data if the parameter `whole.data=TRUE`. This mechanism is useful to handle chunks in the PopGenome framework. Otherwise the regions are scanned separately.

`type=1`: Scanning the SNPs
`type=2`: Scanning the whole data

5.1 Scanning whole data

```
> GENOME.class.slide <- sliding.window.transform(GENOME.class,width=50,
+                                                jump=50,type=1,whole.data=TRUE)

|           :           |           :           | 100 %
|=====|
|=====|

> GENOME.class.slide@region.names

[1] "1 - 50 :"      "51 - 100 :"    "101 - 150 :"   "151 - 200 :"   "201 - 250 :"
[6] "251 - 300 :"   "301 - 350 :"   "351 - 400 :"   "401 - 450 :"

> GENOME.class.slide <- linkage.stats(GENOME.class.slide)

|           :           |           :           | 100 %
|=====|

> get.linkage(GENOME.class.slide)[[1]]

      Wall.B      Wall.Q      Rozas.ZA      Rozas.ZZ      Kelly.Z_nS
1 - 50 :      0.6666667 0.7500000 0.6666667 0.2916667 0.375000000
```

```

51 - 100 :      NaN      NaN 0.00000000 0.00000000 0.00000000
101 - 150 : 0.0000000 0.0000000 0.01851852 -0.05266204 0.071180556
151 - 200 : 0.6250000 0.66666667 0.37847222 0.10206619 0.276406036
201 - 250 : 0.5833333 0.6923077 5.40972222 1.05354208 4.356180145
251 - 300 : 0.0000000 0.0000000 0.01388889 -0.17860000 0.192488889
301 - 350 : 0.0000000 0.0000000 0.01388889 0.00462963 0.009259259
351 - 400 : 0.4000000 0.5000000 3.95688889 2.19704321 1.759845679
401 - 450 : 0.5000000 0.6000000 1.81250000 1.31916667 0.493333333

```

5.2 Scanning the regions separately

```

> GENOME.class.slide <- sliding.window.transform(GENOME.class,width=50,
+                                                jump=50,type=1,whole.data=FALSE)

|           :           |           :           | 100 %
|=====

> GENOME.class.slide@region.names

[1] "1:4CL1tl.fas" "2:4CL1tl.fas" "3:4CL1tl.fas" "4:C4Htl.fas" "5:CADtl.fas"
[6] "6:CADtl.fas"  "7:CADtl.fas"

> GENOME.class.slide <- linkage.stats(GENOME.class.slide)

|           :           |           :           | 100 %
|=====

> get.linkage(GENOME.class.slide)[[1]]

           Wall.B Wall.Q  Rozas.ZA  Rozas.ZZ Kelly.Z_nS
1:4CL1tl.fas 0.6666667 0.75 0.6666667 0.29166667 0.37500000
2:4CL1tl.fas      NaN      NaN 0.00000000 0.00000000 0.00000000
3:4CL1tl.fas 0.0000000 0.00 0.01851852 -0.05266204 0.07118056
4:C4Htl.fas 0.6666667 0.80 0.54086420 -0.09315802 0.63402222
5:CADtl.fas 0.0000000 0.00 2.09259259 -0.04456019 2.13715278
6:CADtl.fas 0.0000000 0.00 0.01388889 -1.37808642 1.39197531
7:CADtl.fas 0.5000000 0.60 0.88888889 -0.27527778 1.16416667

```

6 Coalescent simulation

PopGenome supports the Coalescent simulation program **MS** from Hudson as well as the **MSMS** simulation tool from Greg Ewing. The observed statistics are tested against the simulated values. You have to specify the θ value and the module you want to apply to the simulated data. An new object of class **cs.stats** will be created. The main input is an object of class **GENOME**

```

> MS.class <- MS(GENOME.class,thetaID="Tajima",neutrality=TRUE)

|           :           |           :           | 100 %
|=====

> MS.class

```



```

-----
SLOTS:
-----

```

	Slots	Description
1	prob.less	Prob. that sim.val <= obs.val P(sim <= obs)
2	prob.equal	Prob. that sim.val = obs.val P(sim = obs)
3	valid.iter	number of valid iter. for each test and loci
4	obs.val	obs.values for each test
5	n.loci	number of loci considered
6	n.iter	number of iterations for each loci
7	average	average values of each statistic (across all loci)
8	variance	variance values of each statistic (across all loci)
9	locus list	list of loc.stats objects, (detail stats for each locus)

Lets look at the data of the first region

```
> MS.class@locus[[1]]
```

```

      Length      Class      Mode
      1 loc.stats      S4

```

```

-----
SLOTS:
-----

```

	Slots	Description
1	n.sam	number of samples for each iteration
2	n.iter	number of iteration
3	theta	mutation parameter
4	obs.val	vector with observed values for each test
5	positions	position of each polymorphic site
6	trees	if printtree=1, gene tree in Newick format
7	seeds	random numbers used to generate samples
8	haplotypes	haplotypes in each iteration
9	stats	variety of test stats compiled a matrix
10	loc.prob.less	Prob. that simulated val. <= to observed val. P(Sim <= Obs)
11	loc.prob.equal	Prob. that simulated val = to observed val. P(Sim = Obs)
12	loc.valid.iter	number of valid iteration for each test
13	quantiles	13 quantiles for each test

```

-----
[1] "These are the Slots"

```

7 PopGenome classes

