BC205: Algorithms for Bioinformatics. II. Sequence Analysis

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The biological problems:

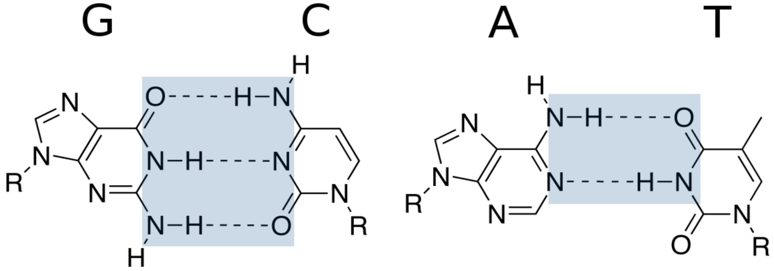
- Compare different species on the basis of DNA composition
- Find evidence of horizontal gene transfer in a bacterial genome
- Locate the Origin of Repication of a Bacterial Genome

Aspects of DNA Composition

- GC content
- genomic signatures
- parity distributions
- k-mer frequencies

GC content

We call GC content (or GC%) the ratio of (G+C) nucleotides of a given DNA sequence * Why is it important:

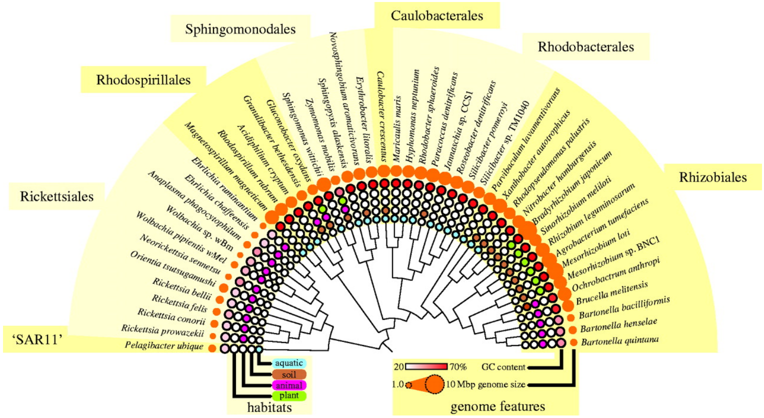


GC is related to:

- Biochemical level: Thermal stability
- Evolutionary level: Organism Phylogeny, Mutational pressures
- Genomic level: Genome size
- Functional level: Functional role of underlying sequences
- and many more

GC content in Genomic Sequences

- Bacteria: GC% is highly variable **between** species
- Bacteria: GC% is rather homogeneous **within** each genome
- Bacteria: GC% can be used in their classification



GC content in Genomic Sequences

- Eukaryotes: Very homogeneous overall GC% (~40-45% in all animals)
- Eukaryotes: Fluctuation of GC contentalong the chromosomes and organization in areas of (rather) stable GC%
- Eukaryotes: Regions of stable high/low GC content that segregate mammalian genomes in isochores

Isochores in a mammalian genome

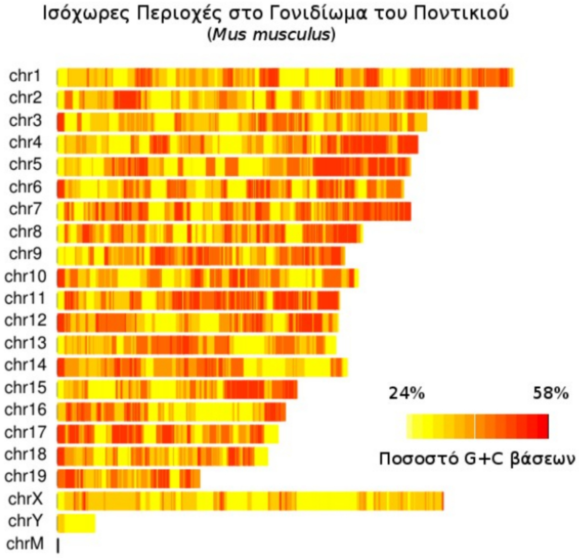


Figure 1: GC content along a mammalian genome

Problem 1: GC content in Bacterial Genomes

- Given the DNA sequence of a Bacterial Genome, calculate its GC content:
  - Read the Sequence
  - Enumerate G
  - Enumerate C
  - Divide (G+C) over length of the sequence

Problem 1: Implementation

```
f = open('ecoli.fa', 'r')
seq = ""
window=1000
total = 0
A=T=G=C=[]
times=0;
for line in f:
    x=re.match(">", line)
    if x == None:
        length=len(line)
        total=total+length
        seq=seq+line[0:length-1]
f.close()
    C=seq.count("C")
    G=seq.count("G")
    print (float(G)+float(C))/len(seq);
```

Hands on #1:

- Download a couple of bacterial genome sequences from ENSEMBL Bacteria (`http://bacteria.ensembl.org/index.html`)
- Implement GC content
- Report the results

Problem 2: Variability of GC content *between* Bacterial Genomes

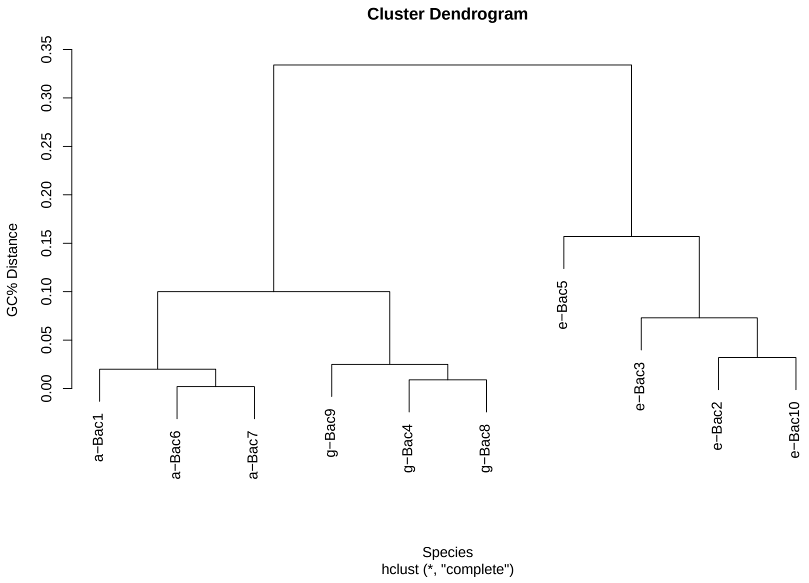
- Given a number of bacterial genomes:
  - Get their genome sequences
  - Calculate the GC contents
  - Calculate differences between the GC contents
  - Rank genomes based on their differences
- Pseudocode:
  - Perform GC_content on each of the genomes you downloaded
  - Calculate D_(i,j)=|GC_i-GC_j| over all i,j
  - Sort D_(i,j)

Problem 2: Approach

- Instead of Sorting Distances, we can do something better
- Use clustering (of any type) on the distance matrix

```r
gc_values=c(0.334, 0.595, 0.668,
            +0.409, 0.511, 0.352,
            +0.354, 0.418, 0.434, 0.627)
species=c("a-Bac1","e-Bac2","e-Bac3",
          +"g-Bac4","e-Bac5","a-Bac6",+
            "a-Bac7","g-Bac8","g-Bac9",+
            "e-Bac10")
# Create distance matrix of values with dist()
gc_dist<-dist(gc_values)
plot(hclust(gc_dist), labels = species, +
       xlab="Species", ylab="GC% Distance")
```

Problem 2: Approach

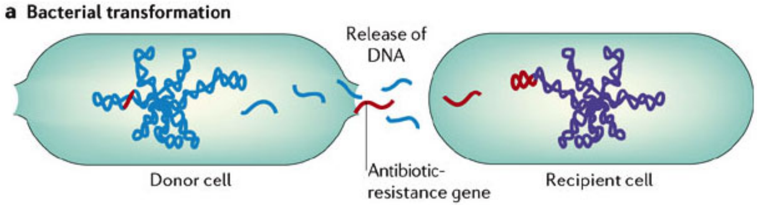


Problem 3: What about different regions of the genome?

- We just saw how genomic GC% values may be used to draw conclusions for bacterial phylogeny
- But: How representative is the GC% value you calculated above?
- And: How efficiently can it be used to describe a genome?

Problem 3: Why should we care?

- We mentioned that GC% is stable within bacterial genomes
- **But** Some areas of bacterial genomes are special



- Parts of the bacterial genome have been “horizontally” (as opposed to vertically, i.e. from their “mom”) transferred from other species.

Problem 3: Stability of GC content *along* Bacterial Genomes

- Regions of “strange”, or “divergent” GC% values in a given genome are red flags of HGT. The problem now is:
  - Given a bacterial genome sequence:
  - Locate regions of the genome where horizontal gene transfer may have occurred.

Problem 3: Approach

- Choose a window to scan your sequence. This will be your resolution
- Calculate GC per window
- Try to locate GC values that deviate from the genome average

Problem 3: The core

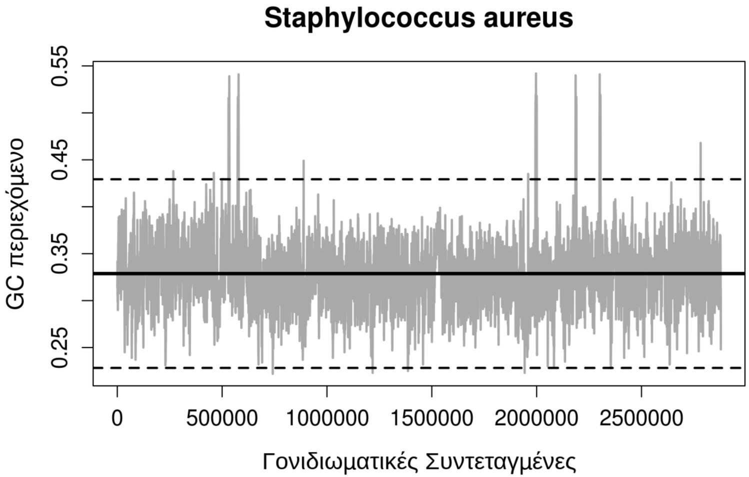
```
window=1000
step=100
times=len(seq)/step;

for i in range(times):
    DNA=seq[i*step:i*step+window]
    A=DNA.count("A")
    T=DNA.count("T")
    C=DNA.count("C")
    G=DNA.count("G")
    print i*step,"\t",i*step+window,"\t",
+(float(G)+float(C))/window;
```

Hands on #2:

- Get the genome sequence of St. aureus
- Implement Sliding GC
- Plot the results in R

GC content along the Genome of St. aureus

- It should look something like this
- Now how do we locate HGT candidates?



Problem 3: Statistics Interlude

- Given a set/sample of values, how can we decide on whether a value could be part of that sample or not?
- In our problem: We know that the GC% of bacteria tends to be characteristic of the genome. Can we "spot" regions of the genome that bear GC% values that are *different* from that characteristic value?
- Q1: How will we define that characteristic value?
- Q2: How will we quantify the *difference* as big enough or not?

Problem 3: Theoretical basis (simplified)

- Central Limit Theorem (simplified):
    - Regardless of the underlying distribution, the means of a large number of samples follow the normal distribution.
    - We can thus model GC values per window based on the normal distribution

Modeling with the Normal Distribution

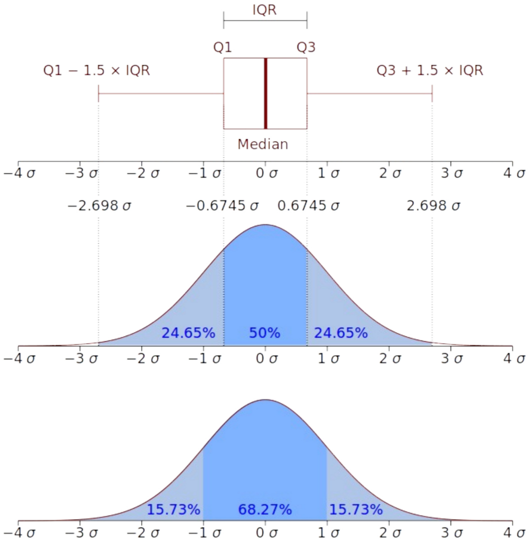


Figure 2: The Bell Curve

Problem 3: The statistics

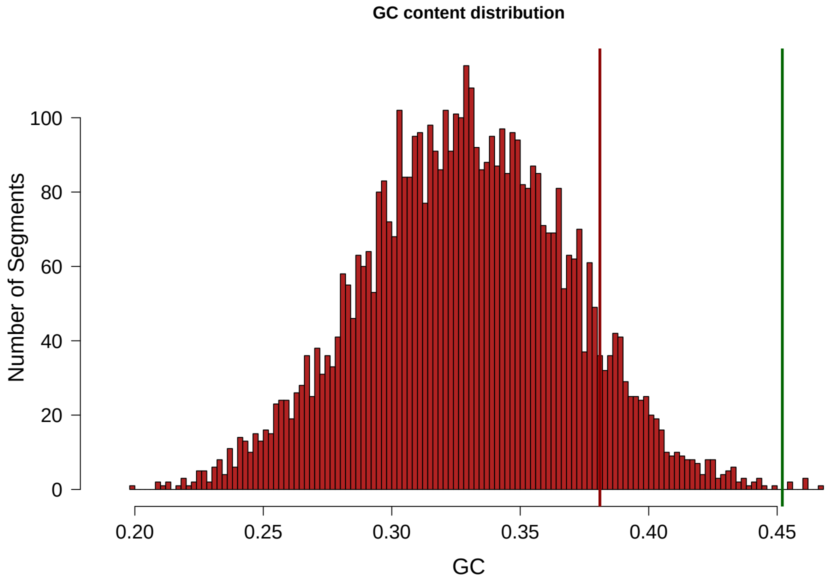
- We will model the “characteristic value” as the mean of GC values for all windows
- We will also calculate the standard deviation of these values to model variance

```
gc_mean=0.33
gc_sd=0.04
x<-rnorm(5000, mean=gc_mean, sd=gc_sd)
my_gc1=0.381
my_gc2=0.452
```

Problem 3: The statistics

```
hist(x, breaks=100, col="firebrick",
     +main="GC content distribution",
     +las=1, xlab="GC", ylab="Number of Segments",
     +xlim=c(gc_mean-3.5*gc_sd, gc_mean+3.5*gc_sd))
abline(v=my_gc1, col="darkred", lwd=3)
abline(v=my_gc2, col="darkgreen", lwd=3)
```

Problem 3: The statistics



Z-transformation

- Notice the difference between the position of the two vertical bars in the previous plot. One is much more “inside” the distribution than the other
- Can we have a quantitative measure of this?
- Given a value x, we can compare x to a normal distribution with mean=m and standard deviation=std with the z-score:
  Z(x)= (x - m)/std
  Z(x) is thus the difference of x from m in units of standard deviation.
  Knowing that in a normal distribution ~99% of the values fall within +/-2*std a value of Z(x)>2 or Z(x)<-2 makes it highly unlikely that x is part of our distribution.

Problem 3: The Statistics

```r
gc_mean=0.33
gc_sd=0.04
x<-rnorm(5000, mean=gc_mean, sd=gc_sd)
my_gc1=0.381
my_gc2=0.452
z1=(my_gc1-gc_mean)/gc_sd
z1
```

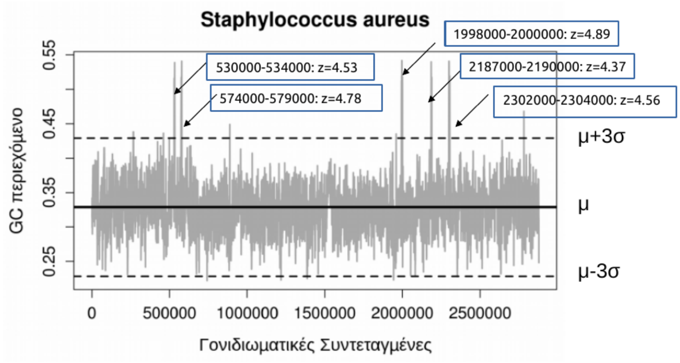
```
## [1] 1.275
```

```r
z2=(my_gc2-gc_mean)/gc_sd
z2
```

```
## [1] 3.05
```

Problem 2: Revisited

Let's take another look at the GC analysis of the St. aureus genome



Θέσεις με πολύ υψηλό z φαίνονται γραφικά στο σχήμα.

Οι θέσεις αυτές είναι οι θέσεις των γονιδίων του ριβοσωμικού RNA (rRNA)!

The problem:

- Background DNA composition has some **functional** role besides simply reflecting mutational pressures
- This means that in some cases we need to know why the local composition is guided by *other* aspects of molecular evolutio. E.g. why would rRNA genes be G+C-rich even in AT-rich genomes?
- We need to find a way to control for *background nucleotide composition*

Problem 2 Revisited: Distinguishing between genomes through their sequence composition

1. Going beyond the GC content
2. Going beyond simple bases (mononucleotides, k=1)
3. Analyzing all dinucleotide frequencies of k=2

- Pseudocode:
  - For each kmer in 4^k k-mers
  - Calculate N(kmer)
  - Create a table

Problem 2 Revisited: K-mer frequencies

```
import re
import math
import itertools

bases=['A','T','G','C']
k=2
kmer=[''.join(p) for p in
+itertools.product(bases, repeat=k)]
counts={}

for i in kmer:
    counts[i]=seq.count(str(i))
    print '%s %.3f' % (str(i),float(counts[i])/len(seq))
```

Problem 2 Revisited: A table of 4^k frequencies of occurrence

Base	A	T	G	C
A	0.090	0.112	0.048	0.053
T	0.095	0.090	0.064	0.053
G	0.052	0.052	0.023	0.034
C	0.066	0.048	0.026	0.023

- Values may be seen as “probabilities” of finding each k-mer in the sequence
- Can we use the notion of the probability to modify the table so that we get rid of the background nucleotide composition?

Problem 2 Revisited: Removing Background Composition

- The problem stated above persists at the level of k-mers: The background DNA composition may affect our results
- At the k-mer level we can remove the background using ratios of observed/expected frequencies
- Which is the expected frequency of a given k-mer?

Problem 2 Revisited: Observed/Expected(o/e) k-mer frequencies

- Mathematics Interlude:
  - Assume two events A, B that are linked with each other
  - We then say tha A and B are dependent (or conditioned) and we have a “conditional probability” of A happening given B is also happening
  - We can think of k-mers the same way: a k-mer is more probable to occur if its constituent mono-mers are occurring
  - Bottomline: Any given k-mer’s frequency of occurrence is dependent on the frequencies of occurrence of its mononucleotides. Thus:

Given a k-mer of length k the o/e-ratio frequency is defined as:
$R[N_1N_2..N_k] = F[N_1N_2..N_k]/(F[N_1]F[N_2]..F[N_k])$

In this way we can define a new table of modified frequencies that is independent of mono-nucleotide composition

Problem 2 Revisited: Observed/Expected K-mer frequencies

```
bases=['A','T','G','C']
k=1
kmer1=[''.join(p) for p in
+itertools.product(bases, repeat=k)]
k=2
kmer2=[''.join(p) for p in
+itertools.product(bases, repeat=k)]
oecounts={}

for i in kmer2:
    bg=list(i)
    oecounts[i]=float(seq.count(str(i)))/len(seq)
    for j in bg:
        oecounts[i]/=(float(seq.count(j))/len(seq))
    print '%s %.3f' % (str(i),oecounts[i])
```

Problem 2 Revisited: A table of o/e 4^k frequencies of occurrence

Base	A	G	C	T
A	0.800	0.997	0.878	0.949
G	0.848	0.799	1.174	0.957
C	0.946	0.955	0.848	1.252
T	1.183	0.872	0.946	0.841

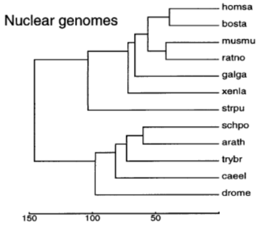
- Notice how values now go >1. What does this mean?
- How is this table better (or not) than the previous one?

Genomic Signatures: Comparing o/e k-mer composition

- Genomic Signatures are defined as the table of o/e k-mers for a given genome
- We can use these tables to analyze distances between genomes. (Hint: even eukaryote genomes!)

$$\rho^*_{XY} = f^*_{XY}/f^*_X f^*_Y$$

$$\delta^*(p,q) = \frac{1}{16}\sum_{XY}|\rho^*_{XY}(p) - \rho^*_{XY}(q)|,$$



Hands on #3:

- Get chromosome 1 from (human, mouse, fly, worm, yeast)
- Use a genomic signature approach to cluster genomic signatures from different genomes
- You can make use of R's dist() function on array of values as well

Problem 4: Finding the DNA Replication in a bacterial genome

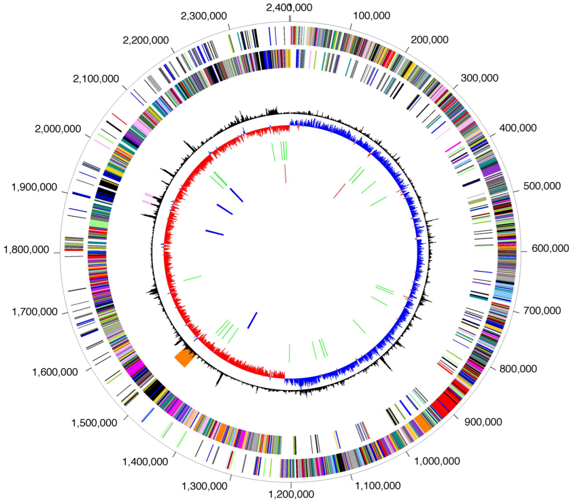


Figure 3

What we know

- Due to the pioneering work of E. Chargaff we know that A∼T and G∼C in **single-stranded DNA**
- We know that this holds for all complete genomes except very few exceptions
- The exceptions are the few genomes that **do not** replicate symmetrically
- DNA-strand parity:
  - Strand X is replicated in-continuously
  - Accumulates more substitutions
  - If substitutions are biased the strand will guide the change in both strands through base-pairing

Why should you care?

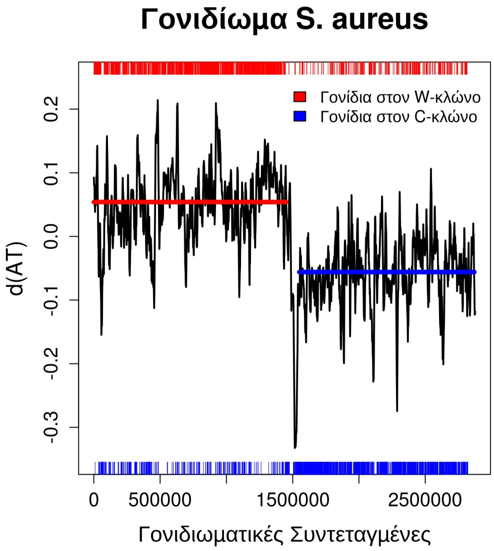


Figure 4: Nucleotide Parity

Approaching the problem

- We thus expect (and observe) the parity to be violated and that this violation occurs symmetrically on either side of the OriC
- We are looking for a way to locate this *phase transition* in the parity violation
- We thus need:
  - A measure of the parity
  - A way to monitor this measure along the genome
  - A way to locate abrupt changes in its values

Breaking the problem into pieces

1. Analyze the DNA composition *along* the genome
2. Calculate a quantity that will be informative
3. Create a condition that will test the location of the Ori

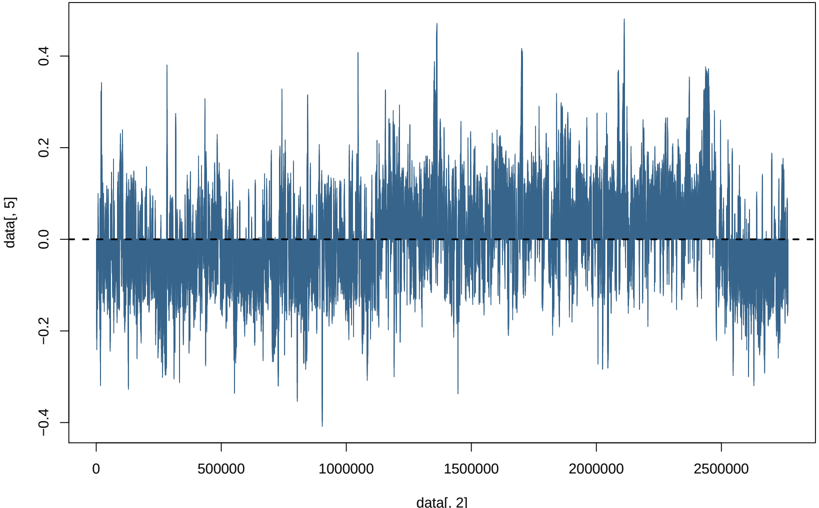
- Pseudocode: Given a bacterial genome:
  - Count nucleotides in windows of N base pairs
  - Calculate the scaled AT-skew as (A-T)/(A+T)
  - Create an array of the skew values along the genome
  - Locate the transition point

Problem 4: Parity Measure Implementation

```
window=1000
step=100
times=len(seq)/step;

for i in range(times):
    DNA=seq[i*step:i*step+window]
    A=DNA.count("A")
    T=DNA.count("T")
    C=DNA.count("C")
    G=DNA.count("G")
    print i*step,"\t",i*step+window,"\t",float(A-T)/(A+T)
```

Problem 4: Plotting the Values

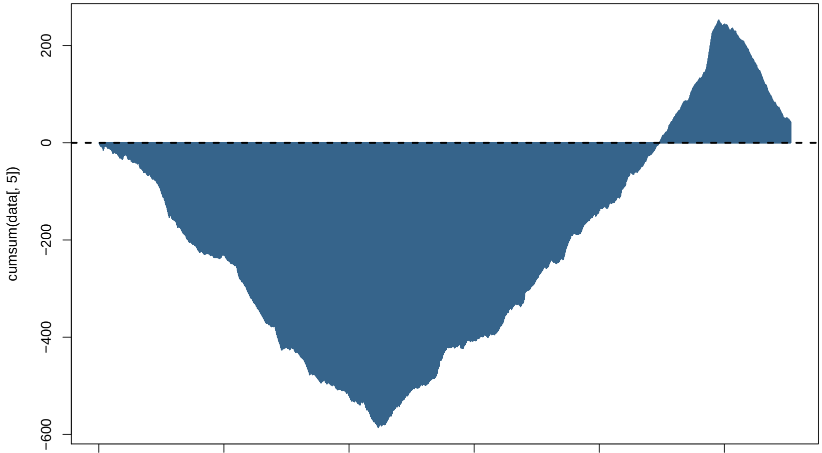
```r
data<-read.delim("out", header=F)
plot(data[,2], data[,5], type="h", col="steelblue4")
abline(h=0, lty=2, lwd=2)
```



Problem 4: Plotting the Values

- Using a cumulative approach often helps

```
data<-read.delim("out", header=F)
plot(data[,2], cumsum(data[,5]), type="h", col="steelblue4'
abline(h=0, lty=2, lwd=2)
```



Problem 4: Locating the breakpoint(s)

- Not a simple problem. In fact one (breakpoint detection) for which research is ongoing in many fields
- Things you could try:
  - Using derivation (checking the difference between each value and the previous one)
  - Density-based approaches: Trying to locate the region around which changes in the sign occur more robustly (i.e. given many different points around it)

Exercises: To think about

- Use a genomic signature approach to locate possible HGT genes in the genome of St. aureus. Do your results of “outliers” differ from those obtained with the GC content appoach?