Regulatory sequence analysis

Sequence models
(Bernoulli and Markov models)

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Why do we need random models ?

- Any pattern discovery relies on an underlying model to estimate the random expectation.
  - This model can be simple (succession of independent and equiprobable nucleotides) or more elaborate (differences in oligonucleotide composition).
  - The choice of an inappropriate model can lead to false conclusions.
  - In practice, a sequence model can be used to generate random sequences, which will serve to validate some theoretical assumptions.
- Example: comparison of observed and expected occurrences with the binomial distribution, as applied with oligo-analysis :
  - Relies on an assumption that successive oligonucleotides are independent from each other.
  - This is clearly not the case: each k-letter word depends on the k-1 neighbour words on both sides. How far does it affect the conclusions ?
  - We could test it by generating random sequences, counting words, and fitting the distribution of observed occurrences with a binomial distribution.

Probability of a sequence segment

- What is the probability for a given sequence segment (oligonucleotide, "word") to be found at a given position of a DNA sequence ?

- Different models can be chosen
  - Independently distributed nucleotides (**Bernoulli model**)
    - The probability of each residue is fixed a priori (*prior residue probability*)
      - Example: P(A) = 0.35; P(T) = 0.32; P(C) = 0.17; P(G) = 0.16
    - Particular case: equiprobable residues
      - P(A) = P(T) = P(C) = P(G) = 0.25
      - simple, but NOT realistic !
  - **Markov model**
    - The probability of each residue depends on the *m* preceding residues.
    - The parameter *m* is called the *order* of the Markov model
    - Remark: a Markov model of order 0 is a Bernoulli model.

- The simplest model : identically and independently (i.i.d.) distributed nucleotides.

  $p = P(A) = P(C) = P(G) = P(T) = 0.25$

$$P(S) = p^L$$

- The probability of a sequence
  - Is the product of its residue probabilities (independence)
  - Equiprobability: since all residues have the same probability, it is simply computed as the residue proba (p) to the power of the sequence length (L)
    - S is a sequence segment (e.g. an oligonucleotide)
    - L length of the sequence segment
    - p nucleotide probability
    - $P(S)$ is the probability to observe this sequence segment at given position of a larger sequence

- Example
  - $P(CACGTG) = 0.25^6 = 2.44e^{-4}$

- A more refined model consists in using residue-specific probabilities. The probability of each residue is assumed to be constant on the whole sequence (Bernoulli schema).

$$P(S) = \prod_{i=1}^{L} P(r_i)$$

- The probability of a sequence is the product of its residue probabilities.
  - $i = 1..k$ is the index of nucleotide positions
  - r_i is the residue found at position I
  - $P(r_i)$ is the probability of this residue
- Example: non-coding sequences in the yeast genome
  - $P(A) = P(T) = 0.325$
  - $P(C) = P(G) = 0.175$
  - $P(CACGTG) = P(C)\,P(A)\,P(C)\,P(G)\,P(T)\,P(G)$
    $= 0.325^4 * 0.175^2$
    $= 9.91E^{-5}$

$$P\left(r_i \mid S_{i-m,i-1}\right)$$

Transition matrix, order 1

	a	c	g	t
A	P(A|A)	P(C|A)	P(G|A)	P(T|A)
C	P(A|C)	P(C|C)	P(G|C)	P(T|C)
G	P(A|G)	P(C|G)	P(G|G)	P(T|G)
T	P(A|T)	P(C|T)	P(G|T)	P(T|T)

Transition matrix, order 2

Pref	A	C	G	T
AA	P(A|AA)	P(C|AA)	P(G|AA)	P(T|AA)
AC	P(A|AC)	P(C|AC)	P(G|AC)	P(T|AC)
AG	P(A|AG)	P(C|AG)	P(G|AG)	P(T|AG)
AT	P(A|AT)	P(C|AT)	P(G|AT)	P(T|AT)
CA	P(A|CA)	P(C|CA)	P(G|CA)	P(T|CA)
CC	P(A|CC)	P(C|CC)	P(G|CC)	P(T|CC)
CG	P(A|CG)	P(C|CG)	P(G|CG)	P(T|CG)
CT	P(A|CT)	P(C|CT)	P(G|CT)	P(T|CT)
GA	P(A|GA)	P(C|GA)	P(G|GA)	P(T|GA)
GC	P(A|GC)	P(C|GC)	P(G|GC)	P(T|GC)
GG	P(A|GG)	P(C|GG)	P(G|GG)	P(T|GG)
GT	P(A|GT)	P(C|GT)	P(G|GT)	P(T|GT)
TA	P(A|TA)	P(C|TA)	P(G|TA)	P(T|TA)
TC	P(A|TC)	P(C|TC)	P(G|TC)	P(T|TC)
TG	P(A|TG)	P(C|TG)	P(G|TG)	P(T|TG)
TT	P(A|TT)	P(C|TT)	P(G|TT)	P(T|TT)

- In a Markov model, the probability to find a letter at position i depends on the residues found at the m preceding residues.

- The tables represent the transition matrices for Markov chain models of order $m=1$ (top) and $m=2$ (bottom).

- Each row specifies one **prefix**, each column one **suffix**.

- The values indicate the probability to observe a given residue (suffix r_i) at position (i) of the sequence, as a function of the m preceding residues (the prefix $S_{i-m,i-1}$)

- Particular case
   - A Bernoulli model is a Markov model of order 0.

Markov model estimation ("training")

- Transition frequencies for a Markov model of order *m* can be estimated from the frequencies observed for oligomers of length *k=m+1* in a reference sequence set.

$$P(r_i \mid S_{1..m}) = \frac{F_{bg}(r_i \mid S_{1..m})}{\sum_{j \in A} F_{bg}(r_j \mid S_{1..m})} = \frac{F_{bg}(S_{1..m}r_i)}{\sum_{j \in A} F_{bg}(S_{1..m}r_j)}$$

Example

The upper table shows dinucleotide frequencies computed from the whole set of upstream sequences of the yeast *Saccharomyces cerevisiae* The bottom table shows the corresponding transition frequencies.

Dinucleotide frequencies

Sequences S	Occurrences N(S)	Frequency F(S)
AA	526,149	0.112
AC	251,377	0.054
AG	275,056	0.059
AT	414,453	0.088
CA	294,423	0.063
CC	178,324	0.038
CG	146,052	0.031
CT	275,859	0.059
GA	277,343	0.059
GC	184,367	0.039
GG	173,404	0.037
GT	239,569	0.051
TA	369,980	0.079
TC	280,475	0.060
TG	279,932	0.060
TT	521,236	0.111

$$P(G \mid T) = \frac{F(G \mid T)}{\sum_{j \in A} F(j \mid T)} = \frac{F(TG)}{F(T*)}$$

$$= \frac{0.070}{0.046 + 0.058 + 0.070 + 0.073} = 0.282$$

Transition matrix: order 1

Prefix \ Suffix	A	C	G	T	P(Prefix)	N(Suffix)
A	0.359	0.171	0.187	0.283	0.313	1,467,035
C	0.329	0.199	0.163	0.308	0.191	894,658
G	0.317	0.211	0.198	0.274	0.187	874,683
T	0.255	0.193	0.193	0.359	0.310	1,451,623
P(Suffix)	0.313	0.191	0.187	0.310		
N(Suffix)	1,467,895	894,543	874,444	1,451,117		

Examples of transition matrices

$$P(r_i \mid S_{i-m,i-1})$$

- The two tables below show the transition matrices for a Markov model of order 1 (top) and 2 (bottom), respectively.
- The two models were trained with the whole set of **non-coding upstream sequences** of the yeast *Saccharomyces cerevisiae*.
- Notice the strong probability of transitions from AA to A and TT to T.

Transition matrix, order 1

Pre/Suffix	A	C	G	T	P(Prefix)	N(Suffix)
A	0.359	0.171	0.187	0.283	0.313	1,467,035
C	0.329	0.199	0.163	0.308	0.191	894,658
G	0.317	0.211	0.198	0.274	0.187	874,683
T	0.255	0.193	0.193	0.359	0.310	1,451,623
P(Suffix)	0.313	0.191	0.187	0.310		
N(Suffix)	1,467,895	894,543	874,444	1,451,117		

Transition matrix, order 2

Prefix/Suffix	A	C	G	T	P(Prefix)	N(Prefix)
AA	0.388	0.161	0.200	0.251	0.112	525,000
AC	0.339	0.198	0.173	0.290	0.054	251,072
AG	0.345	0.204	0.196	0.255	0.059	274,601
AT	0.311	0.184	0.182	0.323	0.088	413,946
CA	0.347	0.178	0.189	0.286	0.063	293,750
CC	0.341	0.190	0.161	0.309	0.038	178,110
CG	0.293	0.221	0.196	0.290	0.031	145,876
CT	0.229	0.195	0.205	0.371	0.059	275,634
GA	0.394	0.155	0.187	0.264	0.059	277,053
GC	0.330	0.205	0.169	0.297	0.039	184,192
GG	0.318	0.217	0.187	0.277	0.037	173,266
GT	0.285	0.175	0.204	0.336	0.051	239,384
TA	0.300	0.193	0.168	0.339	0.079	369,426
TC	0.313	0.203	0.152	0.332	0.060	280,131
TG	0.302	0.209	0.208	0.282	0.060	279,783
TT	0.210	0.208	0.189	0.392	0.111	520,906
P(Suffix)	0.313	0.191	0.187	0.310		
N(suffix)	1,466,075	893,444	873,260	1,449,351		

Markov chains and Bernoulli models

- By extension of the concept of Markov chain, Bernoulli models can be qualified as Markov models of order 0 (the order 0 means that there is no dependency between a residue and the preceding ones).

- The prior probabilities of a Makov model of order $m=0$ can be estimated from the residue of single nucleotides ($k=m+1=1$) in a background sequence set.

- The table below shows the residue frequencies in the genomes of the yeast *Saccharomyces cerevisiae* and the bacteria *Escherichia coli K12*, respectively.

- Notice the strong differences between these genomes.

Markov order 0 = Bernouli

A	C	G	T	Genome
0.310	0.191	0.191	0.309	*Saccharomyces cerevisiae*
0.246	0.254	0.254	0.246	*Escherichia coli K12*

- The example below illustrates the computation of the probability of a seqence segment **P(S|B)** with a background Markov model **B** of order 2, calibrated from 3nt frequencies on the yeast genome.

CCTACTATATGCCCAGAATT

Background model B

Transition matrix, order 2

Prefix/Suffix	A	C	G	T	P(Prefix	N(Prefix
AA	0.388	0.161	0.200	0.251	0.112	525,000
AC	0.339	0.198	0.173	0.290	0.054	251,072
AG	0.345	0.204	0.196	0.255	0.059	274,601
AT	0.311	0.184	0.182	0.323	0.088	413,946
CA	0.347	0.178	0.189	0.286	0.063	293,750
CC	0.341	0.190	0.161	0.309	0.038	178,110
CG	0.293	0.221	0.196	0.290	0.031	145,876
CT	0.229	0.195	0.205	0.371	0.059	275,634
GA	0.394	0.155	0.187	0.264	0.059	277,053
GC	0.330	0.205	0.169	0.297	0.039	184,192
GG	0.318	0.217	0.187	0.277	0.037	173,266
GT	0.285	0.175	0.204	0.336	0.051	239,384
TA	0.300	0.193	0.168	0.339	0.079	369,426
TC	0.313	0.203	0.152	0.332	0.060	280,131
TG	0.302	0.209	0.208	0.282	0.060	279,783
TT	0.210	0.208	0.189	0.392	0.111	520,906
P(Suffix)	0.313	0.191	0.187	0.310		
N(suffix)	1,466,075	893,444	873,260	1,449,351		

Sequence probability given the backgound model

$$P(S \mid B) = P(S_{1,m} \mid B) \prod_{i=m+1}^{L} P\left(r_i \mid S_{i-m,i-1}, B\right)$$

pos	P(R|W)		wR	S	P(S)
1	P(CC)	0.038	cc	CC	3.80E-02
2	P(T|CC)	0.309	ccT	CCT	1.17E-02
3	P(A|CT)	0.229	ctA	CCTA	2.69E-03
4	P(C|TA)	0.193	taC	CCTAC	5.19E-04
5	P(T|AC)	0.290	acT	CCTACT	1.50E-04
6	P(A|CT)	0.229	ctA	CCTACTA	3.45E-05
7	P(T|TA)	0.339	taT	CCTACTAT	1.17E-05
8	P(A|AT)	0.311	atA	CCTACTATA	3.63E-06
9	P(T|TA)	0.339	taT	CCTACTATAT	1.23E-06
10	P(G|AT)	0.182	atG	CCTACTATATG	2.25E-07
11	P(C|TG)	0.209	tgC	CCTACTATATGC	4.69E-08
12	P(C|GC)	0.205	gcC	CCTACTATATGCC	9.61E-09
13	P(C|CC)	0.190	ccC	CCTACTATATGCCC	1.82E-09
14	P(A|CC)	0.341	ccA	CCTACTATATGCCCA	6.21E-10
15	P(G|CA)	0.189	caG	CCTACTATATGCCCAG	1.17E-10
16	P(A|AG)	0.345	agA	CCTACTATATGCCCAGA	4.04E-11
17	P(A|GA)	0.394	gaA	CCTACTATATGCCCAGAA	1.59E-11
18	P(T|AA)	0.251	aaT	CCTACTATATGCCCAGAAT	4.00E-12
19	P(T|AT)	0.323	atT	CCTACTATATGCCCAGAATT	1.29E-12

Background sequences

- The frequencies observed for a *k*-letter word in a reference sequence set (background sequence) can be used to estimate the expected frequencies of the same *k*-letter word in the sequences to be analyzed.
- Typical background models:
  - Whole genome
    - But this will bias the estimates towards coding frequencies, especially in microbial organisms, where the majority of the genome is coding.
  - Whole set of intergenic sequences
    - More accurate than whole-genome estimates, but still biased because intergenic sequences include both upstream and downstream sequences
  - Whole set of upstream sequences, same sizes as the sequences to be analyzed
    - Requires a calibration for each sequence size
  - Whole set of upstream sequences, fixed size (default on the web site)
   - Reasonably good estimate for microbes, NOT for higher organisms.

- The genome shows a strong richness in A and T residues (80%AT).
- This enrichment is even stronger in upstream non-coding sequences (86%AT).

Frequencies

Residue	Genome	Upstream (max 2kb)
a	0.40	0.42
c	0.10	0.07
g	0.10	0.07
t	0.40	0.44

Upstream frequencies
Gray scale

a	c	g	t
0.42419	0.06761	0.06951	0.43870

Plasmodium-falciparum 1nt frequencies
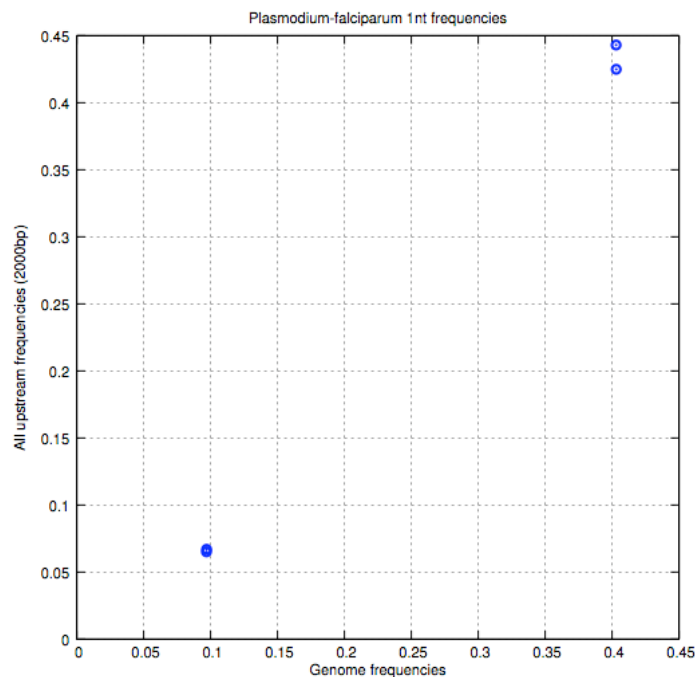
Dinucleotide composition of the Plasmodium upstream sequences

- Dinucleotide frequencies reflect the AT-richness.

Plasmodium-falciparum 2nt frequencies

Residue	Genome	Upstream (max 2 kb)
AT	0.175	0.207
TA	0.159	0.199
TT	0.109	0.120
AA	0.109	0.111
TG	0.044	0.033
CA	0.044	0.031
GT	0.035	0.028
AC	0.035	0.027
GA	0.039	0.025
TC	0.039	0.024
AG	0.032	0.021
CT	0.032	0.021
CC	0.013	0.007
GG	0.013	0.007
GC	0.009	0.006
CG	0.007	0.004
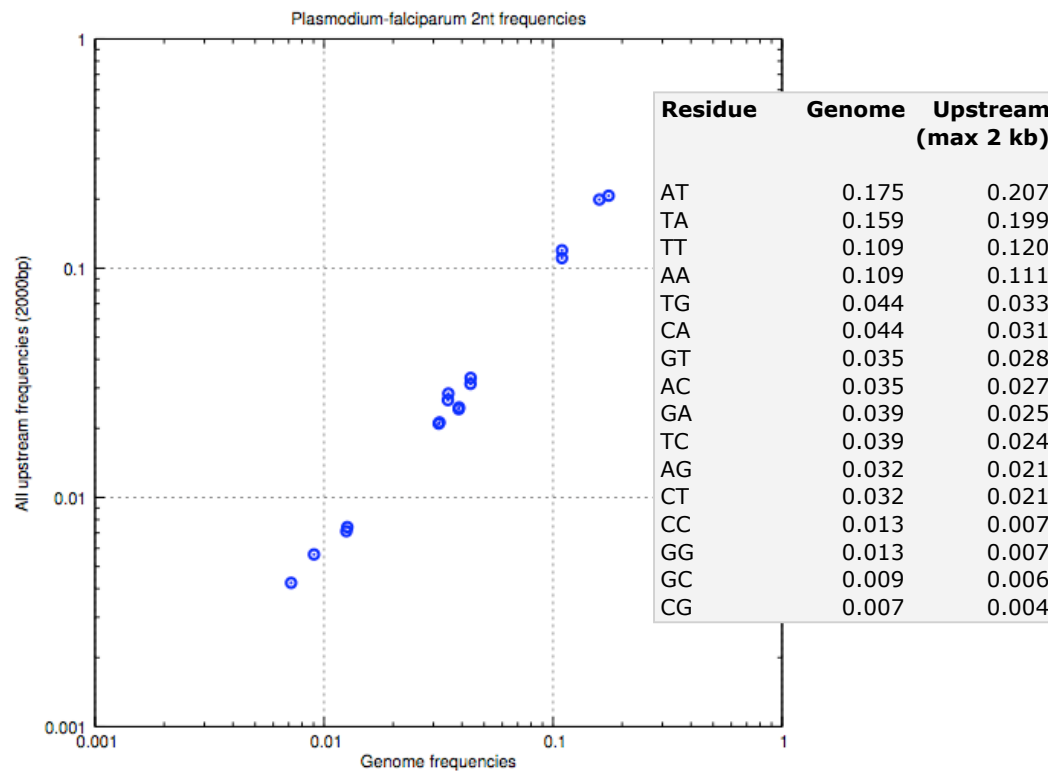
Markov order m=1 (based of dinucleotides k=2)

Pr	a	c	g	t
a	0.39983	0.06522	0.05243	0.48253
c	0.47917	0.13198	0.06745	0.32140
g	0.36686	0.08719	0.12634	0.41961
t	0.44833	0.05728	0.07758	0.41681

Transition frequencies

- On the basis of oligonucleotide frequencies, one can compute Markov models, which indicate the probability to osberve a certain residue (suffix) after a certain oligonucleotide (prefix).
- The Markov model can be represented in the form of a transition table.

**Markov order m=2
(based on trinucleotides k=3)**

**Markov order m=1
(based on dinucleotides k=2)**

pr	a	c	g	t
a	0.39983	0.06522	0.05243	0.48253
c	0.47917	0.13198	0.06745	0.32140
g	0.36686	0.08719	0.12634	0.41961
t	0.44833	0.05728	0.07758	0.41681

pr	a	c	g	t
aa	0.55133	0.05771	0.06855	0.32241
ac	0.59611	0.10571	0.07782	0.22037
ag	0.45875	0.08478	0.16910	0.28737
at	0.60625	0.03631	0.08099	0.27645
ca	0.32382	0.11650	0.04995	0.50973
cc	0.36057	0.16777	0.04837	0.42329
cg	0.32114	0.10703	0.08672	0.48511
ct	0.28437	0.09985	0.07587	0.53991
ga	0.54541	0.06894	0.09101	0.29464
gc	0.49165	0.11509	0.08103	0.31223
gg	0.43450	0.07578	0.16309	0.32663
gt	0.43973	0.06254	0.13930	0.35844
ta	0.26328	0.06301	0.03410	0.63961
tc	0.38894	0.15242	0.05942	0.39921
tg	0.29671	0.08900	0.09461	0.51969
tt	0.29185	0.07502	0.06441	0.56871

pr	a	c	g	t
aaa	0.60977	0.04557	0.06493	0.27973
aac	0.58765	0.10970	0.08361	0.21904
aag	0.48609	0.07425	0.18023	0.25943
aat	0.58314	0.03951	0.09415	0.28320
aca	0.33624	0.11699	0.04732	0.49945
acc	0.42056	0.13956	0.05166	0.38822
acg	0.32069	0.10224	0.09021	0.47886
act	0.33953	0.08427	0.09022	0.48599
aga	0.59498	0.05904	0.09664	0.24934
agc	0.50258	0.12264	0.07180	0.30297
agg	0.47636	0.07224	0.15773	0.29366
agt	0.45732	0.06399	0.13465	0.34404
ata	0.24700	0.05147	0.02792	0.67361
atc	0.44744	0.14610	0.06464	0.34182
atg	0.31388	0.08453	0.09069	0.51090
att	0.36939	0.07058	0.06213	0.49790
caa	0.50256	0.09844	0.08040	0.31860
cac	0.60914	0.10891	0.07457	0.20738
cag	0.41605	0.10501	0.14426	0.33467
cat	0.52552	0.05702	0.09066	0.32679
cca	0.33910	0.12537	0.05311	0.48242
ccc	0.31962	0.24652	0.03963	0.39424
ccg	0.31788	0.11607	0.07717	0.48888
cct	0.26231	0.10092	0.06513	0.57163
cga	0.49791	0.09494	0.08617	0.32098
cgc	0.48094	0.13045	0.08932	0.29929
cgg	0.39413	0.10204	0.14089	0.36294
cgt	0.40734	0.07709	0.13139	0.38418
cta	0.29671	0.12770	0.05561	0.51798
ctc	0.36301	0.16743	0.05648	0.41309
ctg	0.30288	0.10998	0.09812	0.48902
ctt	0.28491	0.10316	0.07477	0.53716
gaa	0.50960	0.09393	0.09571	0.30076
gac	0.54425	0.12584	0.10360	0.22631
gag	0.48604	0.10232	0.17386	0.23777
gat	0.49644	0.07266	0.12443	0.30647
gca	0.29541	0.15864	0.06259	0.48336
gcc	0.36138	0.16281	0.05862	0.41719
gcg	0.32615	0.14755	0.08999	0.43630
gct	0.30894	0.12070	0.08886	0.48150
gga	0.54253	0.07813	0.09408	0.28526
ggc	0.46171	0.11411	0.08953	0.33466
ggg	0.41855	0.07394	0.23191	0.27560
ggt	0.39290	0.08119	0.14861	0.37731
gta	0.29983	0.11245	0.06728	0.52044
gtc	0.41194	0.17140	0.07744	0.33922
gtg	0.26188	0.12997	0.10396	0.50419
gtt	0.32069	0.12418	0.10655	0.44857
taa	0.46627	0.06216	0.06566	0.40592
tac	0.60619	0.09884	0.07071	0.22426
tag	0.41057	0.09261	0.15341	0.34341
tat	0.63289	0.03019	0.07157	0.26535
tca	0.30623	0.10005	0.04961	0.54411
tcc	0.33077	0.15949	0.04747	0.46227
tcg	0.30935	0.09838	0.08339	0.50889
tct	0.25488	0.10510	0.06881	0.57121
tga	0.50400	0.07174	0.08498	0.33929
tgc	0.49330	0.10821	0.08360	0.31489
tgg	0.39794	0.07761	0.14156	0.38289
tgt	0.44502	0.05733	0.14044	0.35722
tta	0.28876	0.07117	0.03837	0.60169
ttc	0.35959	0.15103	0.05488	0.43451
ttg	0.28338	0.07849	0.09647	0.54166
ttt	0.24799	0.06960	0.06044	0.62198

Markov models show strong variations between organisms

Saccharomyces cerevisiae (Fungus)

pr	a	c	g	t
a	0.37000	0.16588	0.17908	0.28504
c	0.32610	0.19058	0.16818	0.31514
g	0.31163	0.21456	0.18957	0.28424
t	0.27256	0.17991	0.17364	0.37389

Escherichia coli K12 (Proteobacteria)

pr	a	c	g	t
a	0.34491	0.18156	0.17676	0.29677
c	0.30806	0.21557	0.22129	0.25507
g	0.27123	0.25972	0.21545	0.25360
t	0.24080	0.19176	0.21144	0.35599

Mycobacterium leprae (Actinobacteria)

pr	a	c	g	t
a	0.23239	0.28694	0.25692	0.22375
c	0.24574	0.24601	0.30574	0.20252
g	0.21748	0.29238	0.25535	0.23479
t	0.18806	0.26081	0.31784	0.23329

Mycoplasma genitalium (Firmicute, intracellular)

pr	a	c	g	t
a	0.45565	0.11743	0.13602	0.29091
c	0.39457	0.13008	0.06403	0.41132
g	0.31505	0.18738	0.12047	0.37710
t	0.32450	0.09573	0.11934	0.46044

Bacillus subtilis (Firmicute, extracellular)

pr	a	c	g	t
a	0.38159	0.13935	0.18767	0.29139
c	0.33699	0.19499	0.16508	0.30293
g	0.34249	0.18100	0.23541	0.24110
t	0.25122	0.17199	0.19402	0.38278

Plasmodium falciparum (Aplicomplexa, intracellular)

pr	a	c	g	t
a	0.39821	0.06446	0.05206	0.48527
c	0.47798	0.13336	0.06695	0.32171
g	0.36764	0.08587	0.12431	0.42217
t	0.44739	0.05676	0.07673	0.41912

Anopheles gambiae (Insect)

pr	a	c	g	t
a	0.34603	0.21388	0.18890	0.25119
c	0.31499	0.21232	0.24159	0.23109
g	0.26036	0.25414	0.20275	0.28275
t	0.20368	0.20710	0.24970	0.33951

Homo sapiens (Mammalian)

| pr	a	c	g	t
a	0.29760	0.19031	0.28856	0.22353
c	0.28019	0.30209	0.11692	0.30080
g	0.24408	0.24738	0.30309	0.20545
t	0.18589	0.23061	0.27491	0.30859

Chaos representation - upstream frequencies
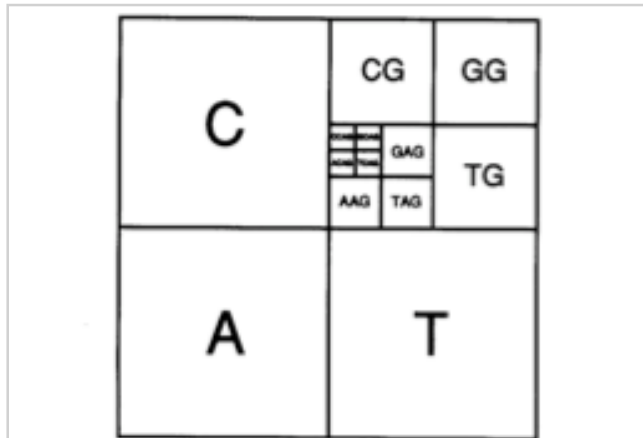
Source: Goldman, 1993

Figure 2. Explanation of the correspondence between oligonucleotides and areas of the CGR of DNA sequences. Each base gives a point in the quadrant labelled with that base; the sub-quadrant is determined by the preceding base, the sub-sub-quadrant (e.g. 'TAG') by the base preceding that, etc. This correspondence continues recursively, but is shown to a maximum of four bases in this figure for clarity.

- The chaos representation (Jeffrey, 1990) permits to visualize oligonucleotide frequencies and detect enrichment in particular ones.

- Plasmodium upstreal sequences are particularly rich for the following motifs
    - A, T nucleotides
    - Oligonucleotides made of As and Ts only
      (last rw pf each chaos map)
    - Poly-A and poly-T oligos (bottom corners of the maps)
    - (TA){n} motifs (the darkest boxes from dinucleotides to tetranucleotides.

Nucleotides

Dinucleotides

Trinucleotides

Tetranucleotides
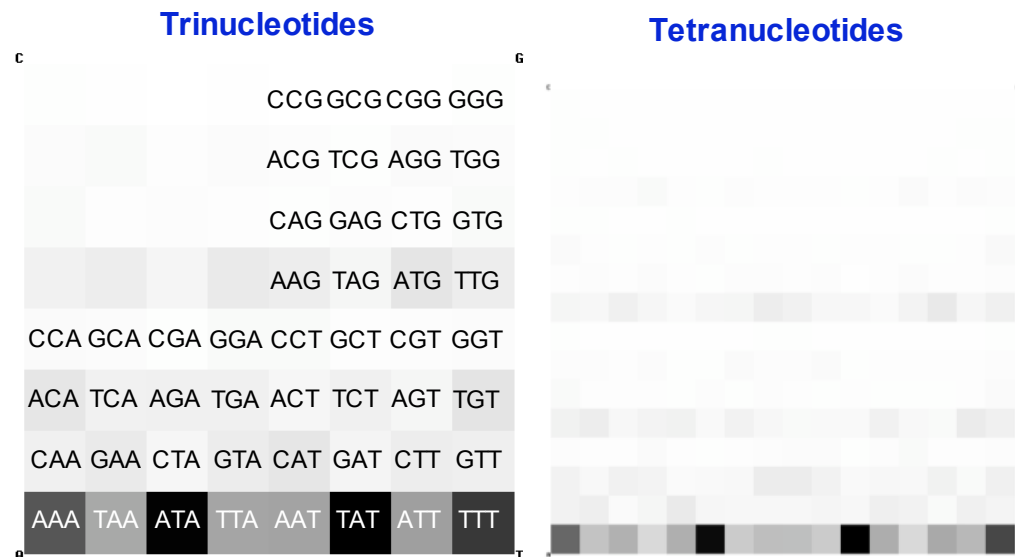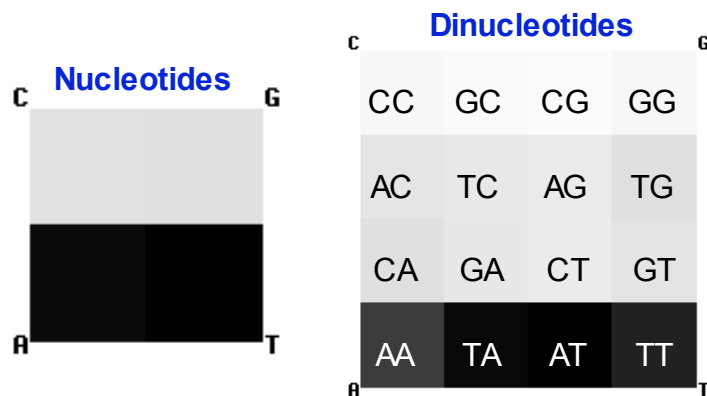
Goldman. Nucleotide, dinucleotide and trinucleotide frequencies explain patterns observed in chaos game representations of DNA sequences. Nucleic Acids Res (1993) vol. 21 (10) pp. 2487-91
Jeffrey. Chaos game representation of gene structure. Nucleic Acids Res (1990) vol. 18 (8) pp. 2163-70

Hexanucleotide frequencies in Plasmodium Genome versus upstream (2Kb)

- Hexanucleotides show a very wide range of frequencies in the whole genome (X axis) as well as in the subset of upstream sequences (max 2kb, Y axis).

Plasmodium-falciparum 6nt frequencies

Plasmodium-falciparum 6nt frequencies