

Identifying CpG islands using hidden Markov models

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CpG islands

On a DNA strand, a cytosine followed by guanine is a dinucleotide called **CpG**. The 'p' is for the *phosphate bond* between them.



Figure: CpG nucleotides on a DNA strand and its complement.

CpG's are often clustered in regions called **CpG islands** (CGIs).

CGIs are often associated with the promoter region of genes (where transcription begins).

Identifying CGIs can help identify new genes, some of which may be involved in cancer.

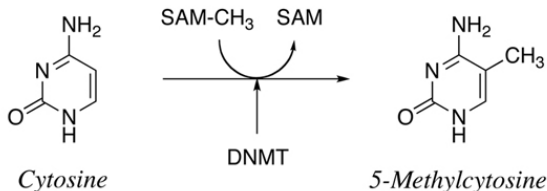
Goal

Given a genome of millions of base pairs, how can one identify the CpG islands?

Cytosine methylation

Almost all cells in an organism have the same DNA sequence. The difference lies in the levels of *gene expression*.

One common way that genes are turned off is by a chemical change called **methylation** at the promoter CGI.



Promoter regions of housekeeping genes are usually unmethylated.

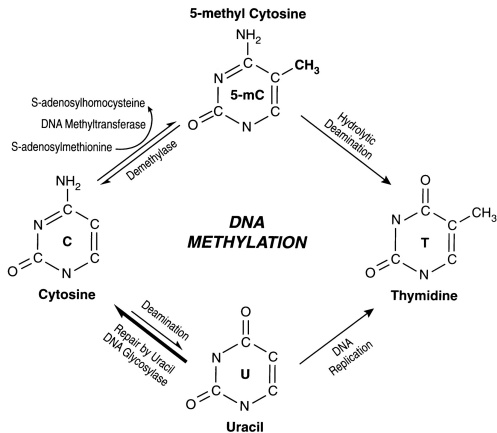
Appropriate methylation of CGIs is needed for normal development. If methylation occurs when it should not in tumor suppressor genes, then problems such as cancer can result.

In mammals, 70–80% of CpG cytosines are methylated, but it depends on the type of cell.

For example, hemoglobin genes should be methylated (and shut off) in skin cells but unmethylated (and expressed) in red blood cell precursors.

Methylation and deamination

5-methyl cytosine can be **deaminated** to produce thymine (T), which is a mutation. As a result, there is a lack of CpG sites in methylated DNA.



Rule of thumb

On an evolutionary timescale, **unmethylated C's tend to persist** and **methylated C's tend to be eliminated**.

CATTCCGCCTTCTCTCCCGAGGTGGCGCGTGGGA
 GGTGTTTTCCTCGGGTCTGTAAAGAATAGGCCAGG
 CAGCTTCCCGCGGATGCCCTCATCCCTCTCGG
 GGTTCGCCTCCACCGCGCGCGTTCCGCCGTT
 CCGCCTGCGAGATGTTTTCCACCGACAATGATTC
 CACTCTCGCGCCTCCCATGTTGATCCAGCTCCT
 CTGCGGGGTCAGGACCCCTGGGCCCGCCCCG
 CTCCACTCAGTCAATCTTTGTCCCGGTATAAGCG
 GATTATCGGGGTGGTGGGGCGGCTGATTCGSA
 CGAATGCCCTTGGGGGTACCCCGGGAGGGAACTC
 CGGGCTCGCGCTTTGGCCACCGCACCCCTGGT
 TGAGCGGCCCGAGGGCACACAGGGGGCGCTCG
 ATGTTCTGACGCCCCCGCAGCAGCCCCACTCC
 CCGGCTCACCCCTCGATTGGCTGGCCCGCCCGAG
 CTCTGTGCTGTGATTGGTCACAGCCCGTGTCCGTC
 CGCGGGCGCGGGGGCGGATCGAGGTGACCGCGCA
 GAGGCCAGCTCGGGCGGTGTCCCGCGCGCGG
 GACTCGGGCGGAGTTTCCCGAGGGCGAAGCG
 GGGCAGTGTGACCGCAGCGGTCTGGGAGGCGC
 CCGCGCGCGTCCGAGCAGCTCCCGTCTCTCCCA
 GCCGTACCCCGCGCGCTCCCGCGCCCTGGCC
 TCCCGCACTCCCGCACTCCTGTCCCGCCACC
 CGCCACCTCCACCTCGATCGGTGCGCGGGCTGC
 TGCGTGATGGGCTGCCGCGCGCCCTGCGG
 CTCGCGGGGGCGCTGCTCGCGCTGAGGTGCGT
 CGGTGCCCGGCCCCCGGCCCGCGCGCGCGCG
 GCTCCTGTTGACCGGTCGCGCGTCCGCTGCTGC
 AGCGCGGCTGAGGTAAGCGCGCGGGCTGGCG
 CGGTTGGCGCGCGCGGTCCCGGGGTTGGGGAGGG
 GGCCTTCCCGCGGGAGGAGCGCGCGGCCGG
 GGTCGCGCGGGGTCTGAGGGGA

CTCTTAGTTTTGGGTGCATTTGTCTGGCTTCCAAA
 CTAGATTGAAAGCTCTGAAAAAAAACCTATCTTGT
 GTTCTACTCTGTTGAGCTCATAGTAGGTACCAGGA
 AGTAGTAGGGTGTACTGCATTGATTTGGGACTACAC
 TGGGAGTTTTCTTCCCATCTCCCTTAGTTTTCTCT
 TTTTTCTTTCTTTCTTTCTTTCTTTTCTTTTTTTTT
 TTGAGATGTCTCTTGTCTCAGTCCCCCAGGCTGGA
 GTGCAGTGGTGCATCTTGGCTCACTGTAGCCCTCC
 ACCTCCAGGTTCAAGCAATTCTACTGCTTAGCCT
 CCGAGTAGTGTGGGATTACAAGCACCGCCACCAT
 TCCTGGCTAATTTTTTTTTTTGTAATTTTAGTTGAGA
 CAGGGTTTACCATGTTGGTGTGCTGATCCAGAG
 CTCCTGGGGCCTAGCATCCCCCTGCCTCAGCCT
 CCCAGAGTGTTAGGATTACAGGCATGAGCCACTGT
 ACCCGCCCTCTCTCCAGTTTCCAGTTGGAATCCAA
 GGGAASTAAGTTTAAGATAAAGTTACGATTTTGAAT
 CTTTGGATTGAGAAGAAATTTGTACCTTTAACACCT
 AGAGTTGAACGTTTATACCTGGAGAGCCTTAACATT
 AAGCCCTAGCCAGCCTCCAGCAAGTGGACATTGGT
 CAGGTTTGGCAGGATTCCCTCCGAAAGTGGACT
 GAGAGCCACACCCTGGCCTGTCCACCATACCCATCC
 CCTATCCTTAGTGAAGCAAACTCCTTTGTTCCCTT
 CTCCTTCTCCTAGTGACGAAATATTTGATCCTA
 AAGAATGAAAATAGTCTAGTCAACTCGTGCCTCAG
 GCCTCTTGACTTCAGGCGTCTGTATTAATCAAGT
 GACATCTTCCCGAGGCTCCCTGAATGTGGGAGATG
 AAAGAGACTAGTTCAACCCTGACTGAGGGGAAAG
 CCTTTGTGAAGGGTCAGGAG

Left: CpG sites at 1/10 nucleotides, constituting a CpG island. The sample is of a gene-promoter, the highlighted ATG constitutes the start codon.

Right: CpG sites present at every 1/100 nucleotides, constituting a more normal example of the genome, or a region of the genome that is commonly methylated.

How to define a CpG island

The human genome has a 42% GC content. Thus, the expected frequency of a CpG $0.21 \cdot 0.21 = 4.41\%$. However, the actual frequency is 1%.

The **percent combined C + G content** ($\%C + G$) is defined “exactly how you would expect.”

If dinucleotides were formed by randomly choosing two nucleotides, then the expected number of CpG's would be

$$\frac{(\# \text{ C's}) \cdot (\# \text{ G's})}{\text{length of sequence}}$$

The **observed over expected CpG ratio** (O/E CpG) is: $\frac{\text{observed } \# \text{ CpG's}}{\text{expected } \# \text{ CpG's}}$

Definition (Gardiner-Garden, Frommer, 1987)

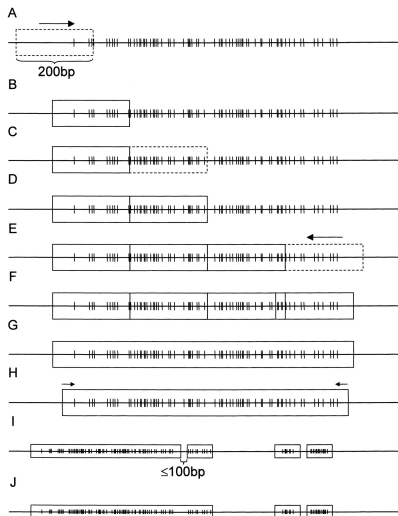
A subsequence in a vertebrate genome is **CpG island** if:

1. it has length at least 200 bp;
2. $\%C + G \geq 50\%$;
3. $\text{O/E CpG} \geq 0.6$;

There is no universal standard for these values. Another paper (Takai & Jones) used 500 bp, $\%C + G \geq 55\%$, and $\text{O/E CpG} \geq 0.65$.

Finding CpG islands

One method for inferring CpG islands is purely algorithmic: using a **sliding window**.



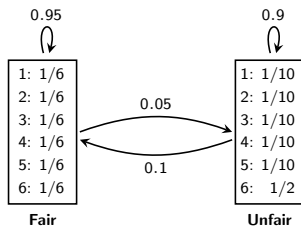
The remainder of this lecture will focus on an alternative approach: **hidden Markov models**.

The occasionally dishonest casino

Suppose a casino hosts a simple game with two dice: one fair and one unfair.

- FAIR: $p(1) = p(2) = p(3) = p(4) = p(5) = p(6) = 1/6$.
- UNFAIR: $p(1) = p(2) = p(3) = p(4) = p(5) = 1/10, p(6) = 1/2$.

The casino switches between fair and unfair die according to the following probabilities:



You cannot tell which die the casino is using. This is a **hidden Markov model** (HMM).

Suppose that the outcome of the game is the following:

- WIN: roll 1, 2, 3, or 4.
- LOSE: roll 5 or 6.

Would you play this game?

The occasionally dishonest casino

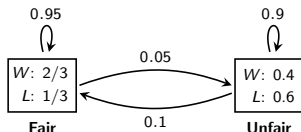
3 canonical questions

Given a sequence of roles by the casino:

123626362511512666122161452152616661611661261626643666262223451612426

one may ask:

1. **Evaluation:** How likely is this sequence given our model?
2. **Decoding:** When was the casino rolling the fair vs. the unfair die?
3. **Learning:** Can we deduce the probability parameters if we didn't know them? (e.g., "how loaded are the die?", and "how often does the casino switch?")



We'll analyze these questions but for simplicity, only record wins vs. losses:

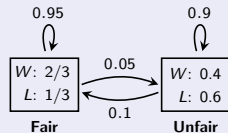
WWWLWLWLWLWWLWWLLLWWWLWWLWWLWLWLLLWLWLLWLLWLLWLWLLWLLWLLLWLWWWLWLWWWL

Two examples of Hidden Markov models

The parameters of an HMM can be encoded in a table.

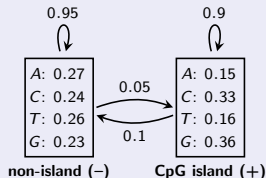
HMM for the occasionally dishonest casino

State	Transitions		Emissions		Initial distribution
	F	U	W	L	
F	.95	.05	2/3	1/3	.5
U	.1	.9	.4	.6	.5



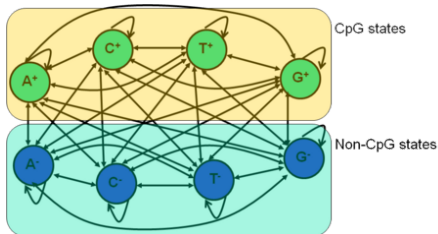
HMM for CpG islands (simple)

State	Transitions		Emissions				Init. dist.
	-	+	A	C	T	G	
-	.95	.05	.27	.24	.26	.23	.5
+	.1	.9	.15	.33	.16	.36	.5



A better hidden Markov model for CpG islands

A “better” HMM model should incorporate the fact that transmission probabilities within CpG islands are much different than the rest of the genome.



The following is from a sequence of annotated human DNA of length $\approx 60,000$.

	Transitions								Emissions				Init.
	A ₋	C ₋	T ₋	G ₋	A ₊	C ₊	T ₊	G ₊	A	C	T	G	
A ₋	.300	.205	.210	.285	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	1	0	0	0	.125
C ₋	.322	.298	.302	.078	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	0	1	0	0	.125
T ₋	.248	.246	.208	.298	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	0	0	1	0	.125
G ₋	.177	.239	.292	.292	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	$(1-q)/4$	0	0	0	1	.125
A ₊	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$.180	.274	.120	.426	1	0	0	0	.125
C ₊	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$.171	.368	.188	.274	0	1	0	0	.125
T ₊	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$.161	.339	.125	.375	0	0	1	0	.125
G ₊	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$	$(1-p)/4$.079	.355	.182	.384	0	0	0	1	.125

Three canonical HMM problems, formalized

Problem #1: Evaluation

Given an observed path $x = x_1x_2x_3 \cdots x_\ell$, what is its probability $P(x)$? That is, compute

$$P(x) = \sum_{\pi} P(x, \pi), \quad \text{where } P(x, \pi) = a_{0\pi_1} \prod_{i=1}^{\ell} e_{\pi_i}(x_i) a_{\pi_i, \pi_{i+1}}$$

and the sum is over all hidden sequences $\pi = \pi_1\pi_2 \cdots \pi_\ell$.

Problem #2: Decoding

Given an observed path $x = x_1x_2x_3 \cdots x_\ell$, what is the most likely hidden path $\pi = \pi_1\pi_2\pi_3 \cdots \pi_\ell$ to emit x ? That is, compute

$$\pi_{\max} = \arg \max_{\pi} P(\pi|x) = \arg \max_{\pi} P(x, \pi)$$

Problem #3: Learning

Given an observed sequence x (or set of sequences), what are the HMM parameters that make x mostly likely to occur?