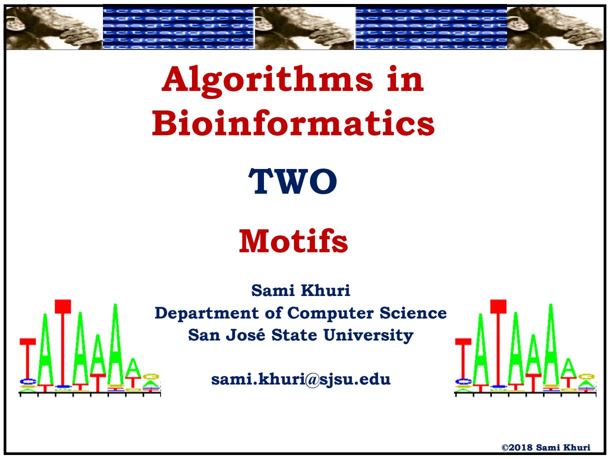

Algorithms in Bioinformatics

TWO

Motifs

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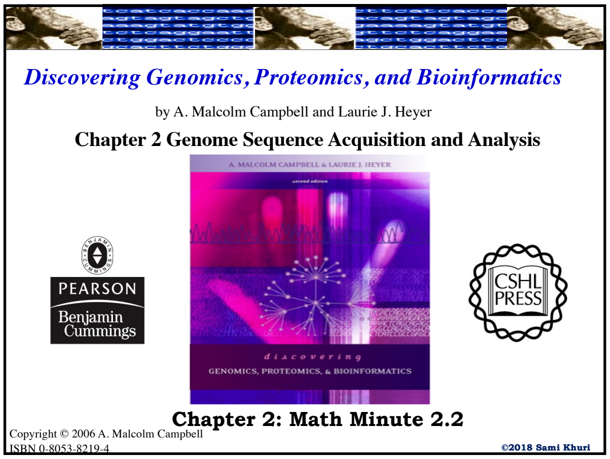

Discovering Genomics, Proteomics, and Bioinformatics

by A. Malcolm Campbell and Laurie J. Heyer

Chapter 2 Genome Sequence Acquisition and Analysis

A. MALCOLM CAMPBELL & LAURIE J. HEYER
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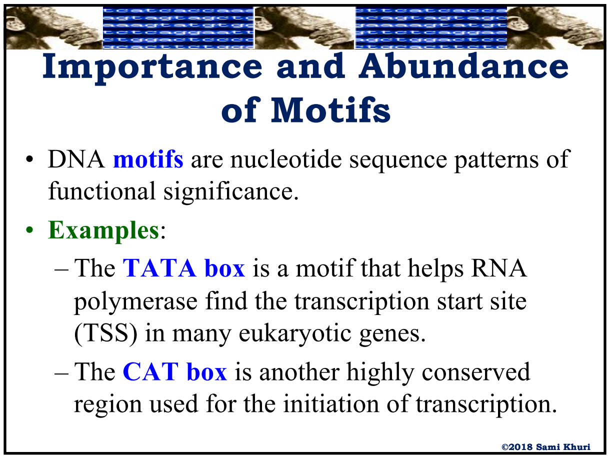
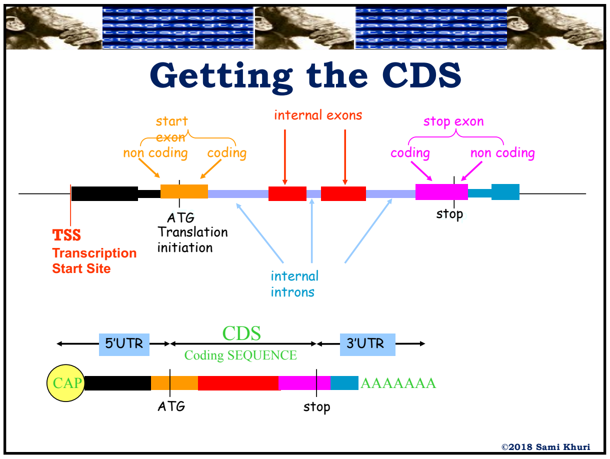
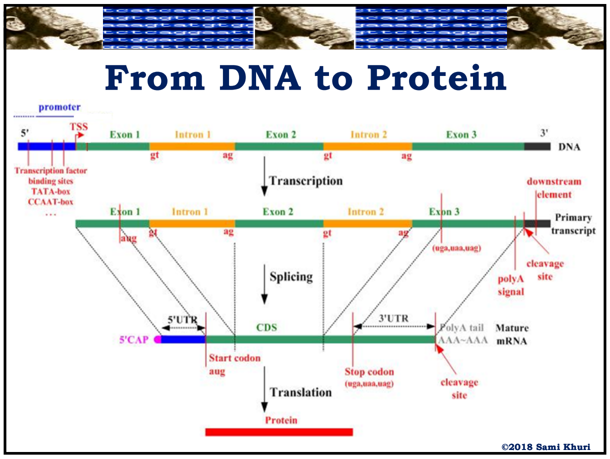


Importance and Abundance of Motifs

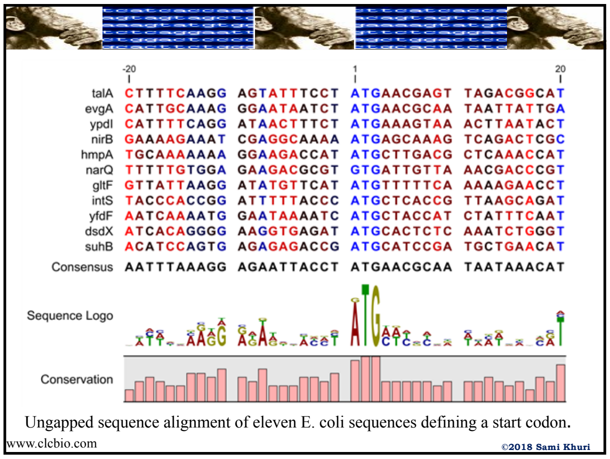
- DNA **motifs** are nucleotide sequence patterns of functional significance.
- **Examples**:
  - The **TATA box** is a motif that helps RNA polymerase find the transcription start site (TSS) in many eukaryotic genes.
  - The **CAT box** is another highly conserved region used for the initiation of transcription.



Getting the CDS



From DNA to Protein



	-20		1	20
talA	CTTTTCAAGG	AGTATTTCCT	ATGAACGAGT	TAGACGGCAT
evgA	CATTGCAAAG	GGAATAATCT	ATGAACGCAA	TAATTATTGA
ypdI	CATTTTCAGG	ATAACTTTCT	ATGAAAGTAA	ACTTAATACT
nirB	GAAAAGAAAT	CGAGGCAAAA	ATGAGCAAAG	TCAGACTCGC
hmpA	TGCAAAAAAA	GGAAGACCAT	ATGCTTGACG	CTCAAACCAT
narQ	TTTTTGTGGA	GAAGACGCGT	GTGATTGTTA	AACGACCCGT
gltF	GTTATTAAGG	ATATGTTCAT	ATGTTTTTCA	AAAAGAACCT
intS	TACCCACCGG	ATTTTTACCC	ATGCTCACCG	TTAAGCAGAT
yfdF	AATCAAAATG	GAATAAAATC	ATGCTACCAT	CTATTTCAAT
dsdX	ATCACAGGGG	AAGGTGAGAT	ATGCACTCTC	AAATCTGGGT
suhB	ACATCCAGTG	AGAGAGACCG	ATGCATCCGA	TGCTGAACAT
Consensus	AATTTAAAGG	AGAATTACCT	ATGAACGCAA	TAATAAACAT

Ungapped sequence alignment of eleven E. coli sequences defining a start codon.

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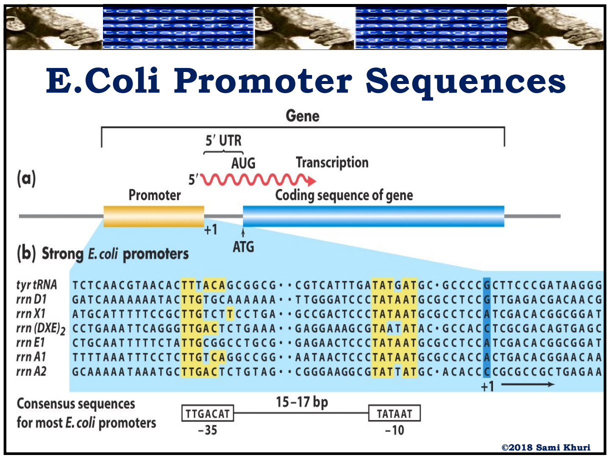
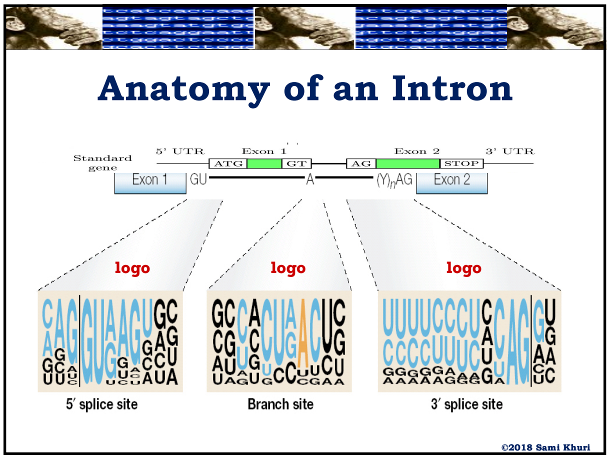


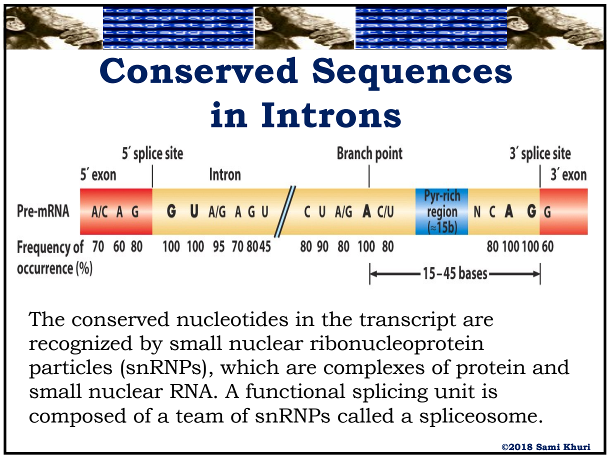
E.Coli Promoter Sequences

tyr tRNA	TCTCAACGTAACACTTTACAGCGGCG••CGTCATTTGATATGATGC•GCCCCGCTTCCCGATAAGGG
rrn D1	GATCAAAAAAATACTTGTGCAAAAAA••TTGGGATCCCTATAATGCGCCTCCGTTGAGACGACAACG
rrn X1	ATGCATTTTTCCGCTTGTCTTCCTGA••GCCGACTCCCTATAATGCGCCTCCATCGACACGGCGGAT
rrn (DXE)$_2$	CCTGAAATTCAGGGTTGACTCTGAAA••GAGGAAAGCGTAATATAC•GCCACCTCGCGACAGTGAGC
rrn E1	CTGCAATTTTTCTATTGCGGCCTGCG••GAGAACTCCCTATAATGCGCCTCCATCGACACGGCGGAT
rrn A1	TTTTAAATTTCCTCTTGTCAGGCCGG••AATAACTCCCTATAATGCGCCACCACTGACACGGAACAA
rrn A2	GCAAAAATAAATGCTTGACTCTGTAG••CGGGAAGGCGTATTATGC•ACACCCCGCGCCGCTGAGAA

Consensus sequences for most *E. coli* promoters: TTGACAT (−35) — 15–17 bp — TATAAT (−10)

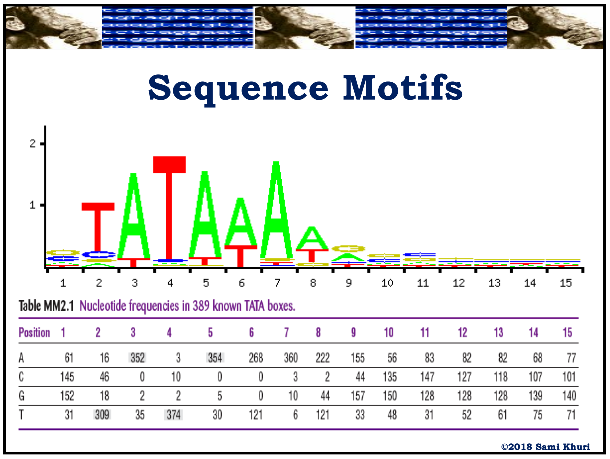


Anatomy of an Intron



Conserved Sequences in Introns

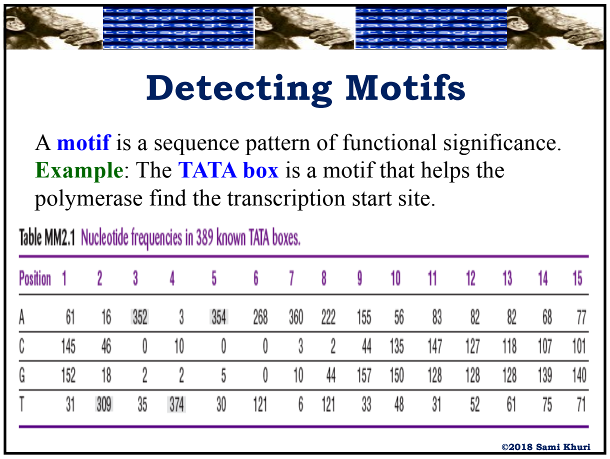
The conserved nucleotides in the transcript are recognized by small nuclear ribonucleoprotein particles (snRNPs), which are complexes of protein and small nuclear RNA. A functional splicing unit is composed of a team of snRNPs called a spliceosome.



Sequence Motifs

Table MM2.1 Nucleotide frequencies in 389 known TATA boxes.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	61	16	352	3	354	268	360	222	155	56	83	82	82	68	77
C	145	46	0	10	0	0	3	2	44	135	147	127	118	107	101
G	152	18	2	2	5	0	10	44	157	150	128	128	128	139	140
T	31	309	35	374	30	121	6	121	33	48	31	52	61	75	71

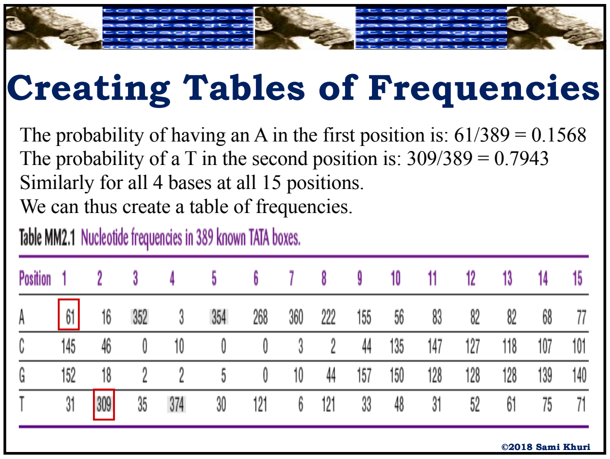


Detecting Motifs

A **motif** is a sequence pattern of functional significance.
Example: The **TATA box** is a motif that helps the polymerase find the transcription start site.

Table MM2.1 Nucleotide frequencies in 389 known TATA boxes.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	61	16	352	3	354	268	360	222	155	56	83	82	82	68	77
C	145	46	0	10	0	0	3	2	44	135	147	127	118	107	101
G	152	18	2	2	5	0	10	44	157	150	128	128	128	139	140
T	31	309	35	374	30	121	6	121	33	48	31	52	61	75	71

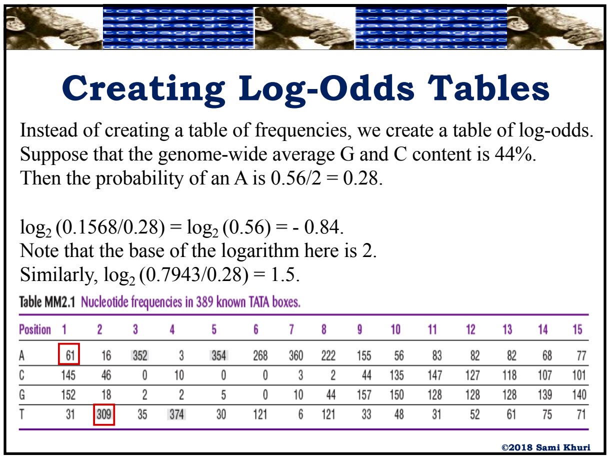


Creating Tables of Frequencies

The probability of having an A in the first position is: 61/389 = 0.1568
The probability of a T in the second position is: 309/389 = 0.7943
Similarly for all 4 bases at all 15 positions.
We can thus create a table of frequencies.

Table MM2.1 Nucleotide frequencies in 389 known TATA boxes.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	61	16	352	3	354	268	360	222	155	56	83	82	82	68	77
C	145	46	0	10	0	0	3	2	44	135	147	127	118	107	101
G	152	18	2	2	5	0	10	44	157	150	128	128	128	139	140
T	31	309	35	374	30	121	6	121	33	48	31	52	61	75	71



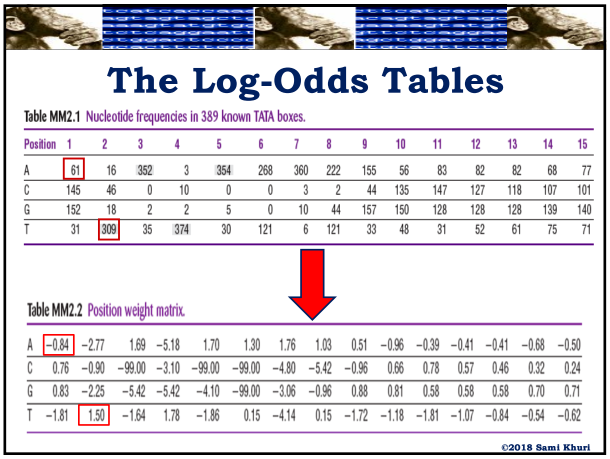
Creating Log-Odds Tables

Instead of creating a table of frequencies, we create a table of log-odds.
Suppose that the genome-wide average G and C content is 44%.
Then the probability of an A is 0.56/2 = 0.28.

$\log_2(0.1568/0.28) = \log_2(0.56) = -0.84$.
Note that the base of the logarithm here is 2.
Similarly, $\log_2(0.7943/0.28) = 1.5$.

Table MM2.1 Nucleotide frequencies in 389 known TATA boxes.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	61	16	352	3	354	268	360	222	155	56	83	82	82	68	77
C	145	46	0	10	0	0	3	2	44	135	147	127	118	107	101
G	152	18	2	2	5	0	10	44	157	150	128	128	128	139	140
T	31	309	35	374	30	121	6	121	33	48	31	52	61	75	71



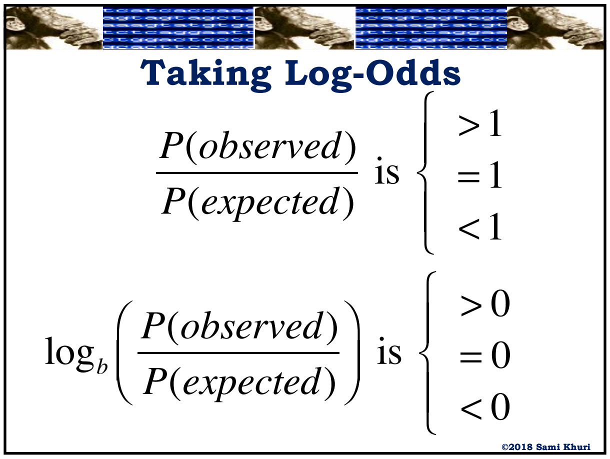
The Log-Odds Tables

Table MM2.1 Nucleotide frequencies in 389 known TATA boxes.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	61	16	352	3	354	268	360	222	155	56	83	82	82	68	77
C	145	46	0	10	0	0	3	2	44	135	147	127	118	107	101
G	152	18	2	2	5	0	10	44	157	150	128	128	128	139	140
T	31	309	35	374	30	121	6	121	33	48	31	52	61	75	71

Table MM2.2 Position weight matrix.

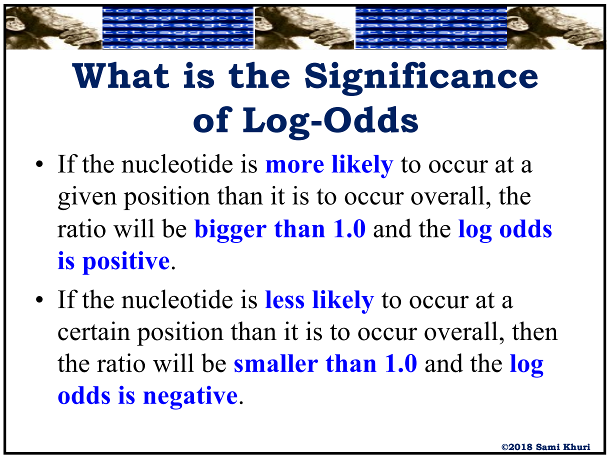
A	−0.84	−2.77	1.69	−5.18	1.70	1.30	1.76	1.03	0.51	−0.96	−0.39	−0.41	−0.41	−0.68	−0.50
C	0.76	−0.90	−99.00	−3.10	−99.00	−99.00	−4.80	−5.42	−0.96	0.66	0.78	0.57	0.46	0.32	0.24
G	0.83	−2.25	−5.42	−5.42	−4.10	−99.00	−3.06	−0.96	0.88	0.81	0.58	0.58	0.58	0.70	0.71
T	−1.81	1.50	−1.64	1.78	−1.86	0.15	−4.14	0.15	−1.72	−1.18	−1.81	−1.07	−0.84	−0.54	−0.62



Taking Log-Odds

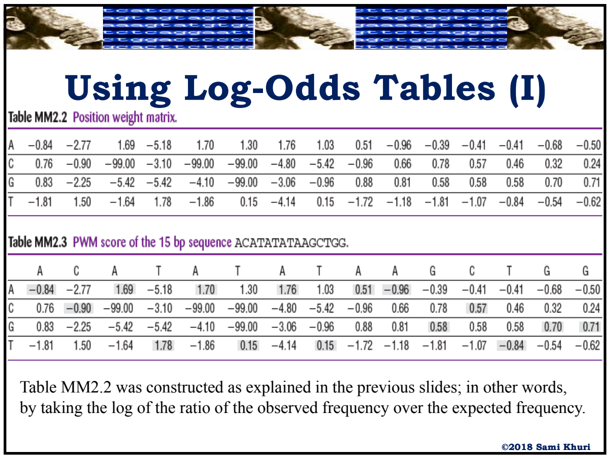
$$\frac{P(observed)}{P(expected)} \text{ is } \begin{cases} >1 \\ =1 \\ <1 \end{cases}$$

$$\log_b\left(\frac{P(observed)}{P(expected)}\right) \text{ is } \begin{cases} >0 \\ =0 \\ <0 \end{cases}$$



What is the Significance of Log-Odds

- If the nucleotide is **more likely** to occur at a given position than it is to occur overall, the ratio will be **bigger than 1.0** and the **log odds is positive**.
- If the nucleotide is **less likely** to occur at a certain position than it is to occur overall, then the ratio will be **smaller than 1.0** and the **log odds is negative**.



Using Log-Odds Tables (I)

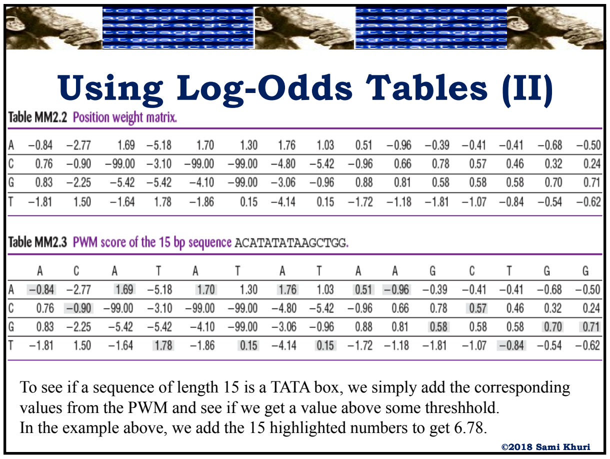
Table MM2.2 Position weight matrix.

A	−0.84	−2.77	1.69	−5.18	1.70	1.30	1.76	1.03	0.51	−0.96	−0.39	−0.41	−0.41	−0.68	−0.50
C	0.76	−0.90	−99.00	−3.10	−99.00	−99.00	−4.80	−5.42	−0.96	0.66	0.78	0.57	0.46	0.32	0.24
G	0.83	−2.25	−5.42	−5.42	−4.10	−99.00	−3.06	−0.96	0.88	0.81	0.58	0.58	0.58	0.70	0.71
T	−1.81	1.50	−1.64	1.78	−1.86	0.15	−4.14	0.15	−1.72	−1.18	−1.81	−1.07	−0.84	−0.54	−0.62

Table MM2.3 PWM score of the 15 bp sequence ACATATATAAGCTGG.

	A	C	A	T	A	T	A	T	A	A	G	C	T	G	G
A	−0.84	−2.77	1.69	−5.18	1.70	1.30	1.76	1.03	0.51	−0.96	−0.39	−0.41	−0.41	−0.68	−0.50
C	0.76	−0.90	−99.00	−3.10	−99.00	−99.00	−4.80	−5.42	−0.96	0.66	0.78	0.57	0.46	0.32	0.24
G	0.83	−2.25	−5.42	−5.42	−4.10	−99.00	−3.06	−0.96	0.88	0.81	0.58	0.58	0.58	0.70	0.71
T	−1.81	1.50	−1.64	1.78	−1.86	0.15	−4.14	0.15	−1.72	−1.18	−1.81	−1.07	−0.84	−0.54	−0.62

Table MM2.2 was constructed as explained in the previous slides; in other words, by taking the log of the ratio of the observed frequency over the expected frequency.



Using Log-Odds Tables (II)

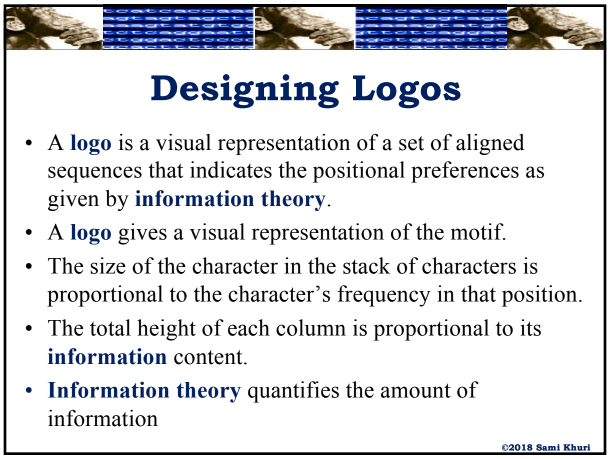
Table MM2.2 Position weight matrix.

A	−0.84	−2.77	1.69	−5.18	1.70	1.30	1.76	1.03	0.51	−0.96	−0.39	−0.41	−0.41	−0.68	−0.50
C	0.76	−0.90	−99.00	−3.10	−99.00	−99.00	−4.80	−5.42	−0.96	0.66	0.78	0.57	0.46	0.32	0.24
G	0.83	−2.25	−5.42	−5.42	−4.10	−99.00	−3.06	−0.96	0.88	0.81	0.58	0.58	0.58	0.70	0.71
T	−1.81	1.50	−1.64	1.78	−1.86	0.15	−4.14	0.15	−1.72	−1.18	−1.81	−1.07	−0.84	−0.54	−0.62

Table MM2.3 PWM score of the 15 bp sequence ACATATATAAGCTGG.

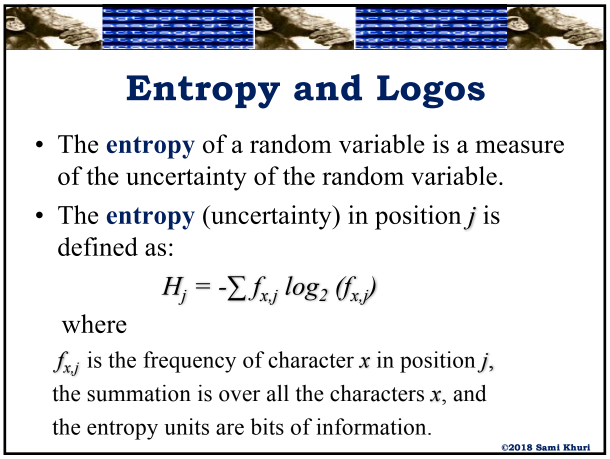
	A	C	A	T	A	T	A	T	A	A	G	C	T	G	G
A	−0.84	−2.77	1.69	−5.18	1.70	1.30	1.76	1.03	0.51	−0.96	−0.39	−0.41	−0.41	−0.68	−0.50
C	0.76	−0.90	−99.00	−3.10	−99.00	−99.00	−4.80	−5.42	−0.96	0.66	0.78	0.57	0.46	0.32	0.24
G	0.83	−2.25	−5.42	−5.42	−4.10	−99.00	−3.06	−0.96	0.88	0.81	0.58	0.58	0.58	0.70	0.71
T	−1.81	1.50	−1.64	1.78	−1.86	0.15	−4.14	0.15	−1.72	−1.18	−1.81	−1.07	−0.84	−0.54	−0.62

To see if a sequence of length 15 is a TATA box, we simply add the corresponding values from the PWM and see if we get a value above some threshhold.
In the example above, we add the 15 highlighted numbers to get 6.78.



Designing Logos

- A **logo** is a visual representation of a set of aligned sequences that indicates the positional preferences as given by **information theory**.
- A **logo** gives a visual representation of the motif.
- The size of the character in the stack of characters is proportional to the character’s frequency in that position.
- The total height of each column is proportional to its **information** content.
- **Information theory** quantifies the amount of information



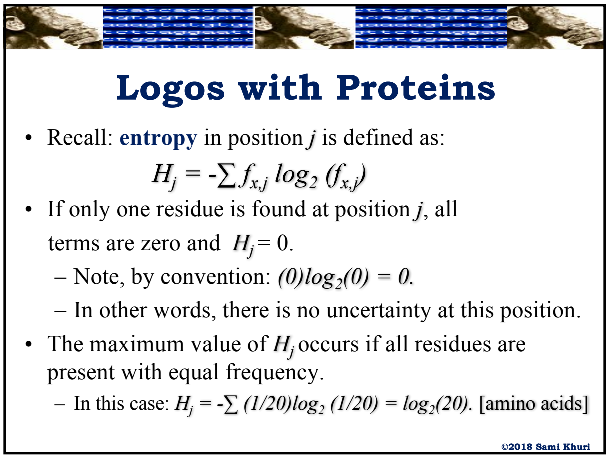
Entropy and Logos

- The **entropy** of a random variable is a measure of the uncertainty of the random variable.
- The **entropy** (uncertainty) in position *j* is defined as:

$$H_j = -\sum f_{x,j} \log_2 (f_{x,j})$$

where

$f_{x,j}$ is the frequency of character x in position j,
the summation is over all the characters x, and
the entropy units are bits of information.

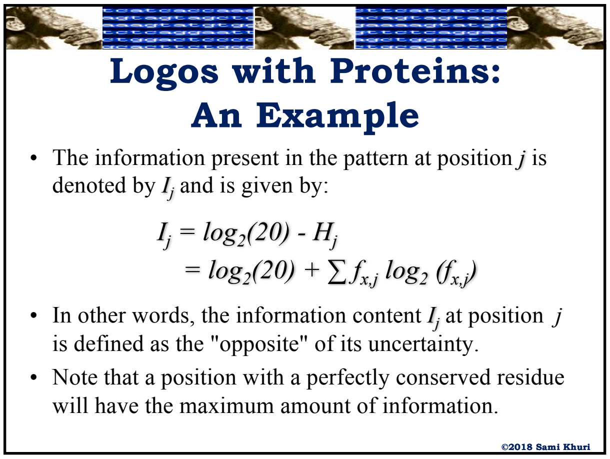


Logos with Proteins

- Recall: **entropy** in position *j* is defined as:

$$H_j = -\sum f_{x,j} \log_2 (f_{x,j})$$

- If only one residue is found at position *j*, all terms are zero and $H_j = 0$.
  - Note, by convention: $(0)\log_2(0) = 0$.
  - In other words, there is no uncertainty at this position.
- The maximum value of H_j occurs if all residues are present with equal frequency.
  - In this case: $H_j = -\sum (1/20)\log_2 (1/20) = \log_2(20)$. [amino acids]

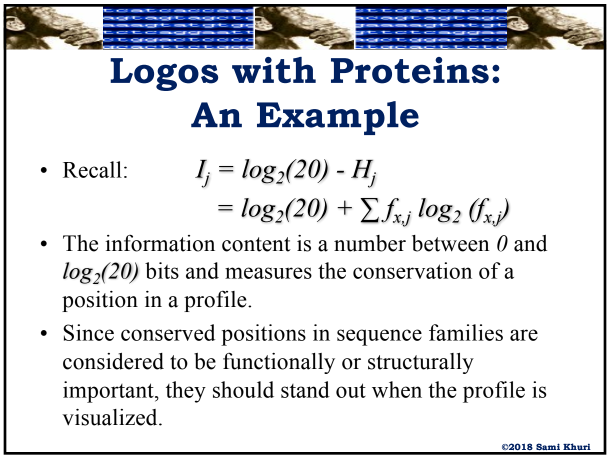


Logos with Proteins: An Example

- The information present in the pattern at position *j* is denoted by I_j and is given by:

$$I_j = \log_2(20) - H_j$$
$$= \log_2(20) + \sum f_{x,j} \log_2 (f_{x,j})$$

- In other words, the information content I_j at position *j* is defined as the "opposite" of its uncertainty.
- Note that a position with a perfectly conserved residue will have the maximum amount of information.

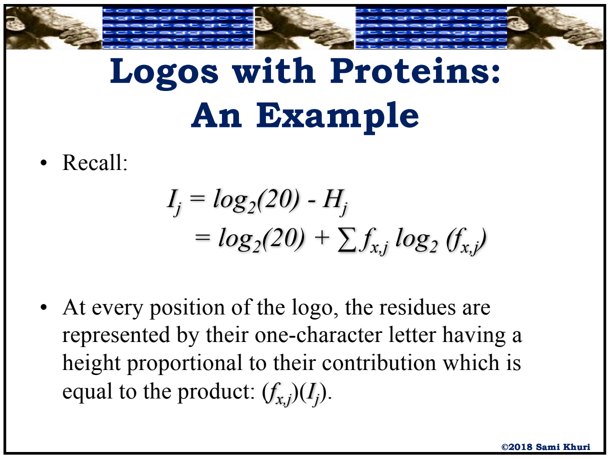


Logos with Proteins: An Example

- Recall: $I_j = \log_2(20) - H_j$

$$= \log_2(20) + \sum f_{x,j} \log_2 (f_{x,j})$$

- The information content is a number between *0* and $\log_2(20)$ bits and measures the conservation of a position in a profile.
- Since conserved positions in sequence families are considered to be functionally or structurally important, they should stand out when the profile is visualized.

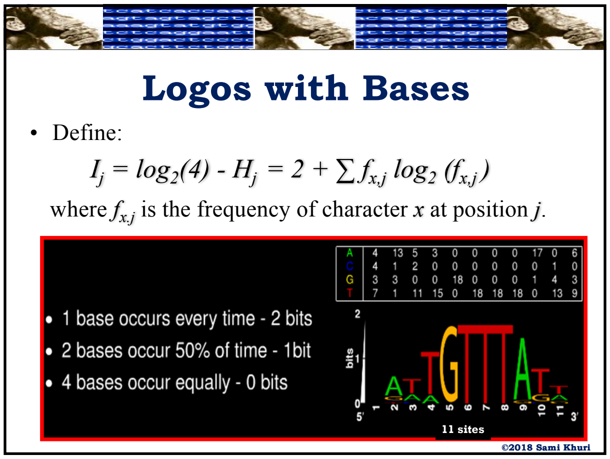


Logos with Proteins: An Example

- Recall:

$$I_j = \log_2(20) - H_j$$
$$= \log_2(20) + \sum f_{x,j} \log_2 (f_{x,j})$$

- At every position of the logo, the residues are represented by their one-character letter having a height proportional to their contribution which is equal to the product: $(f_{x,j})(I_j)$.



Logos with Bases

- Define:

$$I_j = log_2(4) - H_j = 2 + \sum f_{x,j} \, log_2 \, (f_{x,j})$$

where $f_{x,j}$ is the frequency of character x at position j.

A	4	13	5	3	0	0	0	0	17	0	6
C	4	1	2	0	0	0	0	0	0	1	0
G	3	3	0	0	18	0	0	0	1	4	3
T	7	1	11	15	0	18	18	18	0	13	9

- 1 base occurs every time - 2 bits
- 2 bases occur 50% of time - 1bit
- 4 bases occur equally - 0 bits

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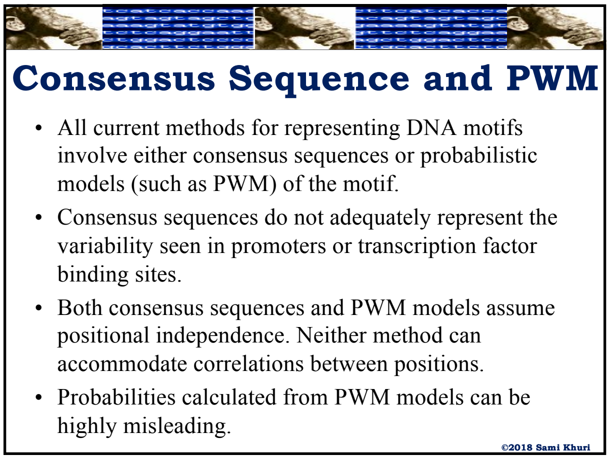

Consensus Sequence and PWM

- All current methods for representing DNA motifs involve either consensus sequences or probabilistic models (such as PWM) of the motif.
- Consensus sequences do not adequately represent the variability seen in promoters or transcription factor binding sites.
- Both consensus sequences and PWM models assume positional independence. Neither method can accommodate correlations between positions.
- Probabilities calculated from PWM models can be highly misleading.

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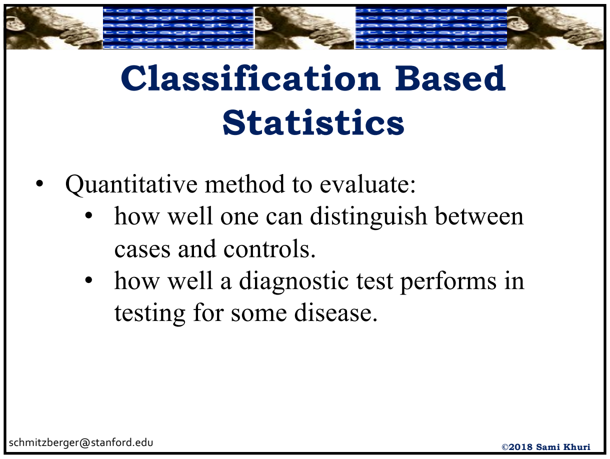

Classification Based Statistics

- Quantitative method to evaluate:
  - how well one can distinguish between cases and controls.
  - how well a diagnostic test performs in testing for some disease.

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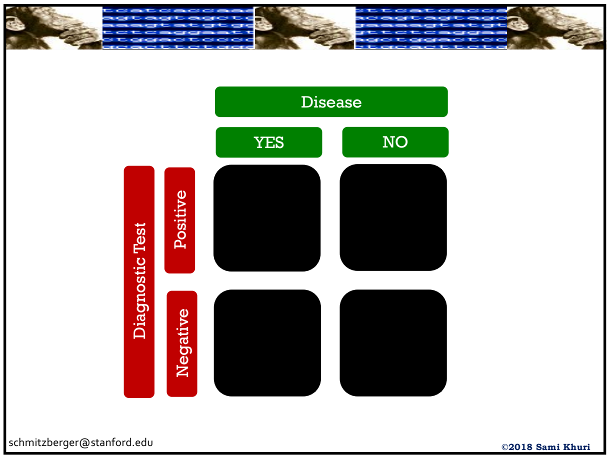

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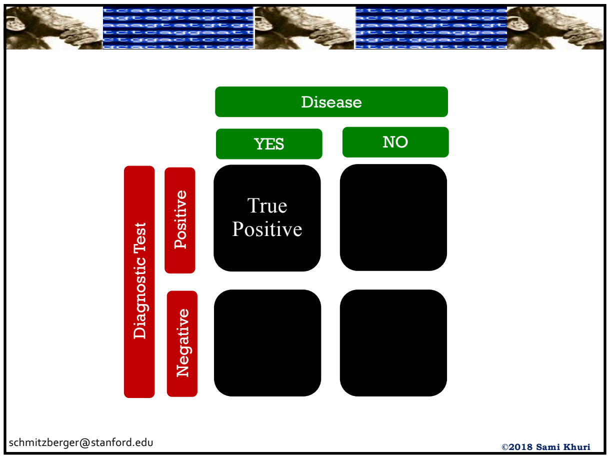

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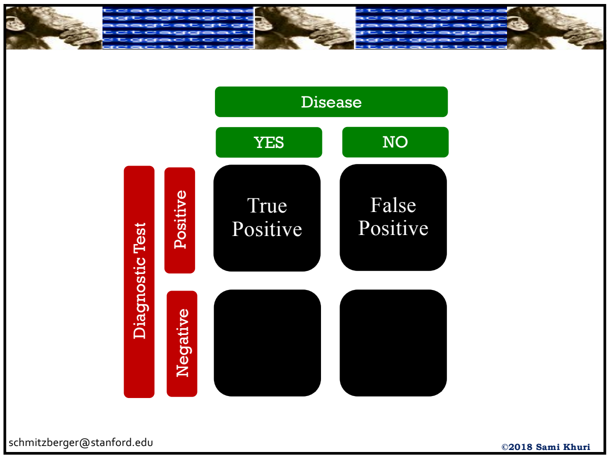

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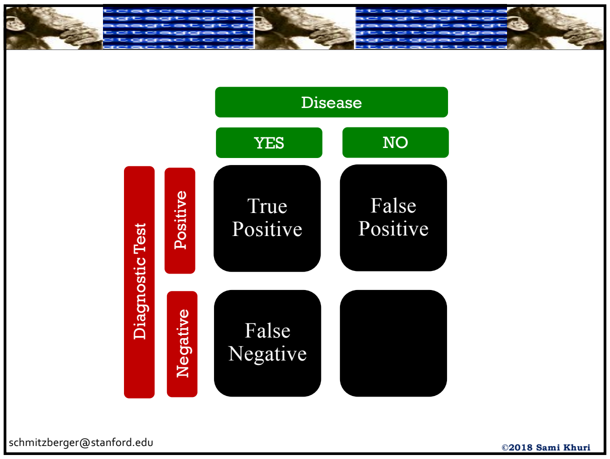

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	

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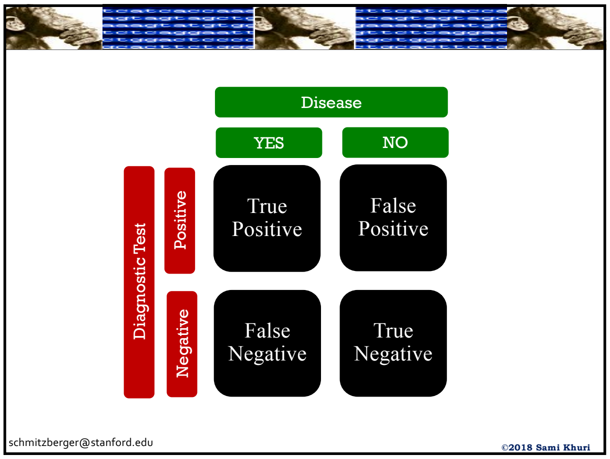

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

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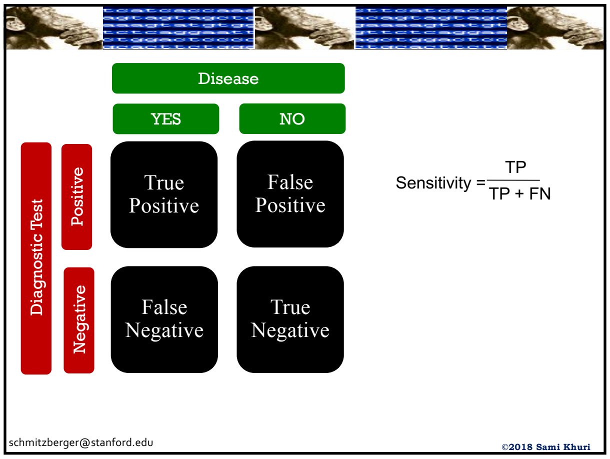

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

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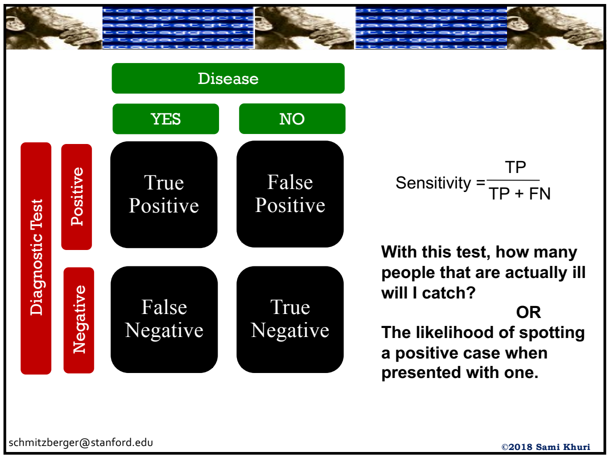

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

With this test, how many people that are actually ill will I catch?

OR

The likelihood of spotting a positive case when presented with one.

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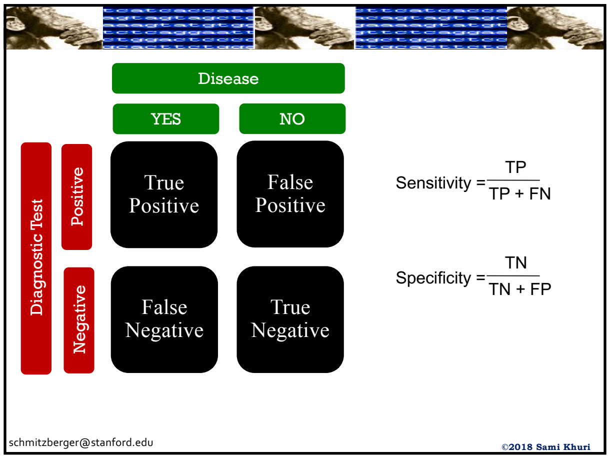

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Specificity} = \frac{TN}{TN + FP}$$

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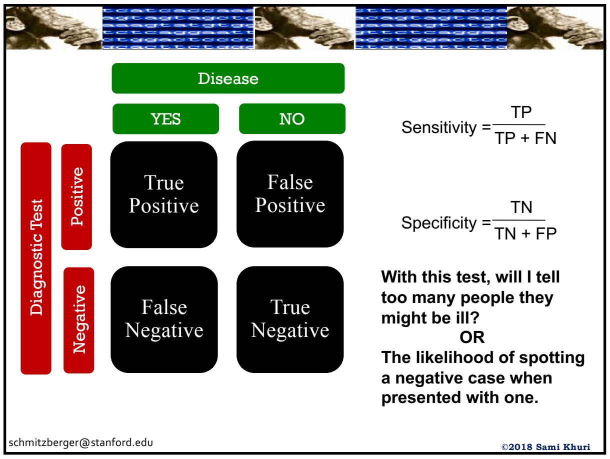

		Disease	
		YES	NO
Diagnostic Test	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Specificity} = \frac{TN}{TN + FP}$$

With this test, will I tell too many people they might be ill?

OR

The likelihood of spotting a negative case when presented with one.

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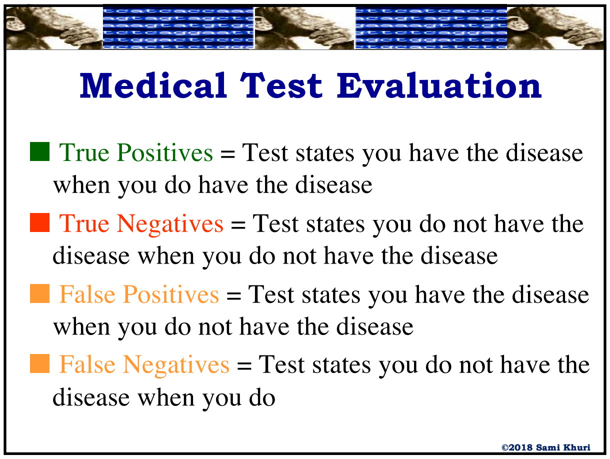

Medical Test Evaluation

- True Positives = Test states you have the disease when you do have the disease
- True Negatives = Test states you do not have the disease when you do not have the disease
- False Positives = Test states you have the disease when you do not have the disease
- False Negatives = Test states you do not have the disease when you do

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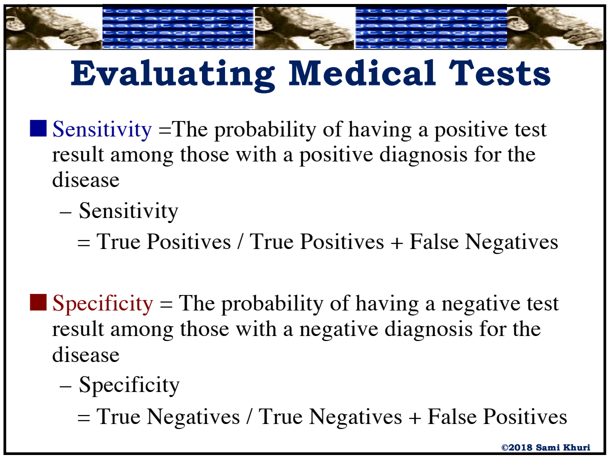

Evaluating Medical Tests

- Sensitivity =The probability of having a positive test result among those with a positive diagnosis for the disease
  - Sensitivity
    = True Positives / True Positives + False Negatives
- Specificity = The probability of having a negative test result among those with a negative diagnosis for the disease
  - Specificity
    = True Negatives / True Negatives + False Positives

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