

# DNA Structure and Properties

Biochemistry Boot Camp 2017  
Session #6  
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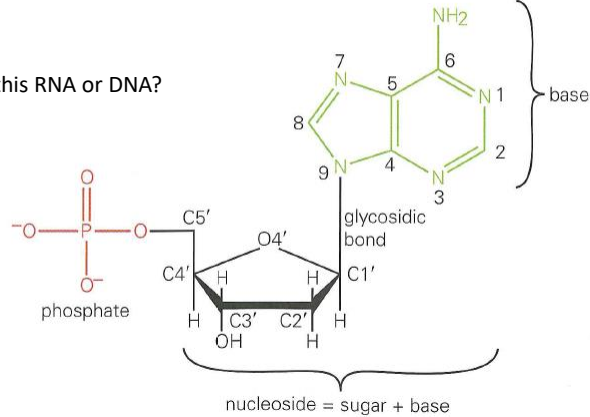
## DNA

- DNA- a polymer of deoxyribonucleotides
- Found in chromosomes, mitochondria and chloroplasts
- Carries the genetic information

# Nucleic Acid Structure

nucleotide = nucleoside + phosphate

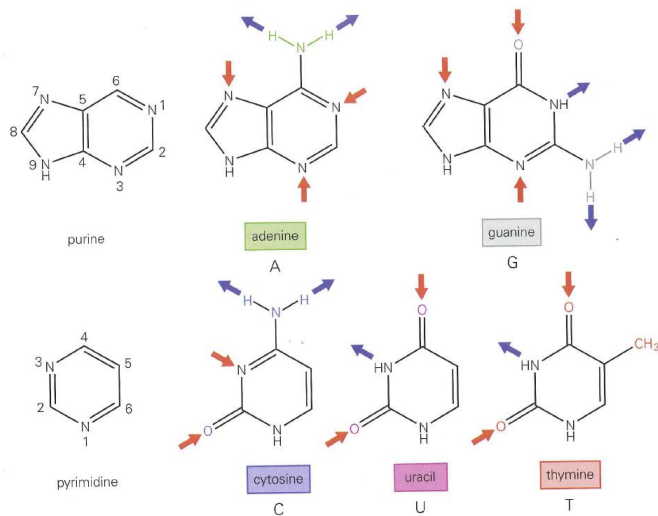
**Question:** Is this RNA or DNA?



*Molecules of Life*, pp. 15

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# Nucleic Acid Bases



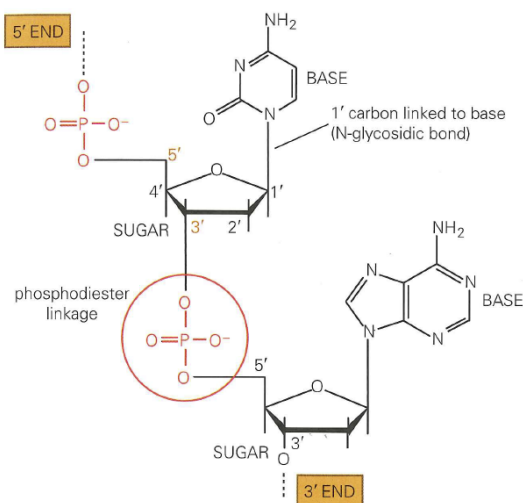
*Molecules of Life*, pp. 20

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# Nomenclature

	Base	Nucleoside	Nucleotide	Nucleic Acid
<b>Purine</b>	Adenine	Adenosine	Adenylate	RNA
		Deoxyadenosine	Deoxyadenylate	DNA
	Guanine	Guanosine	Guanylate	RNA
		Deoxyguanosine	Deoxyguanylate	DNA
<b>Pyrimidines</b>	Cytosine	Cytidine	Cytidylate	RNA
		Deoxycytidine	Deoxycytidylate	DNA
	Thymine	Thymidine	Thymidylate	
		Deoxythymidine	Deoxythymidylate	DNA
	Uracil	Uridine	Uridylate	RNA

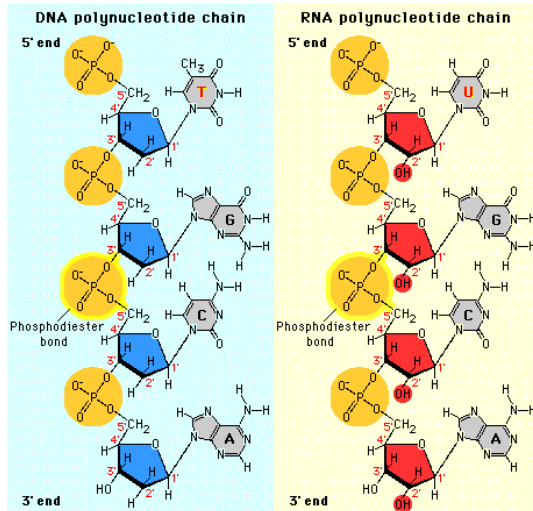
## Nucleic Acids Are Also Polymers



**DNA & RNA Polymerase:** Build up DNA and RNA from nucleoside triphosphates (5' → 3' synthesis)

**Convention:** RNA/DNA typically is read from 5' to 3' direction (e.g. 5'-ATTGCAAC-3')

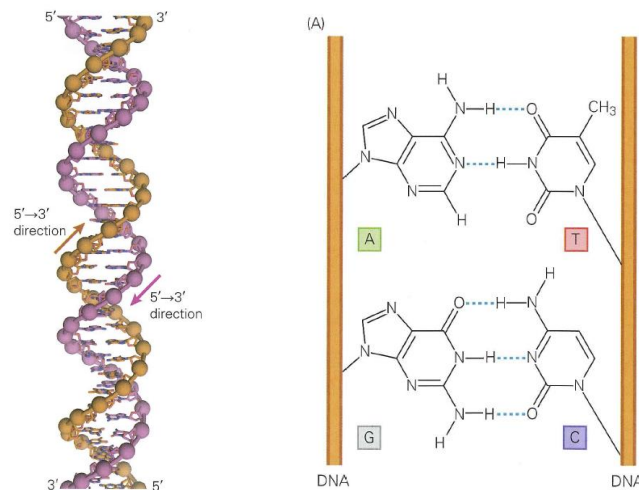
## DNA vs RNA



- DNA less reactive
- RNA is easily attacked by enzymes

Science, [www.phschool.com](http://www.phschool.com) (Accessed on June 02, 2014)

## Watson-Crick Base Pairing in an (Antiparallel) Double Helix



*Molecules of Life*, pp. 23

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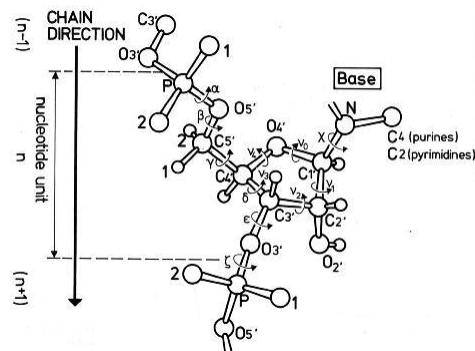
## Reverse Complement

- Watson-Crick base pairing
  - A pairs with T (or U in RNA)
  - G pairs with C
- RNA can “hybridize” with DNA, forming mixed strands
- **Example:** What’s the reverse complement to AUCCGCCTT?

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## Structure in DNA

- Bases are planar
- Torsion angles are shown
  - Much more complex than proteins

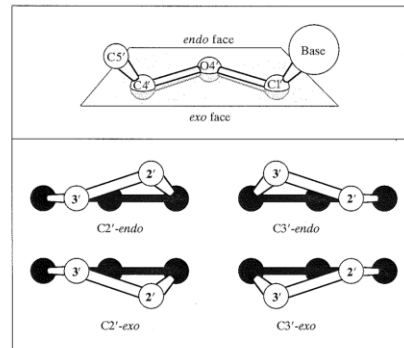


Saenger, W. *Principles of Nucleic Acid Structure*.

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## Simplification: Sugar Pucker

- $\nu$  angles are related, so sugar ring can be simplified
- Think “chair” and “boat” forms of cyclohexane



**Figure 1.38** Sugar conformations of nucleic acids. The pucker of the sugar ring in RNA and DNA is defined relative to the plane formed by the C1'-carbon, C4'-carbon, and O4'-oxygen of the five-member ring. The *endo* face lies above the plane, toward the nucleobase, while the *exo* face lies below the plane.

van Holde, et al. *Principles of Physical Biochemistry*.

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## Nucleic Acid Primary Structure

- **Just like proteins:** the sequence of bases

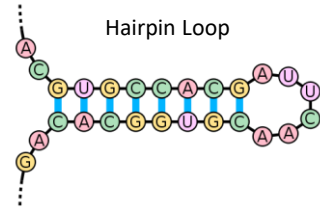
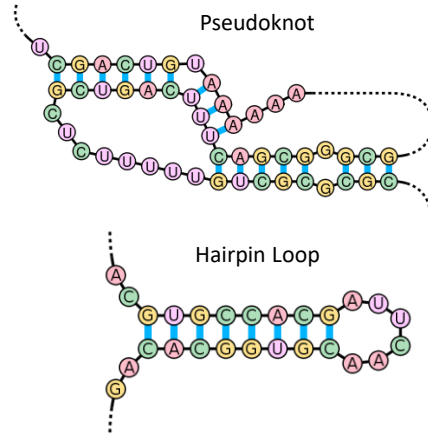
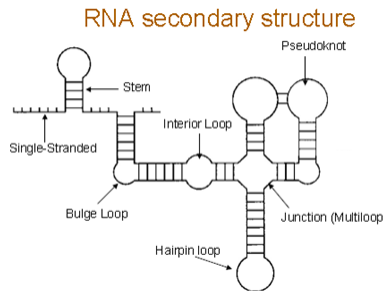
5'-dAdGdTdTdCdAdCdCdC-3' (DNA)

AGTTCACCC

5'-AGUUCACCC-3' (RNA)

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## Secondary Structure

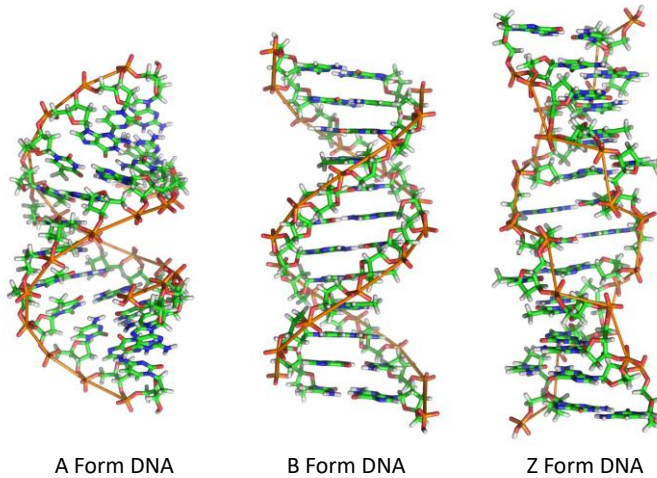


- Base pairing motifs

Source: Wikipedia, "RNA Secondary Structure," "Nucleic Acid Secondary Structure"

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## Tertiary Structure



Source: Steven Carr, [www.mun.ca](http://www.mun.ca)

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## Tertiary Structure

Structure Type	Average Torsion Angles for Nucleic Acid Helices (in °)						
	Alpha	Beta	Gamma	Delta	Epsilon	Zeta	Chi
A-DNA (fibres)	-50	172	41	79	-146	-78	-154
GGCCGGCC	-75	185	56	91	-166	-75	-149
B-DNA (fibres)	-41	136	38	139	-133	-157	-102
CGCGAATTCGCG	-63	171	54	123	-169	-108	-117
Z-DNA (C residues)	-137	-139	56	138	-95	80	-159
Z-DNA (G residues)	47	179	-169	99	-104	-69	68
DNA-RNA decamer	-69	175	55	82	-151	-75	-162
A-RNA	-68	178	54	82	-153	-71	-158

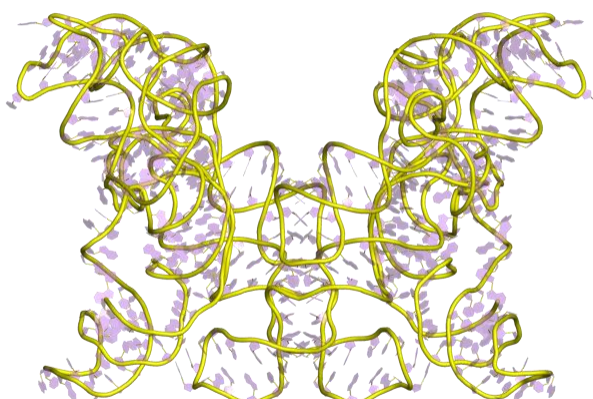
Blackburn and Galt. *Nucleic acids in chemistry and biology*.

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## Tertiary & Quaternary Structure

**Ribozyme:** An RNA capable of catalyzing a chemical reaction

The ribosome contains a significant amount of RNA as well as proteins



**Macromolecules can perform incredibly diverse structures!**  
(And we haven't even mentioned lipids and sugars.)

Wikipedia, "Group I Catalytic Intron." Accessed 8/23/2012.

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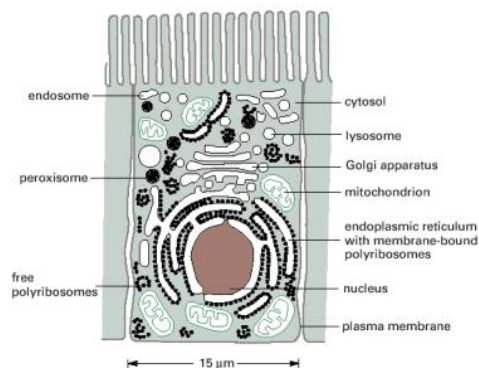
## Think and Discuss

What are the major differences between DNA and protein structures? What are the similarities?

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## Review of Intro Biology

- Parts of a eukaryotic animal cell
- Has a nucleus where DNA is stored
- Membrane-bound organelles

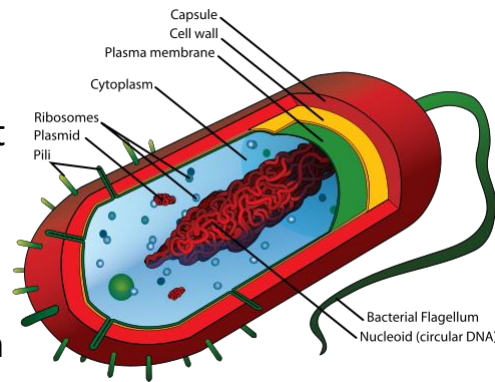


Alberts, et al. *Molecular Biology of the Cell*, 4<sup>th</sup> Edition.

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## Review of Intro Biology

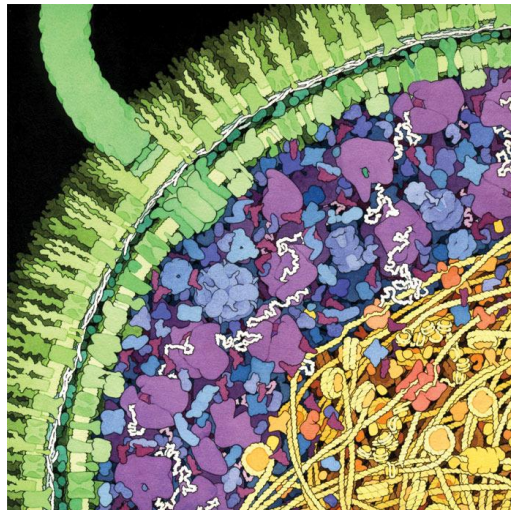
- Parts of a prokaryotic bacterial cell
- No nucleus: DNA is not linear but circular (no ends)
- No organelles, but ribosomes, etc. exist in the cytoplasm



Source: Wikipedia, "Bacterial Cell Structure."

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## It's Crowded in There!

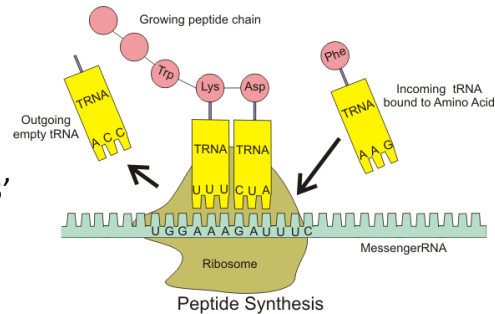


Source: Goodsell, D. <http://mgl.scripps.edu/people/goodsell/illustration/public/>

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# Central Dogma

- DNA → mRNA  
“Transcription”
  - Synthesized RNA Polymerase
  - RNA formed from 5' to 3'
- mRNA → Protein  
“Translation”
  - Synthesized by ribosome
  - New proteins formed from NT to CT



**Trick:** Reading the DNA in the “standard way”, one can easily identify the codons for peptide synthesis.

Source: Wikipedia, “Ribosome”

# Genetic Code

nonpolar polar basic acidic (stop codon)

Standard genetic code						
1st base	2nd base				3rd base	
	U	C	A	G		
U	UUU (Phe/F) Phenylalanine	UCU (Ser/S) Serine	UAU (Tyr/Y) Tyrosine	UGU (Cys/C) Cysteine	U	
	UUC	UCC	UAC	UGC	C	
	UUA	UCA	UAA Stop (Ochre)	UGA Stop (Opal)	A	
	UUG	UCG	UAG Stop (Amber)	UGG (Trp/W) Tryptophan	G	
C	CUU (Leu/L) Leucine	CCU (Pro/P) Proline	CAU (His/H) Histidine	CGU (Arg/R) Arginine	U	
	CUC	CCC	CAC	CGC	C	
	CUA	CCA	CAA (Gln/Q) Glutamine	CGA	A	
	CUG	CCG	CAG	CGG	G	
A	AUU (Ile/I) Isoleucine	ACU (Thr/T) Threonine	AAU (Asn/N) Asparagine	AGU (Ser/S) Serine	U	
	AUC	ACC	AAC	AGC	C	
	AUA	ACA	AAA (Lys/K) Lysine	AGA (Arg/R) Arginine	A	
	AUG <sup>[A]</sup> (Met/M) Methionine	ACG	AAG	AGG	G	
G	GUU (Val/V) Valine	GCU (Ala/A) Alanine	GAU (Asp/D) Aspartic acid	GGU (Gly/G) Glycine	U	
	GUC	GCC	GAC	GGC	C	
	GUA	GCA	GAA (Glu/E) Glutamic acid	GGA	A	
	GUG	GCG	GAG	GGG	G	

Source: Wikipedia, “Genetic Code”

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## Different Reading Frames

reading frame:	123
	acttaccgaggacta
first reading frame	T Y P G L
second reading frame	L T R D
third reading frame	L P G T

Source: <http://www.ncbi.nlm.nih.gov/Class/MLACourse/Original8Hour/Genetics/readingframe.html>

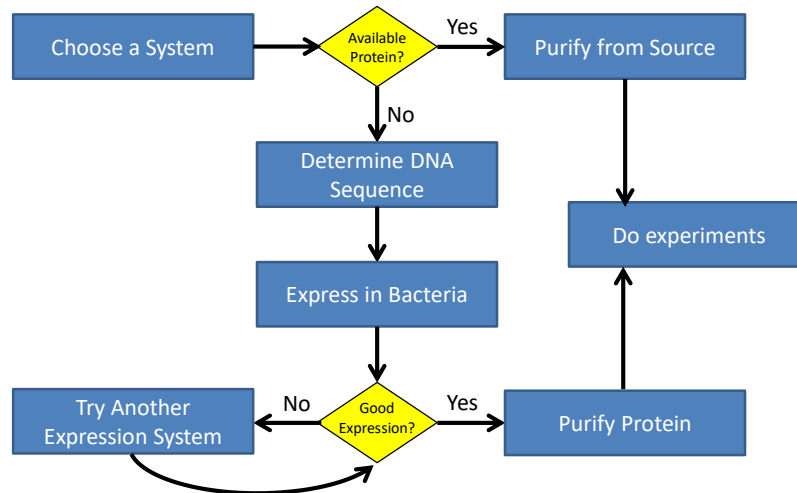
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## *Think and Discuss*

Our biochemistry experiments are normally done in aqueous buffer. Is this a good model for the inside of a cell?

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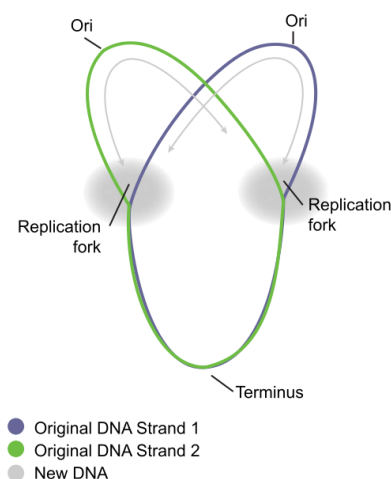
## Biochemistry Research Flow Chart



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## Bacterial DNA: Features

- Chromosome is *circular*
- Replication starts at the *origin of replication* (Ori, TTATCCACA)
- **Plasmid:** Any circular DNA in the bacterial cell can be replicated if it has an Ori



Source: Wikipedia, "Circular Bacterial Chromosome"

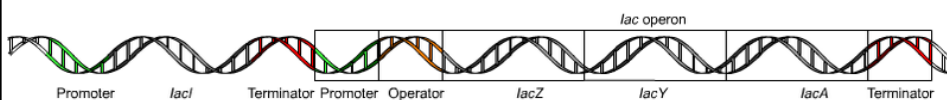
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## The Lactose (lac) Operon

- **Idea:** Bacteria only want to produce proteins if they are needed
- Why metabolize lactose (hard) when glucose (easy) is available?
- **Operon:** A set of genes (proteins) under the control of other genes in the cell

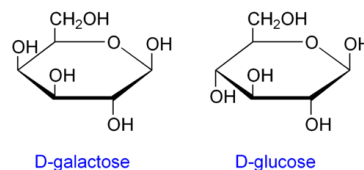
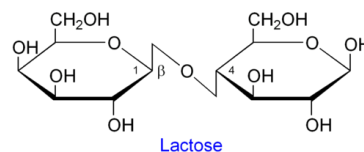
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## The Lactose (lac) Operon



Proteins:

- **lacI** (lac repressor): binds at operator when no lac present; prevents binding of RNA polymerase at promoter
- **lacZ** ( $\beta$ -galactosidase): converts Lac in to Gal and Glc by hydrolyzing glycosidic linkage
- **lacY** ( $\beta$ -galactoside permease): Pumps Lac into the cell

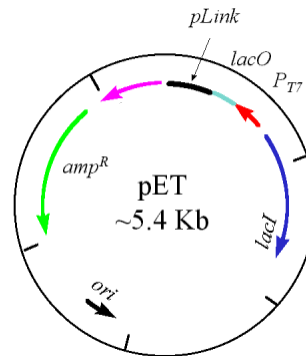


Source: Wikipedia, "Lac Operon"

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## Bacterial Expression Vectors

- pET Plasmid Genes
  - Origin of replication
  - Lac repressor (*lacI*)
  - RNA Pol promoter ( $P_{T7}$ )
  - Lac Operator (*lacO*)
  - Polylinker – where your DNA sequence goes (*pLink*)
  - Ampicillin resistance (*amp<sup>R</sup>*)



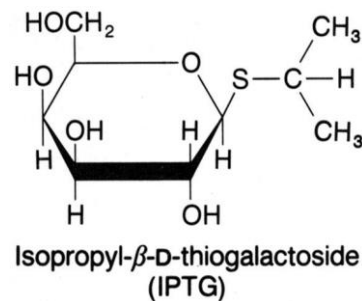
- Is this plasmid persistent?

Source: Mike Blaber, BCH5425 Course Notes

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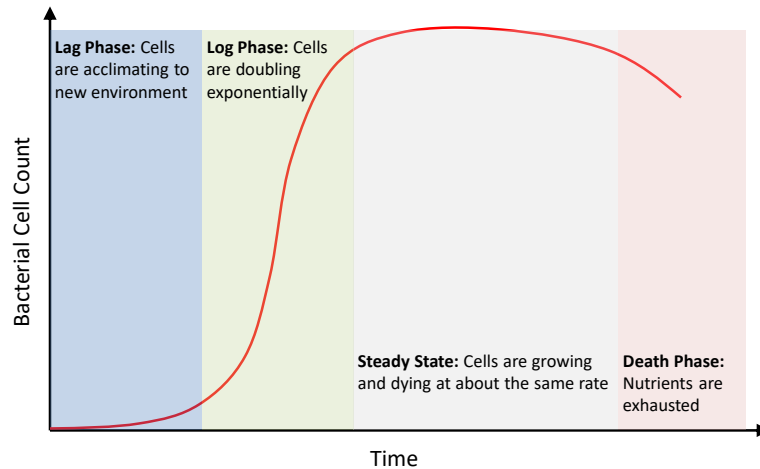
## Inducible Expression

- **IPTG:** Turns on protein expression without being hydrolyzed
- Protein expression can be switched on when desired



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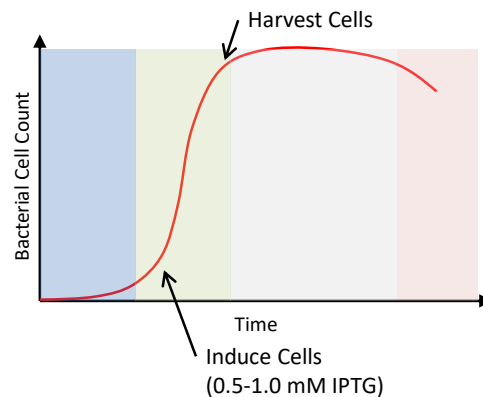
## When Should I Induce?



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## When Should I Induce?

- Protein expression is greatest during log phase
- Inducing at lag phase may unnecessarily cripple your cells
- Typically, induce at an  $OD_{600}$  of 0.5-0.6
- Always follow your lab's protocols!



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## *Think and Discuss*

Why is Ampicillin resistance necessary for the function of the pET vector system?

## Summary

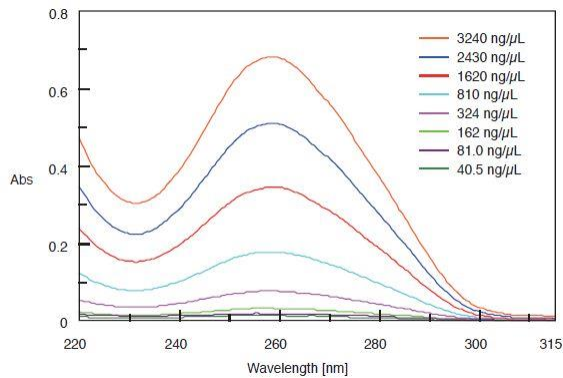
- DNA structure is as varied as protein structure, and nucleic acids can catalyze chemical reactions (“ribozymes”)
- Bacterial and animal cells store and process DNA slightly differently, although both use similar ribosomes and the same genetic code
- Modern molecular biology allows us to express virtually any gene using bacterial expression systems

## Nucleic Acid Extinction Coefficient

**DNA Concentrations:** Often measured in  $\mu\text{g/mL}$  (or the equivalent  $\text{ng}/\mu\text{L}$ ) instead of M, mM, etc. Also sequence isn't exactly known in many cases.

**Rule of Thumb:** For double-stranded, plasmid DNA, the extinction coefficient at 260 nm is

$$0.020 (\mu\text{g/mL})^{-1} \text{cm}^{-1}$$



Source: [www.jascoinc.com](http://www.jascoinc.com)

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## DNA vs. Protein Absorbance

**DNA Concentrations:** At 260 nm, double-stranded DNA has an extinction coefficient of

$$0.020 (\mu\text{g/mL})^{-1} \text{cm}^{-1}$$

**Protein Concentrations:** At 280 nm, the GB3 protein has an extinction coefficient (in equivalent units) of

$$0.0016 (\mu\text{g/mL})^{-1} \text{cm}^{-1}$$

Which is more sensitive?

What are the implications?

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## Other Values for Long, Randomized Sequences

- Single-Stranded RNA:  $0.025 (\mu\text{g/mL})^{-1} \text{ cm}^{-1}$
- Single-Stranded DNA:  $0.030 (\mu\text{g/mL})^{-1} \text{ cm}^{-1}$
- For a pure nucleic acid, the 260/280 nm ratio should be approximately 1.8-2.0

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## Nucleic Acids – Smaller Molecules

The screenshot shows the IDT Biophysics website's 'UV Spectrum of DNA' calculator. At the top is the IDT logo and 'Biophysics' text. Below are navigation tabs: 'DNA Thermodynamics', 'UV Spectrum' (selected), 'Publications', 'Tool Help', and 'Contact Us'. The main heading is 'UV Spectrum of DNA'. Below this is a 'Sequence:' input field with a '5'' label on the left and a '# Bases: 0' label on the right. A large light blue rectangular area is below the input field, with a '3'' label at the bottom right corner. Below the input area are three radio buttons: 'Single stranded DNA' (selected), 'Duplex DNA', and 'Apply Cavaluzzi-Borer Correction' (checked). At the bottom are 'Calculate' and 'Reset' buttons.

- IDT DNA Calculator:  
<http://biophysics.idtdna.com/UVSpectrum.html>

Source: [www.jascoinc.com](http://www.jascoinc.com)

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## Calculating Reverse Complement

The screenshot shows a web interface titled "Reverse Complement". It includes a text area for pasting a DNA sequence, with a sample sequence ">Sample sequence GGGGaaaaaaaaatttatatat" entered. Below the text area are "SUBMIT" and "CLEAR" buttons. A radio button is selected for "Convert the DNA sequence into its reverse-complement counterpart." A "[home]" link is at the bottom.

- Bioinformatics.org Calculator (no-frills):  
[http://bioinformatics.org/sms/rev\\_comp.html](http://bioinformatics.org/sms/rev_comp.html)

Source: [www.jascoinc.com](http://www.jascoinc.com)

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## DNA Translation Tool

- **Site:**  
<http://web.expasy.org/translate/>
- **Input:** DNA or RNA sequence (5' → 3' orientation)
- **Output:** All six possible translation frames

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## Other Databases

- NCBI Databases work for DNA sequences, too (reference sequences start with NM\_)
- PDB also houses a number of RNA/DNA structures in addition to proteins

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## Putting it Together: SDSC Biology Workbench



### ***Biology WorkBench***

- **Site:** <http://workbench.sdsc.edu/>
- **Exercise:** Create an account, try to examine some of the tools. What looks familiar?

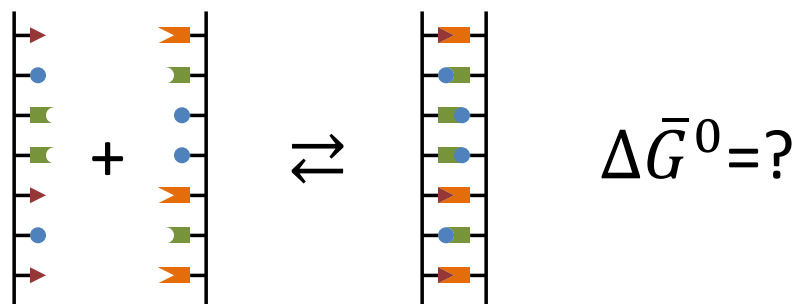
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## Think And Discuss

How can these databases be used to make your lab work easier? What are some practical examples

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## DNA “Melting”



- Two strands come together:
  - How much work can be done?
  - Which side of the reaction does temperature favor?

## Thermal Melts

- Adding heat favors highly random systems, DNA will separate at high temperature
  - Secondary and tertiary structure is lost, primary is maintained
- What will affect the melting temperature?

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## Predicting Melting Temperatures

- To calculate  $T_m$ , add 4 °C for each G-C pair, and 2 °C for each A-T
  - Not terribly accurate
- **Example:** GCCCTGAAGGTCAAGTCCCCC
  - 14 G-C = 56 °C
  - 7 A-T = 14 °C
  - Prediction is 70

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## Predicting Melting Temperatures

- IDT OligoAnalyzer:  
<http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/>
- **Input:** Your DNA sequence of interest, salt concentration
- **Output:**  $T_m$ , extinction coefficient, %GC content

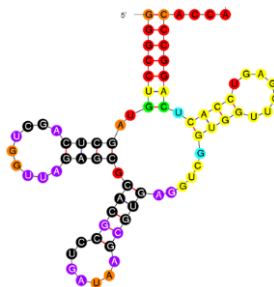
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## Predicting Secondary Structure

- mfold Web Server:  
<http://mfold.rna.albany.edu/?q=mfold>

- **Input:** RNA/DNA sequence

- **Output:**



## Example: HIV TAR RNA


- Trans-Activation Response Element – Binds with a protein (Tat) to promote viral transcription
- Sequence:

GGGUCUCUCUGGUUAGACCAGAUCUGAGCCUGGGAGCUCUCU  
GGCUAACUAGGGAACCCAC



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## Why is this Useful?

- Site-Directed Mutagenesis
- Good Primers:
  - $T_m > 78^\circ\text{C}$  (2 mM  $\text{MgCl}_2$ , 50 mM NaCl)
  - GC content > 40%
  - No secondary structure (< 50 bp)
  - End with G or C

 The Nobel Prize in Chemistry 1993  
Kary B. Mullis, Michael Smith

The Nobel Prize in Chemistry 1993
Nobel Prize Award Ceremony
Kary B. Mullis
Michael Smith

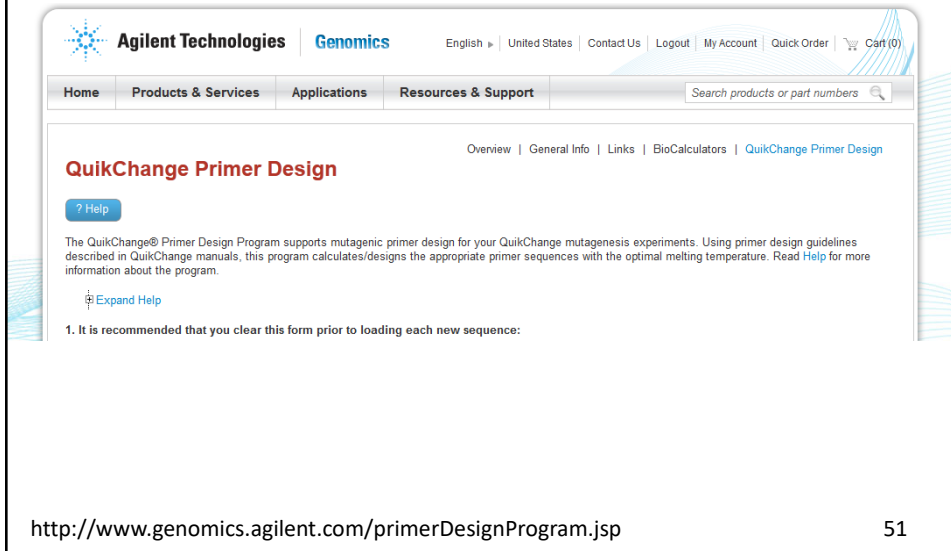
**Kary B. Mullis**      **Michael Smith**

The Nobel Prize in Chemistry 1993 was awarded "for contributions to the developments of methods within DNA-based chemistry" jointly with one half to Kary B. Mullis "for his invention of the polymerase chain reaction (PCR) method" and with one half to Michael Smith "for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies".

Photos: Copyright © The Nobel Foundation

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# Example: Designing Primers



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## QuikChange Primer Design

[? Help](#)

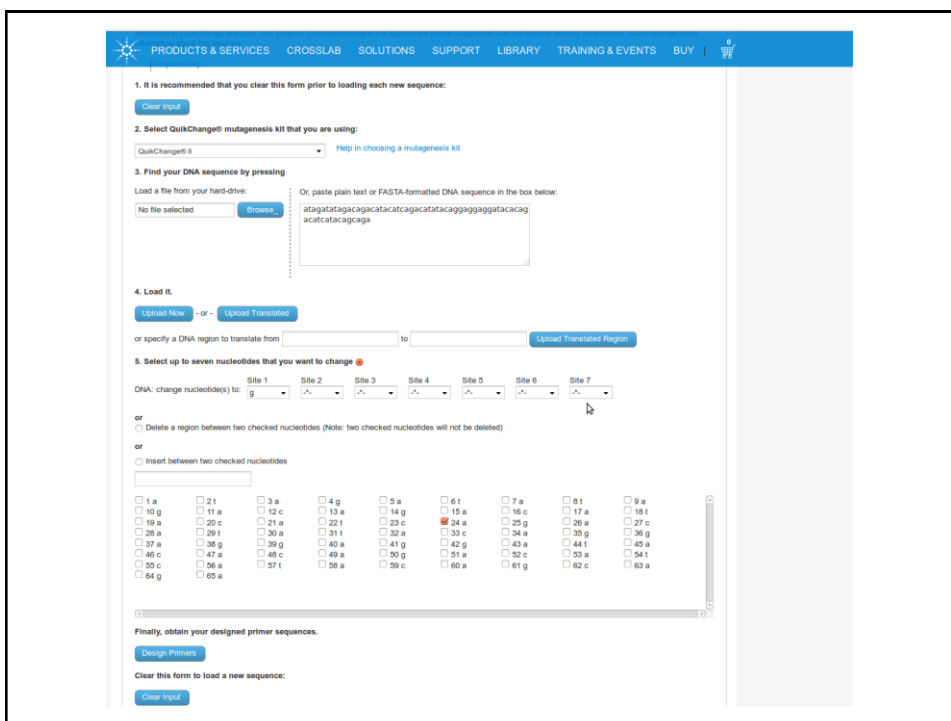
The QuikChange® Primer Design Program supports mutagenic primer design for your QuikChange mutagenesis experiments. Using primer design guidelines described in QuikChange manuals, this program calculates/designs the appropriate primer sequences with the optimal melting temperature. Read [Help](#) for more information about the program.


[Expand Help](#)

1. It is recommended that you clear this form prior to loading each new sequence:

<http://www.genomics.agilent.com/primerDesignProgram.jsp>

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1. It is recommended that you clear this form prior to loading each new sequence:

[Clear Input](#)

2. Select QuikChange® mutagenesis kit that you are using:

QuikChange® II [Help in choosing a mutagenesis kit](#)

3. Find your DNA sequence by pressing

Load a file from your hard-drive: No file selected [Browse...](#) Or, paste plain text or FASTA-formatted DNA sequence in the box below:

```
atagttatagacagacatcacatcacagaggagatcacag
acattatcacagaga
```

4. Load it.

[Upload New](#) - or - [Upload Translated](#)

or specify a DNA region to translate from  to  [Upload Translated Region](#)

5. Select up to seven nucleotides that you want to change

DNA: change nucleotide(s) to: Site 1 Site 2 Site 3 Site 4 Site 5 Site 6 Site 7

g . . . . .

or

☐ Delete a region between two checked nucleotides (Note: two checked nucleotides will not be deleted)

or

☐ Insert between two checked nucleotides


<input type="checkbox"/> 1 a	<input type="checkbox"/> 21	<input type="checkbox"/> 3 a	<input type="checkbox"/> 4 g	<input type="checkbox"/> 5 a	<input type="checkbox"/> 61	<input type="checkbox"/> 7 a	<input type="checkbox"/> 81	<input type="checkbox"/> 9 a
<input type="checkbox"/> 10 g	<input type="checkbox"/> 11 a	<input type="checkbox"/> 12 c	<input type="checkbox"/> 13 a	<input type="checkbox"/> 14 g	<input type="checkbox"/> 15 a	<input type="checkbox"/> 16 c	<input type="checkbox"/> 17 a	<input type="checkbox"/> 18 t
<input type="checkbox"/> 19 a	<input type="checkbox"/> 20 c	<input type="checkbox"/> 21 a	<input type="checkbox"/> 22 t	<input type="checkbox"/> 23 c	<input checked="" type="checkbox"/> 24 a	<input type="checkbox"/> 25 g	<input type="checkbox"/> 26 a	<input type="checkbox"/> 27 c
<input type="checkbox"/> 28 a	<input type="checkbox"/> 29 t	<input type="checkbox"/> 30 a	<input type="checkbox"/> 31 t	<input type="checkbox"/> 32 a	<input type="checkbox"/> 33 c	<input type="checkbox"/> 34 a	<input type="checkbox"/> 35 g	<input type="checkbox"/> 36 g
<input type="checkbox"/> 37 a	<input type="checkbox"/> 38 g	<input type="checkbox"/> 39 g	<input type="checkbox"/> 40 a	<input type="checkbox"/> 41 g	<input type="checkbox"/> 42 g	<input type="checkbox"/> 43 a	<input type="checkbox"/> 44 t	<input type="checkbox"/> 45 a
<input type="checkbox"/> 46 c	<input type="checkbox"/> 47 a	<input type="checkbox"/> 48 c	<input type="checkbox"/> 49 a	<input type="checkbox"/> 50 g	<input type="checkbox"/> 51 a	<input type="checkbox"/> 52 c	<input type="checkbox"/> 53 a	<input type="checkbox"/> 54 t
<input type="checkbox"/> 55 c	<input type="checkbox"/> 56 a	<input type="checkbox"/> 57 t	<input type="checkbox"/> 58 a	<input type="checkbox"/> 59 c	<input type="checkbox"/> 60 a	<input type="checkbox"/> 61 g	<input type="checkbox"/> 62 c	<input type="checkbox"/> 63 a
<input type="checkbox"/> 64 g	<input type="checkbox"/> 65 a							

Finally, obtain your designed primer sequences.


[Design Primers](#)

Clear this form to load a new sequence:

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0

☐ Insert between two checked nucleotides  

☐ 1 a
☐ 2 t
☐ 3 a
☐ 4 g
☐ 5 a
☐ 6 t
☐ 7 a
☐ 8 t
☐ 9 a

☐ 10 g
☐ 11 a
☐ 12 c
☐ 13 a
☐ 14 g
☐ 15 a
☐ 16 c
☐ 17 a
☐ 18 t

☐ 19 a
☐ 20 c
☐ 21 a
☐ 22 t
☐ 23 c
☒ 24 a
☐ 25 g
☐ 26 a
☐ 27 c

☐ 28 a
☐ 29 t
☐ 30 a
☐ 31 t
☐ 32 a
☐ 33 c
☐ 34 a
☐ 35 g
☐ 36 g

☐ 37 a
☐ 38 g
☐ 39 g
☐ 40 a
☐ 41 g
☐ 42 g
☐ 43 a
☐ 44 t
☐ 45 a

☐ 46 c
☐ 47 a
☐ 48 c
☐ 49 a
☐ 50 g
☐ 51 a
☐ 52 c
☐ 53 a
☐ 54 t

☐ 55 c
☐ 56 a
☐ 57 t
☐ 58 a
☐ 59 c
☐ 60 a
☐ 61 g
☐ 62 c
☐ 63 a

☐ 64 g
☐ 65 a

Finally, obtain your designed primer sequences.

Design Primers

Clear this form to load a new sequence:

Clear Input

Primer sequences:

Primer Name
Primer Sequence (5' to 3')

a24c
5'-gatatagcagacatcacctccgacatatacaggaggagat-3'  
5'-gatatagcagacatcacctccgacatatacaggaggagat-3'

Oligonucleotide information:

Primer Name	Length (nt)	Tm	Duplex Energy at 65 °C	Energy Cost of Mismatches
a24c	41	78.60°C	-45.55 kcal/mole	3.05%
a24c	41	78.60°C	-49.02 kcal/mole	3.83%

Primer-template duplexes:

Primer Name
Primer-Template Duplex

a24c\_
gatatagcagacatcacatcacatcagacatatacaggaggagatatac  
|||||  
3'-ctatatctgtctgtatgttaggtctgtatatgtctctctcta-5'  
5'-gatatagcagacatcacatcacatatacaggaggagat-3'  
|||||  
77?ctatatctgtctgtatgttaggtctgtatatgtctctctcta-5'

## Example: General Approach



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## DNA Thermodynamics & Hybridization

*Oligo + Target  $\leftrightarrow$  Duplex*

Sequence:
# Bases: 0

5'-
-3'

☐ Mismatch, Dangling Ends

Oligo Conc
0.25
μM

Target Conc
0
μM

Na<sup>+</sup>, K<sup>+</sup> Conc
50
mM

Mg<sup>2+</sup> Conc
0
mM

dNTPs Conc
0
mM

CALCULATE

RESET

**Base Sequence Symbols**

Native DNA: A, C, G, T

Inosine: I

Locked nucleic acids: +A, +C, +G, +T

# General Primer Design Principles

- **PCR Steps:** Denature (95 °C), anneal (60 °C), extend (70 °C)
- Considerations:
  - **Melting Temperature:** Should be 52-58 °C
  - **GC Content:** 40-60%
  - **Length:** ~30 bp (but longer can be okay)
  - **Secondary Structure:** Avoid if possible
- Lots of software exists (some costs \$\$\$). For more information (some trial and error here):
  - <https://goo.gl/4EwMG3> (Life Technologies)
  - [http://www.premierbiosoft.com/tech\\_notes/PCR\\_Primer\\_Design.html](http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html)

## Example: General Approach

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**DNA Thermodynamics & Hybridization** *Oligo + Target ↔ Duplex*

Sequence:  # Bases:

5' GCC CTG AAG GTC AAG TCC CCC 3'

Oligo Conc:  0.25  $\mu$ M  
 Target Conc:  0  $\mu$ M  
 Na<sup>+</sup>, K<sup>+</sup> Conc:  50 mM  
 Mg<sup>2+</sup> Conc:  2 mM  
 dNTPs Conc:  0 mM

**INTRODUCE MISMATCH**

Unpaired Base	5'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Unpaired Base
5'		G	C	C	C	T	G	A	A	G	G	T	C	A	A	G	T	C	C	C	C	C		3'
3'		C	G	G	G	A	C	T	T	C	A	A	G	T	C	A	G	G	G	G	G		5'	

<http://biophysics.idtdna.com/>

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## *Think And Discuss*

Compared to DNA, why is it harder to calculate melting temperature and dimerization for proteins?

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### **Example:** Sequence Analysis of SH3 Mutants

- Step 1: Design Primers (for T22G)
  - Agilent Web Program (we'll do this)
- Step 2: Do experiments, get sequence of result
- Step 3: Check sequence to see if mutation was successful (we'll do this)

## *Think and Discuss*

What problems could arise when introducing new mutations in to a known sequence?

## Summary

- Advanced computational tools for nucleic acids depend on two things:
  - The simplicity of DNA primary structure (4 bases)
  - The regularity of Watson-Crick base pairing
- Combining DNA and protein tools makes it possible to perform very advanced sequence analysis