

Calculating DNA Properties

Biochemistry Boot Camp 2021

Session #8

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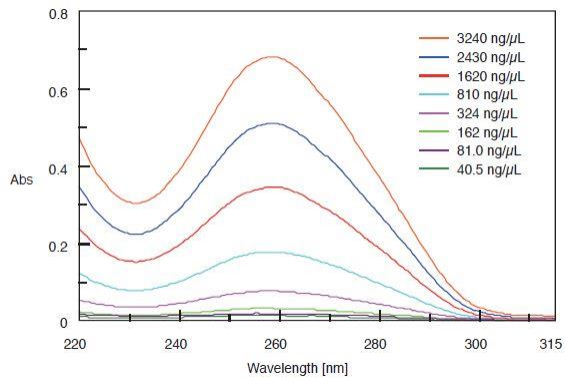
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Nucleic Acid Extinction Coefficient

DNA Concentrations: Often measured in $\mu\text{g}/\text{mL}$ (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

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



Source: www.jascoinc.com

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DNA vs. Protein Absorbance

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

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

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

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

Which is more sensitive?

What are the implications?

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Other Values for Long, Randomized Sequences

- Single-Stranded RNA: $0.025 (\mu\text{g}/\text{mL})^{-1} \text{cm}^{-1}$
- Single-Stranded DNA: $0.030 (\mu\text{g}/\text{mL})^{-1} \text{cm}^{-1}$
- 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

OligoAnalyzer

- IDT DNA Analyzer (extinction coefficient, Tm):
<https://www.idtdna.com/pages/tools/oligoanalyzer>
 – Need to log in, create an account (free)

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Calculating Reverse Complement

- Bioinformatics.org Calculator (no-frills):
http://bioinformatics.org/sms/rev_comp.html

Source: 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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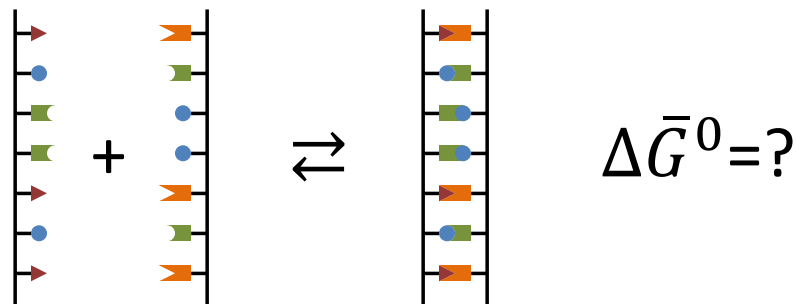
Think And Discuss

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?

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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:
<https://www.idtdna.com/pages/tools/oligoanalyzer>
- **Input:** Your DNA sequence of interest, salt concentration
- **Output:** T_m , extinction coefficient, %GC content

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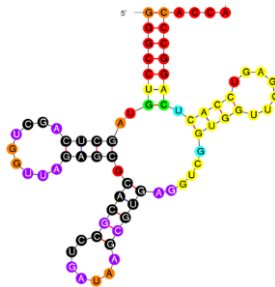
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Predicting Secondary Structure

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

- **Input:** RNA/DNA sequence

- **Output:**



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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:
 - $T_m > 78\text{ }^\circ\text{C}$ (2 mM 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**

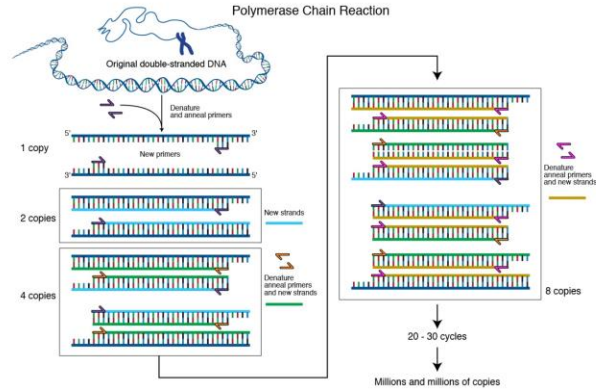
The Nobel Prize in Chemistry 1993 was awarded "for contributions to the developments of methods within DNA-based chemistry" jointly with one half to Kary B. Mullis "for his invention of the polymerase chain reaction (PCR) method" and with one half to Michael Smith "for his fundamental contributions to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

Photos: Copyright © The Nobel Foundation

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Polymerase Chain Reaction (PCR)



- **Key consideration:** Temperature for primer annealing (computational tools)!

<https://www.genome.gov/genetics-glossary/Polymerase-Chain-Reaction>

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Example: Designing Primers

The screenshot shows the Agilent Technologies Genomics website interface. The main content area is titled "QuikChange Primer Design" and includes a "Help" button. The text below the button reads:

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:

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: Or paste plain text or FASTA-formatted DNA sequence in the box below:
 No file selected `atagatagacagacatcacatccgacatatacaggaggatcacag
 acatcatcacgaga`

4. Load it.
 or
 or specify a DNA region to translate from to

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

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.

 Clear this form to load a new sequence:

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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.

 Clear this form to load a new sequence:

Primer sequences:

Primer Name	Primer Sequence (5' to 3')
s24c_	5'-atctctctctgatgatgctgatgctgctatc-3' 5'-gatatagacagacatcacatccgacatatacaggaggat-3'

Oligonucleotide information:

Primer Name	Length (nt)	Tm	Duplex Energy at 68 °C	Energy Cost of Mismatches
s24c_	41	78.60°C	-45.55 kcal/mole	3.05%
s24c_	41	78.60°C	-49.02 kcal/mole	3.85%

Primer-template duplexes:

Primer Name	Primer-Template Duplex
s24c_	gatatagacagacatcacatccgacatatacaggaggagataga 3'-ctatatctgtctgtatgttaggtgtatgtctctccta-5' 5'-gatatagacagacatcacatccgacatatacaggaggat-3' s24c_ ???ctatatctgtctgtatgttaggtgtatgtctctccta-5'

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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

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Example: General Approach

Exact and Single Base Mismatch DNA Thermodynamics

Primary Sequence: 5' to 3'; Target Sequence: 3' to 5'

Additional Target Base

5' CGAAGAACAGGAAGCGGAATTTAAAGAAG
 |||
 3' GCTTCTG T CCTTCGCCTAAATTCTTC

Additional Target Base

A

Hybridization Temperature

Use Exact Complement T_m

User Defined

Target Concentration

ANALYZE

HAIRPIN

SELF-DIMER

HETERO-DIMER

NCBI BLAST

TM MISMATCH

ADD TO ORDER

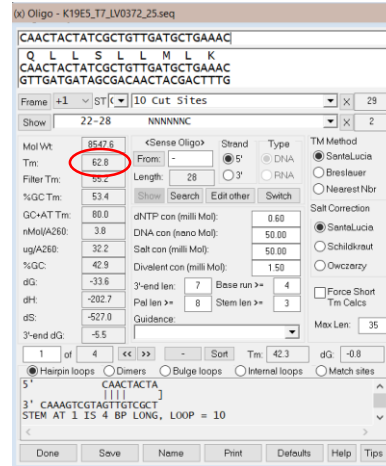
IDT OligoAnalyzer Mismatch Mode – estimate T_m cost of non-matching bases

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Other Calculators: GeneRunner

- Download (free) from <http://generunner.net/>
- Analysis → Oligo brings up window to the right
- Suggested options shown to the right
 - SantaLucia temperature & salt recommended
 - Check your specific dNTP, DNA, Mg²⁺ and salt concentrations



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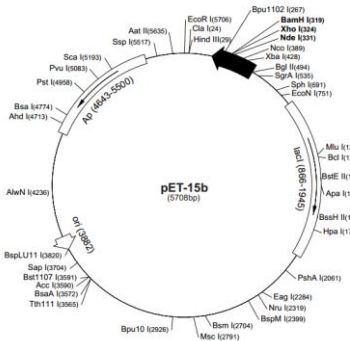
Other Calculators: Phusion Calculator

- Some polymerases have their own calculator specific to buffers, recommendations
- Phusion Polymerase found at NEB website (<https://tmcalculator.neb.com/#!/main>)

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GB3 Protein and Expression Plasmid (pET-15b)



WT GB3 DNA/Protein Sequence:

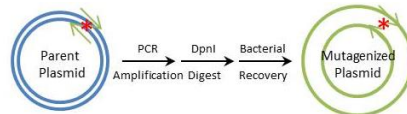
```
(-6) I * E G D I H M Q Y K L V I N G K T L K 13
1 ctttaagaaagagatatacatatgcaatgacaattatgatcaatgtaaaacattaaa...60
14 G E T T T K A V D A E T A E K A F K Q Y 33
61 ggcgaaacaaactactaaagctgttgatgctgaaactgcagaaaaagctttcaacaatac...120
34 A N D N G V D G V W T Y D D A T K T F T 53
121 gctaacgacaaacggtgtgacggtgtttgacttacgacgatgcgactaaqacctttaca...180
54 V T E * D P A A N K A
181 gttactgaatagatccgactgctaacaagcc 213
```

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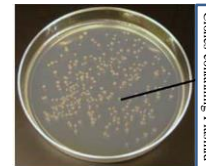
Successful Mutagenesis Primers for GB3 K19I

Primer Name	Primer Sequence (5' to 3')
K19I-Fwd	CAACTACTATCGCTGTTGATGCTGAAAC
K19I-Rev	GCATCAACAGCGATAGTAGTTGTTTCGC



Description	Primer Duplex
K19I -Fwd/ Template	5' -CAACTACTATCGCTGTTGATGCTGAAAC 3' -TAACTTCCGCCTTGTGATGATTTCGACAACTACGACTTTGACGT
K19I -Rev/ Template	CATTGAAAGCGAAACAACACTAAAGCTGTTGATGCTGAAACTGCAGAAA CGCTTTGTTGATGATAGCGACAACACTACG
K19I -Fwd/ K19I -Rev	CAACTACTATCGCTGTTGATGCTGAAAC CGCTTTGTTGATGATAGCGACAACACTACG

After Transformation:



- Annealing temperature used: 53 °C

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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)*

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Think and Discuss

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

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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

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