Calculating DNA Properties

Biochemistry Boot Camp 2022
Session #4
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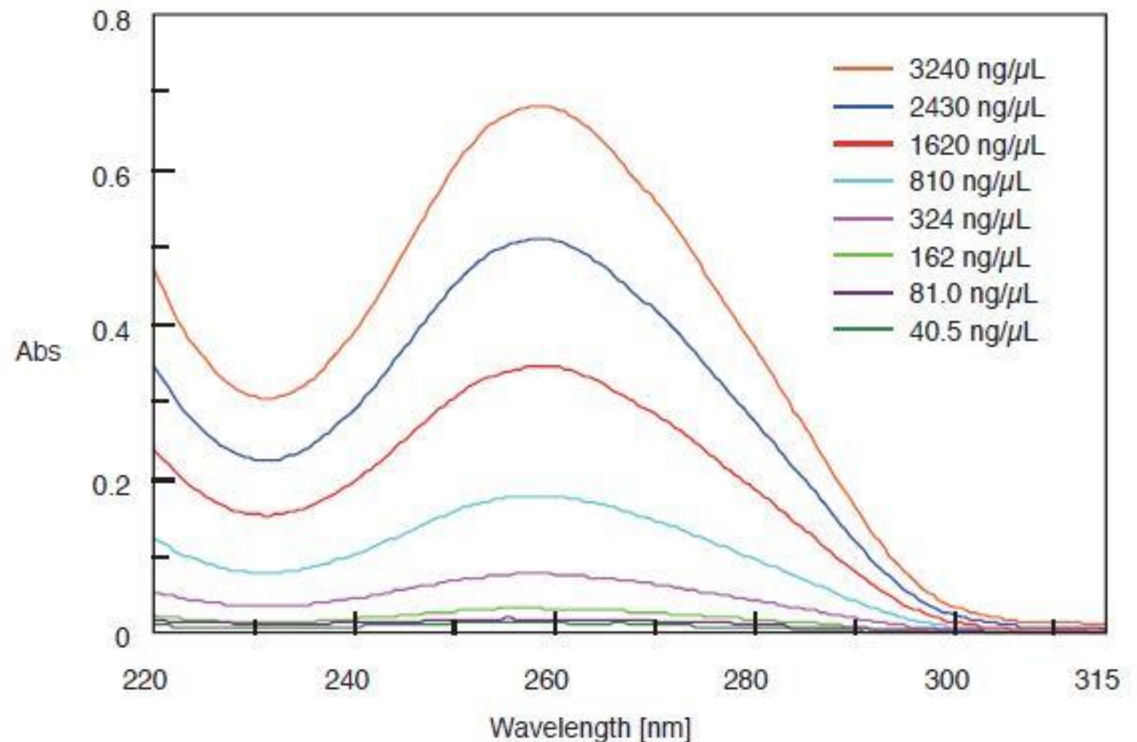
Nucleic Acid Extinction Coefficient

DNA Concentrations: Often measured in μg/mL (or the equivalent ng/μL) 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

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



Source: www.jascoinc.com

DNA vs. Protein Absorbance

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

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

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

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

Which is more sensitive?

What are the implications?

Other Values for Long, Randomized Sequences

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

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

- For a pure nucleic acid, the 260/280 nm ratio should be approximately 1.8-2.0

Nucleic Acids – Smaller Molecules

OligoAnalyzer

Instructions | Definitions | Feedback

Sequence 5' MOD INTERNAL 3' MOD MIXED BASES

CGA AGA ACA GGA AGC GGA ATT TAA AGA AG

Bases 29 CLEAR SEQUENCE

Try the new batch mode here

Parameter sets: SpecSheet (Default)

Target type: DNA

Oligo Conc: 0.25 μM

Na^+ Conc: 50 mM

Mg^{++} Conc: 0 mM

dNTPs Conc: 0 mM

ANALYZE

HAIRPIN

SELF-DIMER

HETERO-DIMER

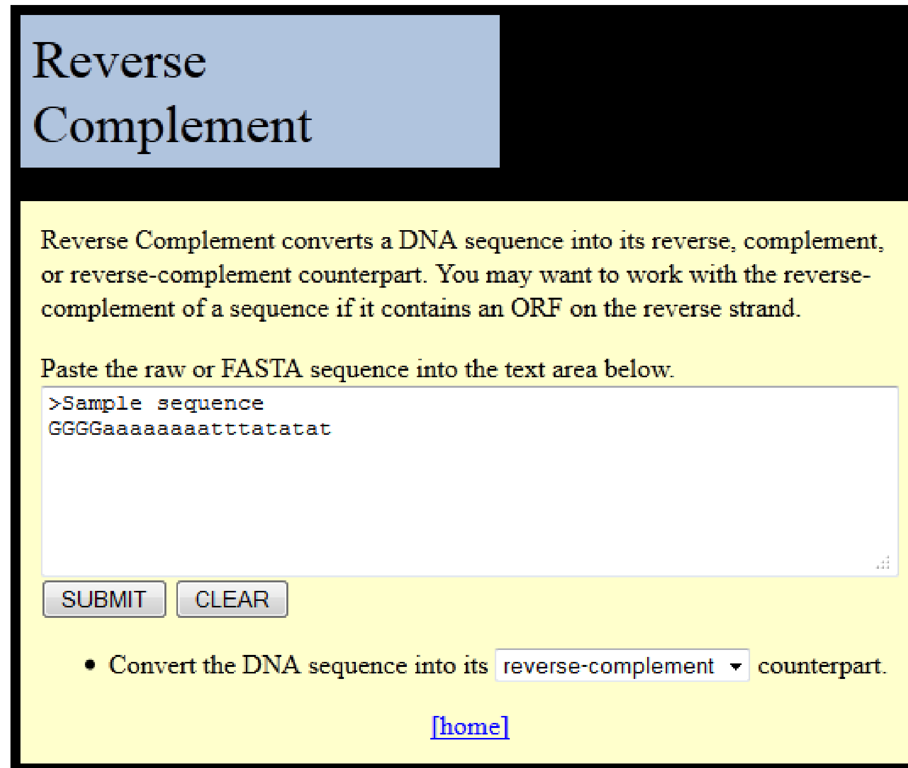
NCBI BLAST

TM MISMATCH

ADD TO ORDER

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

Calculating Reverse Complement



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

Source: www.jascoinc.com

DNA Translation Tool

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

Other Databases

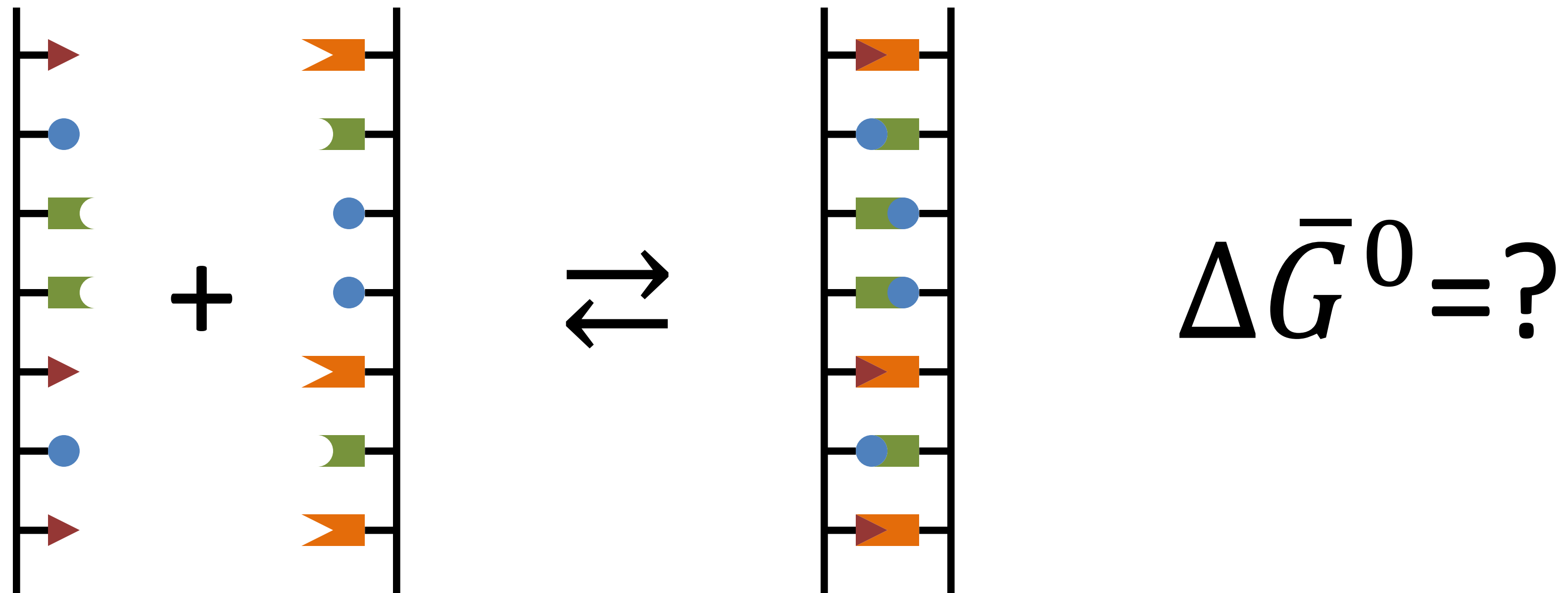
- NCBI Databases work for DNA sequences, too (reference sequences start with NM_)

- PDB also houses a number of RNA/DNA structures in addition to proteins

Think And Discuss

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

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?

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

Predicting Melting Temperatures

- IDT OligoAnalyzer: https://www.idtdna.com/pages/tools/oligoanalyzer

- **Input:** Your DNA sequence of interest, salt concentration

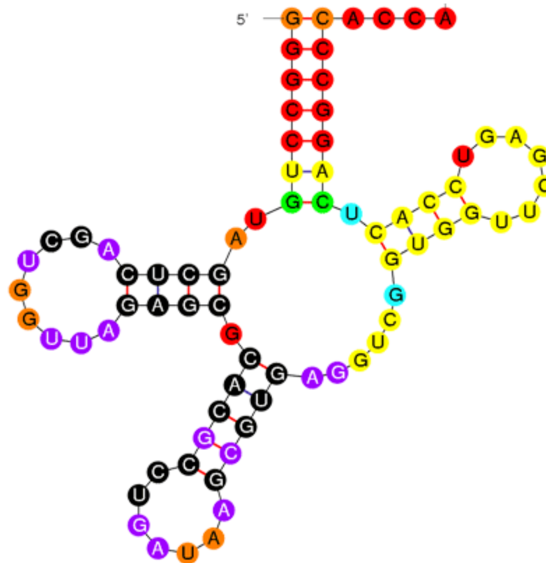
- **Output:** T_m, extinction coefficient, %GC content

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

Why is this Useful?

- Site-Directed Mutagenesis

- Good Primers:
  - T_m > 78 °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

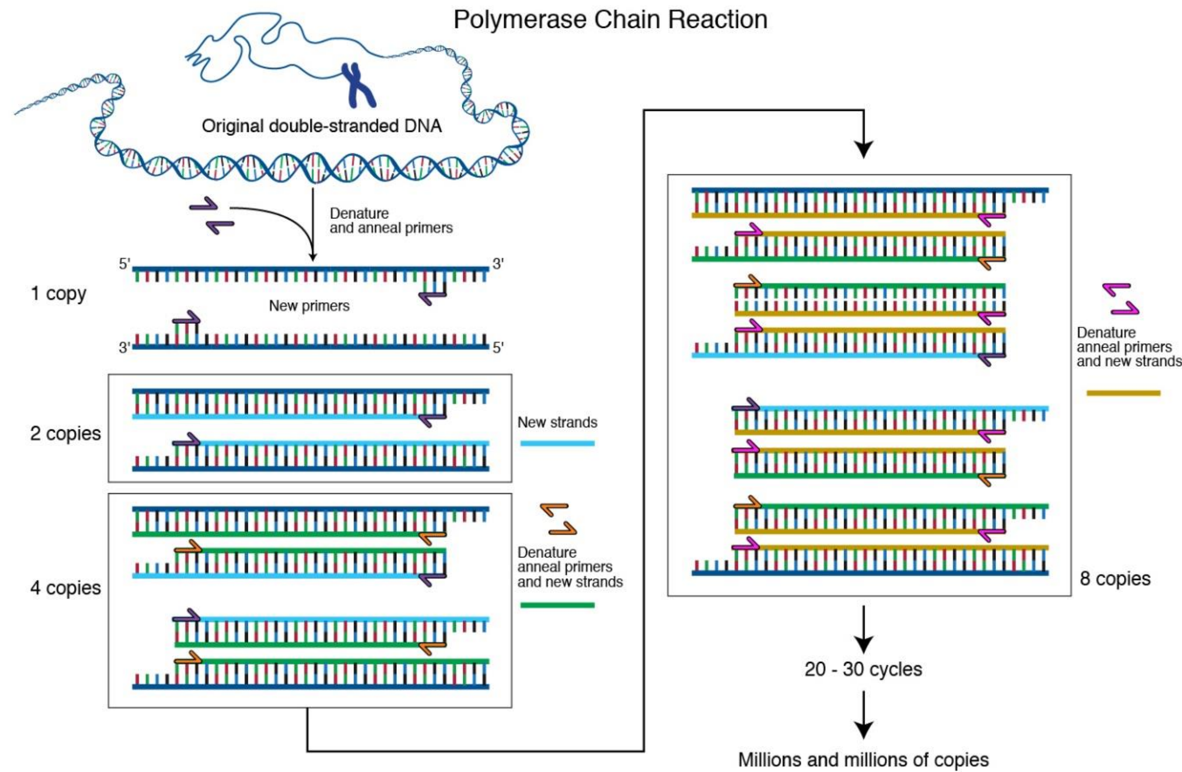
Kary B. Mullis

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

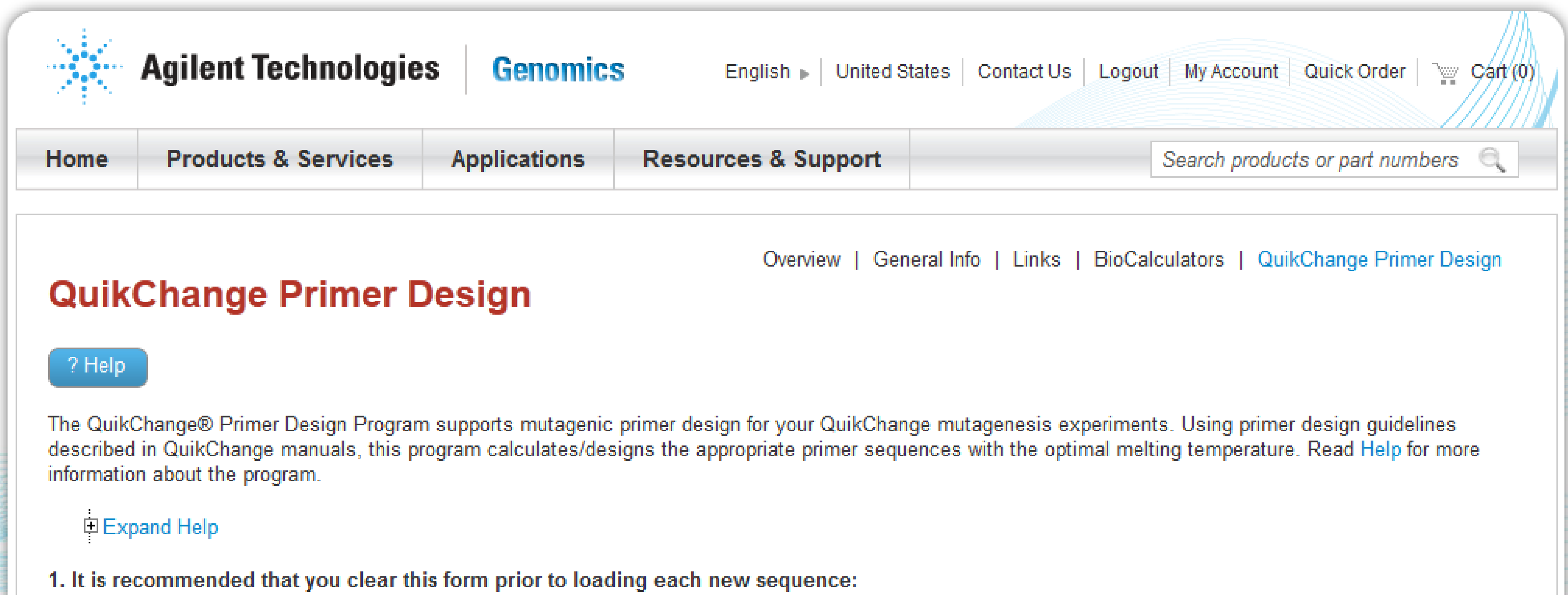
Polymerase Chain Reaction (PCR)



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

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

Example: Designing Primers



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

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:

atagatatagacagacatacatcagacatatacaggaggaggatacacag
acatcatacagcaga

4. Load it.

Upload Now - 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

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

Design Primers

Clear this form to load a new sequence:

Clear Input

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.

Design Primers

Clear this form to load a new sequence:

Clear Input

Primer sequences:

Primer Name	Primer Sequence (5' to 3')
	5'-atcctcctcctgtatatgtcggatgtatgtctgtctatatc-3'
a24c_	5'-gatatagacagacatacatccgacatatacaggaggaggat-3'

Oligonucleotide information:

Primer Name	Length (nt.)	Tm	Duplex Energy at 68 °C	Energy Cost of Mismatches
	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
	gatatagacagacatacatcagacatatacaggaggaggataca
	3'-ctatatctgtctgtatgtaggctgtatatgtcctcctccta-5'
	5'-gatatagacagacatacatccgacatatacaggaggaggat-3'
a24c_	||||||||||||||||||| ||||||||||||||||||||
	???ctatatctgtctgtatgtagtctgtatatgtcctcctcctatgt

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

Example: General Approach

Exact and Single Base Mismatch DNA Thermodynamics

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

Additional Target Base

Additional Target Base

5'- CGAAGAACAGGAAGCGGAATTTAAAGAAG -3'

| | | | | | | | | | | | | | | | | | | | | | | | | | | | |

3'- GCTTCTTG T CCTTCGCCTTAAATTTCTTC -5'

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 Tm cost of non-matching bases

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, Mg^{2+} and salt concentrations

Other Calculators: Phusion Calculator

Product Group
Phusion

Polymerase/Kit
Phusion High-Fidelity DNA Polymerase (HF Buffer)

Primer Concentration (nM)
500

Reset concentration

Primer 1
CAACTACTATCGCTGTTGATGCTGAAAC

Primer 2
GCATCAACAGCGATAGTAGTTGTTTCGC

Switch to batch mode

Clear

Use example input

Anneal at
67 °C

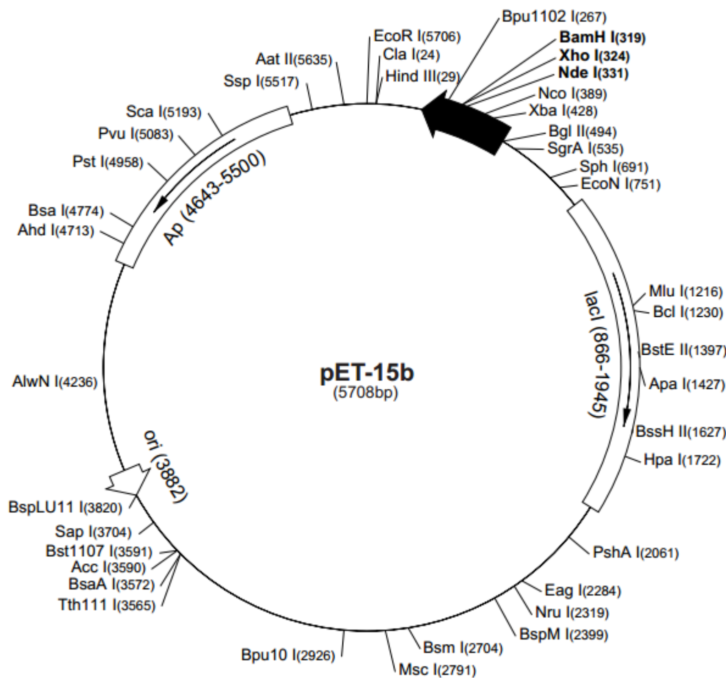
Why is this so high?

Primer 1
28 nt
43% GC
Tm: 64°C

Primer 2
28 nt
46% GC
Tm: 66°C

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

GB3 Protein and Expression Plasmid (pET-15b)

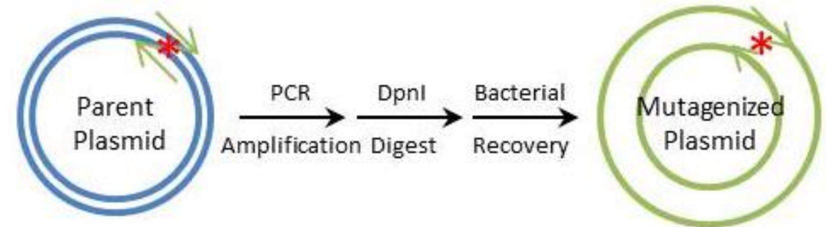


WT GB3 DNA/Protein Sequence:

```
(-6) L  *  E  G  D  I  H  M  Q  Y  K  L  V  I  N  G  K  T  L  K     13
1    ctttaagaaggagatatacatatgcagtacaaattagttatcaatggtaaaacattgaaa  60

14   G  E  T  T  T  K  A  V  D  A  E  T  A  E  K  A  F  K  Q  Y     33
61   ggcgaaacaactactaaagctgttgatgctgaaactgcagaaaaagctttcaaacaatac  120

34   A  N  D  N  G  V  D  G  V  W  T  Y  D  D  A  T  K  T  F  T     53
121  gctaacgacaacggtgttgacggtgtttggacttacgacgatgcgactaagacctttaca  180

54   V  T  E  *  D  P  A  A  N  K  A
181  gttactgaataggatccggctgctaacaaagcc  213
```

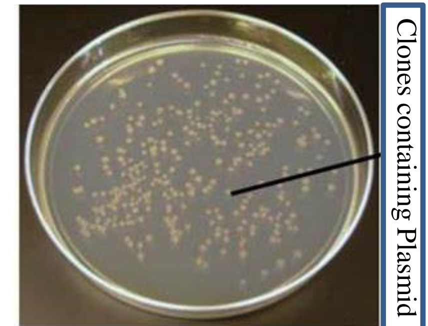
Successful Mutagenesis Primers for GB3 K19I

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



Description	Primer Duplex
K19I -Fwd/ Template	5′-CAACTACTATCGCTGTTGATGCTGAAAC ||||||||| |||||||||||||||| 3′-TAACTTTCCGCTTTGTTGATGATTTCGACAACTACGACTTTGACGT
K19I -Rev/ Template	CATTGAAAGGCGAAACAACTACTAAAGCTGTTGATGCTGAAACTGCAGAAA ||||||||||||||| |||||||||||| CGCTTTGTTGATGATAGCGACAACTACG
K19I -Fwd/ K19I -Rev	CAACTACTATCGCTGTTGATGCTGAAAC CGCTTTGTTGATGATAGCGACAACTACG

After Transformation:



- Annealing temperature used: 53 °C

Think And Discuss

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

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