Calculating DNA Properties

Biochemistry Boot Camp 2021
Session #8
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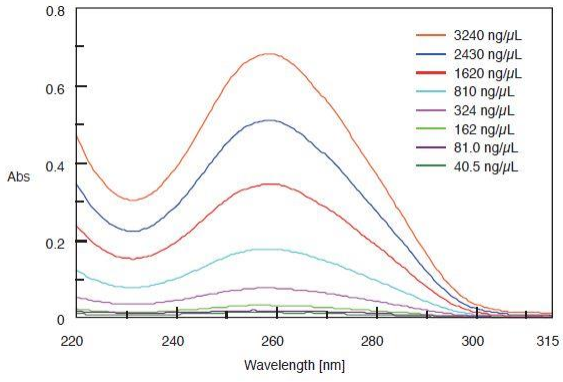
Nucleic Acid Extinction Coefficient

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

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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?

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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

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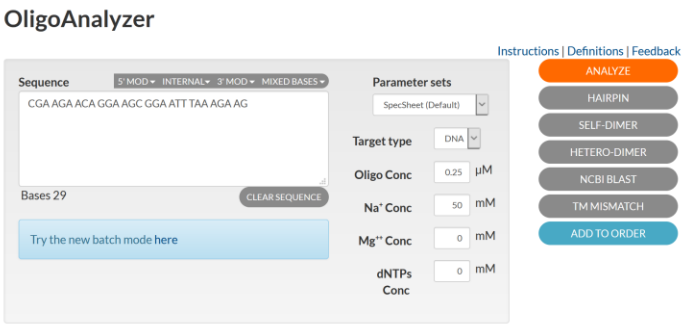
Nucleic Acids – Smaller Molecules



- 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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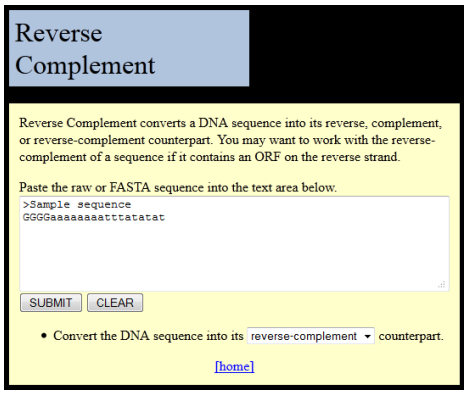
Calculating Reverse Complement



- 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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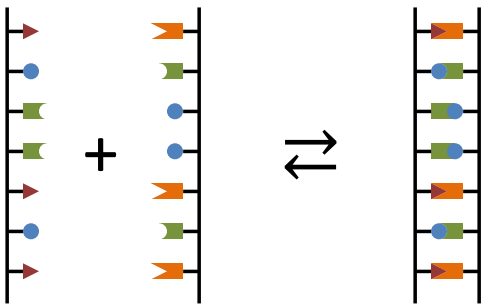
DNA "Melting"



$\Delta \bar{G}^0$=?

- 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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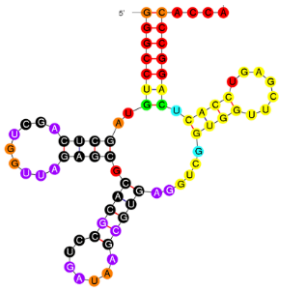
Predicting Secondary Structure

- `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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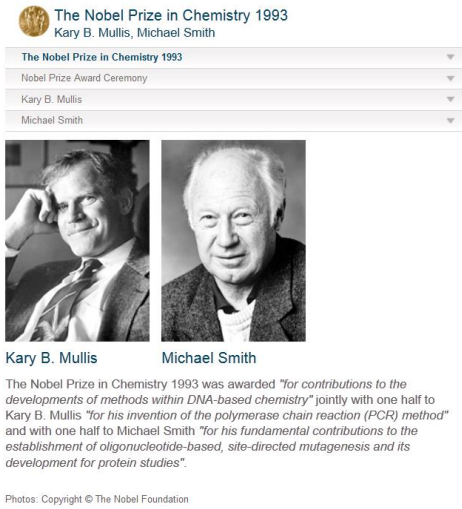
Why is this Useful?

- Site-Directed Mutagenesis

- Good Primers:
  - T_m > 78 °C (2 mM $MgCl_2$, 50 mM NaCl)
  - GC content > 40%
  - No secondary structure (< 50 bp)
  - End with G or C



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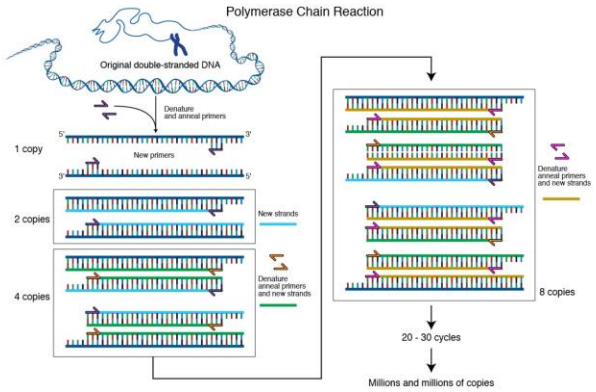
Polymerase Chain Reaction (PCR)



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

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

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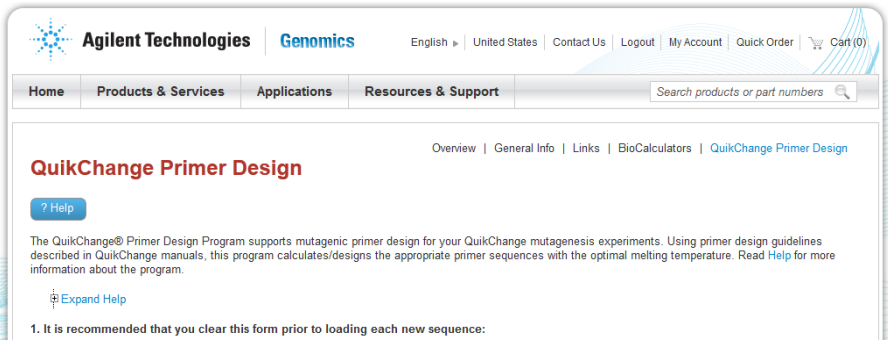
Example: Designing Primers



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

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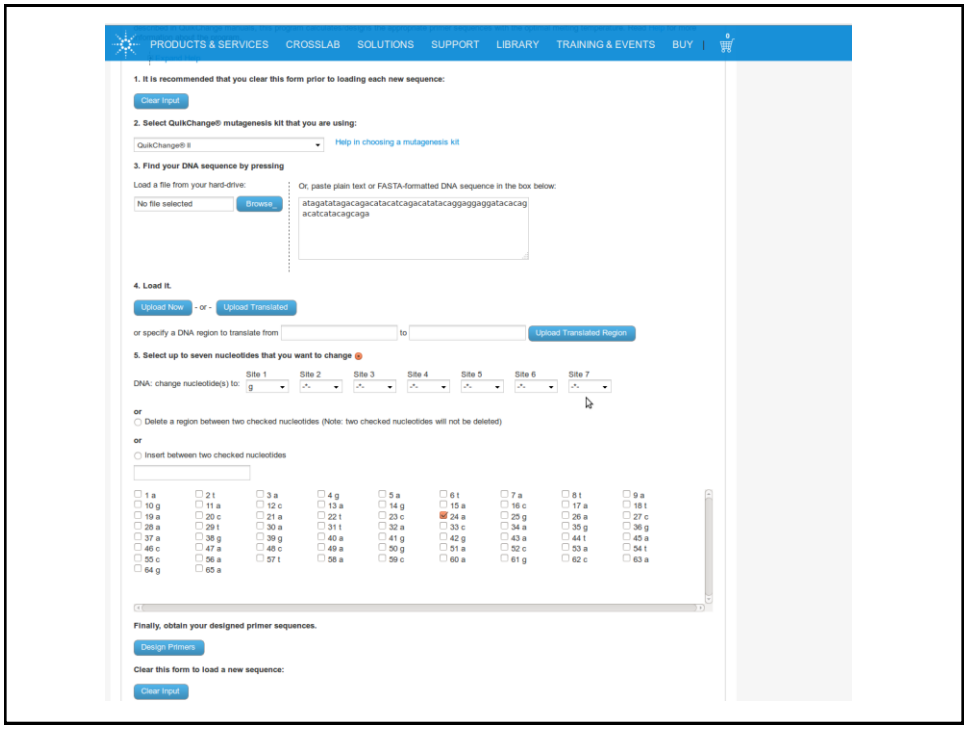
PRODUCTS & SERVICES
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1. It is recommended that you clear this form prior to loading each new sequence:
Clear Input
2. Select QuikChange® mutagenesis kit that you are using:
QuikChange® II
Help in choosing a mutagenesis kit
3. Find your DNA sequence 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:
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
DNA: change nucleotide(s) to:
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
or
Delete a region between two checked nucleotides (Note: two checked nucleotides will not be deleted)
or
Insert between two checked nucleotides
Finally, obtain your designed primer sequences.
Design Primers
Clear this form to load a new sequence:
Clear Input

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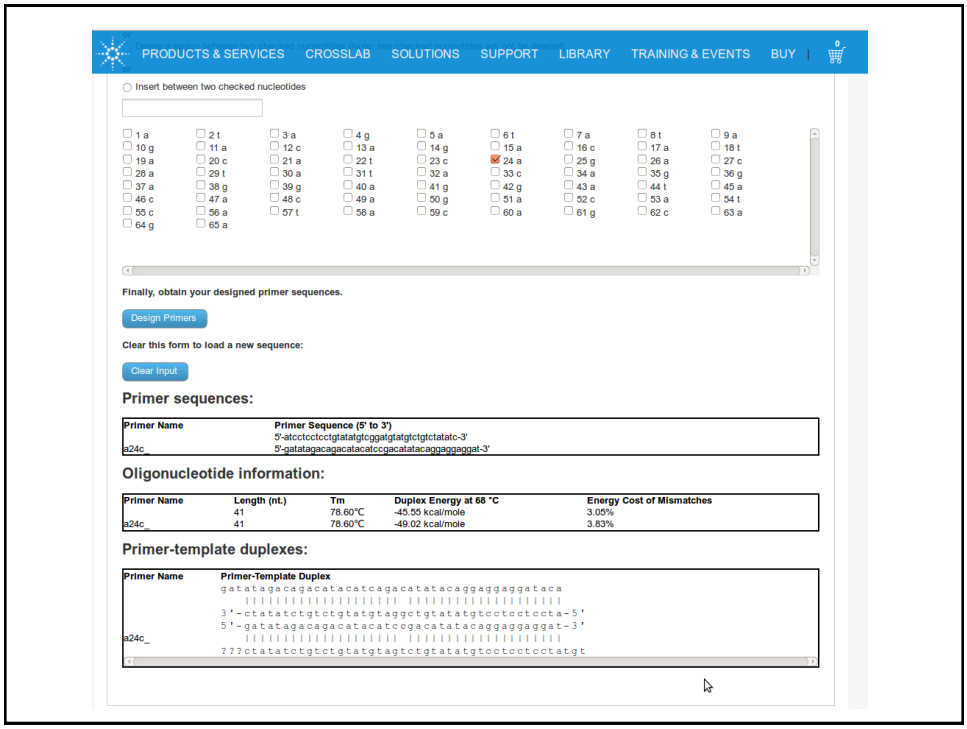
PRODUCTS & SERVICES
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BUY
Insert between two checked nucleotides
Finally, obtain your designed primer sequences.
Design Primers
Clear this form to load a new sequence:
Clear Input
Primer sequences:
Primer Name
Primer Sequence (5' to 3')
5'-atcctcctcctgtatatgtcggatgtatgtctgtctatatc-3'
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

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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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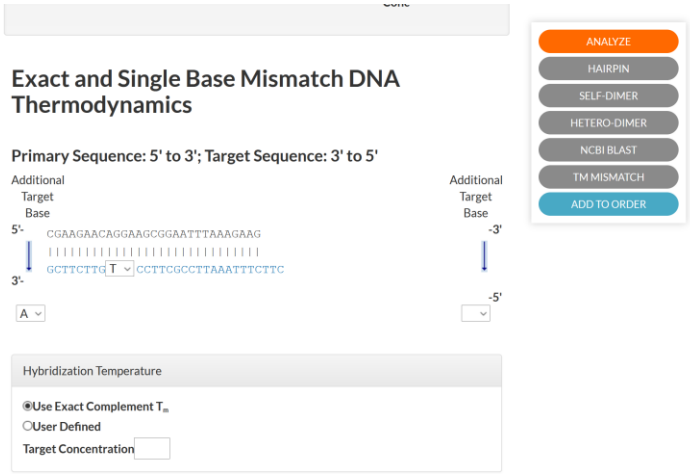
Example: General Approach



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

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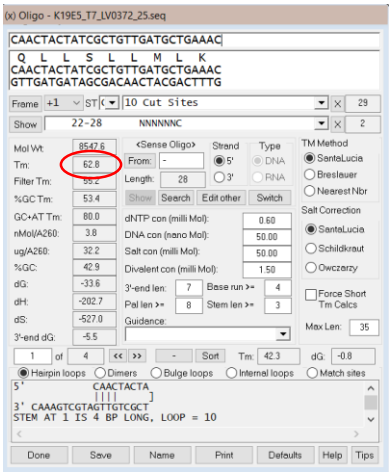
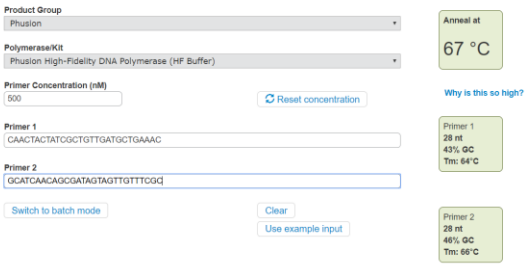
Other Calculators: GeneRunner

- 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, Mg^{2+} and salt concentrations

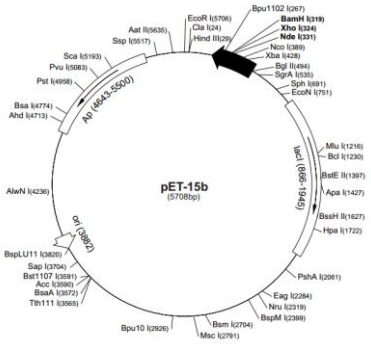


Other Calculators: Phusion Calculator



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

GB3 Protein and Expression Plasmid (pET-15b)

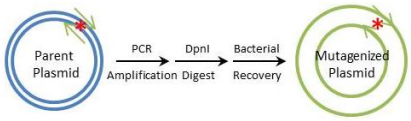


WT GB3 DNA/Protein Sequence:

```
(-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   ggcgaaacaactactaaaagctgttgatgctgaaactgcagaaaaaagctttcaaacaatac 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
```

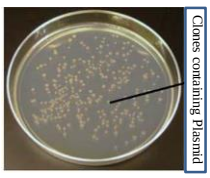

Successful Mutagenesis Primers for GB3 K19I

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?

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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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