Melting DNA

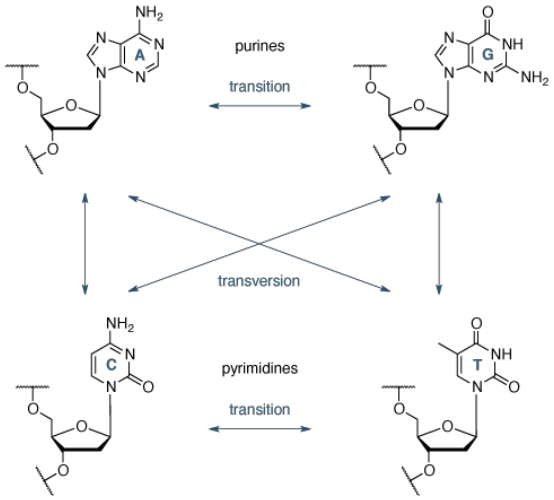
Lab 1

Bio322-2012-Monsoon

DNA Replication Error Rate

- DNA replication based on Watson-Crick base pairing
- Frequency of 10^-2 (1%) incorrect nts per bp incorporated in the absence of external influences
- DNA polymerase holoenzyme error frequency 10^-5 (1 error per 10^5 nts)
- Competition between G-C/A-T and 8 mismatches
- A·A, G·G, A·G, C·C, T·T, C·T, A·C, G·T

Mismatches in DNA



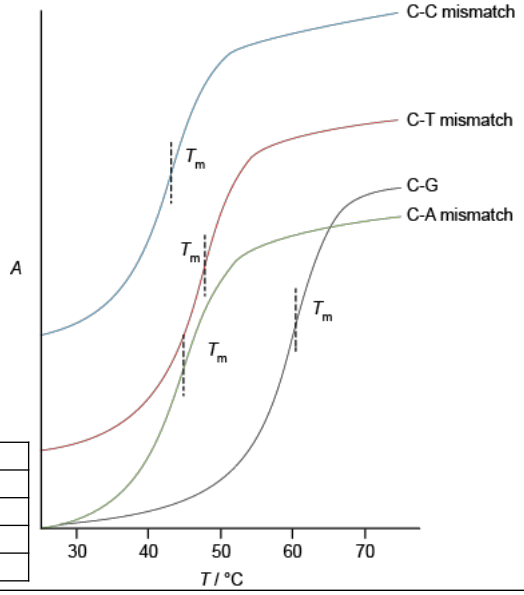
Types of mismatches

http://www.atdbio.com/content/15/Mutagenesis-and-DNA-repair

Stability of DNA Mismatch

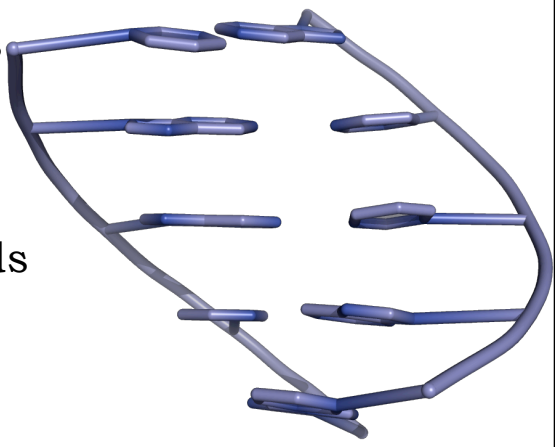
- Measurement of melting temp (Tm) of DNA duplex
- 14 bps DNA
- Complementary sequence with 1 bp mismatch

Base pair	T_m
C·G	60.5
C·C	43.2
C·A	45.2
C·T	47.4



Shape & Stability

- Misshapen bps unable to form stacking interactions
- Hydrogen bonds with water inhibited



Base stacking in DNA

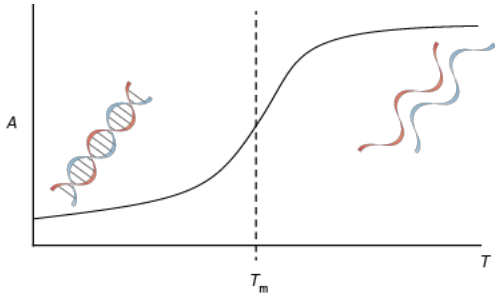
http://www.atdbio.com/

Stabilization

- High salt concentration masks repulsion between two negatively charged backbones- stabilizes
- Divalent Mg2+ more stabilizing than Na+ ions

Determining the Stability of DNA Duplexes

- Heat denaturation
- Hyperchromicity
- Absorbance of dsDNA lower than ssDNA



UV Hyperchromicity

- DNA bases aromatic- absorb in UV
- The λ_{max} is between 250-280nm
- Absorbance (Beer-Lambert)

$$A = \varepsilon \cdot c \cdot l$$

- ε=extinction coeff
- c=conc.
- l=path length through the solution

Extinction Coeff. Of Oligonucleotides

1. Base composition method (extinction coeff. of each nt)

ε_{260}= (nA*15.4 + nC*7.4 + nG*11.5 + nT*8.7)*0.9*1000 M^{-1} cm^{-1}

(0.9 compensates for base stacking, and 1000 for the order of magnitude)

2. Nearest neighbour method (empirically determined parameters)

ε_{260}=ε (end,N1)+ε (N1,N2)+ε (N2,N3)+…ε (Nn,end) M^{-1} cm^{-1}

Measured extinction coeff. of

end-A, end-T,…

AA, AT, AG, AC, TT, TA,…

Molar Extinction Coeffs

DNA		RNA	
Stack or monomer	Extinction coefficient	Stack or monomer	Extinction coefficient
pdA	15400	pA	15400
pdC	7400	pC	7200
pdG	11500	pG	11500
pdT	8700	pU	9900
dApdA	27400	ApA	27400
dApdC	21200	AdC	21000
dApdG	25000	ApG	25000
dApdT	22800	ApU	24000
dCpdA	21200	CpA	21000
dCpdC	14600	CpC	14200
dCpdG	18000	CpG	17800
dCpdT	15200	CpU	16200
dGpdA	25200	GpA	25200
dGpdC	17600	GpC	17400
dGpdG	21600	GpG	21600
dGpdT	20000	GpU	21200
dTpdA	23400	UpA	24600
dTpdC	16200	UpC	17200
dTpdG	19000	UpG	20000
dTpdT	16800	UpU	19600

ε for Short Oligos

- DNA oligonucleotide dAGCGT

1. Base composition ε_{260} = ((1 × 15.4) + (1 × 7.4) + (2 × 11.5) + (1 × 8.7)) × 0.9 × 1000 = 49050 $M^{-1}cm^{-1}$
2. Nearest neighbour ε_{260} = 50200 M^{-1} cm^{-1}

Modelling DNA Melting

- Conc. Of ssDNA strands (A and A')
- Conc. Of dsDNA (A)
- [A] + [A'] <-> [AA']
- Equilibrium constant K_{eq}

$$K_{eq} = \frac{[AA']}{[A][A']}$$

Free Energy

- Standard free energy and equilibrium constant

$$\Delta G^o = \Delta H^o - T\Delta S^o$$

$$\Delta G^o = -RT \ln K_{eq} = -Nk_B T \ln K_{eq}$$

- Solving for K_{eq}

$$K_{eq} = e^{\left[\frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}\right]} \qquad \text{Eq-1}$$

DNA Fraction & Thermodynamic Parameters

Css=[A]=[A']

Cds=[AA']

Total concentration (Ct) of DNA

Ct = 2*Css + 2*Cds

If f is the fraction of dsDNA

$$f = \frac{2 \cdot Cds}{Ct} = \frac{Ct - 2Css}{Ct} = 1 - 2\frac{Css}{Ct}$$

Fraction of ssDNA

- Conc. Of single stranded DNA

$$Css = \frac{(1-f)Ct}{2}$$

- Solving for Keq in terms of Css and Cds

$$K_{eq} = \frac{Cds}{Css^2} = \frac{f \cdot Ct/2}{\left[(1-f)Ct/2\right]^2} = \frac{2f}{(1-f)^2 Ct}$$

Melting Point

$$K_{eq} = \frac{2f}{(1-f)^2 Ct}$$

At the melting point, f=1/2 (by definition) and

$$K_{eq} = \frac{4}{Ct}$$

Substituting for Keq (Eq-1)

$$e^{\left[\frac{\Delta S}{R} - \frac{\Delta H}{RT}\right]} = \frac{2f}{(1-f)^2 Ct} \qquad \text{Eq-2}$$

Melting Point and DNA Fraction

- Solving for T by taking log

$$T(f) = \frac{\Delta H^o}{\Delta S^o - R\ln\left(2f / Ct\left(1-f\right)^2\right)}$$

Taking logs on Eq-2, resubstitution and eliminating non-physical roots

$$f = \frac{1 + Ct \cdot K_{eq} - \sqrt{1 + 2 \cdot Ct \cdot K_{eq}}}{Ct \cdot K_{eq}}$$

Fraction dsDNA and Energy

Substituting from Eq-1

$$f = \frac{1 + Ct \cdot e^{\left[\frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}\right]} - \sqrt{1 + 2 \cdot Ct \cdot e^{\left[\frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}\right]}}}{Ct \cdot e^{\left[\frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}\right]}}$$

Given values DNA conc., calculate change in enthalpy and entropy from nearest neighbour.

Naoki Sugimoto et al. (1996) Improved Thermodynamic Parameters and Helix Initiation Factor to Predict Stability of DNA Duplexes Nucl. Acid Res.

Entropy, Enthalpy: Nearest Neighbour Method

- 5'-CGCTTGA-3'
- 3'-GCGAACT-5'
- Free energy of formation at 37°C
- $\Delta G^{o}_{predict} = \Delta G^{o}_{37}(CG/initiation) + \Delta G^{o}_{37}(CG/GC) + \ldots$

$$\Delta G^{o}_{37} = \Delta G^{o}_{37}(\text{initiation}) + \sum_{i=1}^{N} n_i \cdot \Delta G^{o}_{37}(i)$$

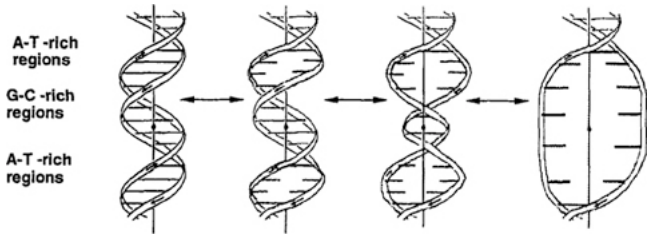
John SantaLucia Jr. (1998). "A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics". Proc. Natl. Acad. Sci. USA 95 (4): 1460–5

Simulated Melting

- Model the process
- Fit existing fraction vs. Temp. data to obtain ΔH and ΔS

Melting dsDNA

The melting of a DNA (or RNA) double helix



A-T regions melt first, then G-C regions

Heat to Melt

Absorbance at 260nm

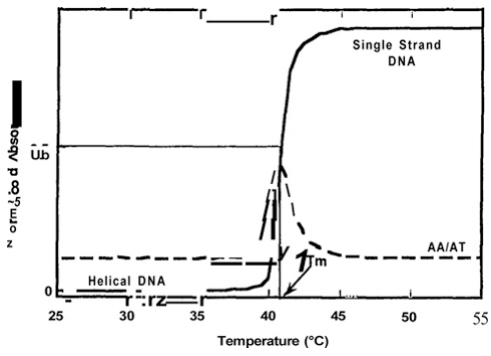
A_{ss}: Abs. single stranded DNA (Max at max temp)

A_h: Abs. helical DNA (Min at lowest temp)

A_T: Abs. at temp T

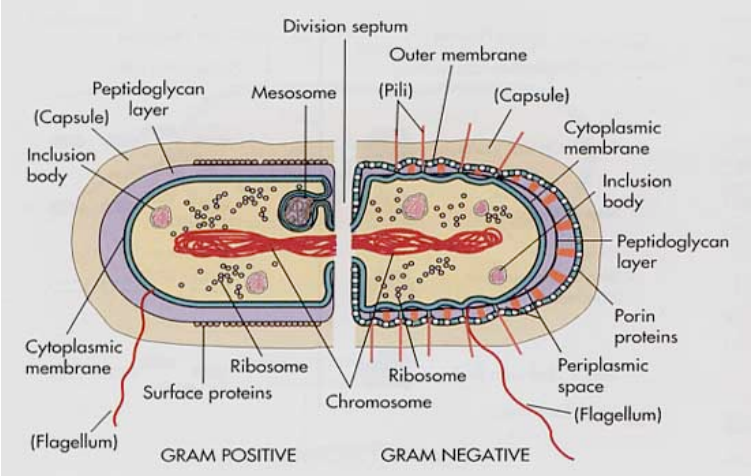
A_{norm} vs. T

dA_{norm}/dT vs. T



Normalized absorbance
$A_{norm} = (A_{ss}-A_T)/(A_T-A_h)$

Gram +/-ve Bacteria



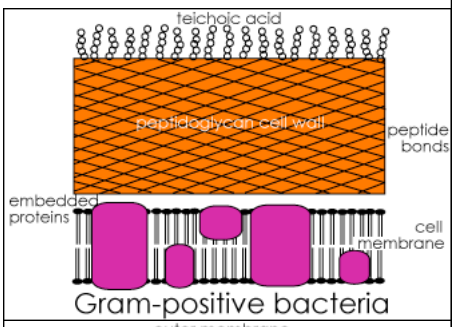
+ve -ve

Medical Microbiol. Proteus

Bacterial Walls

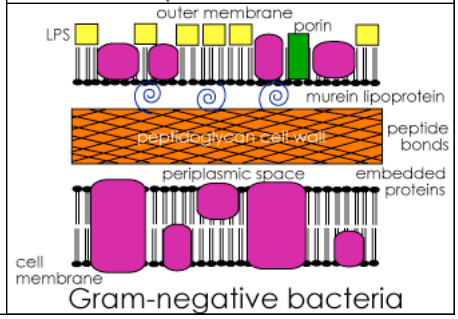
Gram positive

Eg.: Staphylococcus aureus, Bacillus subtilis



Gram negative

Eg.: Escherichia coli



http://www.hhmi.org/biointeractive/

What you will do

- Take E. coli (or S. aureus) DNA in solution (TA: Manasi Gangan)
- Dilute the DNA into clean micro-tubes to 0.4-1 ug/ul of DNA
- 1/2 will add 1M NaCal, 1/2 will add 0.1M MgCl2
- Submit lab report
- Discuss results in class group-wise **30 OCT**