Lab Math
Primer Preparation and Dilution

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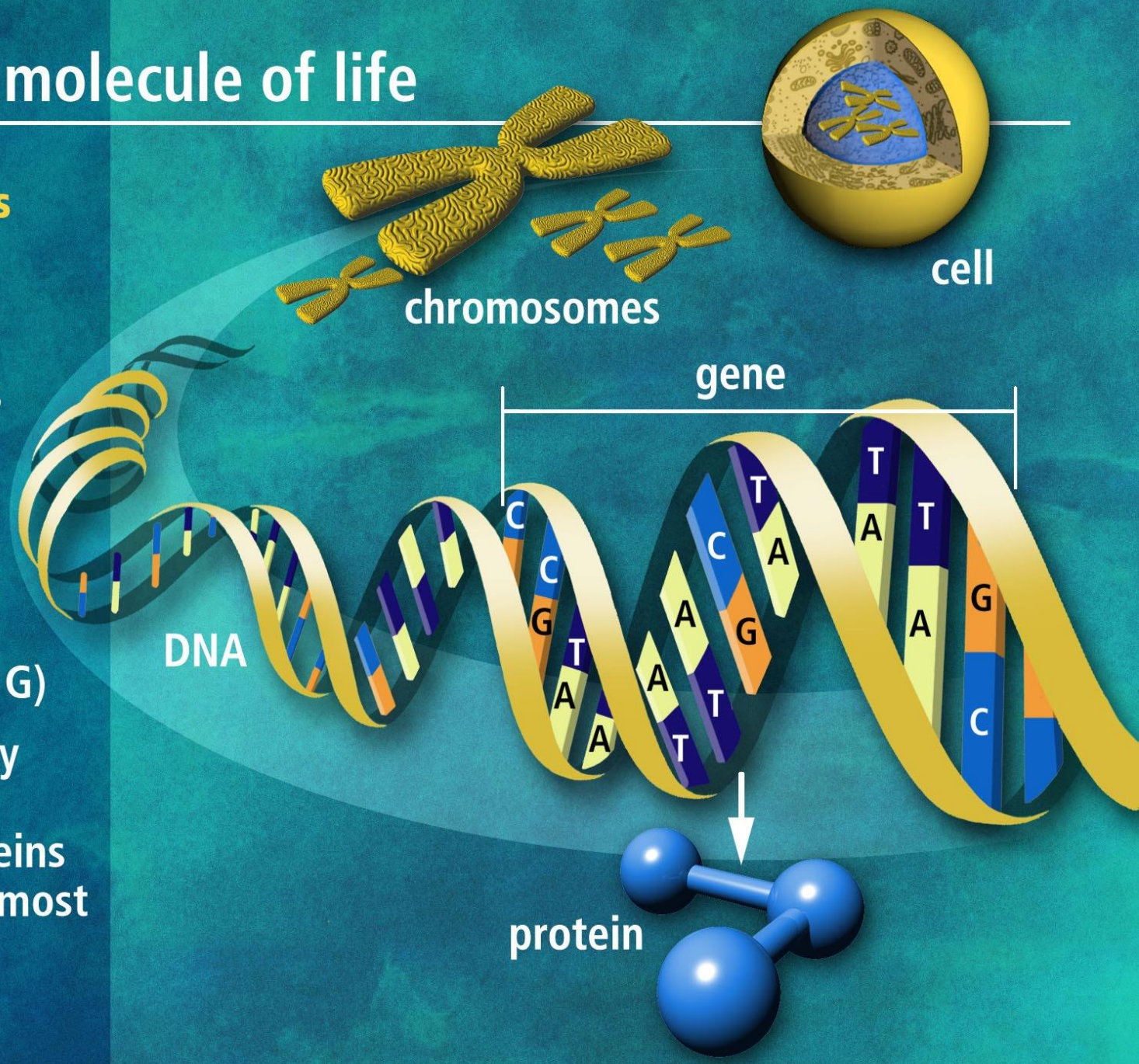
APHL-CDC

DNA the molecule of life

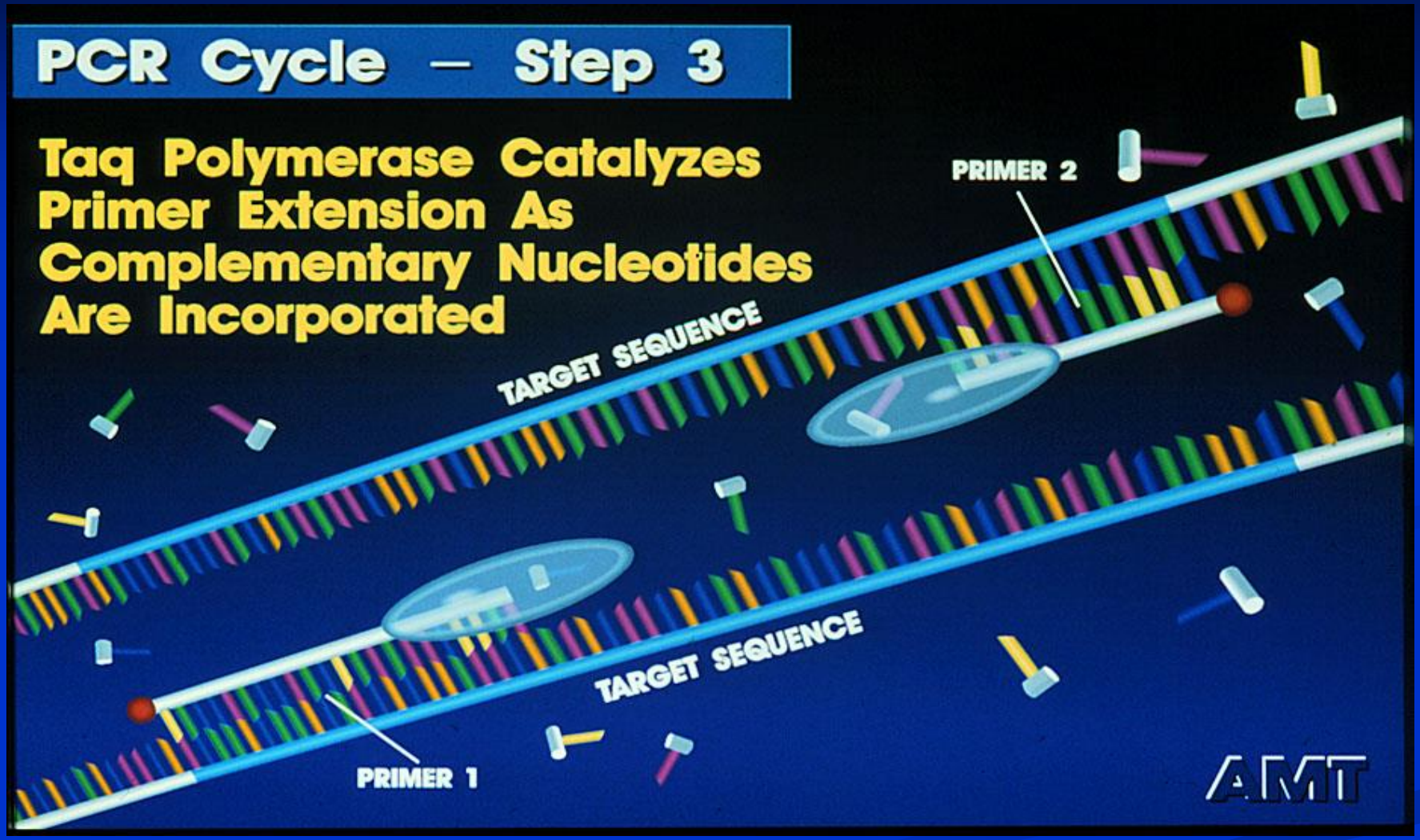
Trillions of cells

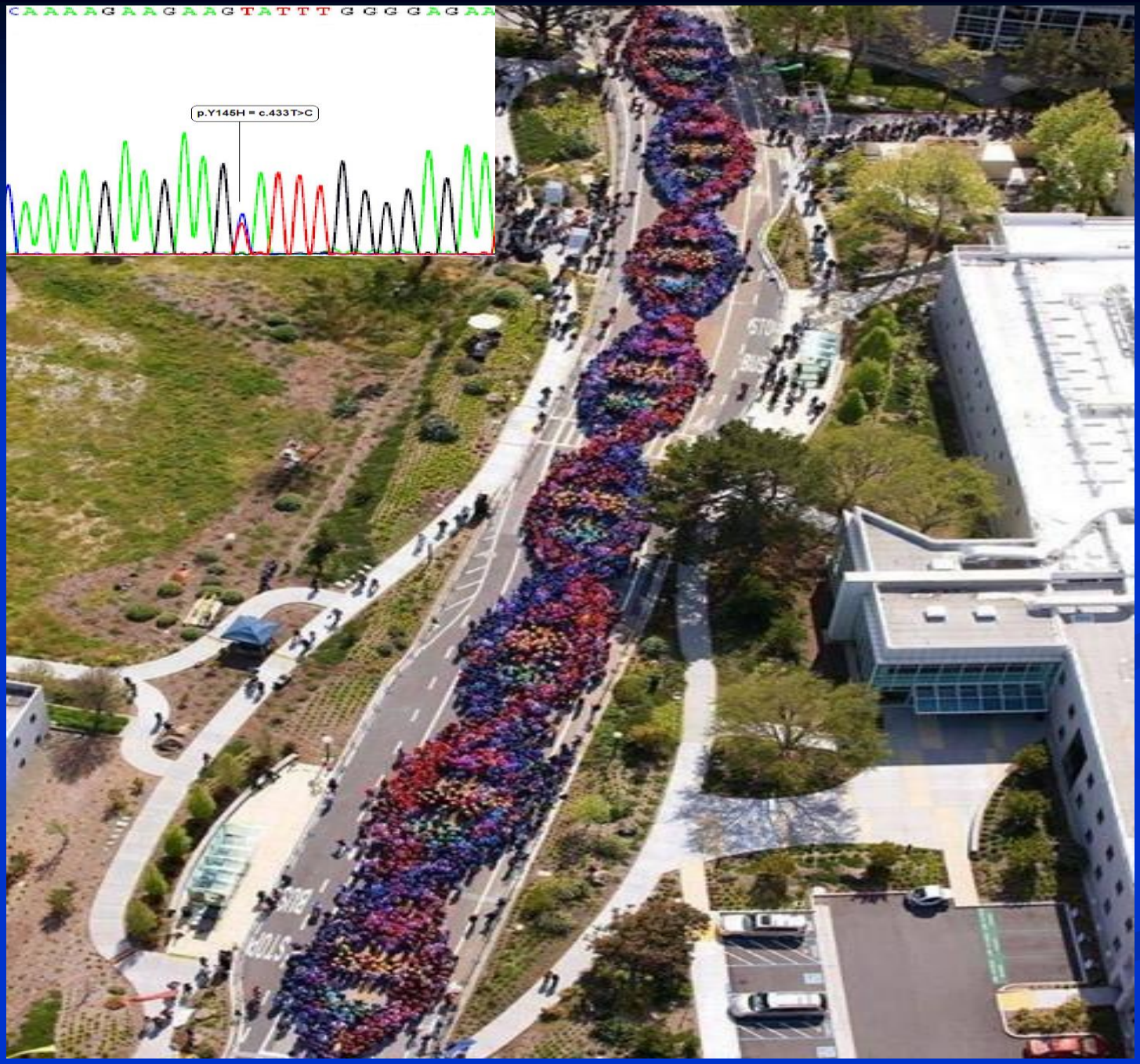
Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 30,000 genes code for proteins that perform most life functions



Y-GG 01-0085


PCR Cycle – Step 3
Taq Polymerase Catalyzes Primer Extension As Complementary Nucleotides Are Incorporated
PRIMER 2
TARGET SEQUENCE
TARGET SEQUENCE
PRIMER 1
AMT


CAAAAGAAGAAGTATTTTGGGGAGAA
p.Y145H = c.433T>C

Concentration of a Solution:

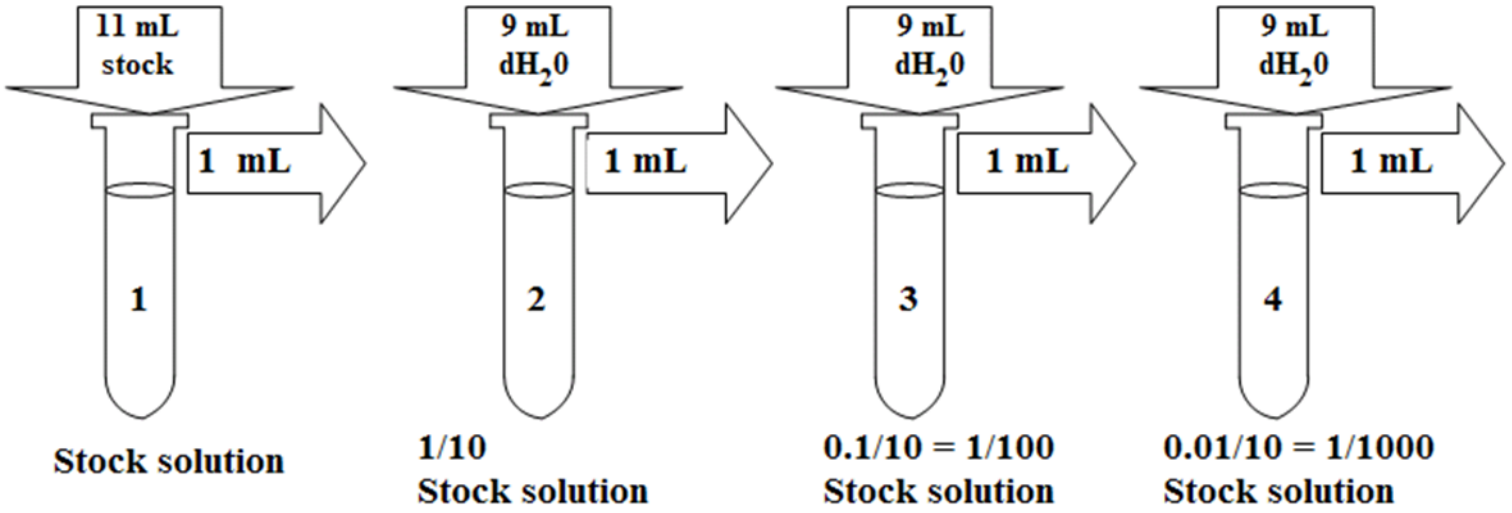
1M solution of **NaOH** ($^{23}Na^{16}O^{1}H$) = 40 g NaOH dissolved in 1 liter of H_2O = **1N**

1mM (miliM) = 1:1000 dilution of 1M or $\mathbf{10^{-3}}$

1µM (microM) = 1:1000 dilution of 1mM or 1:1,000,000 of 1M or $\mathbf{10^{-6}}$

1nM (nanoM) = 1:1000 dilution of 1 µM or 1:1,000,000,000 of 1M or $\mathbf{10^{-9}}$

1pM (picoM) = 1:1000 dilution of 1 nM or 1:1,000,000,000,000 of 1M or $\mathbf{10^{-12}}$



Demonstration: 100 µM = 100 µMoles/L = 0.1 nMoles/µL

100 µM = 100 µMoles/L

= 100 µMoles / 1,000 mL or 100 µMoles / 10^3 mL

= 100,000 nMoles / 1,000 mL or 100x10^3 nMoles / 10^3 mL

= 100 nMoles / mL

= 100 nMoles / 1,000 µL or 100 nMoles / 10^3 µL

= **0.1 nMoles/µL = 100 µM**

5' - LC705-AAgAgTTCggCATCAAggAgCATgg--PH

Amount / Concentration of product in 1 ml	3,0 nmol / 3,0 µM				
Synthesis scale and purification :	Synthesis: 0.00 µmol	Purification: HPLC	Condition: 5LC 3 nmol ly		
Modifications :	no				
Number of bases :	A : 8	G : 9	C 4	T : 4	total 25
Wobble bases and GC content :	Wobbel: 0		Mod.: 0	GC-content 52.0	%

Chemical properties and constant factors of the product :

Molar extinction coefficient ε	291490	l / mol cm
Molecular weight ammonium salt NH_4	8188,9	g / mol
Molecular weight free acid :	7780,2	g / mol
Picomoles per OD $_{260}$	3430,6	pmol / OD
Micrograms per OD $_{260}$	28,1	µg / OD

Delivered amount (per vial when delivered in aliquots)

Amount in optical units OD $_{260}$	0,9	OD
Molar amount :	3,0	nmol
Amount in µg mass units :	24,7	µg

Molar concentration when delivered in 1 ml solution :	3,0	µM	(pmol / µl)
20 µM (20 pmol/µl) requires a volume of :	151	µl	
50 µM (50 pmol/µl) requires a volume of :	60	µl	(To prepare stock solutions of
100 µM (100 pmol/µl) requires a volume of :	30	µl	different concentration)

Mass concentration (for hybridization) :		
Concentration, when dissolved in1 ml :	0,025	µg / µl
Dilution when preparing a solution with 0,5 µg/ml :	1 : 5	dilution factor from a 1 ml solution
To prepare a 0,1 µg / µl solution dissolve the product in :	247	µl

Melting point, thermodynamic approach (TIB MOLBIOL)	64,9	°C
Melting point in the case of a single mutation (-3,5 °C)	61,4	°C
Suggested PCR annealing temperature (<= 72°C) :	72,1	°C
Melting point GC/AT rule (A/T = 2°C, G/C = 4°C)	76,0	°C
Melting point G/C-content rule	61,8	°C

Thermodynamic parameter for the double stranded hybrid :

ΔG / ΔH / Δ S	-189.1	/ -797.0	/ -2040.2	kJ / mol

Code for degenerated base positions (wobble positions IUB Code)

S = G/C	Y = C/T	M = A/C	H = A/C/T	D = A/G/T	N = A/C/G/T	X = Modif.
W = A/T	R = A/G	K = G/T	B = C/G/T	V = A/C/G	I = Inosin	s = Thioate

Sequence - GALC-E2-R

250 nmole DNA oligo, 20 bases

5'- GAC GCA AAA ACA AAA GCA AA -3'

Properties

Tm (50mM NaCl): 50.9 °C
GC Content: 35. %
Molecular Weight: 6,154.1
nmoles/OD260: 4.6
ug/OD260: 28.2
Ext. Coefficient: 218,600 L/(mole·cm)

Amount Of Oligo

34.0 =	155.5	= 0.96
OD 260	*nMoles*	*mg*

Shipped To

NYS DEPARTMENT OF HEALTH WADSWC
ESP -WCLR BIGGS LABS #E224
ALBANY, NY 12237
USA
5184743853
Customer No. [redacted] *PO No.* [redacted]

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -0.31 at 25 °C
Strongest Folding Tm: 29.0 °C

Oligo Base Types

	Quantity
A Bases	20

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Modifications And Services

	Quantity
Standard Desalting	1

Mfg. ID [redacted]

Labels - Peel Here

I N S T R U C T I O N S

- Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo
- Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

Concept: 0.1 nMoles/µL = 100 µM

Primer reported Amount: **155.5 nMoles**

If you want a **Stock Solution** of 100 uM, resuspend oligo in 1555 µL of ddH_2O or TE buffer:

- 155.5 nMoles / 1550 µL = **0.1 nMoles/µL = 100 µM**

If you want a **Working Solution** of 10 uM make a 1:10 dilution from the stock:

- Add 1.0 µL from Stock (100 µM) to 9.0 of ddH_2O or TE buffer (1:10).

- 100 µM x 1 µL / 10 uL = **10 µM**

Concentration in your final PCR mix: $C_1V_1 = C_2V_2$

- $10\ \mu M \times V_1 = 0.3\ \mu M \times 20\ \mu L \longrightarrow V_1 = \frac{0.3\ \mu M \times 20\ \mu L}{10\ \mu M} = 0.6\ \mu L$

http://www.idtdna.com/Calc/resuspension/

Primer reported Amount: 155.5 nMoles = **0.96 mg** = 960 µg

If you want a **Stock Solution** of 1 µg/µL, resuspend oligo in 960 µL of ddH_2O or TE buffer:

- 960 µg / 960 µL = **1.0 µg/µL** = **1000 ng/µL**

If you want a **Working Solution** of 100 ng/µL make a 1:10 dilution from the stock:

- Add 1.0 µL from Stock (1 µg/µL) to 9.0 µL of ddH_2O or TE buffer (1:10).

- 1000 ng x 1 µL / 10 µL = **100 ng/µL**

Concentration in your final PCR mix: $C_1V_1 = C_2V_2$

- $100\ ng/\mu L \times V_1 = 3\ ng/\mu L \times 20\ \mu L \longrightarrow V_1 = \frac{3\ ng/\mu L \times 20\ \mu L}{100\ ng/\mu L} = 0.6\ \mu L$

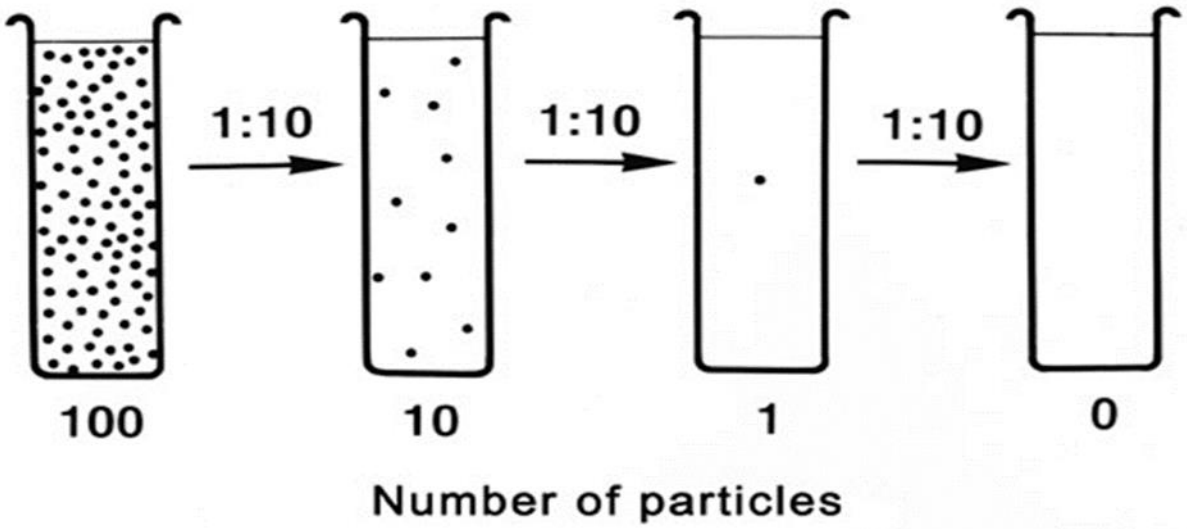
http://www.idtdna.com/Calc/resuspension/

Prepare Stock Solution and Working solution for primers forward and reverse to amplify exon 5 of the ABCD1 gene.

You have received:

ABCD1-E5-F1: 183.5 nMoles
ABCD1-E5-R1: 354.8 nMoles

Once prepared your primers do the calculations needed to run a PCR reaction using a 0.25 uM concentration for each primer in a 20 uL total volume PCR.

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