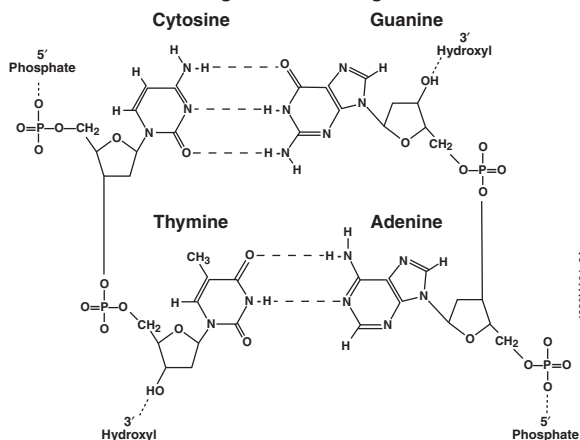


# Nucleotides and Nucleic Acids

## Nucleotide Base Pairing Structural Diagram.



## IUPAC Nucleotide Ambiguity Codes.

- Y = T or C (pyrimidine)
- R = G or A (purine)
- M = A or C (amino)
- K = G or T (keto)
- S = G or C (strong interaction: 3 H bonds)
- W = A or T (weak interaction: 2 H bonds)
- B = G or T or C (not A)
- V = G or C or A (not T, not U)
- D = G or A or T (not C)
- H = A or C or T (not G)
- N = G or A or T or C (unknown nucleotide)

## Physical Constants of NTPs.

Compounds	MW*	$\lambda_{max}$ at pH 7.0 (nm)	Absorbance at $\lambda_{max}$ for 1M Solution (E) (pH 7.0)
$\alpha$ -S dATP	507.3	259	15,400
$\alpha$ -S dCTP	483.3	271	9,100
$\alpha$ -S dGTP	523.3	252	13,700
$\alpha$ -S dTTP	498.3	267	9,650
ATP	507.2	259	15,400
CTP	483.2	271	9,000
dITP	492.2	249	12,200
dUTP	468.2	262	10,200
GTP	523.2	253	13,700
UTP	484.2	260	10,000
dATP	491.2	259	15,400
dCTP	467.2	272	9,100
dGTP	507.2	253	13,700
dTTP	482.2	267	9,600
iso-dC	481.2	260	6,300
iso-dG	507.2	292	11,000

\*Molecular weight of free acid with no waters of hydration.

## Conversion Formula

$$\frac{\text{observed absorbance at } \lambda_{max}}{\text{absorbance at } \lambda_{max} \text{ for 1M solution}} = \text{molar concentration of nucleic acid}$$

## Reference

Sambrook, J. and Russell, D.W. (2001) In: *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

## Copy Number of Commonly Used Plasmids.

Plasmid	Plasmid Size (approx.)	Origin of Replication*	Copy Number	**Yield per ml of Culture	Reference
pGEM® series	2,700bp	mutated pMB1	300-700	1.8-4.1 $\mu$ g	1
pUC	2,700bp	mutated pMB1	500-700	2.9-4.1 $\mu$ g	1
pBR322	4,400bp	pMB1	>25	>0.23 $\mu$ g	2
ColE1	4,500bp	ColE1	>15	>0.15 $\mu$ g	3
pACYC	4,000bp	p15A	~10	~0.09 $\mu$ g	4
pSC101	9,000bp	pSC101	~6	~0.12 $\mu$ g	5
HaloTag®	5,000bp	mutated pMB1	300-700	3.3-7.6 $\mu$ g	1
pGL series	5,000bp	mutated pMB1	300-700	3.3-7.6 $\mu$ g	1
pRL series	4,000bp	mutated pMB1	300-700	2.7-6.0 $\mu$ g	1
pMGFP	4,700bp	mutated pMB1	300-700	3.1-7.1 $\mu$ g	1
pGEM®-T/ T Easy	3,000bp	mutated pMB1	300-700	2.0-4.6 $\mu$ g	1
pGeneClip™ series	5,000bp	mutated pMB1	300-700	3.3-7.6 $\mu$ g	1
psiCHECK™- 1/2	4,000- 6,000bp	mutated pMB1	300-700	2.7-9.1 $\mu$ g	1
pTnT™	2,900bp	mutated pMB1	300-700	1.9-4.4 $\mu$ g	1
pCMVTnT™	4,000bp	mutated pMB1	300-700	2.7-6.0 $\mu$ g	1
pACT and pBIND series	6,000bp	mutated pMB1	300-700	4.0-9.1 $\mu$ g	1
pALTER®-1/Ex1	5,800bp	pMB1	>25	>0.3 $\mu$ g	3
pALTER®-Ex2	5,800bp	p15A	~10	~0.13 $\mu$ g	4
pSP	2,500bp	mutated pMB1	300-700	1.6-3.8 $\mu$ g	1
pCI, pSI	3,600bp	mutated pMB1	300-700	2.4-5.5 $\mu$ g	1

\* Plasmids carrying the pMB1, mutated pMB1 and ColE1 belong to the same incompatibility group, so they are not compatible with one another, but they are fully compatible with those carrying p15A and pSC101 replicons.

\*\* Theoretical plasmid yields were calculated from the reported copy number and size of each plasmid, assuming  $2.0 \times 10^8$  cells per milliliter of culture grown for 16 hours at 37°C.

## References

- Summerton, J., Atkins, T. and Bestwick, R. (1983) *Anal. Biochem.* **133**, 79.
- Holmes, D.S. and Quigley, M. (1981) *Anal. Biochem.* **114**, 193.
- Jansz, H.S., Pouwels, P.H. and Schiphorst, J. (1966) *Biochim. Biophys. Acta* **123**, 626.
- Birnboim, H.C. and Doly, J. (1979) *Nucl. Acids Res.* **7**, 1513.
- Birnboim, H.C. (1983) *Meth. Enzymol.* **100**, 243.

## Lengths and Molecular Weights of Common Nucleic Acids.

Nucleic Acid	Number of Nucleotides	Molecular Weight*
lambda DNA	48,502 (dsDNA)	$3.2 \times 10^7$
pBR322 DNA	4,361 (dsDNA)	$2.8 \times 10^6$
28S rRNA	4,800	$1.6 \times 10^6$
23S rRNA ( <i>E. coli</i> )	2,900	$1.0 \times 10^6$
18S rRNA	1,900	$6.5 \times 10^5$
16S rRNA ( <i>E. coli</i> )	1,500	$5.1 \times 10^5$
5S rRNA ( <i>E. coli</i> )	120	$4.1 \times 10^4$
tRNA ( <i>E. coli</i> )	75	$2.5 \times 10^4$

\*Molecular weights based on actual sequence.

## Standards

- Average MW of a dsDNA base pair = 660.
- Average MW of a ssDNA base = 330.
- Average MW of an RNA base = 340.

## References

- Daniels, D.L. et al. (1983) *Appendix II: Complete annotated lambda sequence*. In: *Lambda II*, ed., R.W. Hendrix et al., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 519.
- Sutcliffe, J.G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3737.
- Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.* **43**, 77.