

July 2008

There is an expanding array of help for scientists on the web if you know where to look. One of the best sites for information on methodologies is <http://www.protocol-online.org/>. In this month's article I've picked a few of the most useful general sites. If there are any others you think are helpful and would like to tell other people about or can't find what you're looking for then drop us a line (j.m.williams.med@bham.ac.uk). Happy experimenting!

Molecular Biology

[Codon Usage Database](#) (Yasukazu Nakamura at The First Laboratory for Plant Gene Research, Kazusa DNA Research Institute)

Codon Usage Database is an extended WWW version of CUTG (Codon Usage Tabulated from GenBank). The frequency of codon use in each organism is made searchable through this World Wide Web site

[Preparation of DNA ladder for agarose gels](#) (IzziD wetlab)

When you first start in a lab, it can be tricky to learn how to appropriately dilute the concentrated DNA ladders provided by experimental biology suppliers into a form suitable for agarose gel electrophoresis. You must dilute the DNA ladder stock with the appropriate amounts of TE and agarose gel loading dye. If you don't want to spend time preparing your DNA ladder, many biosupply companies provide ready-to-load ladders for about 2x the cost of concentrated ladder.

[Genetic Code and Nucleotide Ambiguity Codes](#) (Qiagen)

[Molecular-Weight Conversions for Nucleic Acids](#) (Qiagen)

Useful conversions of nucleic acids

[DNA Gel Electrophoresis](#) (Qiagen)

Useful data about:

Recommended agarose concentrations for resolution of linear dsDNA

DNA markers for gel electrophoresis (bp)

Recommended nondenaturing acrylamide concentrations for resolution of linear dsDNA

Dye migration in denaturing acrylamide gels

[The Restriction Enzyme Database \(REBASE\)](#) (REBASE)

contains a complete listing of all known restriction endonucleases, including: recognition sequences, methylation sensitivity, commercial availability and references. This work has been funded for many years by grants from the NSF and NIH to Dr. Richard J. Roberts.

[Alphabetic List of Commercial Restriction Endonucleases and Their Isoschizomers](#) (Fermentas)

[Commercial Restriction Endonucleases Sorted by Generated DNA Ends](#) (Fermentas)

[Factors that Influence Restriction Enzyme Activity](#) (Dr. Bowen et al, Colorado State University)

At times, the DNA does not appear to cut at all and sometimes it cuts only partially. This is a good summary of factors that may influence restriction activities.

[Star Activity](#) (NEB)

Conditions that contribute to Star Activity and how to inhibit star activity.

[Star Activity of Restriction Enzymes](#) (Takara)

There are some restriction enzymes which cleave partially different base sequence than recognition sequence as a result of reduced specificity when used in a large excess to substrate DNA. This phenomenon is called Star Activity. The page lists enzymes for which Star Activity is noticed, the conditions to accelerate its appearance, and the base sequences cleaved by Star Activity for which such reports are available.

[Plasmid Vectors, Viral DNAs and Bacteriophage](#) (New England Biolabs)

NEB's vector sequences and maps

[Spectrophotometric measurement of nucleic acids concentration](#) (Practical Molecular Biology)

This program helps you to calculate the concentration of nucleic acids according to their optical density. (DNA, RNA, oligonucleotides).

[Conversion: radioactivity - biological amount of dNTP](#) (Practical Molecular Biology)

The calculation of weight of particular amount of radioactive dNTPs. It may be helpful for estimation of how much template should be taken for DNA or RNA labeling. The specific activity [kCi/mmol] should be taken from the vendor catalog.

[Conversion: weight - moles \(for nucleic acids\)](#) (Practical Molecular Biology)

This program helps you to convert the weight (weight concentration) in the molar quantity (molar concentration) and vice versa.

[OligoAnalyzer](#) (IDT)

Cool tool for oligo analysis. Features include analysis of hairpin, self-dimer, heter-dimer, and other basic parameters.

[Primer3](#) (Whitehead Institute/MIT Center for Genome Research)

Very popular primer design tool for designing primers for PCR, hybridization.

[MethPrimer](#) (Long-Cheng Li, UCSF)

A free online program for designing primers for methylation specific PCR (MSP) and bisulfite sequencing PCR.

[Primerfinder](#) (Univ. of Texas-SW Med. Ctr)

Easy to use, free, online primer design program

[Primo](#) (Chang Bioscience)

Primo online is a friendly PCR primer design tool. It reduces PCR noise by lowering the probability of random priming. Batch mode allows designing for multiple sequences. Users can also calculate the melting temperature using the nearest neighbor model.

[The PCR Suite](#) (Van Baren MJ, Heutink P, Erasmus Medical Center)

a collection of programs that interact with the Whitehead's Primer3 program. The original Primer3 program designs primersets for one sequence and it allows you to change almost every parameter. The PCR Suite has more stringent settings (to allow more working primersets) and a smaller range of parameters (because you won't change most of them anyway). The suite consists of four programs: Overlapping_Primers is for creating multiple overlapping PCR products in one sequence. Genomic_Primers designs primers around exons in genomic sequence. All you need is a GenBank file containing your gene. SNP_Primers designs primers around every SNP in a GenBank file. cDNA_Primers designs primers around open reading frames. Simply upload a GenBank file containing your genes.

Tissue culture

[Cell Culture Media and Solutions](#) (Donis-Keller lab)

[The Cell Environment \(including types of culture medium\)](#) (Sigma)

In general terms cultured cells require a sterile environment and a supply of nutrients for growth. In addition the culture environment should be stable in terms of pH and temperature. A good introduction here to the purpose and types of various of media, supplements.

[Can you autoclave liquid media?](#) (Invitrogen)

[Cell Culture Media and Solutions](#) (Donis-Keller lab)

[Components and Supplementation of Various Culture Media](#) (Allan Bradley's Lab, Baylor College of Medicine, Houston, Texas)

Mostly ES culture media.

[Fetal bovine serum vs. Fetal calf serum](#) (Invitrogen)

What is the difference between fetal bovine serum and fetal calf serum?

[Heat Inactivation of Fetal Bovine Serum](#) (The Donelson Lab, University of Iowa)

Heat inactivating fetal calf serum

[primary human keratinocytes \(PHKs\) raft culture media](#) (Matt Lewis, Department of Pathology, University of Liverpool)

[Recipes for Tissue Culture Selective Drug Stocks](#) (Allan Bradley's Lab, Baylor College of Medicine, Houston, Texas)

[Serum Thawing & Heat Inactivation](#) (Dr. Volker Briese, Universitäts-Frauenklinik Rostock)

How to thaw serum and how to heat inactivate serum

[Solutions for Tissue/Cell Digestion](#) (Yu-li Wang's Lab, University of Massachusetts Medical School)

Trouble Shootings and FAQs

[How to make charcoal-stripped serum?](#)

[Why is 2-mercaptoethanol sometimes added to RPMI medium for culturing T-cells](#)

[Filtering for cell culture stuff](#)

Statistics

[Online Calculators for Scientists](#) (GraphPad Software)

Provides calculators for basic statistics such as student's t test, chi square test, sign test, generating random numbers and calculators for chemical and radiochemical data.

[Simple Interactive Statistical Analysis \(SISA\)](#) (SISA)

Provides many online tools for statistical analysis

[Statistical Calculators](#) (Department of Statistics, UCLA)

A collection of online statistical tools written in PHP.

[Online Statistic Tools](#) (College of Saint Benedict, Saint John's University)

Tools include:

Graphical Displays

Display of Statistics

Display of Distributions

Mean, Standard Deviation, etc

Student's *t*-Tests

Kolmogorov-Smirnov test

chi-square distribution test

contingency tables

Fisher Exact Test

ANOVA: ANalysis Of VAriance between groups

Ordinary Least Squares (Regression)

Beyond Ordinary Least Squares

WAPP: Fit to data with *y*-errors

Fit to data with errors in both coordinates

[Power Calculators](#) (Department of Statistics, UCLA)

Power calculators for different studies

[Sample Size and Power Calculation](#) (Russ Lenth, The University of Iowa)

Power and sample size estimation for planning a number of statistical studies

[Sample Size Calculation](#) (SISA)

The sample size calculation procedure provides a basic method of calculating sample sizes for two group comparisons.

[t-Tests](#) (Dr. Richard Lowry, Vassar College)

t-Tests for the significance of the difference between the means of two samples, including:

t-Test for Independent or Correlated Samples

t-Test for Independent Samples [cell-entry format]

t-Test for Correlated Samples [cell-entry format]
Single Sample t-Test.
.95 and .99 Confidence Intervals for the Estimated Mean of a Population

[T-Test](#) (College of Saint Benedict, Saint John's University)
Enter two sets of data so that Student's t-test can be used to determine if the averages of your two samples are significantly different.

Making solutions

[Compound solutions](#) (Practical Molecular Biology)
Composition for commonly used compound solutions with calculating tool.

[Solution Calculators](#) (GraphPad Software)
Create and dilute molar solutions.

[Ammonium Sulfate Calculator](#) (EnCor Biotechnology)
Calculate how much Ammonium Sulfate to weigh out to make a solution of a specific level of saturation.

[Buffer Calculator](#) (Dr. Simon Dawson, University of Nottingham Medical School)
For buffer preparation

[Buffers for pH Control](#) (Department of Biomolecular Sciences, UMIST)
for the calculation of thermodynamically correct buffer solutions

[Calculator for Making up Solutions](#) (EnCor Biotechnology)
All the time Scientists have to calculate how much of a compound to weigh out to make a certain volume of solution at a certain molarity. It is quite easy to make mistakes doing this, and this generally has negative consequences for the experiment. This little program will do the calculation for you

[Recipe Calculator](#) (Chang Bioscience)
A simple web-based tool for making solutions

[Compound solutions](#) (Practical Molecular Biology)
Composition for commonly used compound solutions with calculating tool.

[How to Make Simple Solutions and Dilutions](#) (Department of Biology, Bates College)
Simple Dilution (Dilution Factor Method)/ Serial Dilution / Making fixed volumes of specific concentrations from liquid reagents

[Reto's Buffers&Media Book](#) (Private Page)
Recipes for most common solutions, buffers and media indexed alphabetically

[Salt solutions](#) (Practical Molecular Biology)
Recipes and calculating tool for commonly used salt solutions

[Single Component Solutions](#) (Practical Molecular Biology)
Recipes for many single component solutions.

[Buffers](#) (Gerard R. Lazo)
Recipes for commonly used buffer solutions.

[Receipes](#) (Universitäts-Frauenklinik Rostock)
Receipes for commonly used reagents, culture media, solutions including: Acetone / Formaldehyde Fixative, Acrylamide Gel, denaturing, Acrylamide Gel, nondenaturing, Alkaline Phosphatase Buffer 1 (Glycine Buffer, 0.1 M, pH 10.4), Alkaline Phosphatase Buffer 2 (Tris-HCl, 100 mM, pH 9.5), Alkaline Phosphatase Stain (NBT + BCIP), Amido Black Destain, Ammonium Bicarbonate Buffer, 100x, Annealing Buffer, Antibiotics for Microbiology, Aprotinin Stock Solution, BLOTTO 1 x, Bouins Fixative, Butanol, water saturated, Cadmium Chloride Stock Solution, Calcium Chloride for Competent Cells, Calcium Chloride Stock Solution, CIP Buffer (10x), Coomassie Destain, Coomassie Stain, Crystal Violet Stock Solution, Cytosol Preparation Buffer, DEAE DNA Elution Buffer I, DEAE DNA Elution Buffer II, DEAE Membrane Wash Buffer, Denhardt's, 50 x, DMEM/MCDB-302, DMEM, DTT Stock Solution, EDTA Stock Solution, 0.5

M, EMSA Binding Buffer, Fischer's Medium (for Nb2 cells), Glycerol Stock Solution, 80%, HBS (HEPES buffered saline), HBSS (Hanks Balanced Salt Solution), HBHA (Hanks Balanced Salt Solution with BSA, HEPES, NaN₃), HeBS (Hepes buffered saline) buffer for electroporation, HEPES Stock Solution, IL-2 Stock Solution, Immunoprecipitation buffer (cell lysis buffer 2), Immunoprecipitation dilution buffer, Immunoprecipitation wash buffer, IPTG Stock, KCl Stock Solution, 2 M, LB Agar, LB Medium, Lower Tris Buffer, 4x, Magnesium Chloride Stock Solution, MEM, NaCl Stock, 5 M, NaOH Stock, 10 N, NCTC (medium for RCHO-1 cells), NZY broth, NZY plates, PBS / EDTA solution, PBS Stock Solution, PIPES Stock Solution, PMSF Stock 0.1 M, R10 (medium for KY1 and KY2 cells), RIPA buffer (cell lysis buffer 1), RNK (medium for RNK-16 cells), RPMI 1640 (medium for YAC-1 cells), SDS-PAGE, 4 x Running Buffer, SDS-PAGE, 4 x Sample Buffer, SDS Stock Solution, 10%, 20%, SM buffer, SOB-medium, SOC-medium, Sodium Acetate, 3 M, SSC, 20 x, SSPE, 20 x, Stacking Gel, STE, 1 X (for Nuc Trap), STE for Genomic DNA Isolation, TAE Buffer Stock Solution, 50 x, TBE Stock Solution, 5 x, TBS/TBST buffer, 10x, TDMN Buffer, TE Buffer, Top Agar, Transfer Buffer (for Western Blotting), Tris Stock Solution, 1 M, Upper Tris Buffer, 4x, Zinc Chloride Stock Solution,

[Antibiotics](#) (Gerard R. Lazo)

[Buffer Listing](#) (Salmon Lab, Department of Biology, University of North Carolina at Chapel Hill)

Trouble Shootings and FAQs

[Why use imidazole as a buffer?](#)

[Why SDS is used in so many places?](#)

[Why is TRIS used in DNA extraction?](#)

[How to adjust pH of TRIS-HCl solution without adding novel ions?](#)

[Any other reagents could substitute DTT?](#)

[How to dissolve EDTA?](#)

[How to make nuclease free water?](#)

[How to making TE buffer?](#)

[Science behind commonly used solutions](#)

[Dilution calculations](#)

[Calculation on dilutions](#)

General

[General Reference](#) (Dr. Volker Briese, Universitäts-Frauenklinik Rostock)

General information on: Units conversions/ Formulas/ DNA mobility in gels / Amino acid symbols / Restriction enzymes / Universal primers / Listing of M13 (pUC) cloning sites /

[Amino Acids](#) (Qiagen)

Basic information for amino acids

[Protein Analysis](#) (Qiagen)

[Common protein markers for gel electrophoresis](#)

[Substrates for ELISA, immunoblotting, and immunohistology](#)

[Radii of commonly used rotors](#) (Qiagen)

Commonly used rotors from various vendors and conversions

[Codon Optimization Calculator](#) (EnCor Biotechnology Inc.)

This simple program will translate a protein sequence into nucleic acids. The user can select to translate using the most frequently found codons in yeast, mammalian, insect or bacterial expression systems. It is used to optimize protein expression, particularly of human genes in bacteria and yeast.

[Molecular Weight Markers](#) (BioProtocol)

List of commonly used molecular weight markers

[Nucleic Acids and Proteins: Bacterial Cells](#) (Qiagen)

[Nucleic Acids and Proteins: Mammalian Cells and Tissues](#) (Qiagen)

Including:

Nucleic acid content of a typical human cell

RNA distribution in a typical mammalian cell

mRNA classification based on abundance

RNA content in various cells and tissues
Human blood: DNA, RNA, and protein content
Growth areas and numbers of HeLa cells in various culture vessels

[Online Calculators for Scientists](#) (GraphPad Software)

Provides calculators for basic statistics such as student's t test, chi square test, sign test, generating random numbers and calculators for chemical and radiochemical data.

[Blood Volume Calculator for Rodents](#) (EnCor Biotechnology)

Blood volume can be related to body weight using the experimentally determined equation of Lee and Blafox; $BV = 0.06 \times BW + 0.77$. In which blood volume = BV in mL and BW = body weight in grams. The equation was developed for the rat, but is reasonably accurate for mice, rabbits, guinea pigs etc. Original reference Lee HB and Blafox MD. Blood volume in the rat.

[Capillary Electrophoresis Calculator](#) (Beckman)

[Conversion: DNA - protein](#) (Practical Molecular Biology)

This program helps you to estimate the size of the gene by the protein size and vice versa: the size of the protein by the size of the gene.

[Conversion: molar quantity - molar concentration](#) (Practical Molecular Biology)

This program helps you to convert the molar quantity in the molar concentration and vice versa.

[Conversion: weight - moles \(for proteins\)](#) (Practical Molecular Biology)

This program helps you to convert the weight (weight concentration) in the molar quantity (molar concentration) and vice versa.

[Moles <> Grams converter](#) (GraphPad Software)

Convert between moles and grams