# Table of Contents

**Bio 385L - Molecular Biology Lab**
**Spring 2013**

**Learning Priorities for This Year's Lab**

**Priority One:** technical proficiency in bench-based tools used in recombinant DNA technology. Working with DNA, DNA, and more DNA!

-isolating DNA
-handling DNA
-manipulating DNA
-analyzing DNA

**Priority Two:** molecular bio lab bench math - conversions, calculations for setting up reactions.

**Priority Three:** using bioinformatic tools

-using computer based software for deciphering DNA and protein sequences
-working with bioinformatics databases

**Bio 385L Spring 2014**
**Guidelines for Lab Notebooks in Molecular Lab**

For the Molecular Lab, our goal for keeping a lab book is to have a form of chronological "diary" for recording raw data, gel pictures, and notes that will enable you to keep track of your experiments on a week to week basis.

It should not be as extensive or detailed as a research lab notebook so we will have a kind of stripped down version according to the following format:

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✓**Date**

✓**Brief title** of the lab days activities or experiments

✓I**. Introduction**

- a few sentences on what it is you are going to do or accomplish that lab day

✓**II. Notes During Experiments**

-information on set that accompanies an experimental task or special instructions from the instructor are recorded here.
-measurements, calculations, preliminary data
-quick notes and reminders - anything that you have to jot down

✓**III. Results**

-any final outcome of a lab activity or experiment is posted in this section
-examples include gel photos, numerical data, spectral diagrams from nanodrop instrument

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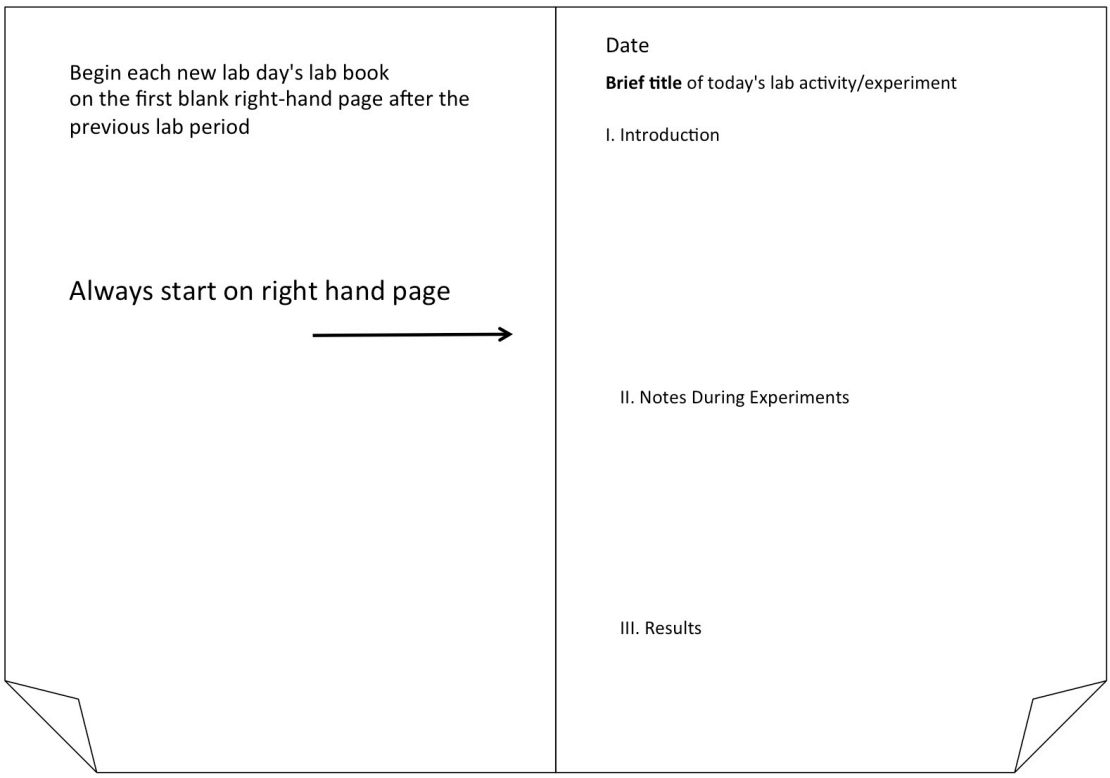
How to set up lab books each new lab day:



It will not be necessary to rewrite detailed protocol steps in your lab book. Any procedures we do will usually be included in your lab manual as a printed protocol. On the other hand, you are welcome to make your lab book as detailed as you want if this is helpful to you. Many students who go on to interview for laboratory positions are sometimes asked to show one of their lab notebooks. In these cases, students who compose a polished lab book have a ready credential for getting a job.

What I will be looking for is your ability to collect key data and notes that you can retrieve later for a an assignment based on your experiments. Usually, it should be no problem to get full credit each time the lab books are reviewed.

For each new lab day, you will make the following entries as follows

**Here are some guidelines on the minimum that should appear in the lab book on a week to week basis.**

✓Each new lab period, start another new page, on the right hand side of the open lab book.
✓Record the date at the top for each new lab.
✓Record bench notes as the lab goes on rather than waiting to record them at the end. For example, if you are assembling a restriction digest, set up a recipe table with brief notes on what and why you are digesting a DNA. Make a notes on how long you incubated the reactions, how you terminated the reactions and if you stored it directly in the freezer or ran it on a gel.

-very minor notes are helpful as you repeat procedures from week to week:

for example, how much you loaded into a gel, the voltage setting you used to run the gel,

✓Make a note if you did anything unusual happen during your experiment that could effect the outcome later.....for example, if you felt you lost your DNA pellet in the washing step.

✓record raw data such as nanodrop numbers with enough notes so you know what sample/procedure those numbers are from.

✓scotch tape gel pics into your lab books and label as directed on the "example" picture in the lab manual.

In the end of the day, the lab book should have a record of all the unique details of the days activities. Hopefully, it will serve the purpose of giving the continuity you will need for learning at the bench despite having only a once a week access.

**General Protocol for precipitation of nucleic acids (DNA and RNA) from aqueous solutions**

**1. determine the starting volume of the DNA solution you wish to subject to EtOH ppt.**

-for example, suppose we have a starting volume of 20 μL

**2. add 1/10 volume of 3M Sodium Acetate (NaAc) to the DNA solution.**

-this means you multiply the starting volume by 0.1 to arrive at the volume of 3 M NaAc you need to add.

-in the example above, our starting volume is 20 μL of DNA solution, how much 3 M NaAc do we need to add?

20 μL x 0.1 = 2 μL.....we will need to add 2 μL of 3 M NaAc to the 20 μL of DNA.

**3. next, add 2 volumes of 100% EtOH to the 1 volume of DNA solution.**

-this means you multiply the current volume of the DNA solution X 2 to arrive at the volume of 100% EtOH you need to add.

-in the example above, our current volume of DNA + NaAc solution is 22 μL. How much 100% EtOH do we need to add?

22 μL x 2 = 44 μL .....we will need to add 44 μL of 100% EtOH to the 22 μL DNA+NaAc solution.

**4.** mix briefly by finger flick or inverting tube.

**5.** microfuge on top speed for 10 min.

**6.** remove and decant supernatant

**7.** "wash" DNA pellet with 1 mL 70% EtOH - add, cap tube, invert, and remove 70% EtOH using a fine tip transfer pipet.

**8.** let DNA pellet air dry for 3-5 min. The DNA pellet is now ready for resuspension in new buffer.

**DNA-Agarose Gel Electrophoresis**

Agarose gels are used to separate DNA fragments following a diagnostic or preparative DNA. Agarose gels are made to a concentration range of 0.5% to 2.0%. The standard concentration for general use is 0.8%. The following are some guidelines on what concentration of agarose you may want to use depending on the size of DNA fragment you want to resolve.

In general, the smaller the fragment you want to resolve the higher the concentration of agarose you will need in the gel.

**Gel Percentages: Resolution of Linear DNA on Agarose Gels.**

| Recommended % Agarose | Optimum Resolution for Linear DNA (Size of fragments in base pairs) |
|---|---|
| 0.5 | 1,000–30,000 |
| 0.7 | 800–12,000 |
| 1.0 | 500–10,000 |
| 1.2 | 400–7,000 |
| 1.5 | 200–3,000 |
| 2.0 | 50–2,000 |

**Casting the gel**

1. Set up gel tray-mold

2. pour molten agarose from stock bottle into the gel mold so the level just reaches almost the top of the teeth of comb (don't overfill) & let cool. (you may want to cast the gel in the fridge to hurry things along)

3. Use when gel solidifies (i.e.-when gel turns cloudy).

**Running the gel**

1. gently remove comb by pulling straight out – DO NOT squeegee the comb back and forth to get out of gel!
2. Place gel in the gel box so wells are at the black electrode end of the box
3. Pour 1X TBE running buffer into gel box - make sure the buffer covers the gel – just covering the surface of the gel is best
4. Load samples and DNA standard ladder
5. Hook up electrodes, black to black and red to red, and turn power on to 100 volts
6. Run gel for about an 45 min to 1 hour or until blue tracking dye band reaches at least 2/3 into the gel or to the bottom (max) of the gel
7. Visualize gel on the on the gel doc imager

**Restriction Digests - General Protocol**

There are generally 2 kinds of restriction digests: a diagnostic digest and a preparative digest:

**diagnostic restriction digest**- performed in a small volume - the end use is for analytical gel electrophoresis - no further use for the digested DNA other than viewing in a gel.

**preparative restriction digest**-is performed in a larger reaction volume and with a higher amount of DNA ....DNA from the digest will be physically isolated for an application.

**I. diagnostic restriction digest - typical volume = 25 μL, Typical amount of DNA used = about 1-2 μg**

**1. First, write out the recipe table for your reaction -** Always write out this standard recipe table in your lab books before putting together your reaction:

be sure to indicate the name of the DNA sample and the name of the restriction enzyme

| | ingredient | volume to add |
|---|---|---|
| ➡ | DNA (i.e., a plasmid) | 2.5 μl (i.e. from a miniprep) |
| | 10X Fast Digest buffer | 2.5 μl |
| | *RNAse | 1 μl (from stock tube)----add this only if the DNA fragment you want to see is less than 700 BP |
| ➡ | Restriction Enzyme | 2 μl |
| | $H_2O$ | 17 μl |
| | Total vol | 25 μl |

**2. ADD IN ORDER:** In a 1.5 mL microfuge tube, add contents *in the following order:* $H_2O$…….10X buffer…… DNA…….RNase .....restriction enzyme last )

**3. Mix** contents by finger flick or quick vortex

**4. Incubate** the reaction in the 37°C water bath for a standard time of 10 minutes

Minimum time for incubation = 10 min

**5. STOP** by adding 4 μL of EZ vision blue stop-buffer, mix as above, and proceed to load 20 μL into a single well of an agarose gel ***OR*** store the tube in the freezer until you are ready to run the gel.

**Plasmid DNA Mini Prep**

You will need extra for this procedure:

-an ice bucket
-a tube of phenol/chloroform
-a tube of SDS/NaOH

**1.** Transfer *E. coli* culture into a 1.5 ml cfg tube directly from culture tube flask.

**2.** Cap and spin in microfuge set on max for 2 min.

**3.** Remove clear amber broth from pellet by pouring out broth in a waste container or sink. Try to get as much of the broth out of the tube by gently flicking tube in sink or waste container.

**4.** Add 100 μL of GTE solution (Glucose-Tris-EDTA), and resuspend cells using a p200 pipetman set for 100 μL. Do not suck the cells into the pipet shaft.

**5.** Add 200 μL of the SDS/NaOH solution (this is in a 15 mL conical tube on your bench). Cap, mix gently by GENTLE inversion so the contents eventually turns a clear amber. This means all the cells are lysed and the solution becomes syrupy.

**6.** Incubate tubes on ice for 5 min.

**7.** Add 150 μL of 5M K-Acetate solution to tubes. Mix again by *gentle* inversion several times so a white precipitate forms.

**8.** Incubate on ice for 5 min.

**9.** Spin tubes for 5 min - max speed, in microfuge.

**10.** Using a p1000 pipetman, carefully remove the supernatant to a new tube WITHOUT transferring any of the white precipitate.....should be about 400-500 μL of supernatant

WATCH OUT !

Be sure to KEEP THE SUPERNATANT.....this contains the plasmid DNA fraction

*and* DISCARD the TUBE WITH THE WHITE GUNK PELLET

**11.** Next, add 500μl of phenol/chloroform to the supernatant

**12.** Cap tightly and vortex briefly (few seconds) so contents form a uniform dispersed sol.

**13.** Microfuge for ~3 min - max speed.

**14.** Recover the upper aqueous phase:

Carefully remove the upper, clear phase (about 500 μL at this point) to a new tube. Use either a p1000 pipetman or p200 pipette man to transfer. Be careful not to suck any of the gunk from the phenol-chloroform interface. If you get a little, its OK. If you accidentally get a lot of this, re-spin the tube for another minute and try again.

**15.** Add 1 ml (1000 μL) of 100% ethanol to the tube with the transferred supernatant.

If you have an excess of 500 μL of supernatant, add an equal volume of 100% isopropanol instead (i.e. 700uL of super, add 700 μL of isopropanol.)

**16.** Cap and mix contents of tubes by hand via quick inversion of the tube several times.

Pellet the plasmid DNA: Centrifuge tubes (HINGE UP) at high speed for 10 min.

**17.** Carefully pour supernatant in waste container. The DNA, which should appear as a tiny white pellet, may get unstuck so you have to take care and not dump it out along with the supernatant. Its OK to leave a little super behind.

**18.** Wash the plasmid DNA pellet: Add 1 ml 70% ETOH, cap and gently invert tube to mix. If your DNA pellet is still stuck at this point, you can pour the super out directly into the waste container. Be careful, the pellet may come loose during this decanting step ..... watch the tube as you pour out the 70% EtOH so the pellet does not get dumped out.

If the pellets are loose OR you want to ensure that your pellets are stuck to the tube, spin the tubes in the microfuge on high for 2 min and then decant ETOH.

**19.** Use a fine tip transfer pipet to suck out the last traces of ETOH. You can use the same pipette for all tubes so long as you don't gouge the DNA pellet with the tip.

air dry the pellets for ~5 min by leaving the caps open.

**20.** Resuspend the plasmid pellet by adding 40 μL of TE to each tube. Try using a p200 pipetman set at 30 μL and gently pumping up and down to so the pellet dissolves.

**21.** Store in your the fridge or freezer box.

**The Alkaline Lysis Plasmid DNA Miniprep Method|Principles of:**

**1. Cell Growth and Harvesting**

The procedure starts with the growth of the bacteria in cell culture harboring your plasmid. When sufficient growth has been achieved, the cells are pelleted by centrifugation to remove them from the growth medium.

**2. Re-suspension - GTE**

**G**lucose - a sugar that keeps the osmotic pressure even so the cells don't burst until the next step

**T**ris buffer - keeps the pH at neutral

**E**DTA - EDTA chelates (removes) divalent cations ($Mg^{2+}$, $Ca^{2+}$) from the solution. This inhibits DNA nucleases (DNases) from degrading the plasmid since these enzymes require $Mg^{2+}$ & $Ca^{2+}$ to work. The bacterial cell wall is also weakened by the removal of $Mg^{2+}$ & $Ca^{2+}$, thus aiding in the next step when the cells are bursted to release plasmid.

**3. Lysis Step - SDS-NaOH solution**

SDS - Sodium Dodecyl Sulfate, a soap detergent. SDS helps to dissolves the cell wall and cell membrane thereby releasing plasmid DNA to the solution. SDS also denatures most of the proteins in the cells thereby rendering them insoluble, which helps in the separation of the proteins from the plasmid DNA later in the process.

NaOH - sodium hydroxide, as strong base. NaOH increases the pH to be extremely alkaline (high pH).

The high pH disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the cell's chromosomal DNA (gDNA) and the plasmid DNA, to single stranded DNA (ssDNA). This process is called **denaturation** and is central part of the procedure, which is why it's called alkaline lysis method.

**4. Neutralization Step - 5 M KAc, pH 5.5**

-causes the precipitation of chromosomal DNA and protein away from the soluble plasmid DNA.

Addition of potassium acetate (KAc) decreases the alkalinity of the mixture, restoring the pH to near neutral. Under these conditions the hydrogen bonding between the bases of the single stranded DNA can be re-established. This allows the DNA that was made single-stranded in step 3 to re-nature back into dsDNA. However, while the tiny circular plasmid DNA can quickly re-nature back into soluble dsDNA, the gigantic single stranded chromosomal DNA molecule re natures very slowly. This is why it's important to mix the tubes gently during step 3 because vigorous mixing or vortexing will shear the chromosomal DNA into small fragments that will co-purify with the smaller plasmid DNA molecules.

-The white precipitate is a complex of single-stranded chromosomal DNA, SDS, and denatured cellular protein. The white precipitate is then separated from the soluble plasmid DNA by centrifugation.

**5. Phenol/Chloroform extraction step**

Phenol, chloroform - organic solvents - immiscible with aqueous solutions - when vortexed with the soluble plasmid DNA, can denature and precipitate away contaminating proteins that may interfere with use of the plasmid DNA. During centrifugation, the water-soluble DNA partitions into the UPPER aqueous phase, while the proteins are denatured by the organic solvents and stay in the LOWER organic phase. The aqueous phase containing the protein-free DNA can then be collected.

**6. Ethanol Precipitation**

Ethanol precipitation of DNA is a tried and tested method for concentrating DNA. Ethanol changes the DNA structure so that the DNA molecules aggregate and precipitate from solution. Ethanol precipitation only works if

monovalent salt ions ($Na^+$, $K^+$) is also in the solution. In the case of the miniprep, these were added as KAc and NaOH in the steps before. So, there is a high concentration of $Na^+$, $K^+$ required of the EtOH ppt procedure.

**8. Washing the DNA pellet**

70% EtOH - After removal of the supernatant above the plasmid DNA pellet, 70% ethanol is added. Since most salts and small organic molecules are soluble in 70% ethanol they are "washed" away from the DNA pellet, which remains insoluble in 70% EtOH

This step is important since salts, if they were to remain in the DNA, can inhibit further manipulation of the DNA (termed downstream uses).

**Protocol for the Purification of DNA from gel bands using Zymo brand spin-columns**

**1.** Excise desired DNA band from gel:

-place gel on light-box
-using a clean razor blade, excise the DNA band out of the agarose gel much cutting a block of ice out of a frozen pond
- cut and trim away agarose as close to the DNA band as possible.
-transfer cube of agarose to a 1.5 ml microcentrifuge tube.

**2.** Extract DNA from agarose gel slice:

✓weigh agarose slice

- place an empty microfuge tube on the pan balance and tare so the tube is ZEROed
- next, place the tube containing the excised band on the balance and record the weight..... the weight in grams is also the liquid volume of your gel slice in mL's, since your agarose gel is ~99.2 % water and 1 metric gram = the weight of 1 mL of $H_2O$.

for example, if your gel slice weighs 0.23 g, then this is 0.23 mL which is 230 μL

*If your gel slice weighs more than 0.300 grams, split it in two and do two tubes.*

✓liquefy the gel slice

-using a p1000, add an amount of MELT BUFFER in a proportion of 3 X the volume of your gel slice

for example, if your gel slice volume is 0.23 mL (230 μL), add 3 X 230 μL melt buffer [total 690 μL]

- incubate tube in the 50 °C water bath until the gel slice is melted (takes 5-10 min)
.....***tip***: try inverting the tube occasionally to hasten melting.

✓bind DNA to the matrix of a Zymo DNA-binding spin-column

- transfer the liquefied gel slice into a Zymo spin-column pre-inserted into a clean 1.5 mL microfuge tube
- centrifuge on high for 60 sec, DISCARD flow-through, replace microfuge tube.

✓wash DNA bound in the Zymo spin column

- using a p200, add 200 μL of WASH BUFFER to the spin column insert and centrifuge on high for ~30 sec.
- discard flow-through and repeat wash-step once more.

✓elute the bound DNA off the matrix of the spin column

- transfer the spin-column to a new 1.5 mL microfuge tube
- using a p20, add 10 μL of TE buffer* to the column, let sit 30 sec
- centrifugation on high for 60 sec, SAVE flow through....this is your DNA

*TE is 10 mM Tris, pH 7.5 - 1 mM EDTA buffer: a universal storage buffer used for DNA

✓ place the recovered DNA on ice & measure the eluted DNA using the nanodrop.

**Protocol for Genomic DNA isolation using Zymo Genomic DNA spin columns**

**Part I.** Isolation of squamous cheek cells

have ready at your bench:

-two 50 mL falcon tubes and a tube holder
-0.9% saline solution
-each table has a set of tubes: 2X digest buffer, genomic lysis buffer, DNA pre-wash buffer, g-DNA wash buffer, DNA elution buffer.
-one zymo-spin IIC column + collection tube

**1.** swish mouth out with saline into a 50 mL falcon tube as follows:

-get two 50 mL falcon tubes
-pour ~ 40 mL saline solution into one of the tubes
-sip a mouthful (about 15-20 mL) of the saline, swish back and forth for 5-10 secs and spit into the clean tube
-repeat again so you have collect 30 mL of mouth cells

-screw cap onto tube and proceed to centrifuge in SL 203

(we will do the following steps as a class)

**2.** centrifuge tubes at 7000 rpm for 5 min to pellet cells

**3.** remove tubes and carefully decant supernatant into the sink and then further remove super from the pellet with a fine-tip dispo pipet

**4.** take tube back into the lab and park on ice, and proceed to the attached ZYMO genomic DNA miniprep protocol below.

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**Part II.** Isolation of gDNA using Zymo spin-column/miniprep method

**1.** Add digest buffer to your cell pellet:

**Hard pellet or Soft pellet?**

✓if you had a hard cell pellet after centrifugation and were able to remove most of the supernatant

add **100 µL** of cell digest buffer

✓if you had a soft cell pellet after centrifugation and had significant supernatant remaining in the tube,

add **200-300 µL** of cell digest buffer

**2.** gently resuspend cells with pre-wrapped dispo transfer pipet and transfer to a 1.5 mL microfuge tube.

**3.** incubate in the 55°C water bath for at least 1 hour.

**4.** have ready a Zymo genomic DNA spin column inserted into a collection tube - labeled with your initials

**5.** Add genomic lysis buffer (on side bench)

➔if you used 100 μL of digest buffer in step 1, **ADD 700 μL** genomic lysis buffer

➔if you used 200 μL or more of digest buffer in step 1, transfer to a 2.0 mL microfuge tube and **ADD 1400 μL** genomic lysis buffer

**6.** cap and mix contents vigorously by briefly vortexing

**7.** using a pre-wrapped dispo transfer pipet, load and run entire tube contents through the Zymo genomic DNA spin column - to get it all passed through the column, it may take two spins.

PLEASE NOTE! SET CENTRIFUGES FOR **G-FORCE 10,000**-----NOT for RPMs!

-centrifuge loaded spin columns for 1 min. at 10,000 X *g*

-discard flow through, the genomic DNA is bound to glass fiber matrix in the columns spout

**8.** Transfer spin column to a new collection tube.

- then, add 200 μL of DNA Pre-Wash buffer
- centrifuge for 1 min. at 10,000 X *g*
- discard flow through

**9.** Next, add 400 μL of g-DNA Wash buffer - use same collection tube

- centrifuge for 1 min. at 10,000 X *g*
- discard flow through

**10.** Elute genomic DNA from spin column:

-transfer column to a regular 1.5 mL microfuge tube
-elute DNA by add 50 μL of TE buffer to the glass fiber matrix in the column and let sit for 2-3 minutes

(TE buffer loosens the DNA from the glass fiber matrix)

-centrifuge on MAX RPM for 30 sec

Your genomic DNA is now contained in the 50 μL TE.

**11.** Lastly, do a nanodrop assay of your isolated DNA

**-record the concentration and $A_{260}/A_{280}$ purity ratio**

**12.** Store your genomic DNA in your freezer box for next weeks experiment.

## Protocol for Transforming Competent *E. coli* Cells with Plasmid DNA

The rule of thumb when handling frozen competent cells is **KEEP IT COOL!**
They are very sensitive to being warmed and will not work as well if not kept on ice.

Here is a paragraph concerning this from the Zymo instructions that was sent with the *E. coli* cells:

> **Important!** Since chemically competent cells are extremely sensitive to changes in temperature, transformation should be performed immediately after thawing. The cells should avoid being exposed to room temperature for more than a few seconds. To mix cells after DNA addition, gently tap the tube with your fingers and then shake the tube downwards in a single motion from the elbow to collect the mixture at the bottom of the tube. Avoid repeated pipetting. Immediately transfer the transformation mixture(s) to ice or an ice bath ($0^{o}$C).

### Protocol - have ready at your bench:

**✓ice bucket**
**✓your ligation reaction**
**✓two petri plates per person**
**✓sterile plastic petri plate spreaders**
**✓15 mL tube of SOC broth**
**✓1 tube of competent cells per person**

**-IMPORTANT! competent cells - keep on ice at all times until just before spreading them onto a petri plate**

1. Add 2 µL of ligation reaction to a tube of Z-Competent™ cells on ice, mix gently for a few seconds (see boxed-note above for how to do this)

2. Incubate on ice for at least 5 minutes (maximum 60 minutes).

3. Remove cells from ice and QUICKLY add 400 µL of SOC broth [a 15 mL tube is on your bench) cap, and invert gently.

4. - open the lids on both petri-plates Using a p200 aliquot 30 µL of cells into the middle of one plate and 150 µL into the other plate.

-spread uniformly with a dispo-plate spreader, replace lids, and let sit on bench for 3-5 min (let the cells soak into the agar)

5. label your plates (your names) on the agar part of the plate, and place into the 37°C incubator AGAR SIDE UP. - incubate O/N

# Visual Violet™ Gel Kit

**General Information:**

The Visual Violet™ Gel Kit provides an in-gel stain that eliminates the need for UV irradiation of agarose gels for visualization of DNA bands following electrophoresis. Since UV light introduces nicks as well as other lesions in DNA, it can impact downstream applications sensitive to DNA damage. With Visual Violet™, cloning efficiency can increase 3 to 5 fold over that obtained with Ethidium Bromide stained gels.

Electrophoresis can be monitored in real time with Visual Violet™ and the run can be stopped as soon as the desired band resolution is obtained. Bands are visible immediately after electrophoresis with the naked eye or on a white (visible spectrum) light box without destaining. Fragments may be excised and purified for use in downstream applications by conventional methods including spin-column chromatography and alcohol precipitation.

The sensitivity of Visual Violet™ is less than Ethidium Bromide. While DNA amounts of 50 ng per band can be visualized for 1000 bp fragments, concentrations of 200 ng or higher are recommended for optimal visualization. Visual Violet™ is ideal for detection of medium to large DNA fragments (500 to >40,000 bp).

## Our modified Protocol

**1.** Casting the agarose gel

-set up agarose gel tray with a 6-tooth comb

Use molten agarose located in water bath...this is 0.8% agarose in 1X TBE buffer:

-pour molten agar into a 50 mL falcon tube to about the 50 mL mark
-add 250 µL Visual Violet Gel Stain
-screw cap on tube, invert to mix into molten agar
-pour into gel mold

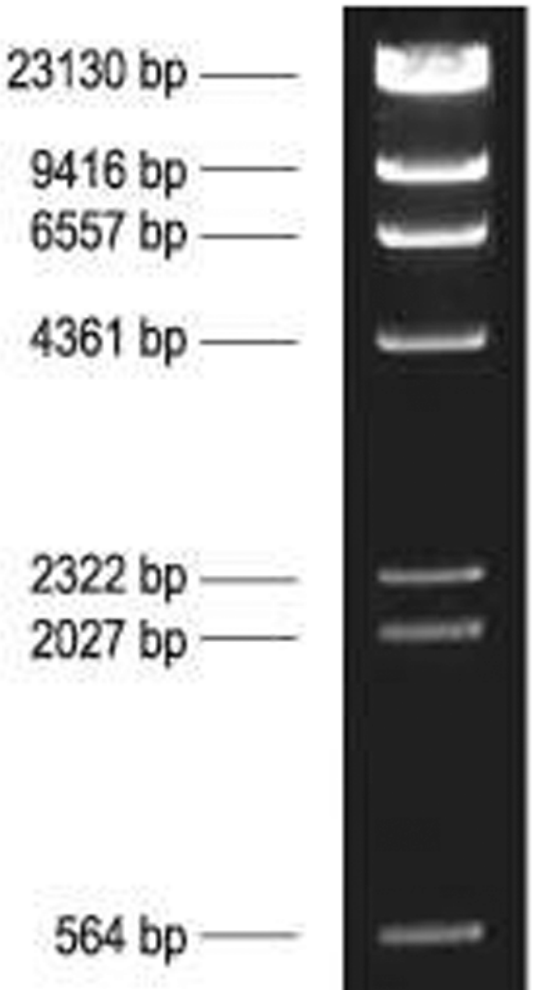
**2.** we wish to load 40 µL of your 50 µL PCR reaction into one lane of the gel. Follow the directions in (#2) on the right for adding visual violet loading buffer to your PCR sample.

*The visual violet loading buffer is different than the the visual violet gel stain!*

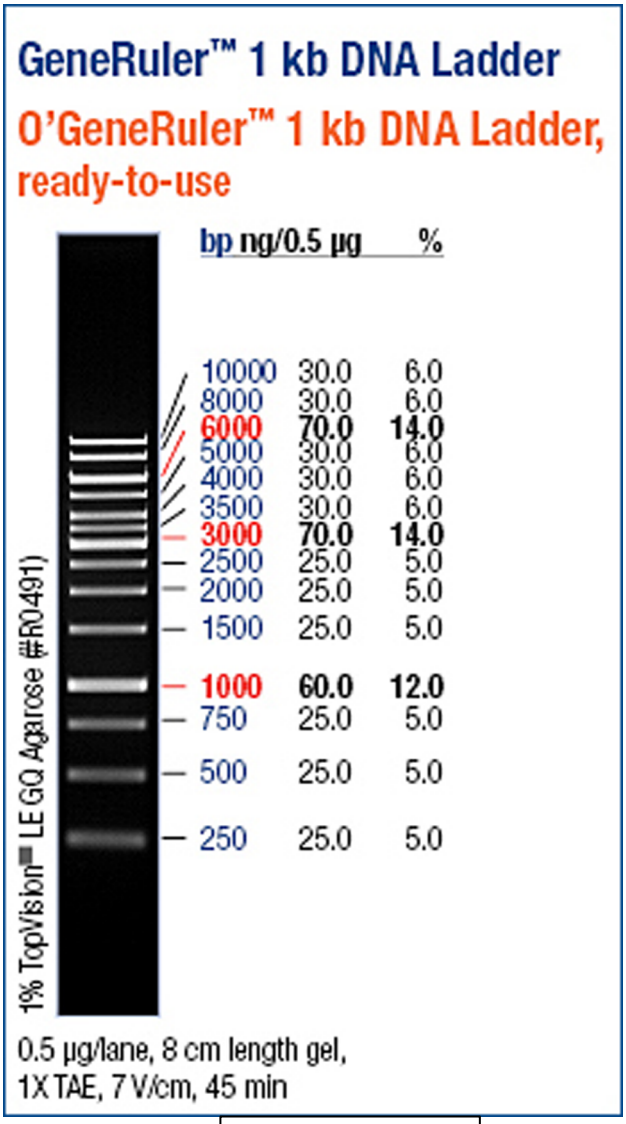
## Protocol:

1. Visual Violet™ Gel Stain, supplied as a 200X solution, should be added to the melted agarose immediately before gel casting.
   (Example:)
   - Melt 1 g of Agarose I™ (Code #: 0710) in 100 mL of 1X TAE
   - Cool agarose to 60°C
   - Add 0.5 mL of Visual Violet™ Gel Stain and mix
   - Pour agarose into gel casting system, add comb and let gel solidify
   - After gel solidifies, remove comb and submerge gel in 1X TAE running buffer. Gel should just be submerged to maximize staining sensitivity
2. Mix 1 volume of Visual Violet™ Loading Buffer, 6X, with 5 volumes of each DNA sample.
3. Load DNA samples and resolve DNA at 5 – 8 V/cm
4. Important, DO NOT let the DNA migrate beyond the Visual Violet™ Dye front.
5. After the run, remove gel and place on a light box for optimum visualization of the DNA.

# DNA standard ladders we will use in this lab




Lambda Hind III ladder ($\lambda H_3$)




1 kb ladder

**Most of the time, we will use three sizes:**

**20 µl**

**200 µl**

**1000 µl = 1 ml**

1 µl = micro liter = 1 millionth of a liter = 1 x $10^{-6}$ liters

1 µl = 1 thousandth of a ml = 1 x $10^{-3}$ ml

**What we use pipet men for:**

(i) aliquoting

(ii) mixing/suspending - for small volumes

**How to set pipetman for a given volume:** always dial just above the desired volume and then dial down to it. DO NOT dial directly from a lower volume setting to a higher volume setting.

The confusing thing about using micropipettors is what you read on the dial or setting is different for each of the pipettors. For example, 100 on the on the p1000 pipettor is actually 1000 µl. And 020 on the p20 pipettor is not 20 µl but 2 µl. So the best way to learn how to set each one for a given volume is just to practice with them enough until it becomes intuitive.

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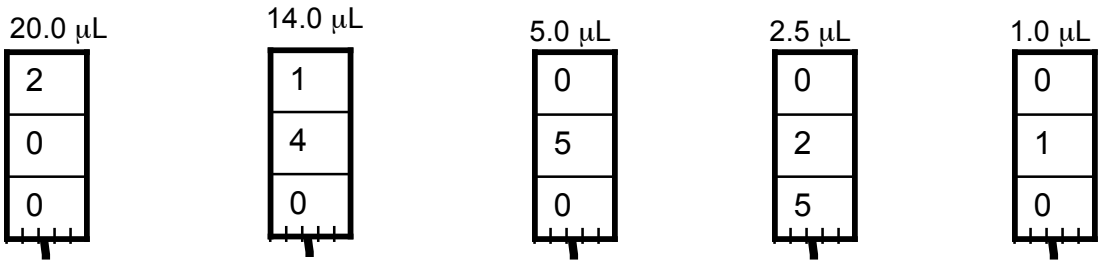
example settings **p20, 20 µl** pipettor

**p 20**

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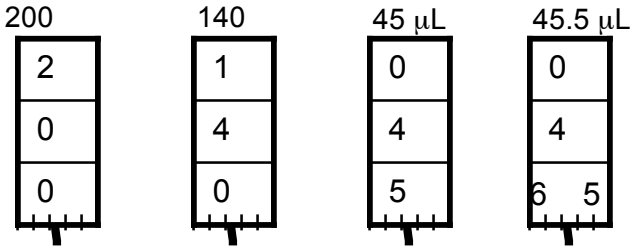
example settings **p200, 200 µl** pipettor

**p 200**

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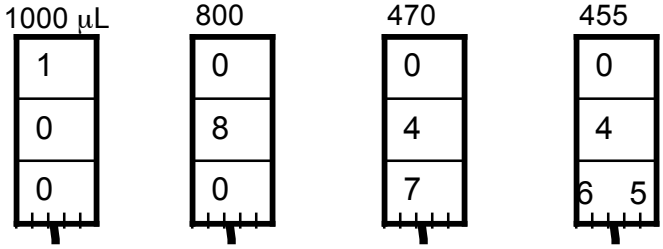
example settings **p1000, 1000 µl** pipettor

**p 1000**



***Rules of thumb on how to choose the correct size pippetor to use for a given volume are:***

-for pippeting volumes from 0.5 to 20 μl use the p20 μl pipettor

-for pippeting volumes from 21 to 200 μl use the p200 μl pippetor

-for pippeting volumes from 201 to 1000 μl use the p1000 μl pipettor

**Don't,** for example, use the 200 μl pipettor for 15 μl volumes.

**Some other pointers:**

**1.** When dipping your pipette tip into liquid to suck up fluid, just stick the tip a little below the surface. Don't plunge it way to the bottom. Otherwise, you will not get a very accurate volume.

**2.** When letting the button up to bring fluid into the pipet tip, let it up slowly and carefully. If you let it up to fast, then you end up sucking fluid into the pipettor.

**3.** When using the p200 or p1000 pipettor to mix or resuspend something, don't set the volume any higher than about a 150 μl. When you resuspend and suck up and down repetitively, the fluid will tends to creep up into the pipettor shaft.

**4.** When using the p200 or p1000 pipettor to mix or resuspend something, set the volume so it is less than the volume of what you are trying to mix. This prevents air bubbles and foaming in your sample.

# How I want you to label your gel photos in your lab book

**Rule 1 – Mount** Trim excess photo paper from gel pic - & tape the gel pic using scotch tape seamlessly to the page. ***Important!*** Mount the gel pic on the page so it is not sideways in your lab book but reads up-down like the page of a book

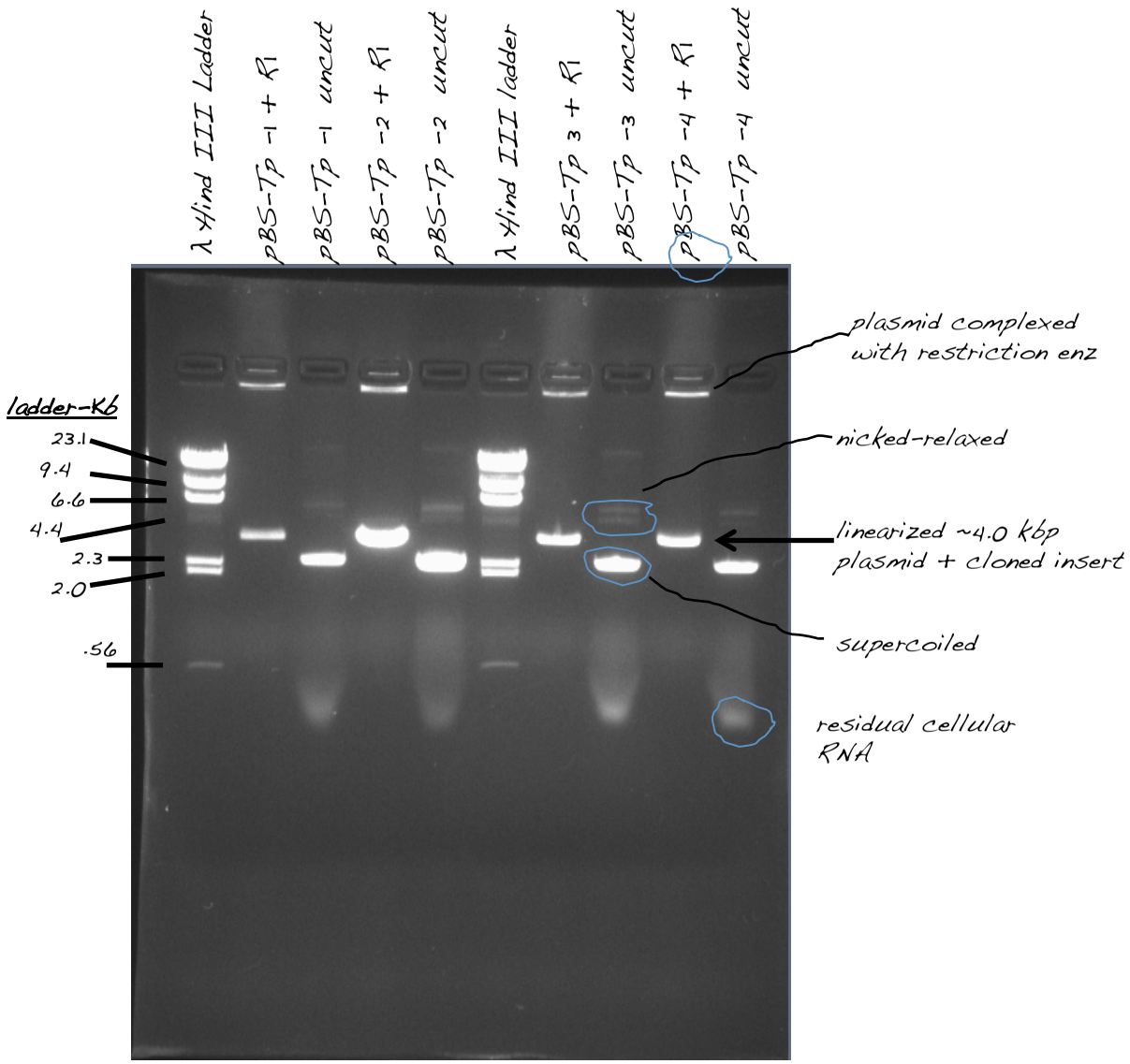
**Rule 2 - DNA standard ladder** - label each band in of the DNA ladder as to it size in base-pairs (bp) or kilo-bp (kb)

**Rule 3 - Record** the identity of the DNA sample in each lane above the gel wells - write vertically (see example gel pic) using short-hand abbreviations.

**Rule 4 - Identify** with a label what all bands in your sample lanes are as best you can by either circling and drawing extending just outside the gel pic with a short label as to the bands identity OR drawing an arrowed line to the band and connecting a label as to its identity just outside the gel pic.

**Rule 5 – Estimate** size of all bands in your sample lanes s as to their size in bp based on the DNA ladder in the gel or what from a predicted size you calculated from a diagrammatic map of the plasmid or DNA sample in question.

**Rule 6 - Anomolies** – note any anomolies in your gel (such as the residual RNA, smears , etc. ) label or mark as needed.



## Rules for labeling gel pictures in your lab book

**Rule 1** - Mount the gel pic on the page so it is not sideways in your lab book but reads up-down like the page of a book
Tape the gel pic using scotch tape seamlessly to the page.

**Rule 2 -** DNA standard ladder - label each band in of the DNA ladder as to it size in base-pairs (bp) or kilo-bp (kb) (see example gel pic)

**Rule 3** record the identity of the DNA sample in each lane above the gel wells - write vertically (see example gel pic)
using short-hand abbreviations:

Examples of various DNA samples and how to label

***1 - HOW TO LABEL*** plasmids: pUC 19, pBS II, pGlo, etc. ---- all plasmids begin with lower case p and then the abbreviated name of the plasmid.

***2 - HOW TO LABEL*** plasmid clones: these are plasmids with a segment of foreign DNA inserted. These are labeled beginning with the plasmid name followed by the abbreviation of the name of the foreign DNA insert.

examples

pUC 19 - ACHE....stands for plasmid UC 19 with a acetyl cholinesterase gene (abbreviated ACHE) insert

pUC 18 - pdk.....stands for plasmid UC 18 with a plant gene insert named pdk (pyruvate dikinase gene).

pGem3 - EK.....stands for plasmid Gem 3 with a gene named enterokinase (EK) inserted

***3 - HOW TO LABEL*** plasmid DNA that has been digested with a restriction enzyme (see example gel pic)

-simply tack "+ " and the name of the restriction enzyme on the end of the DNA sample label.

examples

pUC 19 + Eco R1.....stands for plasmid UC 19 that has been incubated with (e.g., digested with) Eco R1 restriction enzyme

pGem3-EK + Hind III.....stands for plasmid Gem 3 that has been incubated with (e.g., digested with) Hind III restriction enzyme

***4 - HOW TO LABEL*** control DNA sample lanes

if you are electrophoresing some DNA as a control to contrast the same DNA that has been restriction digested simply tack on the term "uncut" to the DNA sample label:

examples

pUC 19 - uncut

pGem3-EK - uncut

***5 - HOW TO LABEL*** non-plasmid DNA samples (PCR DNA, genomic DNA)

-use a handy abbreviation that is derived from the sequence of DNA that was the target of the PCR reaction

example

PCR generated DNA from the human chromosome locus named Alu I .....use the label "Alu I PCR"

**Rule 4 -** Label all bands in your sample lanes as best you can by either circling and drawing extending just outside the gel pic with a short label as to the bands identity OR drawing an arrowed line to the band and connecting a label as to its identity just outside the gel box see (example gel pic).

-if you don't know what it is, place a ? next to it.

**Rule 5 -** All bands in your sample lanes should have an estimate as to their size in bp based on the DNA ladder in the gel or what from a predicted size you calculated from a diagrammatic map of the plasmid or DNA sample in question.

-if you can't estimate the size of the band, place a ? next to it.

## DNA spectrophotometric assays

## Quantity and Quality

✓Quantity = The concentration of DNA in a solution
✓Quality = The purity of DNA in a solution (i.e., the lack of contaminating substances in your DNA solution)

*Quantity* is important since applications you wish to use your DNA for such as a restriction digest require that you add exact amounts of DNA to the reaction.

*Quality* is important since contaminating substances can interfere or inhibit reactions used for manipulating DNA.

Thus, it is important to determine both the quantity and quality of a preparation of DNA for further use in downstream applications.

**Part I. Assaying for Quantity of DNA**

**A.** The basis of the $A_{260}$ DNA spec assay is the same for all spectrophotometric based molecule assays:

"Beer's Law"

Beer's law states that the absorbance by a molecule of a specific wavelength of light is proportional to its concentration *in the solution in which it is assayed.*

Formally Beer's law is stated as:

$$\text{Absorbance}_{@\text{wavelength n}} = \text{extinction coefficient} \times \text{concentration} \times \text{light path length through the DNA solution}$$

$$A_{\lambda n} = e \times C \times p$$

The extinction coefficient is the most confusing entity in the equation. What it is an empirically determined number specific to the molecule that is being measured. Empirically means that someone actually had to do an experiment in a lab to get this number so now everyone else can use it without having to do the same experiment.

For example, the extinction coefficient for DNA @ $A_{260\ nm}$ is $\frac{\text{"50 μgrams DNA / ml solution"}}{\text{1 Absorbance unit}}$

This means if you sprinkled 50 μgrams pure DNA powder into 1 mL of $H_2O$, dissolved it, transferred it into a spectrophotometer cuvette that had a 1 cm light path, beamed light at 260 nm, it would have an absorbance of EXACTLY 1.0, assuming you pre-blanked the spectrophotometer with H2O.

This would be true for not only YOUR spec but anyone else's spec, so long as it has been pre-blanked with $H_2O$.

SO------Always be sure to blank the spectrophotometer with the same solution used for dilution.

**II. Assay for Purity of DNA**

**A. purity analysis of DNA**

Why do we need to know the purity of a DNA solution?

**one,** if the DNA is too contaminated with "dirt molecules" it will not be usable for downstream applications.

**two,** if the DNA is too contaminated, the quantitation of the DNA concentration will be inaccurate.

**1.** The A260/A280 absorbance DNA purity ratio

Is based on the comparison of the peak absorbance wavelengths of DNA and protein.

λ 280 nm = absorbance peak for protein
λ 260 nm = absorbance peak for DNA

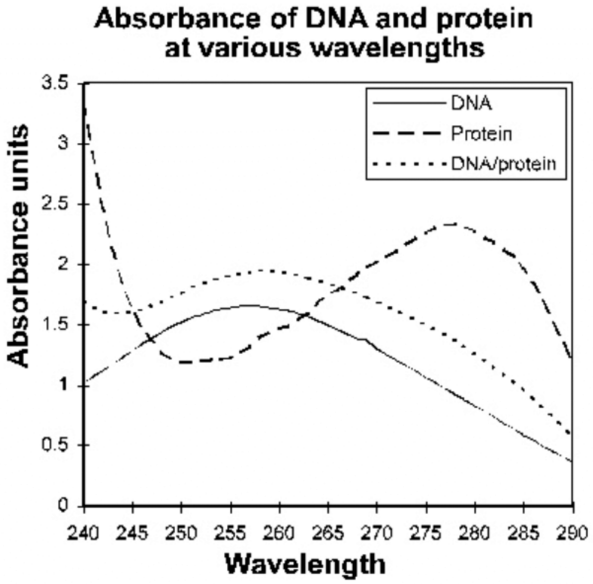



***Figure 1. Absorbance profiles of DNA and protein samples from 240 to 290 nm.*** *The absorbance of purified (-) plasmid DNA (80 mg/ml); (------) 3 mg/ml aqueous bovine serum albumin (BSA) solution ; or a (- - - - - -) 10:1 (w/w) DNA to protein mixture in aqueous solution was determined in 1 nm increments from 240 nm to 290 nm using a Perkin Elmer Lambda 3B spectrophotometer. Similar measurements were made using a 3 mg/ml aqueous bovine serum albumin (BSA) solution.*

Notice how close these two wavelengths are to each other! So, if there is any protein in your DNA solution, some of the absorbance due to the protein will spill over into the DNA absorbance peak (see Fig. 1). For example, pure DNA free of protein has an $A_{260}/A_{280}$ ratio of 2.0 (+/- 1.0). If there is any protein in the DNA solution, this ratio will be always be less than 2.0. A DNA solution that has a lot of protein can have an $A_{260}/A_{280}$ ratio of 1.2.

- in general, if protein is co-purifying with your DNA, we can assume other contaminating molecules that can't be detected at 280 nm are also co-purifying as well. SO by co-assaying for contaminating protein, you get a general index of DNA purity, i.e., the 260/280 ratio:

✓a <u>great</u> DNA prep will have a 260/280 ratio of 1.9-2.1

✓an <u>acceptable</u> level of DNA purity is 260/280 ratio ≥ 1.7…..this also means your quantitative assay is reasonably accurate.

**The Alkaline Lysis Plasmid DNA Miniprep Method|Principles of:**

**1. Cell Growth and Harvesting**

The procedure starts with the growth of the bacteria in cell culture harboring your plasmid. When sufficient growth has been achieved, the cells are pelleted by centrifugation to remove them from the growth medium.

**2. Re-suspension - GTE**

**G**lucose - a sugar that keeps the osmotic pressure even so the cells don't burst until the next step

**T**ris buffer - keeps the pH at neutral

**E**DTA - EDTA chelates (removes) divalent cations ($Mg^{2+}$, $Ca^{2+}$) from the solution. This inhibits DNA nucleases (DNases) from degrading the plasmid since these enzymes require $Mg^{2+}$ & $Ca^{2+}$ to work. The bacterial cell wall is also weakened by the removal of $Mg^{2+}$ & $Ca^{2+}$, thus aiding in the next step when the cells are bursted to release plasmid.

**3. Lysis Step - SDS-NaOH solution**

SDS - Sodium Dodecyl Sulfate, a soap detergent. SDS helps to dissolves the cell wall and cell membrane thereby releasing plasmid DNA to the solution. SDS also denatures most of the proteins in the cells thereby rendering them insoluble, which helps in the separation of the proteins from the plasmid DNA later in the process.

NaOH - sodium hydroxide, as strong base. NaOH increases the pH to be extremely alkaline (high pH).

The high pH disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the cell's chromosomal DNA (gDNA) and the plasmid DNA, to single stranded DNA (ssDNA). This process is called **denaturation** and is central part of the procedure, which is why it's called alkaline lysis method.

**4. Neutralization Step - 5 M KAc, pH 5.5**

-causes the precipitation of chromosomal DNA and protein away from the soluble plasmid DNA.

Addition of potassium acetate (KAc) decreases the alkalinity of the mixture, restoring the pH to near neutral. Under these conditions the hydrogen bonding between the bases of the single stranded DNA can be re-established. This allows the DNA that was made single-stranded in step 3 to re-nature back into dsDNA. However, while the tiny circular plasmid DNA can quickly re-nature back into soluble dsDNA, the gigantic single stranded chromosomal DNA molecule re natures very slowly. This is why it's important to mix the tubes gently during step 3 because vigorous mixing or vortexing will shear the chromosomal DNA into small fragments that will co-purify with the smaller plasmid DNA molecules.

-The white precipitate is a complex of single-stranded chromosomal DNA, SDS, and denatured cellular protein. The white precipitate is then separated from the soluble plasmid DNA by centrifugation.

**5. Phenol/Chloroform extraction step**

Phenol, chloroform - organic solvents - immiscible with aqueous solutions - when vortexed with the soluble plasmid DNA, can denature and precipitate away contaminating proteins that may interfere with use of the plasmid DNA. During centrifugation, the water-soluble DNA partitions into the UPPER aqueous phase, while the proteins are denatured by the organic solvents and stay in the LOWER organic phase. The aqueous phase containing the protein-free DNA can then be collected.

**6. Ethanol Precipitation**

Ethanol precipitation of DNA is a tried and tested method for concentrating DNA. Ethanol changes the DNA structure so that the DNA molecules aggregate and precipitate from solution. Ethanol precipitation only works if

monovalent salt ions ($Na^+$, $K^+$) is also in the solution. In the case of the miniprep, these were added as KAc and NaOH in the steps before. So, there is a high concentration of $Na^+$, $K^+$ required of the EtOH ppt procedure.

**8. Washing the DNA pellet**

70% EtOH - After removal of the supernatant above the plasmid DNA pellet, 70% ethanol is added. Since most salts and small organic molecules are soluble in 70% ethanol they are "washed" away from the DNA pellet, which remains insoluble in 70% EtOH

This step is important since salts, if they were to remain in the DNA, can inhibit further manipulation of the DNA (termed downstream uses).

# Plasmid and Protein Quantitation

## Nucleic Acid and Protein Calculations.

An online calculator for these values is available at: **www.promega.com/techserv/biomath/**

### Metric Prefixes

| Prefix | Symbol | Factor |
|---|---|---|
| kilo | k | $10^{3}$ |
| centi | c | $10^{-2}$ |
| milli | m | $10^{-3}$ |
| micro | µ | $10^{-6}$ |
| nano | n | $10^{-9}$ |
| pico | p | $10^{-12}$ |
| femto | f | $10^{-15}$ |
| atto | a | $10^{-18}$ |
| zepto | z | $10^{-21}$ |

### Spectrophotometric Conversions

1 $A_{260}$ unit of double-stranded DNA = 50µg/ml
1 $A_{260}$ unit of single-stranded DNA = 33µg/ml
1 $A_{260}$ unit of single-stranded RNA = 40µg/ml

### DNA Molar Conversions

1µg of 1,000bp DNA = 1.52pmol (3.03pmol of ends)
1µg of pBR322 DNA = 0.36pmol DNA
1pmol of 1,000bp DNA = 0.66µg
1pmol of pBR322 DNA = 2.8µg

### Formulas for DNA Molar Conversions

**For dsDNA:**

To convert pmol to µg:

$$\text{pmol} \times N \times \frac{660\text{pg}}{\text{pmol}} \times \frac{1\mu\text{g}}{10^{6}\text{pg}} = \mu\text{g}$$

To convert µg to pmol:

$$\mu\text{g} \times \frac{10^{6}\text{pg}}{1\mu\text{g}} \times \frac{\text{pmol}}{660\text{pg}} \times \frac{1}{N} = \text{pmol}$$

where N is the number of nucleotide pairs and 660pg/pmol is the average MW of a nucleotide pair.

**For ssDNA:**

To convert pmol to µg:

$$\text{pmol} \times N \times \frac{330\text{pg}}{\text{pmol}} \times \frac{1\mu\text{g}}{10^{6}\text{pg}} = \mu\text{g}$$

To convert µg to pmol:

$$\mu\text{g} \times \frac{10^{6}\text{pg}}{1\mu\text{g}} \times \frac{\text{pmol}}{330\text{pg}} \times \frac{1}{N} = \text{pmol}$$

where N is the number of nucleotides and 330pg/pmol is the average MW of a nucleotide.

### Protein Molar Conversions

100pmol of 100kDa protein = 10µg
100pmol of 50kDa protein = 5µg
100pmol of 10kDa protein = 1µg
100pmol of 1kDa protein = 100ng

### Protein/DNA Conversions

1kb of DNA = 333 amino acids of coding capacity
= 37kDa protein
270bp DNA = 10kDa protein
810bp DNA = 30kDa protein
1.35kb DNA = 50kDa protein
2.7kb DNA = 100kDa protein
average MW of an amino acid = 110 daltons

Dalton (Da) is an alternate name for the atomic mass unit, and kilodalton (kDa) is 1,000 daltons. Thus a peptide with a mass of 64kDa has a molecular weight of 64,000 grams per mole.

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PART #GE635

# Technical Appendix

## Composition of Solutions

**Note:** Solution compositions for the Cloning Enzyme 10X reaction buffers are listed in Table 15.

**Antibiotic Stock Solutions**

**Ampicillin**: 100mg/ml in deionized water (filter-sterilized).

**Tetracycline**: 10mg/ml in 80% ethanol.

**Kanamycin**: 30mg/ml kanamycin sulfate in deionized water (filter-sterilized).

**Chloramphenicol**: 20mg/ml in 80% ethanol.

Store at –20°C. **Note:** Cell growth in liquid culture is inhibited by tetracycline concentrations greater than 10mg/ml.

**Cell Lysis Solution**

0.2N NaOH, 1% SDS in deionized water. Prepare fresh for each use.

**Cell Resuspension Solution**

| | |
|---|---|
| 25mM | Tris-HCl (pH 8.0) |
| 10mM | EDTA |
| 50mM | glucose |

**IPTG Stock Solution (0.1M)**

| | |
|---|---|
| 1.2g | isopropyl β-D-thiogalacto-pyranoside (IPTG) (Cat.# V3951) |

Add deionized water to 50ml final volume. Filter-sterilize (0.2µm) and store at 4°C.

**LB Medium**

| | |
|---|---|
| 10g | Bacto®-tryptone |
| 5g | Bacto®-yeast extract |
| 5g | NaCl |

Add deionized water to approximately 1L. Adjust pH to 7.5 with 10N NaOH and autoclave.

**LB/ Antibiotic Plates (1L)**

Add 15g of agar to 1 liter of LB medium and autoclave. Allow the medium to cool to 55°C before adding antibiotic to the specified final concentration (ampicillin: 100µg/ml; tetracycline: 12.5µg/ml; kanamycin: 30µg/ml; chloramphenicol: 20µg/ml). Pour 30–35ml of medium into 85mm petri dishes. If necessary, flame the surface of the medium with a Bunsen burner to eliminate bubbles. Let the agar harden overnight. Store at 4°C for <1 month. Tetracycline is light-sensitive; LB/tetracycline plates should be covered with foil.

**M-9 Plates**

| | |
|---|---|
| 6g | $Na_2HPO_4$ |
| 3g | $KH_2PO_4$ |
| 0.5g | NaCl |
| 1g | $NH_4Cl$ |
| 15g | agar |

Add deionized water to approximately 1L. Adjust pH to 7.4 with 10N NaOH. Autoclave. Cool to 50°C. Then add:

| | |
|---|---|
| 2.0ml | 1M $MgSO_4$ |
| 0.1ml | 1M $CaCl_2$ |
| 10.0ml | 20% glucose |
| 1.0ml | 1M thiamine-HCl |

Filter the medium through a 0.2µm filter unit.

**Phenol:Chloroform:Isoamyl Alcohol (25:24:1)**

Mix equal parts of TE buffer and phenol and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

**Potassium Acetate Solution (pH 4.8)**

Prepare 60ml of 5M potassium acetate. Add 11.5ml of glacial acetic acid and 28.5ml of deionized water. This solution will be 3M with respect to potassium and 5M with respect to acetate. Store at 4°C.

**SOC Medium**

| | |
|---|---|
| 2.0g | Bacto®-tryptone |
| 0.5g | Bacto®-yeast extract |
| 1ml | 1M NaCl |
| 0.25ml | 1M KCl |
| 1ml | 2M $Mg^{2+}$ stock (1M $MgCl_2 \cdot 6H_2O$, 1M $MgSO_4 \cdot 7H_2O$), filter-sterilized |
| 1ml | 2M glucose, filter-sterilized |

Add Bacto®-tryptone, Bacto®-yeast extract, NaCl and KCl to 97ml deionized water. Stir to dissolve. Autoclave and cool to room temperature. Add 2M $Mg^{2+}$ stock and 2M glucose stock, each to a final concentration of 20µM. Filter the complete medium through a 0.2mm filter unit. The pH should be 7.0.

**TAE 10X Buffer**

| | |
|---|---|
| 400mM | Tris base |
| 200mM | Sodium acetate |
| 10mM | EDTA |

Adjust pH to 8.2 with glacial acetic acid.

**TBE 10X Buffer**

| | |
|---|---|
| 890mM | Tris base |
| 890mM | Boric acid |
| 19mM | EDTA |

Adjust pH to 8.3.

**TE Buffer**

| | |
|---|---|
| 10mM | Tris-HCl (pH 8.0) |
| 1mM | EDTA |

**X-Gal Stock Solution (50mg/ml)**

| | |
|---|---|
| 100mg | 5-bromo-4-chloro-3-indolyl β–D-galactoside (X-Gal) |

Dissolve in 2ml of N,N′-dimethylformamide. This stock solution is available from Promega (Cat.# V3941).

# Abbreviations

| | |
|---|---|
| | **A** |
| α | alpha |
| Ab | antibody |
| Ac-DEVD-AMC | acetyl-Asp-Glu-Val-Asp-7-amino-4-methyl coumarin (fluorogenic substrate for caspase-3/7) |
| Ac-DEVD-CHO | acetyl-Asp-Glu-Val-Asp-1-aldehyde (reversible aldehyde inhibitor of caspase-3/7) |
| Ac-DEVD-pNA | acetyl-Asp-Glu-Val-Asp-pNA (colorimetric substrate for caspase-3/7) |
| Ac-YVAD-AMC | acetyl-Tyr-Val-Ala-Asp-amino methyl coumarin (fluorogenic substrate for caspase-1) |
| Ac-YVAD-CHO | acetyl-Tyr-Val-Ala-Asp-1-aldehyde (reversible aldehyde inhibitor of caspase-1) |
| ADP | adenosine diphosphate |
| Ad-2 | adenovirus-2 |
| AKAP | A-kinase anchoring protein |
| a.m.u. | atomic mass unit |
| AMC | 7-amino-4-methyl coumarin |
| amol | attomole ($10^{-18}$ mole) |
| AMP | adenosine monophosphate |
| AMV | avian myeloblastosis virus |
| AP | alkaline phosphatase |
| AP1, 2 | activator protein 1, 2 |
| APC film | automatic processor-compatible film |
| ARE | AU-rich element |
| ATP | adenosine triphosphate |
| | **B** |
| β | beta |
| BCIP | 5-bromo-4-chloro-3-indolyl phosphate |
| BDNF | brain-derived neurotrophic factor |
| BMV | brome mosaic virus |
| bp | base pairs |
| BRET | bioluminescence resonance energy transfer |
| BSA | bovine serum albumin |
| BYDV | barley yellow dwarf virus |
| | **C** |
| CaM KII | calcium/calmodulin-dependent protein kinase II |
| cAMP | adenosine-3′,5′-cyclic monophosphate (cyclic AMP) |
| CAT | chloramphenicol acetyltransferase |
| CBZ | benzyloxycarbonyl |
| CCD | charge-coupled device (camera) |
| CCLR | Cell Culture Lysis Reagent |
| cdc2 | cell division cycle 2 protein |
| cDNA | complementary DNA |
| cfu | colony forming unit |
| cGMP | guanosine-3′,5′-cyclic monophosphate (cyclic GMP) |
| Ci | Curie |
| CIAP | calf intestinal alkaline phosphatase |
| CKI, CK-1 | casein kinase I |
| CKII, CK-2 | casein kinase II |
| cm | centimeter |
| CMM | canine pancreatic microsomal membranes |
| CMV | cytomegalovirus |
| CN | 4-chloro-1-naphthol (a horseradish peroxidase substrate) |
| CNBr | cyanogen bromide |
| CNS | central nervous system |
| CNTF | ciliary neurotrophic factor |
| CODIS | COmbined DNA Index System |
| cpm | counts per minute |
| CPP32 | caspase-3 (DEVDase) |
| CREB | cAMP response element binding protein |
| $C_t$ | cycle threshold |
| CTP | cytidine triphosphate |
| CXR | carboxy-X-tetramethylrhodamine |
| | **D** |
| Da | daltons |
| DAB | diaminobenzidine |
| DAG | diacylglycerol |
| DAPI | 4′,6-diamidino-2-phenylindole |
| DEPC | diethyl pyrocarbonate |
| DEVDase | caspase protease activity on the DEVD peptide |
| ddRNAi | DNA-directed RNA interference |
| diAcFAM | diacetyl carboxyfluorescein |
| DLR | Dual-Luciferase® Reporter |
| DMSO | dimethyl sulfoxide |
| DNA | deoxyribonucleic acid |
| DNA-PK | DNA-dependent protein kinase |
| DNase | deoxyribonuclease |
| dNTP | deoxynucleotide triphosphate |
| DOGS | dioctadecylamidoglycyl spermine |
| DOPE | L-dioleoyl phosphatidylethanolamine |
| DPPIV | dipeptidyl peptidase IV |
| dsDNA | double-stranded DNA |
| dsRNA | double-stranded RNA |
| DTT | dithiothreitol |
| | **E** |
| ED50 | effective dose (for 50% of effect) |
| EDTA | ethylenediaminetetraacetic acid |
| EGF | epidermal growth factor |
| EGFR | epidermal growth factor receptor |
| EGTA | ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid |
| ELISA | enzyme-linked immunosorbent assay |
| em | emission |
| ERK1, 2 | extracellular signal-regulated protein kinase 1, 2 |
| EtBr | ethidium bromide |
| EtOH | ethanol |
| ex | excitation |
| | **F** |
| FAB/MS | fast atomic bombardment mass spectrometry |
| FACS | fluorescence-activated cell sorting |
| FGF | fibroblast growth factor |
| FITC | fluorescein isothiocyanate |
| FITC-VAD-FMK | FITC-carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (fluorescent marker for caspase activity) |
| FL | fluorescein |
| fmol | femtomole ($10^{-15}$ mole) |
| | **G** |
| γ | gamma |
| g | gram |
| GAPDH | glyceraldehyde-3 phosphate dehydrogenase |
| GDNF | glial cell line-derived neurotrophic factor |
| GFAP | glial fibrillary acidic protein |
| GFP | green fluorescent protein |
| GLB | Glo Lysis Buffer |
| GMO | genetically modified organism |
| GMP | guanosine monophosphate |
| GPDH | glycerol 3-phosphate dehydrogenase |
| GQ | genome qualified |
| GST | glutathione-S-transferase |
| GTP | guanosine triphosphate |
| | **H** |
| $^3$H | tritium |
| h | human |
| HC | high concentration |
| hCL1 | synthetic CL1, a protein degradation signal |
| HCV | hepatitis C virus |
| HDPE | high-density polyethylene |
| HIV | human immunodeficiency virus |
| h*luc*+ | codon-optimized firefly luciferase gene |
| h*luc*CP+ | h*luc*+ with 3′ hCL1 and hPEST sequences |
| h*luc*P+ | h*luc*+ with 3′ hPEST sequence |
| hPEST | synthetic PEST, a protein degradation signal |
| HPLC | high-pressure or high-performance liquid chromatography |
| hR*luc* | synthetic *Renilla* luciferase gene |
| hR*luc*CP | hR*luc* with 3′ hCL1 and hPEST sequences |
| hR*luc*P | hR*luc* with 3′ hPEST sequence |
| HRP | horseradish peroxidase |
| HSV | herpes simplex virus |
| HTP | high throughput |
| HTS | high-throughput screening |
| | **I** |
| $IC_{50}$ | inhibitory concentration (50% inhibition) |
| ICC | immunocytochemistry |
| ICE | interleukin-1β-converting enzyme (caspase-1) |
| IGF | insulin-like growth factor |
| IgG | immunoglobulin G |
| IgY | immunoglobulin Y (chicken egg yolk immunoglobulin) |
| IHC | immunohistochemistry |
| IL-4 | interleukin-4 |
| IPTG | isopropyl β-D-thiogalactopyranoside |
| iso-dC | isodeoxycytosine |
| iso-dG | isodeoxyguanosine |
| IVEC | in vitro expression cloning |
| IVT | in vitro transcription |
| | **J** |
| JNK | c-Jun N-terminal kinase |
| JOE | 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxy-fluorescein |
| | **K** |
| kb | kilobase; kilobase pairs |
| kDa | kilodalton |
| $K_m$ | Michaelis-Menten constant |

# Abbreviations

| | |
|---|---|
| | **L** |
| λ | lambda |
| L | liter |
| LAR | Luciferase Assay Reagent |
| LDH | lactate dehydrogenase |
| LDPE | low-density polyethylene |
| LNGFR | low-affinity NGF receptor (p75 neurotrophin receptor) |
| LSC | liquid scintillation counting |
| *luc* | native firefly luciferase gene |
| *luc+* | synthetic firefly luciferase gene |
| LY 294002 | 2-(4-morpholinyl)-8-phenyl-4 H-1-benzopyran-4-one (PI-3 kinase inhibitor) |
| | **M** |
| m | murine; milli |
| M | molar |
| mAb | monoclonal antibody |
| MALDI-TOF | matrix-assisted laser desorption/ionization-time of flight mass spectrometry |
| MAO | monoamine oxidase |
| MAPK | mitogen-activated protein kinase |
| MCS | multiple cloning site |
| MEK | MAPK kinase |
| mg | milligram ($10^{-3}$g) |
| MGFP | Monster Green® Fluorescent Protein |
| µg | microgram ($10^{-6}$g) |
| min | minute |
| µl | microliter ($10^{-6}$L) |
| µM | micromolar ($10^{-6}$M) |
| miRNA | micro RNA |
| ml | milliliter ($10^{-3}$L) |
| MLCK | kinase 338, myosin light chain kinase |
| M-MLV | Moloney murine leukemia virus |
| mm | millimeter ($10^{-3}$m) |
| mM | millimolar ($10^{-3}$M) |
| mRNA | messenger RNA |
| MTS | 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| MW | molecular weight |
| | **N** |
| N | Normal |
| NaOAc | sodium acetate |
| NBT | nitro blue tetrazolium |
| NED | N-1-napthylethylenediamine dihydrochloride |
| NF | N-terminal fusion |
| NF-κB | nuclear factor kappa B |
| NGF | nerve growth factor |
| nmol | nanomole ($10^{-9}$ mole) |
| NMR | nuclear magnetic resonance |
| nt | nucleotide |
| NT-3, NT-4 | neurotrophin-3, neurotrophin-4 |
| NTP | nucleotide triphosphate (same as rNTP) |
| | **O** |
| OCT1 | octamer-binding transcription factor 1 |
| ONPG | *o*-nitrophenyl β-D-galactopyranoside |
| | **P** |
| P450 | cytochrome P450 |
| pAb | polyclonal antibody |
| PAGE | polyacrylamide gel electrophoresis |
| PARP | poly (ADP-ribose) polymerase |
| PCR | polymerase chain reaction |
| PD 98059 | 2′-amino-3′-methoxyflavone, MEK1 inhibitor |
| PES | phenazine ethosulfate |
| PEG | polyethylene glycol |
| pg | picogram ($10^{-12}$g) |
| Pgp | P-glycoprotein |
| Pi | inorganic phosphate |
| PKA | cAMP-dependent protein kinase |
| PKC | protein kinase C |
| PKG | cGMP-dependent protein kinase |
| PI 3-K | phosphatidylinositol 3-kinase |
| PLB | Passive Lysis Buffer |
| PMA | phorbol 12-myristate 13-acetate (TPA), PKC activator |
| pmol | picomole ($10^{-12}$ mole) |
| PMP | paramagnetic particle |
| PMS | phenazine methosulfate |
| PMSF | phenylmethylsulfonyl fluoride |
| pNA | *p*-nitroaniline |
| PNGase F | peptide N-glycosidase F |
| PNPP | p-nitrophenyl phosphate |
| Poly(A) | polyadenylation sequence |
| PPase | protein phosphatase |
| PTPase | protein tyrosine phosphatase |
| PTK | protein tyrosine kinase |
| PTT | protein truncation test |
| PVDF | polyvinylidene fluoride |
| PVP | polyvinylpyrrolidone |
| | **Q** |
| qPCR | quantitative PCR |
| qRT-PCR | quantitative reverse transcriptase PCR |
| | **R** |
| r | recombinant |
| RAPD | random amplified polymorphic DNA |
| RF DNA | replicative form DNA |
| RISC | RNA-induced silencing complex |
| RLB | Reporter Lysis Buffer |
| RLU | relative light units |
| R*luc* | native *Renilla* luciferase gene |
| RNA | ribonucleic acid |
| RNAi | RNA interference |
| RNase | ribonuclease |
| rNTP | ribosylnucleotide triphosphate, (same as NTP) |
| ROS | reactive oxygen species |
| rRNA | ribosomal RNA |
| RT | reverse transcriptase |
| RT-PCR | reverse transcription PCR |
| | **S** |
| SAM | S-adenosylmethionine |
| SAM | streptavidin matrix |
| SAP | shrimp alkaline phosphatase |
| SB 203580 | (4-[4′-fluorophenyl]-2-[4′-methylsulfinylphenyl]-5-[4′-pyridyl] imidazole), a p38 MAP kinase inhibitor |
| SDS | sodium dodecyl sulfate |
| shRNA | short hairpin RNA |
| siRNA | short interfering RNA |
| SNP | single nucleotide polymorphism |
| SP1 | specificity protein 1 |
| SSB | single-stranded DNA binding protein |
| ssDNA | single-stranded DNA |
| St | stearated |
| STR | short tandem repeat |
| SV40 | simian virus 40 |
| | **T** |
| TAE | tris acetate EDTA |
| TAP | tobacco acid pyrophosphatase |
| TBE | tris borate EDTA |
| TCA | trichloroacetic acid |
| TdT | terminal deoxynucleotidyl transferase |
| TE | Tris-EDTA buffer |
| TEMED | N,N,N′,N′-tetramethylethylenediamine |
| TFIIB | transcription factor IIB |
| $TGF\beta_{1,2}$ | transforming growth factor β1, 2 |
| TK | thymidine kinase |
| TLC | thin-layer chromatography |
| $T_m$ | melting temperature |
| TMB | 3,3′,5,5′-tetramethylbenzidine |
| TMR | carboxy-tetramethylrhodamine |
| TNFα | tumor necrosis factor-α |
| TPA | 12-O-tetradecanoylphorbol 13-acetate |
| TPCK | N-p-tosyl-L-phenylalanine chloromethyl ketone |
| Trk | tyrosine kinase neurotrophin receptor family |
| tRNA | transfer RNA |
| TTP | thymidine triphosphate |
| TUNEL | TdT-mediated dUTP nick-end labeling |
| | **U** |
| u | unit |
| UTP | uracil triphosphate |
| | **V** |
| (v/v) | volume:volume ratio |
| VAChT | vesicular acetylcholine transporter |
| $V_{max}$ | maximum velocity (enzyme kinetics) |
| | **W** |
| (w/v) | weight:volume ratio |
| | **X** |
| X-Gal | 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside |
| | **Z** |
| Z-DEVD-R110 | bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-aspartic acid amide) rhodamine 110 (fluorogenic substrate for caspase 3/7) |
| Z-VAD-FMK | carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone (pan caspase inhibitor) |
| zmol | zeptomole ($10^{-21}$ mole) |

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PART #GE624

**Isoschizomers.** **The enzymes in boldface type are available from Promega.**

| Enzyme | Isoschizomer(s) | Recognition Sequence |
|---|---|---|
| *mAat* I | ***Stu* I**, *Eco*147 I, *Pme*55 I, *Sse*B I | AGG▼CCT |
| ***Aat* II** | — | GACGT▼C |
| ***Acc* I** | *Fbl* I, *Xmi* I | GT▼(A/C)(G/T)AC |
| ***Acc* III** | *Bsp*E II, *Mro* I | T▼CCGGA |
| ***Acc*65 I** | *Asp*718 I | G▼GTACC |
| | ***Kpn* I*** | GGTAC▼C |
| *Acc*B1 I | ***Ban* I**, *Bsh*N I, *Eco*64 I | G▼G(C/T)(G/A)CC |
| ***Acc*B7 I** | *Pfl*M I, *Van*91 I | CCAN$_4$▼NTGG |
| *Acl*N I | ***Spe* I** | A▼CTAGT |
| *Acl*W I | *Alw* I | GGATCNNNN▼ |
| *Acy* I | *Bbi* II, *Hin*1 I, ***Hsp*92 I**, *Bsa*H I, *Msp*171 I | G(A/G)▼CG(T/C)C |
| *Acs* I | *Apo* I | (G/A)▼AATT(C/T) |
| *Afa* I | *Csp*6 I*, ***Rsa* I** | GT▼AC |
| *Afe* I | ***Eco*47 III** | AGC▼GCT |
| *Afl* II | ***Bst*98 I** | C▼TTAAGG |
| ***Age* I** | *Pin*A I | A▼CCGGT |
| *Aha* III | ***Dra* I** | TTT▼AAA |
| *Ahd* I | ***Ecl*HK I** | GACNNN▼NNGTC |
| ***Alu* I** | — | AG▼CT |
| *Alw* I | *Acl*W I | GGATCNNNN▼ |
| ***Alw*26 I**[1] | *Bsm*A I | GTCTC(1/5) |
| ***Alw*44 I** | *Apa*L I | G▼TGCAC |
| *Aoc* I | ***Bsu*36 I**, *Cvn* I | CC▼TNAGG |
| ***Apa* I** | *Bsp*120 I | GGGCC▼C |
| *Apa*L I | ***Alw*44 I**, *Vne* I | G▼TGCAC |
| *Apo* I | *Acs* I | (G/A)▼AATT(C/T) |
| *Ase* I | ***Vsp* I**, *Asn* I | AT▼TAAT |
| *Asn* I | ***Vsp* I**, *Ase* I | AT▼TAAT |
| *Asp* I | ***Tth*111 I** | GACN▼NNGTC |
| *Asp*E I | *Ahd* I, *Eam*1105 I, ***Ecl*HK I** | GACNNN/NNGTC |
| *Asp*700 I | ***Xmn* I** | GAANN▼NNTTC |
| *Asp*718 I | ***Acc*65 I** | G▼GTACC |
| | ***Kpn* I*** | GGTAC▼C |
| *Asu* I | *Sau*96 I, *Cfr*13 I | G▼GNCC |
| *Asu* II | *Csp*45 I, *Bst*B I | TT▼CGAA |
| *Asu*HP I | *Hph* I | GGTGAN$_8$▼ |
| ***Ava* I** | *Ama*87 I, *Bco* I, *Bso*B I, *Eco*88 I | C▼(C/T)CG(G/A)G |
| ***Ava* II** | ***Sin* I**, *Eco*47 I, *Hgi*E I | G▼G(A/T)CC |
| *Axy* I | ***Bsu*36 I** | CC▼TNAGG |
| ***Bal* I** | *Msc* I, *Mlu*N I | TGG▼CCA |
| ***Bam*H I** | — | G▼GATCC |
| ***Ban* I** | *Acc*BI, *Bsh*N I, *Eco*64 I | G▼G(T/C)(A/G)CC |
| ***Ban* II** | *Eco*24 I | G(A/G)GC(T/C)▼C |
| *Bbe* I | — | GGCGC▼C |
| | ***Nar* I*** | GG▼CGCC |
| *Bbr*P I | *Eco*72 I, *Pml* I | CAC▼GTG |
| *Bbs* I[1] | *Bsc*91 I, *Bpi* I | GAAGAC(2/6) |
| ***Bbu* I** | *Pae* I, ***Sph* I** | GCATG▼C |
| ***Bcl* I** | *Bsi*Q I, *Fba* I | T▼GATCA |
| *Bcn* I | ***Nci* I** | CC▼(C/G)GG |
| *Bfr* I | ***Bst*98 I** | C▼TTAAG |
| ***Bgl* I** | — | GCCNNNN▼NGGC |
| ***Bgl* II** | — | A▼GATCT |
| *Bmy* I | ***Bsp*1286 I** | G(G/A/T)GC(C/A/T)▼C |
| *Bpm* I | *Gsu* I | CTGGAG(16/14) |
| *Bsa*H I | ***Hsp*92 I** | G(A/G)▼CG(T/C)C |
| ***Bsa*M I** | *Bsm* I | GAATGC(1/–1) |
| *Bsa*O I | *Bsh*1285 I, *Bsi*E I | CG(A/G)(T/C)▼C |
| *Bse*A I | ***Acc* III** | T▼CCGGA |
| *Bse*N I | ***Bsr*S I**, *Bsr* I | ACTGGN (1/–1) |
| *Bse*P I | ***Bss*H II**, *Pau* I | G▼CGCGC |
| *Bsh*1285 I | *Bsa*O I | CG(A/G)(T/C)▼CG |
| *Bsh*N I | ***Ban* I**, *Acc*B1 I, *Eco*64 I | G▼G(T/C)(A/G)CC |
| *Bsh*1365 I | *Bsr*BR I | GATNN▼NNATC |
| *Bsi*E I | *Bsa*O I | CG(A/G)(T/C)▼CG |

| Enzyme | Isoschizomer(s) | Recognition Sequence |
|---|---|---|
| *Bsm* I | ***Bsa*MI** | GAATGCN▼ |
| *Bsm*A I[1] | ***Alw*26 I** | GTCTC(1/5) |
| *Bso*B I | ***Ava* I**, *Ama*87 I, *Bco* I, *Eco*88 I | C(C/T)CG(G/A)G |
| *Bsp*19 I | ***Nco* I** | C▼CATGG |
| *Bsp*68 I | ***Nru* I** | TCG▼CGA |
| *Bsp*106 I | ***Cla* I**, *Bsa*D I | AT▼CGAT |
| *Bsp*119 I | ***Csp*45 I**, *Nsp* V, *Bst* B I | TT▼CGAA |
| *Bsp*120 I | ***Apa* I** | G▼GGCCC |
| *Bsp*143 I | ***Mbo* I**, ***Sau*3A I**, ***Nde* II** | ▼GATC |
| *Bsp*143 II | ***Hae* II** | (A/G)GCGC▼(T/C) |
| ***Bsp*1286 I** | *Bmy* I, *Sdu* I | G(G/A/T)GC(C/A/T)▼C |
| *Bsp*C I | ***Pvu* I** | CGAT▼CG |
| *Bsp*D I | ***Cla* I** | AT▼CGAT |
| *Bsp*E I | ***Acc* III** | T▼CCGGA |
| *Bsr* I[1] | ***Bsr*S I**, *Bse*N I | ACTGGN(1/-1) |
| ***Bsr*S I**[1] | *Bse*N I, *Bsr* I | ACTGGN(1/-1) |
| ***Bss*H II** | *Bse*P I, *Pau* I | G▼CGCGC |
| ***Bst*98 I** | *Afl* II, *Bfr* I | C▼TTAAG |
| *Bst*B I | ***Csp*45 I**, *Nsp* V, *Bsp*119 I | TT▼CGAA |
| ***Bst*E II** | *Bst*P I, *Eco*91 I, *Psp*E I | G▼GTNACC |
| *Bst*N I | ***Bst*O I**, *Mva* I, *Eco*R II | CC▼(A/T)GG |
| ***Bst*O I** | *Bst*N I, *Eco*R II, *Mva* I | CC▼(A/T)GG |
| ***Bst*X I** | – | CCANNNNN▼NTGG |
| *Bst*Y I | ***Xho* II**, *Mfl* I | (A/G)▼GATC(T/C) |
| ***Bst*Z I** | ***Eco*52 I**, *Eag* I, *Xma* III, *Ecl*X I | C▼GGCCG |
| *Bsu*15 I | ***Cla* I** | AT▼CGAT |
| ***Bsu*36 I** | *Cvn* I, *Aoc* I, *Eco*81 I | CC▼TNAGG |
| *Bsu*R I | ***Hae* III**, *Pal* I | GG▼CC |
| ***Cfo* I** | ***Hha* I** | GCG▼C |
| | *Hin*6 I | GCG▼C |
| | *Hin*P1 I* | G▼CGC |
| *Cfr*9 I | ***Xma* I** | C▼CCGGG |
| | ***Sma* I*** | CCC▼GGG |
| *Cfr*13 I | *Sau*96 I | G▼GNCC |
| *Cfr*42 I | ***Sac* II** | CCGC▼GG |
| ***Cla* I** | *Ban* III, *Bsp*106 I, *Bsp*D I, *Bsu*15 I | AT▼CGAT |
| *Cpo* I | ***Csp* I**, *Rsr* II | CG▼G(A/T)CCG |
| ***Csp* I** | Cpo I, *Rsr* II | CG▼G(A/T)CCG |
| *Csp*6 I | ***Rsa* I***, *Afa* I* | GT▼AC |
| ***Csp*45 I** | *Bst*B I, *Nsp* V, *Bsp*119 I | TT▼CGAA |
| *Cvn* I | ***Bsu*36 I** | CC▼TNAGG |
| ***Dde* I** | *Bst*DE I | C▼TNAG |
| ***Dpn* I**[2] | *Dpn* II* | G$^{me}$A▼TC |
| *Dpn* II | ***Mbo* I**, ***Sau*3A I**, ***Nde* II**, ***Dpn* I*** | ▼GATC |
| ***Dra* I** | — | TTT▼AAA |
| *Eag* I | ***Eco*52 I**, ***Bst*Z I**, *Ecl*X I, *Xma* III | C▼GGCCG |
| *Eam*1105 I | ***Ecl*HK I**, *Ahd* I, *Asp*E I | GACNNN▼NNGTC |
| *Ecl*136 II | ***Eco*ICR I** | GAG▼CTC |
| | ***Sac* I*** | GAGCT▼C |
| ***Ecl*HK I** | *Ahd* I, *Eam*1105 I, *Asp*E I | GACNNN▼NNGTC |
| *Ecl*X I | ***Bst*Z I**, *Eag* I, ***Eco*52 I**, *Xma* III | C▼GGCCG |
| *Eco*24 I | ***Ban* II**, *Fri*O I | G(AG)GC(TC)▼C |
| *Eco*32 I | ***Eco*R V** | GAT▼ATC |
| *Eco*47 I | ***Ava* II**, ***Sin* I** | G▼G(A/T)CC |
| ***Eco*47 III** | *Afe* I | AGC▼GCT |
| ***Eco*52 I** | ***Bst*Z I**, *Xma* III, *Eag* I, *Ecl*X I | C▼GGCCG |
| *Eco*64 I | ***Ban* I**, *Bsh*N I, *Eco*64 I | G▼G(TC)(AG)CC |
| *Eco*81 I | ***Bsu*36 I** | CC▼TNAGG |
| *Eco*88 I | ***Ava* I** | C▼(TC)CG(AG)G |
| *Eco*91 I | ***Bst*E II** | G▼GTNACC |
| *Eco*105 I | ***Sna*B I** | TAC▼CTA |
| *Eco*130 I | ***Sty* I** | C▼C(A/T)(T/A)GG |
| *Eco*147 I | ***Stu* I** | AGG▼CCT |
| ***Eco*ICR I** | *Ecl*136 II | GAG▼CTC |
| | ***Sac* I*** | GAGCT▼C |
| | *Sst* I* | GAGCT▼C |

**Isoschizomers (continued).** **The enzymes in boldface type are available from Promega.**

| Enzyme | Isoschizomer(s) | Recognition Sequence |
|---|---|---|
| ***Eco*R I** | — | G▼AATTC |
| *Eco*R II | ***Bst*O I**, *Bst*N I, *Mva* I | CC▼(A/T)GG |
| ***Eco*R V** | *Eco*32 I | GAT▼ATC |
| *Eco*T14 I | ***Sty* I** | C▼C(A/T)(A/T)GG |
| *Eco*T22 I | ***Nsi* I** | ATGCA▼T |
| *Ehe* I | ***Nar* I*** | GG▼CGCC |
| ***Fok* I**[2] | — | GGATG(9/13) |
| ***Hae* II** | *Bsp*143 II | (A/G)GCGC▼(T/C) |
| ***Hae* III** | *Bsu*R I, *Pal* I | GG▼CC |
| *Hap* II | ***Hpa* II**, ***Msp* I** | C▼CGG |
| *Hgi*E I | *Eco*47 I, ***Sin* I**, ***Ava* II** | G▼G(A/T)CC |
| ***Hha* I** | ***Cfo* I** | GCG▼C |
| | *Hin*P1 I*, *Hin*6 I* | G▼CGC |
| *Hin*1 I | *Acy* I, ***Hsp*92 I** | G(A/G)▼CG(T/C)C |
| ***Hin*c II** | *Hin*d II | GT(T/C)▼(A/G)AC |
| *Hin*d II | ***Hin*c II** | GT(T/C)▼(A/G)AC |
| ***Hin*d III** | — | A▼AGCTT |
| ***Hin*f I** | — | G▼ANTC |
| *Hin*P1 I | — | G▼CGC |
| | ***Hha* I***, ***Cfo* I*** | GCG▼C |
| ***Hpa* I** | *Ksp*A I | GTT▼AAC |
| ***Hpa* II**[3] | ***Msp* I**, *Hap* II | C▼CGG |
| ***Hsp*92 I** | *Acy* I, *Bsa*H I, *Hin*1 I | G(A/G)▼CG(C/T) |
| ***Hsp*92 II** | *Nla* III | CATG▼ |
| ***I-Ppo* I** | — | CTCTCTTAA▼GGTAGC |
| *Kas* I | ***Nar* I*** | GG▼CGCC |
| ***Kpn* I** | — | GGTAC▼C |
| | ***Acc*65 I***, *Asp*718 I* | G▼GTACC |
| *Ksp* I | ***Sac* II** | CCGC▼GG |
| ***Mbo* I** | ***Sau*3A I**, ***Nde* II**, *Dpn* II | ▼GATC |
| ***Mbo* II**[1] | — | GAAGA(8/7) |
| *Mfl* I | ***Xho* II** | (A/G)▼GATC(T/C) |
| ***Mlu* I** | — | A▼CGCGT |
| *Mlu*N I | ***Bal* I**, *Msc* I | TGG▼CCA |
| *Mro* I | ***Acc* III** | T▼CCGGA |
| *Msc* I | ***Bal* I**, *Mlu*N I | TGG▼CCA |
| *Mse* I | ***Tru*9 I** | T▼TAA |
| ***Msp* I**[3] | ***Hpa* II**, *Hap* II | C▼CGG |
| ***Msp*A1 I** | *Nsp*B II | C(A/C)G▼C(G/T)G |
| *Mst* II | ***Bsu*36 I** | CC▼TNAGG |
| *Mva* I | ***Bst*O I**, *Eco*R II, *Bst*N I | CC▼(A/T)GG |
| ***Nae* I** | ***Ngo*M IV** | G▼CCGGC |
| ***Nar* I** | — | GG▼CGCC |
| | *Ehe* I* | GGC▼GCC |
| | *Kas* I* | G▼GCGCC |
| | *Bbe* I* | GGCGC▼C |
| ***Nci* I** | *Bcn* I | CC▼(C/G)GG |
| ***Nco* I** | *Bsp*19 I | C▼CATGG |
| ***Nde* I** | — | CA▼TATG |
| ***Nde* II** | ***Mbo* I**, ***Sau*3A I**, *Dpn* II | ▼GATC |
| ***Ngo*M IV** | ***Nae* I** | G▼CCGGC |
| ***Nhe* I** | — | G▼CTAGC |
| *Nla* III | ***Hsp*92 II** | CATG▼ |
| ***Not* I** | — | GC▼GGCCGC |
| ***Nru* I** | *Bsp*68 I | TCG▼CGA |
| ***Nsi* I** | *Eco*T22 I, *Mph*1103 I | ATGCA▼T |
| *Nsp* V | ***Csp*45 I**, *Bst*B I, *Bsp*119 I | TT▼CGAA |
| *Nsp*B II | ***Msp*A1 I** | C(A/C)G▼C(G/T)G |
| *Pae* I | ***Bbu* I**, ***Sph* I** | GCATG▼C |
| *Pae*R7 I | ***Xho* I** | C▼TCGAG |
| *Pal* I | ***Hae* III**, *Bsu*R I | GG▼CC |
| *Pfl*M I | ***Acc*B7 I**, *Vau*91 I | CCAN$_4$▼NTGG |
| *Pin*A I | ***Age* I** | A▼CCGGT |
| ***Pst* I** | — | CTGCA▼G |
| ***Pvu* I** | *Bsp*C I | CGAT▼CG |
| ***Pvu* II** | — | CAG▼CTG |
| ***Rsa* I** | *Afa* I | GT▼AC |
| *Rsr* II | ***Csp* I**, *Cpo* I | CG▼G(A/T)CCG |
| ***Sac* I** | *Sst* I | GAGCT▼C |
| | *Ecl*136 II*, ***Eco*ICR I*** | GAG▼CTC |
| ***Sac* II** | *Sst* II, *Ksp* I, *Cfr*42 I | CCGC▼GG |
| ***Sal* I** | — | G▼TCGAC |
| ***Sau*3A I** | ***Mbo* I**, ***Nde* II**, *Dpn* II | ▼GATC |
| *Sau*96 I | *Cfr*13 I | G▼GNCC |
| ***Sca* I** | — | AGT▼ACT |
| *Sdu* I | ***Bsp*1286 I** | G(G/A/T)GC(C/A/T)▼C |
| ***Sfi* I** | — | GGCCNNNN▼NGGCC |
| *Sfu* I | ***Csp*45 I** | TT▼CGAA |
| ***Sgf* I** | — | GCGAT▼CGC |
| ***Sin* I** | ***Ava* II**, *Eco*47 I | G▼G(A/T)CC |
| ***Sma* I** | — | CCC▼GGG |
| | ***Xma* I***, *Cfr*9 I* | C▼CCGGG |
| ***Sna*B I** | *Eco*105 I | TAC▼GTA |
| ***Spe* I** | *Acl*N I | A▼CTAGT |
| ***Sph* I** | ***Bbu* I**, *Pae* I | GCATG▼C |
| ***Ssp* I** | — | AAT▼ATT |
| *Sst* I | ***Sac* I** | GAGCT▼C |
| | ***Eco*ICR I*** | GAG▼CTC |
| *Sst* II | ***Sac* II** | CCGC▼GG |
| ***Stu* I** | *Aat* I, *Eco*147 I | AGG▼CCT |
| ***Sty* I** | *Eco*T14 I | C▼C(A/T)(A/T)GG |
| ***Taq* I** | *Tth*HB8 I | T▼CGA |
| ***Tru*9 I** | *Mse* I | T▼TAA |
| ***Tth*111 I** | *Asp* I | GACN▼NNGTC |
| *Tth*HB8 I | ***Taq* I** | T▼CGA |
| *Van*91 I | ***Acc*B7 I**, *Pfl*M I | CCAN$_4$▼NTGG |
| *Vne* I | *Apa*L I, ***Alw*44 I** | G▼TGCAC |
| ***Vsp* I** | *Ase* I, *Asn* I | AT▼TAAT |
| ***Xba* I** | — | T▼CTAGA |
| ***Xho* I** | *Pae*R7 I | C▼TCGAG |
| ***Xho* II** | *Bst*Y I, *Mfl* I | (A/G)▼GATC(T/C) |
| ***Xma* I** | *Cfr*9 I, *Xma*C I, | C▼CCGGG |
| | ***Sma* I*** | CCC▼GGG |
| *Xma* III | ***Eco*52 I**, ***Bst*Z I**, *Eag* I, *Ecl*X I | C▼GGCCG |
| *Xma*C I | ***Xma* I** | C▼CCGGG |
| | ***Sma* I*** | CCC▼GGG |
| ***Xmn* I** | *Asp*700 I | GAANN▼NNTTC |

**Key:**

N = A, C, G or T
* = neoschizomer

**Notes:**

1. The locations of cleavage sites falling outside the recognition site are indicated in parentheses. For example, GTCTC(1/5) indicates cleavage at:

   5′...GTCTCN▼...3′
   3′...CAGAGNNNNN▲...5′

2. *Dpn* I is unique among commercially available restriction enzymes in requiring methylation of a nucleotide (adenine) in its recognition sequence in order to cut. Therefore, *Dpn* I cannot be substituted for other enzymes recognizing the GATC sequence (e.g., *Mbo* I and *Sau*3A I).
3. Although *Hpa* II and *Msp* I recognize the same nucleotide sequence, *Hpa* II is sensitive to methylation of either cytosine in its recognition sequence, while *Msp* I is sensitive only to methylation of the external cytosine. These enzymes may not be interchanged for all applications.

**Reference**

Roberts, R.J. (1991) *Nucl. Acids Res.* **19** (supp), 2077–109.

## Compatible Ends.

### Promega Restriction Enzymes That Generate 5′ Overhangs

| Overhang | Definite Compatible Ends | Possible Compatible Ends |
|---|---|---|
| 5′-N | | *Tth*111 I |
| 5′-S | | *Nci* I |
| 5′-W | | *Bst*O I |
| 5′-AT | *Acc* I | |
| 5′-CG | *Nar* I, *Msp* I, *Hsp*92 I, *Taq* I, *Cla* I, *Csp*45 I, *Hpa* II | |
| 5′-GN | | *Bsr*S I |
| 5′-MK | | *Acc* I |
| 5′-TA | *Vsp* I, *Nde* I, *Tru*9 I | |
| 5′-ANT | | *Hin*f I |
| 5′-GNC | | *Sau*96 I |
| 5′-GWC | | *Ava* II, *Csp* I, *Sin* I |
| 5′-TNA | | *Dde* I, *Bsu*36 I |
| 5′-AATT | *Eco*R I | |
| 5′-AGCT | *Hin*d III | |
| 5′-CATG | *Nco* I | *Sty* I |
| 5′-CCGG | *Age* I, *Xma* I, *Acc* III, *Ngo*M IV | *Ava* I |
| 5′-CGCG | *Mlu* I, *Bss*H I | |
| 5′-CTAG | *Spe* I, *Nhe* I, *Xba* I | *Sty* I |
| 5′-CWWG | | *Sty* I |
| 5′-GATC | *Mbo* I, *Sau*3A I, *Bam*H I, *Bgl* II, *Xho* II, *Bcl* I, *Nde* II | |
| 5′-GCGC | *Ban* I | |
| 5′-GGCC | *Not* I, *Bst*Z I, *Eco*52 I | |
| 5′-GTAC | *Acc*65 I | *Ban* I |
| 5′-GTNAC | | *Bst*E II |
| 5′-GYRC | | *Ban* I |
| 5′-TCGA | *Sal* I, *Xho* I | *Ava* I |
| 5′-TGCA | *Alw*44 I | |
| 5′-TTAA | *Bst*98 I | |
| 5′-YCGR | | *Ava* I |

### Promega Restriction Enzymes That Generate 3′ Overhangs

| Overhang | Definite Compatible Ends | Possible Compatible Ends |
|---|---|---|
| N-3′ | | *Ecl*HK I |
| AT-3′ | *Sgf* I, *Pvu* I | |
| CG-3′ | *Cfo* I, *Hha* I | |
| CN-3′ | | *Bsa*M I |
| GC-3′ | *Sac* II | *Bsa*O I |
| NNN-3′ | | *Acc*B7 I, *Bgl* I, *Sfi* I |
| ACGT-3′ | *Aat* II | |
| AGCT-3′ | *Sac* I | *Ban* II, *Bsp*1286 I |
| CATG-3′ | *Hsp*92 II, *Sph* I, *Bbu* I | |
| DGCH-3′ | | *Bsp*1286 I |
| GCGC-3′ | *Hae* II | |
| GGCC-3′ | *Apa* I | *Ban* II, *Bsp*1286 I |
| GTAC-3′ | *Kpn* I | |
| NNNN-3′ | | *Bst*X I |
| RGCY-3′ | | *Ban* II, *Bsp*1286 I |
| TGCA-3′ | *Nsi* I, *Pst* I | *Bsp*1286 I |
| TTAA-3′ | I-*Ppo* I | |

**Key:**

D = A or G or T
H = A or C or T
K = G or T
M = A or C
N = A or C or G or T
R = A or G
S = C or G
W = A or T
Y = C or T

# Agarose and Polyacrylamide Gels

### Dye Migration: Polyacrylamide Denaturing Gels.

Dyes will migrate to the same point as double-stranded DNA of the indicated size in a denaturing polyacrylamide gel.

| Gel % | Bromophenol Blue | Xylene Cyanol |
|---|---|---|
| 5.0 | 35bp | 140bp |
| 6.0 | 26bp | 106bp |
| 8.0 | 19bp | 75bp |
| 10.0 | 12bp | 55bp |
| 20.0 | 8bp | 28bp |

Adapted from Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

### Dye Migration: Polyacrylamide Nondenaturing Gels.

Dyes will migrate to the same point as double-stranded DNA of the indicated size in a nondenaturing polyacrylamide gel.

| Gel % | Bromophenol Blue | Xylene Cyanol |
|---|---|---|
| 3.5 | 100bp | 460bp |
| 5.0 | 65bp | 260bp |
| 8.0 | 45bp | 160bp |
| 12.0 | 20bp | 70bp |
| 15.0 | 15bp | 60bp |
| 20.0 | 12bp | 45bp |

Adapted from Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989)
In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

### Dye Migration in 0.5–1.4% Agarose Gels.

Dyes will migrate to the same point as double-stranded DNA of the indicated size in an agarose gel. Sizes are approximate.

| Gel | Size |
|---|---|
| GoTaq® blue dye | 4kb |
| Xylene cyanol FF | 4kb |
| Bromophenol Blue | 300bp |
| Orange G | 50bp |
| GoTaq® yellow dye | 10bp |

Some information adapted from Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989)
In: *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

### Gel Percentages: Resolution of Linear DNA on Agarose Gels.

| Recommended % Agarose | Optimum Resolution for Linear DNA (Size of fragments in base pairs) |
|---|---|
| 0.5 | 1,000–30,000 |
| 0.7 | 800–12,000 |
| 1.0 | 500–10,000 |
| 1.2 | 400–7,000 |
| 1.5 | 200–3,000 |
| 2.0 | 50–2,000 |

### Gel Percentages: Resolution of Proteins on Polyacrylamide Gels.

| Recommended % Acrylamide | Protein Size Range |
|---|---|
| 8 | 40–200kDa |
| 10 | 21–100kDa |
| 12 | 10–40kDa |

Dalton (Da) is an alternate name for the atomic mass unit, and kilodalton (kDa) is 1,000 daltons. Thus a peptide with a mass of 64kDa has a molecular weight of 64,000 grams per mole.

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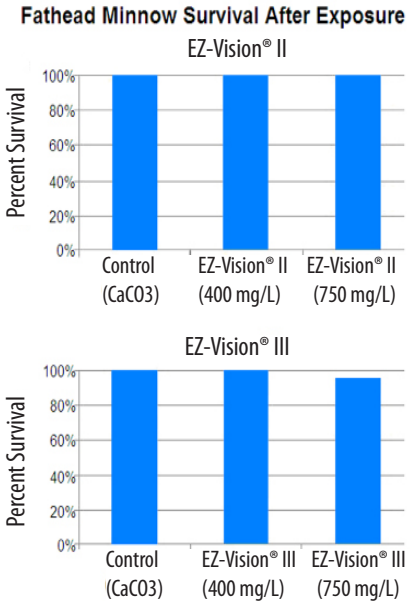
PART #GE625

## Safer Alternatives from AMRESCO

*Instant DNA visualization with non-toxic, non-mutagenic formula*




**Environmental Toxicity Testing of EZ-Vision® Products.** EZ-Vision® environmental hazard testing was determined by the CCR Title 22 Fathead Minnow Hazardous Waste Screen Bioassay. Both EZ-Vision® Two and EZ-Vision® Three were determined non-hazardous with LC50 > 750 mg/l.

***"It [EZ Vision® DNA Dye] is easy to use, doesn't slow down separation (unlike ethidium bromide) . . . [EZ-Vision®] allows an extremely easy gel extraction process - this product truly makes lab life easier"***

***Robert Jedrzejczak, PhD***
*Midwest Center for Structural Genomics*
*Argonne National Laboratory*
*Argonne, IL*

# EZ-Vision® DNA Dye as Loading Buffer

AMRESCO's non-toxic, non-mutagenic alternative to ethidium bromide.

### Convenient Solution
All-in-one solution contains a fluorescent DNA dye in a 6X loading buffer which eliminates the need for post-staining and destaining.

### Instant Visualization
Fluorescent dye allows for immediate visualization of DNA bands on a standard UV transilluminator.

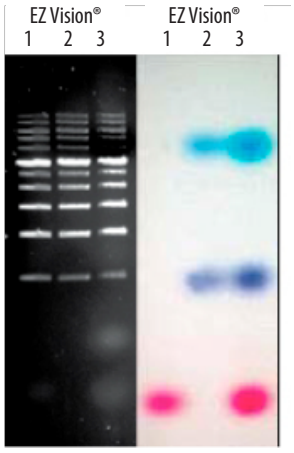
### Safer to Use
Formula is both non-toxic and non-mutagenic and eliminates the need to use ethidium bromide.

Available with three different tracking dye options, each containing the same non-hazardous DNA dye and loading buffer.

**EZ-Vision® One**
One Tracking Dye

**EZ-Vision® Two**
Two Tracking Dyes

**EZ-Vision® Three**
Three Tracking Dyes




**EZ-Vision® One, Two and Three.** Left image: 1% TAE agarose gel showing the fluorescence of AMRESCO's 1 kb Ladder (K180-250UL) stained with EZ-Vision® One (lane 1), EZ-Vision® Two (lane 2) and EZ-Vision® Three (lane 3). Right image: Digital camera photograph of the same gel as left image, showing the colors and migration position of the one fast migrating tracking dye of EZ-Vision® One (lane 1), the two tracking dyes of EZ-Vision® Two (lane 2), and the three tracking dyes of EZ-Vision® Three (lane 3).

| Description | Product Code | Pack Size | List Price |
|---|---|---|---|
| **EZ Vision® One** | N472-KIT | 5 X 1.0 ML | $115.40 |
| **EZ Vision® Two** | N650-KIT | 5 X 1.0 ML | $115.40 |
| **EZ Vision® Three** | N313-KIT | 5 X 1.0 ML | $115.40 |

# Genotypes of Frequently Used Bacterial Strains

## Genotypes of Frequently Used Bacterial Strains.

All genes in the bacterium are presumed to be in the wildtype state, except for those listed, which are mutant alleles carried by that bacterium. Genes listed on the F′ episome, however, represent wildtype alleles unless specified otherwise. Strains are λ– unless specified otherwise.

| Strain | Genotype |
|---|---|
| BL21(DE3) | F⁻, *ompT*, *hsdS*$_B$ ($r_B^-$, $m_B^-$), *dcm*, *gal*, λ(DE3) |
| *__BL21(DE3)pLysS__ | F⁻, *ompT*, *hsdS*$_B$ ($r_B^-$, $m_B^-$), *dcm*, *gal*, λ(DE3), pLys*S* ($Cm^r$) |
| *__BMH 71-18 *mutS*__ | *thi*, *supE*, Δ(*lac-proAB*), [*mutS*::Tn10($tet^r$)] [F′, traD36, *proAB*, *lacI*$^q$ZΔM15] |
| C600 (1) | *thi*-1, *thr*-1, *leuB*6, *lacY*1, *tonA*21, *supE*44 |
| C600*hfl* (1) | *thi*-1, *thr*-1, *leuB*6, *lacY*1, *tonA*21, *supE*44, *hfl*A150::Tn10($tet^r$) |
| DH1 (2) | *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 ($r_K^-$, $m_K$+), *supE*44, *relA*1 |
| DH10B | F⁻, *mcr*A Δ(*mrr-hsdRMS-mcrBC*) ϕ80*lacZ*ΔM15, Δ*lacX*74, *deoR*, *recA*1, *endA*1, *araD*139, Δ(*ara*, *leu*)7697, *galU*, *galK*, λ-, *rpsL*($str^r$), *nupG* |
| DH5α™ | ϕ80d*lacZ*ΔM15, *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 ($r_K^-$, $m_K$+), *supE*44, *relA*1, *deoR*, Δ(*lacZYA-argF*) U169, *phoA* |
| DM1 (3) | F′, *dam*⁻13::Tn9($Cm^r$) *dcm*, *mcrB*, *hsdr-M*$^+$, *gal*1, *gal*2, ara-, *lac*-, *thr*-, *leu*-, *ton*$^R$, *tsx*$^R$, *Su*$^o$ |
| **ES1301 *mutS*** | *lacZ*53, *thyA*36, *rha*-5, *metB*1, *deoC*, IN(*rrn*D-*rrn*E), [*mutS*201::Tn5] |
| *HB101 (4) | *thi*-1, *hsdS*20 ($r_B^-$, $m_B^-$), *supE*44, *recA*13, *ara*-14, *leuB*6, *proA*2, *lacY*1, *galK*2, *rpsL*20($str^r$), *xyl*-5, *mtl*-1 |
| JM101 (5) | *supE*, *thi*, Δ(*lac-proAB*), F′ (*traD*36, *proAB*, *lacI*$^q$ZΔM15) |
| *__JM109__ (5) | *endA*1, *recA*1, *gyrA*96, *thi*-1, *hsdR*17 ($r_K^-$, $m_K$+), *relA*1, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*$^q$ZΔM15] |
| **JM109(DE3)** (5) | *endA*1, *recA*1, *gyrA*96, *thi*-1, *hsdR*17 ($r_K^-$, $m_K$+), *relA*1, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*$^q$ZΔM15], λ(DE3) |
| JM110 (5) | *rpsL*($str^r$), *thr*, *leu*, *thi*, *hsdR*17 ($r_K^-$, $m_K$+), *lacY*, *galK*, *galT*, *ara*, *tonA*, *tsx*, *dam*, *dcm*, *supE*44, Δ(*lac-proAB*), [F′, *traD*36, *proAB*, *lacI*$^q$ZΔM15] |
| KRX | [F′, *traD*36, Δ*ompP*, *proAB*$^+$, *lacI*$^q$, Δ(*lacZ*)M15] Δ*ompT*, *endA*I, *recA*I, *gyrA*96 ($Nal^r$), *thi*-I, *hsdR*17 ($r_K$–, $m_K$+), eI4$^-$ (*mcrA*), *relA*I, *supE*44, Δ(*lac-proAB*), Δ(*rhaBAD*)::T7 RNA polymerase |
| KW251 | *supE*44, *galK*2, *galT*22, *metB*1, *hsdR*2, *mcrB*1, *mcrA*, [*argA*81::Tn10($tet^r$)], *recD*1014 |
| **LE392** (6) | *hsdR*514, ($r_K^-$, $m_K$+), *supE*44, *supF*58, *lacY*1 or Δ(*lacIZY*)6, *galK*2, *galT*22, *metB*1, *trpR*55 |
| **NM522** (7) | *supE*, *thi*, Δ(*lac-proAB*), Δ*hsd*5 ($r_K^-$, $m_K^-$), [F′, *proAB*, *lacI*$^q$ZΔM15] |
| NM538 (8) | *supF*, *hsdR* ($r_K^-$, $m_K$+), *trpR*, *lacY* |
| NM539 (8) | *supF*, *hsdR* ($r_K^-$, $m_K$+), *lacY*, (P2) |
| *Single Step (KRX) | [F′, *traD*36, Δ*ompP*, *proAB*$^+$, *lacI*$^q$, Δ(*lacZ*)M15] Δ*ompT*, *endA*I, *recA*I, *gyrA*96 ($Nal^r$), *thi*-I, *hsdR*17 ($r_K$–, $m_K$+), eI4$^-$ (*mcrA*), *relA*I, *supE*44, Δ(*lac-proAB*), Δ(*rhaBAD*)::T7 RNA polymerase |
| Stbl2™ | F⁻, *mcrA*, Δ(*mcrBC-hsdRMS-mrr*), *recA*1, *endA*1, *gyrA*96, *thi*-1, *supE*44, *relA*1, λ⁻, Δ(*lac-proAB*) |
| Stbl4™ | *mcrA*, Δ(*mcrBC-hsdRMS-mrr*), *recA*1, *endA*1, *gyrA*96, *thi*-1, *supE*44, *relA*1, λ⁻, Δ(*lac-proAB*), *gal*, F′{*proAB*$^+$, *lacI*$^q$, ZΔM15, Tn10(*tet*$^R$)} |
| SURE® | *e*14–, (*mcrA*–) Δ(*mcrCB-hsdSMR-mrr*)171, *endA*1, *supE*44, *thi*-1, *gyrA*96, *relA*1, *lac*, *recB*, *recJ*, *sbcC*, *umuC*::Tn5 (*kan*$^r$), *uvrC*, [F′ *proAB*, *lacI*$^q$ZΔM15::Tn10 (*tet*$^r$)] |
| TOP10 | F⁻, *mcrA*, Δ(*mrr-hsdRMS-mcrBC*), ϕ80*lacZ*ΔM15, Δ*lacX*74, *deoR*, *recA*1, *araD*139, Δ(*ara*, *leu*)7697, *galU*, *galK*, *rpsL* ($str^R$), *endA*1, *nupG* |
| TOP10F′ | F′{*lacI*$^q$ Tn10 ($tet^R$)}, *mcrA*, Δ(*mrr-hsdRMS-mcrBC*), ϕ80*lacZ*ΔM15, Δ*lacX*74, *deoR*, *recA*1, *araD*139, Δ(*ara-leu*)7697, *galU*, *galK*, *rpsL* ($str^r$), *endA*1, *nupG* |
| XL1-Blue | *recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17($r_K^-$, $m_K$+), *supE*44, *relA*1, *lac*, [F′, *proAB*, *lacI*$^q$ZΔM15::Tn10($tet^r$)] |
| XL10-Gold® | $Tet^r$, Δ(*mcrA*)183, Δ(*mcrCB-hsdSMR-mrr*)173, *endA*1, *supE*44, *thi*-1, *recA*1, *gyrA*96, *relA*1, *lac* Hte [F′ *proAB lacI*$^q$ZΔM15, *Tn*10 (*Tet*$^r$) Amy $Cam^r$] |
| Y1089 (9) | Δ(*lac*U169), *proA*+, Δ(*lon*), *araD*139, *strA*, *hflA*150, [chr::Tn10($tet^r$)], (pMC9) |
| Y1090 (9) | Δ(*lac*U169), *proA*+, Δ(*lon*), *araD*139, *strA*, *supF*, rpsL($str^r$), [*trpC*22::Tn10 ($tet^r$)], (pMC9), *hsdR* ($r_K^-$, $m_K$+) |

*Strains available from Promega as competent cells are indicated by an asterisk. Strains shown in **bold** are available from Promega as glycerol freezer stocks.

### Miscellaneous

F′ host contains an F′ episome with the stated features.

λ(DE3) bacteriophage λ carrying the gene for T7 RNA polymerase is integrated into the host genome.

pMC9 is pBR322 with *lacI*$^q$ inserted and confers ampicillin and tetracycline resistance.

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PART #GE632

## Host Gene Descriptions

| Host Gene Descriptions | | | |
|---|---|---|---|
| ara | Mutation causes inability to utilize arabinose | recBCD | Exonuclease V. Mutation in recB or recC reduces general recombination to one hundredth of its normal level and affects DNA repair |
| endA | DNA-specific endonuclease I. Mutation shown to improve yield and quality of DNA from plasmid minipreps | recJ | Exonuclease involved in alternate recombination pathways of *E. coli*. Mutation impairs recombination. |
| galK | Mutation causes inability to utilize galactose | relA | Relaxed phenotype; mutation permits RNA synthesis in the absence of protein synthesis |
| gyrA | DNA gyrase subunit A; mutation results in resistance to naladixic acid | rpsL | 30S ribosomal subunit protein S12. Mutation makes cells resistant to streptomycin; also written strA |
| hfl | High frequency lysogeny. Mutation increases λ lysogeny by inactivating a specific protease | sbcBC | Exonuclease I. Permits general recombination in recBC mutant hosts. Mutation impairs recombination. |
| lacI | Repressor protein of lac operon. LacIq is a mutant of lacI that overproduces the repressor protein | supE | Suppressor of amber (UAG) mutations. Some phage require a mutation in this gene in order to grow |
| lacY | Galactoside permease (M protein). Mutation causes inability to utilize lactose | supF | Suppressor of amber (UAG) mutations. Some phage require a mutation in this gene in order to grow |
| lacZ | ß-D-galactosidase; lactose utilization. Cells with lacZ mutations produce white colonies in the presence of X-gal; wild-type produces blue colonies | thi-1 | Mutants require vitamin B1 (thiamin) for growth in minimal media |
| lacZDM15 | A specific N-terminal deletion which permits the α-complementation segment present on the pBluescript phagemid or Lambda ZAP II vector to make a functional lacZ protein | traD36 | Mutation inactivates conjugal transfer of F′ episome |
| malA | Mutations causes inability to utilize maltose | umuC | Component of SOS repair pathway. Mutation increases stability of DNA containing long inverted repeats |
| proAB | Mutants require proline for growth in minimal media | uvrC | Component of UV excision pathway. Mutation increases stability of DNA containing long inverted repeats |
| recA | Gene central to general recombination and DNA repair. Mutation eliminates general recombination and renders bacteria sensitive to UV light | xylA | Mutation causes inability to utilize xylose |

| Other Descriptions | |
|---|---|
| $Amp^r$ | Ampicillin resistance |
| $Cam^r$ | Chloramphenicol resistance |
| $Kan^r$ | Kanamycin resistance |
| $Rif^r$ | Rifamycin resistance |
| $Tet^r$ | Tetracycline resistance |
| $Str^r$ | Streptomycin resistance |
| Δ | Indicates a deletion of the genes following it |
| Tn10 | A transposon that normally codes for $Tet^r$ |
| Tn5 | A transposon that normally codes for $Kan^r$ |
| spi $^-$ | Red$^-$gam$^-$ mutant derivatives of λ with the ability to form plaques on *E. Coli* P2 lysogens |
| amy | Strains with this phenotype express amylase |

| Restriction & Modification Systems | |
|---|---|
| *dam* | DNA adenine methylase. Mutation blocks methylation of adenine residues in the recognition sequence 5′- G*ATC -3′ (*methylated). |
| *dcm* | DNA cytosine methylase. Mutation blocks methylation of internal cytosine residues in the recognition sequences 5′- C*CAGG -3′ or 5′- C*CTGG -3′ (*methylated). |
| *hsdM* | *E. coli* (or EcoK) DNA methylase. Mutation blocks sequence-specific adenine methylation in the sequence $A^{N6}$*ACNNNNNNGTGC OR GC$^{N6}$*ACNNNNNNGTT (*methylated). DNA isolated from a $HsdM^-$ strain will be restricted by a $HsdR^+$ host. |
| *hsdR* | *E. coli* (or EcoK) restriction endonuclease. Absense of this activity permits the introduction of DNA propagated from non-*E. coli* sources. |
| *hsdS* | Specificity determinant for *hsdM* and *hsdR*. Mutation eliminates HsdM and HsdR activity. |
| *mcrA* | *E. coli* restriction system. Mutation prevents McrA restriction of methylated DNA of sequence 5′- C*CGG (*internal cytosine methylated). Formerly known as *rglA*. |
| *mcrCB* | *E. coli* restriction system. Mutation prevents McrCB restriction of methylated DNA of sequence 5′- $G^5$*C, 5′- $G^{5h}$*C, or 5′- $G^{N4}$*C (*methylated cytosine). Formerly known as *rglB*. |
| *mrr* | *E. coli* restriction system. Mutation prevents Mrr restriction of methylated DNA of sequence 5′- G*AC or C*AG (*methylated adenine). Mutation also prevents McrF restriction of methylated cytosine sequences. |

# Glossary

**5′-RACE:** Rapid Amplification of cDNA 5′-Ends.
**Activated Calf Thymus DNA:** Nicked and gapped dsDNA prepared by treatment with DNase I; a substrate for many DNA polymerases.
**Alkaline Phosphatase (AP):** An enzyme that catalyzes the removal of a phosphate group from a substrate. This property is used in colorimetric and chemiluminescent detection reagents.
**Alpha Complementation:** The process by which a functional β-galactosidase (*lac*Z) gene is generated when the *lac*Z α-peptide (N-terminus) complements the ω fragment of *lac*Z (C-terminus). In general, a cloning vector contributes the *lac*Z α-peptide and the host bacterial strain provides the ω fragment. See also blue/white cloning.
**Blue/White Cloning:** A technique used to identify recombinant (positive) clones in cloning experiments. Specially constructed cloning vectors will produce β-galactosidase by α-complementation when transformed into the appropriate host strain after exposure to the chemical IPTG. This will cause the cells to exhibit a blue color in the presence of the substrate X-Gal. Foreign DNA inserted into the cloning vector will interrupt the *lac*Z gene, preventing α-complementation and producing white colonies.
**Blunt Ends:** DNA ends that do not possess short, single-stranded overhangs. See also sticky ends.
**Cloning:** The production of a large number of identical DNA molecules from a single DNA molecule through replication of the DNA within a cell. Cloning also refers to the production of large numbers of identical cells from a single cell.
**Cohesive Ends:** Two DNA ends, in the same or different molecules, which have short, single-stranded overhangs that are complementary to one another. Cohesive ends allow comparatively efficient ligation of DNA molecules. See also sticky ends.
**Competent Cells:** Bacterial cells that are able to take in exogenous DNA.
**Distributive:** Enzyme dissociates from template after a single nucleotide addition.
**DNA (Deoxyribonucleic Acid):** A polymeric molecule composed of deoxyribonucleotide units joined in a specific sequence through the formation of 3′→5′ phosphodiester bonds.
**DNase (Deoxyribonuclease):** An enzyme that breaks down DNA into small fragments or deoxyribonucleotides.
**DNA Ligase:** An enzyme that joins two DNA molecules (or two ends of the same DNA molecule) by forming a phosphodiester bond between the 3′- and 5′-ends.
**dNTP:** Deoxyribonucleotide 5′-triphosphate.
**ds:** Double-stranded.
**DTT:** Dithiolthreitol.
**End-Labeling:** The addition of a labeled group (radioactive or nonradioactive) to the 5′- or 3′-end of DNA or RNA. This is typically accomplished by using a kinase to label the 5′-end, or a DNA polymerase or terminal deoxyxnucleotidyl transferase to label the 3′-end.
**Endonuclease:** An enzyme that hydrolyzes phosphodiester bonds at internal locations within a DNA or RNA molecule.
**Exonuclease:** An enzyme that hydrolyzes phosphodiester bonds at the ends of DNA or RNA molecules, resulting in the stepwise removal of nucleotides.
**Gene:** A segment of DNA that encodes a polypeptide, protein or RNA molecule.
**Genotype:** The specific genes (which may or may not be expressed) that are present in an organism.
**Heat-Inactivation:** Destroying an enzyme's activity by heating to a high temperature (typically 60–70°C) for an extended length of time. Not all enzymes can be heat-inactivated.
***In vitro:*** A reaction or experiment performed in the absence of living cells, typically using conditions that attempt to mimic those found within cells.
***In vivo:*** A reaction or experiment performed in a living organism or cell.
**kb:** Kilobase or kilobase pairs.
**$k_{cat}$:** Maximum number of substrate molecules converted to products per active site per unit time.
**kDa:** KiloDalton.
**$K_m$:** The Michaelis constant; the concentration of substrate that an enzyme can convert to product at half its maximal rate.
**Labeling:** A process in which nucleic acids or proteins are tagged with a radioactive or nonradioactive marker.
**Ligase:** An enzyme that catalyzes DNA or RNA linkage, generally splitting off a pyrophosphate group from ATP concurrently.
**Multiple Cloning Site (MCS):** The region of a DNA vector that contains unique restriction enzyme recognition sites into which foreign DNA can be inserted; also called a polylinker.
**Nuclease:** An enzyme that degrades nucleic acids.
**Nucleotide:** A molecule composed of an organic base, sugar and phosphate group, which constitutes the "building blocks" of nucleic acids (DNA and RNA).
**Oligonucleotide (Oligo):** A short (typically <50 nucleotides), single-stranded DNA or RNA molecule.
**PCR:** Polymerase Chain Reaction.
**PEG:** Polyethylene glycol.
**Phosphatase:** An enzyme that removes a phosphate group from a protein, nucleic acid or other molecule.
**$PP_i$:** Inorganic pyrophosphate.
**Primer:** An oligonucleotide or short single-stranded nucleic acid that acts as a starting point for the synthesis of nucleic acids from a template.
**Promoter:** DNA sequence for the initiation of RNA transcription by RNA polymerase.
**RNA (Ribonucleic Acid):** A polymeric molecule composed of ribonucleotide units joined in a specific sequence through the formation of 3′→5′ phosphodiester bonds.
**RNase (Ribonuclease):** An enzyme that breaks down RNA into smaller RNA fragments or ribonucleotides.
**rNTP:** Ribonucleotide 5′-triphosphate.
**ss:** Single-stranded.
**Sticky Ends:** Two DNA ends, in the same or different molecules, that have short, single-stranded overhangs that are complementary to one another. Sticky ends allow comparatively efficient ligation of DNA molecules. See also Cohesive Ends.
**TAE:** Tris Acetate EDTA .
**TCA:** Trichloroacetic acid.
**Terminator:** DNA sequence for the termination of RNA transcription by RNA polymerase.
**Transformation:** The process during which a plasmid DNA is inserted into a bacterial cell.
**Turnover Rate ($k_{cat}$):** Maximum number of substrate molecules converted to products per active site per unit time.
**Vector:** A DNA molecule that can replicate within a host cell and that allows the insertion of foreign DNA sequences. Vectors commonly used may be plasmids, phagemids or bacteriophage.