

Biology 305 Laboratory

Protocols
2017 Edition

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Guidelines for Safety

Objectives

Students in teaching laboratories should be aware that there are risks of personal injury through accidents, e.g. fire or explosion, exposure to bio-hazardous materials, corrosive chemicals, fumes, and cuts. These guidelines are meant to minimize injury risk by emphasizing safety precautions, and to clarify emergency procedures in the event of an accident.

Emergency Equipment

Make sure you know where to find and how to use the

- Closest emergency exit.
- Numbers to call in an emergency (911 and security 306-585-4999)
- Closest fire alarm and pull station.
- Fire extinguishers.
- Safety shower.
- Eyewash.
- First aid kit.

General safety regulations

- **Eating, drinking and smoking are prohibited** in all laboratories.
- **Do not bring backpacks or coats or other bulky items into the lab; only bring the things you will need.** The labs get very congested and these items are tripping hazards. Do not hang coats over the back of your chairs. Lockers and locks are provided for storing these items during lab time.
- Always **wash your hands** prior to leaving the laboratory.
- Laboratory coats and safety glasses are required for all laboratories, unless otherwise indicated by your instructor.
- Students are not allowed to work in a laboratory unless a lab instructor is present.
- Confusion can cause accidents. Before starting an experiment, establish a plan of action and list of required materials. Compile all materials before starting.
- **Report equipment problems** to the instructor immediately.
- **Report all spills** to the lab instructor immediately.
- **Report any injuries** immediately to your lab instructor.
- **Long hair** must be tied back to keep from being caught in equipment, Bunsen burners, chemicals, etc.
- We recommend that contact lenses not be worn in laboratories.
- Enthusiasm is encouraged but pranks and horseplay can be hazardous.

Spills

Minor SOLUTION/CHEMICAL spill: Put on gloves and wipe up the spill with paper towels as indicated by the lab instructor.

Major ACID/BASE/TOXIN spill: Immediately contact your lab instructor. DO NOT TOUCH!

Blood/Bacterial/Fungal/Virus spill: Immediately tell your lab instructor and the students around the spill area. If necessary, take off contaminated protective and personal clothing. Prevent anyone from going near the spill. The lab instructor must supervise the clean up.

Health concerns

Allergies

Students who have allergies (chemical, plant, animal or antibiotic) that may be relevant to any laboratory exercise in this course should inform the lab instructor. If the student should not be present during the experiment, a doctor's note will be required.

Pregnancy

Some chemicals used in laboratory teaching may cause fetal deformities. If you are pregnant, please inform your lab instructor so that the appropriate precautions are taken.

Disposal of wastes

Some wastes produced by laboratories cannot be disposed of in the sink or regular garbage cans. Please pay close attention to the following procedures.

Sharps: needles, scalpels, syringes, razor blades and other sharp items are placed in a special sharps container.

Glassware: clean lab glassware, broken glass, Pasteur pipettes are disposed of in the glass disposal bucket, near the entrance to the lab.

Petri plates: containing any microbial growth: Place in biohazard waste bucket.

Bacterial or fungal liquid: Place tubes and flasks in marked trays for autoclaving.

Liquid or solid chemicals: Place in disposal containers as indicated by the lab instructor.

If you are uncertain, please ask your lab instructor.

Welcome to the 305 lab

The majority of the information you will need to prepare for the lab each week will **only be available online**. This book contains most of the protocols you will need. I have also included frequently needed reference materials such as plasmid maps and DNA ladders.

Please see the 305 website for all lab policies including attendance policies.

Preparing for the lab

In the 305 lab we need the majority of our weekly 3 h to complete experiments. Thus, there is very little time for pre-labs and **you are expected to come to lab fully prepared each week**. Please leave yourself at least one hour per week for preparation.

The weekly guides, available online, will guide your preparation. Each week there is background material to read and protocols to go over ahead of time. If you do not understand something in this material you need to ask questions before you start the lab – preferably before the lab time begins. There are several ways to get clarification on the material.

- look it up – Google is very handy for simple explanations (for example – if the protocol says “decant the supernatant” and you don't know what decant or supernatant mean – look it up).
- ask a question in the comment section of the weekly guide
- post a question on a forum (see the website)
- contact the lab instructor directly for clarification
- ask in the lab during the pre-lab time

All labs should be completed in the allotted time. If you need to stay late or come back outside the lab time, your lab mark for the week could be reduced.

How to keep a notebook

Keeping good notes in the lab is vital. Your notebook **is your only complete record** of what you have done. Your notebook is **also a tool** to keep you **organized** as you plan, carry out and evaluate your experiments and studies. Good notes will allow you and others to understand and replicate your experiments. Unlike published research, every experiment and procedure you do should be in your notebook. It is just as important to keep track of things that don't work as things that do.

In research, good original documentation is important for publication; not only for writing a paper, but also to prove the data you publish is accurate (charges of falsification do happen). Good notebooks are essential in patent applications (dates of discovery are critical). Finally Good Laboratory Practice (GLP) requires keeping accurate and complete experiment notes.

There is no single way to keep a good notebook and no single style will work for all experiments. Below is the general layout and approach you are expected to use in the 305 lab.

Please do use your notebook for:

- experiment planning,
- prelab notes
- experiment execution notes,
- data collection,
- data analyses and
- data interpretations
- anything else that is useful to you

When making notes keep the following questions in mind.

- What do I need to know to complete this procedure today?
- Why am I doing this procedure?
- What will I need to know if I am to repeat this experiment a year from now?
- If someone else needed to repeat what I have done using **only** these notes as a source, what information would they need?
- What details could influence the results? For example, the time of day is not usually important in controlled laboratory experiments, but in a field study it could be very important.

How to keep notes

- Lab notebooks must be **bound** with no pages removed (i.e. no spiral books, no perforated pages). However, they don't need to be expensive. A simple composition book from a dollar store is fine. You will need at least 80 pages.
- ALL **PAGES MUST BE NUMBERED** BEFORE YOU WRITE ANY NOTES. I usually only number the odd pages, that way every page has a number but I save a little time.
- A **table of contents** must be at the front of the notebook, leave the first 3 pages blank for this purpose.
- Each project should have a separate book.
- Always use permanent **ink** for note keeping (no pencil).
- Notes must be clear, **succinct, logical and legible**. Leave lots of white space so your notes are not crowded. Use **point form** wherever possible and avoid repeating information. For example, if all steps were done on ice, at the top of the procedure state "all steps were performed on ice".



- If you need to make a change, cross it out with a single stroke, **never obscure your notes**.
- Differentiate between what you did and your interpretations.
- Do not write out entire procedures before you start, protocols are often adapted as you go, instead write notes as you complete the protocol. **Ideally write out each step just before you do it**. It is okay to write some additional notes after completing the experiment but you should not be writing out what you did from memory.

Procedure/experiment notes - guidelines

- Many experiments/studies will run over multiple days, even weeks or months. Introduce each project by summarizing the overall goal before you get into the minutia.
- Start each **new protocol/experiment** on a **new page**. While a notebook is recorded chronologically, don't list all things done on a particular day as a single entry. Some days you may complete multiple experiments/protocols, and some protocols will take more than one day.
- At the start of each entry note the **date** (including year) and write a **descriptive title**. For example, "plasmid isolation" is not specific "pUC18 plasmid isolation" would be better.
- If a single protocol/experiment runs over multiple days, indicate "continued on pg#__" at the end of each entry and "continued from pg# __" at the start of every entry. This makes discontinues experiments easy to follow.
- All experiment titles and page numbers must be listed in the table of contents.
- Start each entry with a **description of the purpose/reason** you conducted the described experiment/procedure. For example, "Isolate pUC18 plasmid DNA to use as a vector in subcloning Gene A".
- Indicate the source of materials (where did the cells/DNA etc. come from). When someone other than yourself performs procedure steps for you, clearly indicate the when/how/who of the steps not completed by you.
- **Indicate the protocol source**. If the protocol is from a kit, note the kit name, the company name and the version date (if you can find it), if it is from a journal, reference it. If it is your own protocol, make reference to the title and version.
- Indicate how/where all materials produced are stored. For example, if you isolate a plasmid you will be keeping it somewhere for further use – indicate how it is stored as this could influence your results (and you need to find it).
- **In your own words** (point form) describe what you do. Even if you have referenced a protocol, you still need to record how you implement it because you will make changes that are important. Note what you do every time, even if you have used the protocol dozens of time before, do not write, "as was done on pg X". This type of cross-referencing leads to many mistakes.
- Where appropriate, **use tables or drawings to simplify your notes**. For example, describing how you set up an enzyme digest in long hand is confusing but the same information in a table is much easier to follow.
- **All solution recipes should be in the notebook**. It is acceptable to have them all in one place (for example at the back of your notebook) and reference them by page number.
- Note name and model number for specialized equipment (not necessary for standard equipment like pipettors and micro-centrifuges).
- All data should be in your book. If the data are printouts or photographs, attach them to your book. All attached data must be labelled with the **date collected, your name** and the **page number** where the data is supposed to live (photographs can fall out of notebooks). If it's not possible to attach the data, clearly reference where they are stored.

- **Describe your analysis, interpretations and thoughts**, and clearly distinguish interpretations from facts. For example, "plasmid yield was low, perhaps due to lower cell density as cultures were not as turbid as usual". You never know when an observation like this might be important.
- It is vital that others be able to easily follow your notes. This means the notes must be **logically organized, succinctly presented** and **legible**. However, it does not mean they need to be perfect! Strikeouts are common in lab notebooks. See my example notebook pages from my real notebook on the 305 lab website.

Layout for 305 notebooks

In addition to meeting the guidelines for procedure and experiment notes you must layout your 305-lab notebook as follows:

- **Write your in-lab notes on the right hand pages only**. Use the left hand side for attached items such as gel photos and unanticipated notes you need to add later. If you add notes at a later date indicate when they were added.
- Leave the first 3 to 4 pages for a table of contents and keep this up to date.
- Your first notebook entry should be a brief description of the overall project goals. Give this entry a descriptive title.
- Attach printed copies of the plasmid maps to your notebook. You could either put these on the left hand page facing the project description, making them easy to find later, or you could put them in on the pages following your project description.
- Do not prewrite protocols before coming to lab. Instead, briefly note each step in a protocol **immediately prior** to doing it.

Notebook assessment

We will check your notebooks every week look for the following.

- Each experiment/entry has a descriptive title.
- The purpose for the experiment/entry is clearly stated (sometimes the title fully encapsulates the purpose but if not it must be clearly stated just below the title).
- All pages are numbered.
- Changes are made using strike out and the original notes are clearly readable.
- Experimental procedures are complete such that a reader could repeat the experiment using the notebook only. USE POINT FORM.
- If someone other than the notebook owner did steps, this is clearly indicated.
- Data tables are clearly laid out and legible.
- Attached data items are clearly labelled with the notebook owners name and the date.
- Results are interpretations are briefly summarized and are clearly identified as such (i.e. one can easily distinguish between interpretations and factual information).
- The table of contents are up to date.
- Materials are described as they are used – don't make a separate list.
- You do not need to include pre-lab notes, but if you do make sure we can distinguish them from in-lab notes (give pre-lab notes their own title).

Please see the 305 lab website for the notebook marking rubric and for some example notebook pages.

General guidelines for molecular biology labs

Please review this guide before every lab until the techniques become second nature.

Following protocols

Protocols in this manual are presented as they would be in a research lab. Protocols used in research labs are written much more concisely than they would be for a student teaching lab. For example, a protocol will not include details like "label your tubes" as it is generally understood that unlabelled tubes will lead to disaster.

You are expected to **think** and **anticipate** while you use the protocol.

Using a protocol effectively and efficiently involves 4 steps.

1. **Read** the entire protocol carefully, making a list of any equipment or components needed. Make sure you **understand** what you need to do in each step – look up words you do not understand.
2. **Check** to make sure you have all the needed components and equipment and that you know how to use them.
3. As you work through the protocol, **read** each step in its entirety, right before you **do** it.
4. **Anticipate**. For example, if you will need another set of labelled tubes in step 5, prepare these tubes during the 5 min spin in step 3.

Sterility

In molecular biology we frequently use sensitive biological agents such as enzymes and naked DNA molecules. Biological molecules can be damaged or degraded by enzymes that are found on non-sterile surfaces and even floating in the air. Consequently, all manipulations are done with sterile materials (e.g. sterile tips and tubes). In our lab, sterile tube and tip packages have autoclave tape attached; this tape has dark stripes if the materials have been autoclaved.

To protect the sterility of your materials please:

- clean your bench with the provided 70% ethanol at the beginning of every laboratory;
- keep tip and tub containers closed, except when using;
- do not touch the materials with your hands or any other non-sterile surfaces.

Biohazards

We will frequently work with living organisms, many of which have artificially modified DNA and; therefore, are classified as Genetically Modified Organisms (GMOs). GMOs are considered biohazards until their potential effects on humans and the environment are assessed (this is rarely done for products produced for research purposes). DNA and proteins extracted from such organisms are also classified as biohazards. The organisms we are working with have been used by 1000s of researchers for decades with no evidence of ill effects, none-the-less it is appropriate and required by law that we take the following precautions.

- GMO cultures and materials derived from them must be destroyed before disposal. The materials we use are destroyed using high-pressure steam (see general protocols in your lab manual). Your job is to make sure all materials you use – tips, tubes, gloves etc. are placed in the bio-hazardous waste buckets – I will take care of the waste from there.
- Rather than trying to track which tubes came in contact with the bio-hazardous material – assume everything you use is bio-hazardous and treat accordingly.
- Please report any spills to your lab instructor immediately – you will not be in trouble – we simply need to ensure you clean up properly.
- Always wash your hands thoroughly before leaving the lab with soap and hot water.

Safety

General lab safety is discussed elsewhere, the following points apply specifically to this lab. Besides the biohazards mentioned above please be aware of the following hazards in the lab:

- Bunsen burners – tie back long hair and avoid loose clothing.
- Bunsen burners and ethanol – ethanol is very flammable and we often use it with an open flame. Do not panic if you accidentally set ethanol on fire. If the ethanol is in a container put the lid on the container, if the ethanol fire is on the bench and is small – turn off the burner and move away any flammable objects – the fire will quickly burn itself out. If the fire seems large, notify your instructor immediately.
- We will occasionally use hazardous chemicals such as ethidium bromide – please wear gloves and eye protection when instructed to do so and always wash your hands before leaving the lab.
- We regularly use equipment such as centrifuges and vortexes that can be hazardous when used incorrectly – make sure you are using all equipment correctly and be aware of what is going on around you.
- Watch out for heat blocks that have very hot surfaces.

Proper use of equipment and reagents

Pipettors

Instructions for proper use and care of pipettes is available on the 305 website. Your pipetting skills will be tested in the first lab. Remember to keep tips sterile.

Micro-centrifuges

A centrifuge must always be **balanced** before being run. This means that every tube must be directly across from another tube that has an equal weight.

When using a low speed micro centrifuge (max rpm \leq 15000), it is not necessary to weigh the tubes provided that the tubes have identical contents and volumes (e.g. if you are doing two plasmid preps side by side the tubes should have the same components and volumes).

If the tubes do not have identical components or do not have the same volume you will have to weigh the tubes and adjust the tube weights to within 0.1 g.

Once the centrifuge is loaded

1. place the rotor lid on the rotor
2. close the centrifuge lid
3. set the speed (make sure you know what the speed should be)
4. set the timer (setting the timer usually starts the centrifuge)

Always place your tubes with the hinge facing the outer rim of the rotor. If you are spinning something down this will make it easier to find the pellet.

Working with reagents

When repeatedly pipetting the same reagent into multiple tubes you must change tips between tubes – this prevents contaminating the reagent or cross contamination between samples (one exception – if the tube is empty you can use one tip to add the reagent to all tubes).

Working with small volumes (1 µl to 1 ml)

- Before opening a tube containing a small volume (e.g. a 2 µl aliquot of enzyme) **quick spin** the tube.
- After setting up a small volume reaction (< 50 µl), mix the reaction by pipetting up and down 2 or 3 times (set the volume on the pipetter to ~ 80% of the total volume and avoid foaming the reaction as many enzymes are destroyed by foaming). Then quick spin the tube.

QUICK SPIN This technique pushes small volumes to the bottom of a tube where they are easier to find. Place the tube and a balance tube (for volumes < 50 µl you can use an empty tube) in the centrifuge. Hold down the 'quick' button for 3 or 4 sec. The drop should be at the bottom of the tube on the outside edge.

Molecular stock reagents: how to use them

The small volumes used in molecular biology create a problem for making solutions. For example, the working concentration for MgCl₂ in *Hind* III digest is 10 mM. The molecular weight of MgCl₂ is 203.30, so to make 10 µl of 10 mM MgCl₂ you would need

$$203.3 \text{ g} / \times 0.01 \text{ M} \times 0.000 01 \text{ L} = 2.3 \times 10^{-5} \text{ g} \quad \text{of MgCl}_2.$$

Since weighing such a small amount is extremely difficult, we usually make stock solutions at concentrations 10 times (10X) or greater than the working concentration. We then simply add the appropriate volume to the reaction we are setting up, to get the desired final concentration (and bring to the correct final volume using H₂O). For example, restriction enzyme buffers are usually made as a 10X stock. The working concentration is usually 1X (but always check the instructions for a particular enzyme).

This means you need to regularly calculate dilution factors. See the 305 website for some practice problems.

A note on water and molecular grade reagents

Biological molecules can be negatively affected by organic and inorganic compounds frequently found in tap and deionized water. **Use only 15 megohm (MΩ) water or distilled water** when preparing solutions.

For similar reasons, **use only high quality molecular grade reagents** (tested for the presence of DNases, RNases and Proteases).

Alkaline lysis & plasmid purification using EconoSpin®

Plasmid DNA is separated from unwanted cell macromolecules using alkaline lysis and neutralization. Then the DNA is purified away from soluble contaminants using a silica column purchased from Epoch Life Science™.

E. coli cells are grown to stationary phase in a liquid culture. Cells can be started from a single isolated colony grown on a plate, or directly from a glycerol stock if you are confident in your glycerol stock strain. Cells are generally grown in Luria-Bertani Broth with the appropriate selection to insure that the bacteria maintain the plasmids during growth. Most recombinant plasmid vectors will have at least one antibiotic resistance gene that can be used for selection.

E. coli take about 12 – 16 h at 37 °C with vigorous agitation (~ 250 – 300 rpm in a shaking incubator) to reach stationary phase so you need to plan ahead to have your cultures ready for plasmid isolation. Generally cultures are started around 4 pm the day before you want to isolate the plasmids.

1. Harvest the cells by centrifugation at 10 000 rpm for 1 min.

Table 1. Amount of stationary culture to harvest

Plasmid copy number	Culture volume	Elution Volume
Low (< 100 copies per cell)	1 – 5 mL	50 µl
High (> 100 copies per cell)	1 – 10 mL	50 – 100 µl

2. Discard supernatant by decanting; give the tube a little shake to get all the broth out, leaving broth behind will interfere with the lysis step.
3. Resuspend the cells in a neutral buffer by adding 250 µl of MX1 (containing RNase A at 100 µg/ml) and resuspend the pellet by vortexing. Good lysis depends on the cells being completely resuspended. After this step, all mixing is done gently, so put the vortex away.
4. Lyse the cells by adding 250 µl of MX2. Mix by inverting the tube 4 - 6 times. The solution should get goopy and clear slightly. DO NOT VORTEX – if you do you will get genomic contamination in your final prep.

MX2 is an alkaline solution that contains a surfactant. The high pH causes all the macromolecules in the cell and the cell wall (proteins and nucleic acids) to denature while the surfactant gently disrupts the the cell membrane allowing smaller macromolecules like plasmids, to leak out of the cell, while the larger chromosomal DNA a most of the proteins remain inside the leaky cell. If you vortex at this stage you will completely disrupt the membrane and everything will leak out, resulting in chromosomal DNA contamination of your plasmid prep.

5. Neutralize the lysate by adding 350 µl of MX3. Mix by inverting 4 – 6 times. The solution will immediately form stringy white gunk.

MX3 instantly brings the solution back to a neutral pH. The chromosomal DNA and the proteins attempt to renature but because the neutralization is so rapid the large chromosomal DNA can't properly align and the proteins and the chromosomal DNA end up sticking to each other and the cell membrane, forming massive molecules that precipitate out as white, stringy gunk. However, when plasmid DNA is denatured, the two strands remain together because they are concatenated, thus, when returned to a neutral pH, the two single strands can easily renature by base pairing. Additionally, most of the plasmid molecules have leaked out of the cell they don't end up getting caught up in the super molecules that form with the cells. The rapid renaturing of the plasmid molecules, combined with their physical separation from the rest of the cell contents results in the plasmid molecules staying in solution when the rest of the macromolecules precipitate.

6. Pellet the cell debris by centrifugation at >12 000 rpm for 10 min.
7. Using a 1 ml pipette, transfer the supernatant to a spin column that is sitting in a collection tube. Avoid transferring any of the white precipitate.
MX3 contains the conditions that will ensure the nucleic acids remaining in the supernatant stick to the silica column. However, most other soluble contaminants, such as divalent cations, will not stick to the column.
8. Centrifuge at 4000 rpm for 1 min. Discard the flow through.
In column chromatography language the flow through contains the stuff you probably don't want, while the eluent contains the molecules you do want.
9. Wash the column to remove contaminants by adding 500 μ l of WS and centrifuge > 12 000 rpm for 1 min. Discard the flow through.
10. Repeat the wash step (2 washes total).
11. Spin the column for an additional minute to ensure no wash buffer remains in the column.
12. Elute the nucleic acids by place the column in a new 1.5 ml tube and add 50 μ l of EB to the centre of the membrane (do not touch the membrane with the pipette tip). Incubate at room temperature for 2 – 5 min.
13. Centrifuge at 12 000 rpm for 1 min. Remove and discard the column. Store the eluted plasmid DNA at -20 °C. For very high copy number plasmids (>500 per cell) repeat the elution step and pool the elutes.

Restriction Enzyme digestion

Generally we use commercially available Type II restriction enzymes to digest DNA into discrete fragments. There are two main reasons digestion with these enzymes is useful in molecular biology. First Type II restriction enzymes recognize short, specific sequences of DNA and then cut the DNA at a predictable position within or close to the recognition site. Secondly, the DNA fragments produced can be re-joined using DNA ligase. Thus, if you digest two different DNA strands with the same restriction enzyme, mix the distinct fragments together, and add ligase, you can create new DNA molecules.

Many bacterial species have independently evolved a restriction-modification system. Scientists have identified and cloned hundreds of different restriction enzyme from diverse bacterial species. These enzymes, like all enzymes, require a specific set of conditions in order properly catalyze their reaction. For example, there will be an optimal pH, temperature and salt concentration for activity, and many enzymes require at least one cofactor. Because they have all evolved independently, there is no one set of conditions that can be universally applied so it is important that you use the optimal conditions for the enzyme(s) you have chosen. Fortunately, manufactures include optimal buffers with the enzyme and describe the optimal conditions for setting up digests.

General restriction enzyme digestion set up

component	amount (in μl)
DNA	Depends on amount of digested product needed and the [DNA].
restriction enzyme	Variable but usually in the range of 5 – 10 units per digest (enzymes are described as unit amounts rather than [protein] because it is enzyme activity that is important).
restriction enzyme buffer	Generally supplied as a 10X concentration and is used at a final concentration of 1X.
additional components	For some applications an additional component might be need but this is rare.
molecular grade water	Used to achieve the desired final volume. The final volume will vary depending on how much digested DNA you need and the [DNA]. Final volumes usually range from 10 – 50 μ l.

Most commercial restriction enzymes work optimally at 37 °C and will completely digest the DNA in 1 h.

Note that these are general guidelines; you must refer to the manufactures specific instructions for the enzyme(s) you are using.

Agarose gel electrophoresis of DNA

Reference: Most of the information in the protocol below is adapted from FMCs BioProducts web page of technical information.

Purpose

Agarose electrophoresis is used to separate DNA molecules by size. DNA, a negatively charged molecule, is moved by electric current through a matrix of agarose towards the positive electrode.

Part 1: Dissolving agarose and pouring the gel

Materials	<ul style="list-style-type: none"> • Microwave oven • Flask that is 4-5 times the gel volume • gel tray, comb and casting apparatus
Reagents	<ul style="list-style-type: none"> • Distilled water • 1X TAE or 1X TBE or 0.5X TBE electrophoresis buffer • Agarose powder (electrophoresis grade) • Ethidium bromide (EtBr) - if adding to the gel, final concentration is 0.5 µg/ml
Precautions	<ul style="list-style-type: none"> • Always wear eye protection, handle hot flasks with mitts, guard yourself and others against scalding • Ethidium bromide is a known carcinogen, wear gloves at all times and dispose of contaminated materials as indicated • All components produced are biohazards and must be autoclaved before disposal. Dispose of all tips and tubes in biohazardous waste buckets.

Table 2. Electrophoresis buffer recipes - final volume for all is 1 L. Usually these buffers are used at a 1X concentration.

50x TAE (Tris-acetate)¹	5X TBE (Tris-borate)¹	10X SB (Sodium Borate)²
Working concentrations (1X): 40 mM Tris, 40 mM acetic acid, 1 mM EDTA.	Working concentrations (1X): 89 mM Tris, 89 mM boric acid, 2 mM EDTA	
242.0 g Tris-base	54.0 g Tris-base	4 g NaOH
57.1 mL Glacial acetic acid	27.5 g Boric acid	24 g Boric Acid
18.61 g Na ₂ EDTA · 2H ₂ O	3.72 g Na ₂ EDTA · 2H ₂ O	
Bring to final volume of 1 L with H ₂ O	Bring to final volume of 1 L with H ₂ O	pH to 8.5 and bring to final volume of 1 L with H ₂ O

¹From Sambrook and Russell, 2001

²Brady and Kern, 2004

Table 3. Gel volumes for BioRad electrophoresis apparatus.

apparatus	volume of gel
mini-gel	25 – 40 ml
mini-wide	50 – 80 ml
double-wide	100 – 160 ml

Table 4. DNA fragment size resolving ranges for various agarose concentrations.¹

percent agarose	DNA size range resolved (in base pairs)²
0.5	1000 – 30 000
0.7	800 – 12 000
1.0	500 – 10 000
1.2	400 – 7000
1.5	200 – 3000
2.0	50 - 2000

¹Adapted from Qiagen News, 1999.

²The best resolution will be at the bottom of the range.

Microwave instructions for agarose preparation

Follow the steps below to prepare agarose in the microwave for gel concentrations of < 2% w/v.

1. Add buffer and agarose to an Erlenmeyer flask that is 2-4 times the volume of the solution. For 40 ml gel use a 250 ml flask, for 80 or 160 ml gel use 500 ml flask.
2. Weigh the flask before heating.
3. Cover the flask to prevent evaporation. Heat the flask in the microwave oven on HIGH power until bubbles appear.

CAUTION: Microwaved solution may become superheated and foam over when agitated.



4. Remove the flask from the microwave oven. Remove the cover and GENTLY swirl the flask to suspend any settled powder and gel pieces.

Watch that the agarose doesn't boil over.

5. Reheat the flask on HIGH power until the solution comes to a boil. Hold at boiling point for 45 s or until all of the particles are dissolved.
6. Remove the flask from the microwave and remove the cover. GENTLY swirl the flask to thoroughly mix the agarose solution.
7. After dissolution, add sufficient distilled water to obtain the initial weight (volume). Mix thoroughly.

e.g. if you have lost 10 g of weight, add 10 ml of H₂O.

- Cool the solution to 50-60 °C prior to casting. Add ethidium bromide (final concentration 0.5 µg/ml) before casting if desired.

When you can hold the flask in the palm of your hand, it is the right temperature for pouring.

Gel Casting

- Assemble the appropriate gel tray and comb in the casting apparatus according to the manufacturer's instructions. Make sure gel tray is level.
- Pour cooled agarose into the casting tray. Remove any air bubbles. Allow to solidify for 30 to 40 min. As the gel solidifies it will become more opaque and take on a bluish tinge, the more concentrated the gel is the more bluish it will become.
- If the gel will not be used within 2 hours, cover it with a small amount of running buffer and saran wrap to prevent it from becoming too dry. (To store longer place the wrapped gel at 4 °C.)

Part 2: Loading DNA and Running the Gel

Materials	<ul style="list-style-type: none"> • prepared agarose gel • micro-pipettors • sterile disposable tips • electrophoresis power-pac
Reagents	<ul style="list-style-type: none"> • DNA in solution • Loading buffer • electrophoresis buffer (use the same buffer as the gel was made with, see part 1 for recipe) • ethidium bromide (if not already added to the gel)
Precautions	<ul style="list-style-type: none"> • Ethidium bromide is a known carcinogen, wear gloves at all times and dispose of contaminated materials as indicated. • Potential shock hazard. The safety lid must be in place on the gel box before connecting the power leads to the power supply. Turn all power supply controls off and disconnect the power leads before removing the safety lid.

Table 5. Loading buffer recipes.

Loading Buffer	6X Recipe	Storage Temperature
Sucrose Based	40% (w/v) Sucrose 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4 °C
Glycerol Based	30% Glycerol in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	4 °C
Ficoll® Based	15% Ficoll (type 400) polymer in distilled water 0.25% Bromophenol Blue 0.25% Xylene cyanol FF	Room Temperature

Sample preparation and Gel Loading

1. Remove gel/gel tray from casting stand and place it in the appropriate gel box. Make sure the wells are closest to the negative (black) electrode.
2. Add electrophoresis buffer to fill gel box and cover the gel to a depth of 3 – 5 mm.

Too little buffer and the gel may dry out during electrophoresis. Excessive buffer depth will decrease DNA mobility, promote band distortion and can cause excessive heating within the system.

3. Remove comb (can also be removed before placing gel in gel box).
4. DNA solution is mixed with the appropriate amount of loading dye (we usually use the Ficoll based buffer) in a 1.5 ml tube (or on a piece of Parafilm).

The volume of DNA to use is determined by the concentration of the DNA (see note "Optimal DNA loading amount") and limited by the volume a well will hold (dependent upon comb size and thickness of gel – determine empirically by loading a well with loading dye + water). Normally we use 1 µl of loading dye per 10 µl of DNA sample.

5. Use only the pipette designated for gel loading to load DNA samples. Set the pipetter to the volume to be loaded. Draw up the DNA/loading dye and load the well by placing the tip midway down into the well (not all the way to the bottom as you may pierce the well). Gently expel the tip contents; the DNA/loading dye will sink to bottom of the well. Repeat until all samples are loaded.

Remember: load at least 1 well with an appropriate size range marker.

6. Place lid on electrophoresis tank. Hook up colour-coded leads to matching colours on the power pack. Set voltage required (see note "Determining voltage to use for running agarose gels") and set timer on power pack if desired.

NOTES

Optimal DNA loading amount

The amount of DNA that may be loaded on a gel depends on several factors:

- Well volume
- Fragment size: The capacity of the gel drops sharply as the fragment size increases, especially over a few kb
- Distribution of fragment sizes
- Voltage gradient: Higher voltage gradients are better suited to DNA fragments < 1 kb, lower voltages are better suited to fragments > 1 kb
- Total amount of DNA versus the number of bands. The least amount of DNA in a single band that can be reliably detected with ethidium bromide is approximately 10 ng. The most DNA compatible with a sharp, clean band is about 100 ng. Overloaded DNA results in trailing and smearing, a problem that is more severe with larger DNA molecules.

Loading Buffers

Gel loading buffers serve three purposes in DNA electrophoresis:

- Increase the density of the sample - this ensures that the DNA will drop evenly into the well.
- Add colour to the sample - simplifies loading
- Add mobility dyes - the dyes migrate in an electric field towards the anode at predictable rates. This enables one to monitor the electrophoretic process.

We usually use the Ficoll® based dye for DNA samples (Table 5. Loading buffer recipes.).

The use of the lower molecular weight glycerol in the loading buffer allows DNA to stream up the sides of the well before electrophoresis has begun and can result in a U-

shaped band. In TBE gels, glycerol also interacts with borate, which can alter the local pH.

Sample preparation

Loading buffer that is too high in ionic strength causes bands to be fuzzy. Ideally, the DNA sample should be resuspended in the same solution as the running buffer. If this is not possible, use a sample buffer with a lower ionic strength than the running buffer. TE (10 mM Tris, 1 mM EDTA, pH 7.5 – 8.0) is a good choice for DNA suspension buffer.

Determining voltage to use for running agarose gels

The voltage gradient is determined using the distance between the electrodes. If the voltage is too high, band streaking, especially for DNA >12-15 kb, may result. When the voltage is too low, the mobility of small (< 1 kb) DNA is reduced and band broadening will occur due to dispersion and diffusion. Buffer also plays a role in band sharpness. Band sharpness for small DNA tends to be better in TBE gels, whereas TAE is better for large (>12 kb) DNA. Guidelines for optimal voltage and length of time to run your gel are given in Table 6.

Table 6. Optimal Voltage and Electrophoretic Times.

Fragment Size	Optimal Voltage	Buffer to use	
		Recovery	Analytical
< 1 kb	5 V/cm	TAE	TBE
1 to 12 kb	4-10 V/cm	TAE	TAE/TBE
> 12 kb	1-2 V/cm	TAE	TAE

Table 7. Allowable voltage range for each buffer system.

Buffer system	Voltage Range (V/cm)
TAE	5 – 10
TBE	4 - 10
TB	5 - 35

Table 8. Dye Mobility Table: Migration of double-stranded DNA in relation to Bromophenol Blue (BB) and Xylene Cyanol (XC) in SeaKem LE Agarose Gels (in base pairs).

<i>1X TAE Buffer</i>		<i>% Agarose</i>	<i>1X TBE Buffer</i>	
<i>XC</i>	<i>BB</i>		<i>XC</i>	<i>BB</i>
24,800	2,900	0.30	19,400	2,850
11,000	1,650	0.50	12,000	1,350
10,200	1,000	0.75	9,200	720
6,100	500	1.00	4,100	400
3,560	370	1.25	2,500	260
2,800	300	1.50	1,800	200
1,800	200	1.75	1,100	110
1,300	150	2.00	850	70

Taken from Cambrex Resource Center WWW site.

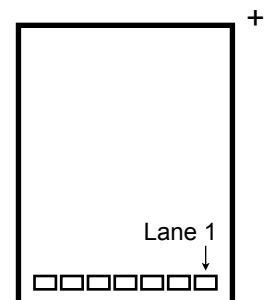
Quick Guide to Pouring a Gel

Also view the photo tutorial available on the website.

- weigh agarose, add to flask
- measure and add buffer
- weigh and record the total weight of the flask
- swirl gently
- cover flask opening with weigh boat
- place in microwave
- cook for 45 – 60 s (for mine use high power – which is the default, for a better microwave oven, use medium power)
- protecting your hand with a glove, take out flask and swirl contents (watch out for boiling over from superheating)
- cook for an additional 45 – 60 s
- weigh flask again, bring to starting volume by adding H₂O (1 ml H₂O per 1 g of lost weight)
- tape the ends of the gel tray
- run a bead of agarose to seal the tape/tray join (to keep you gel from leaking) – wait about 5 min to let this harden
- put a comb in the gel tray
- add 0.1 µl of 0.5 mg/ml EtBr per ml of gel (i.e. 1/10000 dilution) WEAR GLOVES
- swirl gently to mix and pour gel
- immediately rinse flask with running water, and place in bucket for wash up

Quick guide to preparing and loading DNA samples

- If you will only be loading a portion of your total sample you must set up for loading in a new tube.
- If your sample is less than 8 µl add 2 µl of loading dye and bring the volume to 10 µl using molecular grade water.
- If your sample is 8 – 12 µl add 2 µl of loading dye.
- If your sample is > 12 µl add loading dye at a rate of 1 µl of loading dye per 6 µl of sample.
- Gels are loaded with the wells closest to you. In this position lane 1 will be the lane closest to the right side of the gel.
- Load an appropriate amount of DNA ladder into lane 1.
- Load your prepared samples – generally the entire amount.
- Make sure you know what was loaded in each lane.



Viewing gels and labelling photos

To see the DNA, following electrophoresis the gel must be exposed to UV light. Ethidium bromide complexed with DNA fluoresces under UV light, producing orange bands. The gel is placed on a UV transilluminator (essentially a box of UV lamps with a glass top). For a permanent record, the gel is photographed.

Caution – the UV light coming from a transilluminator is extremely intense. You can burn your skin and permanently damage your eyes in seconds if you are not protected. Always use a UV shield between your exposed skin and the UV transilluminator.



Gels pictures should always be orientated in your notebook with the wells at the top. Thus, the largest fragments are said to be at the top of the gel. The individual samples on the gel are referred to as lanes, and the lanes are numbered from left to right. Each distinct DNA fragment on the gel is referred to as a band (see Figure 1).

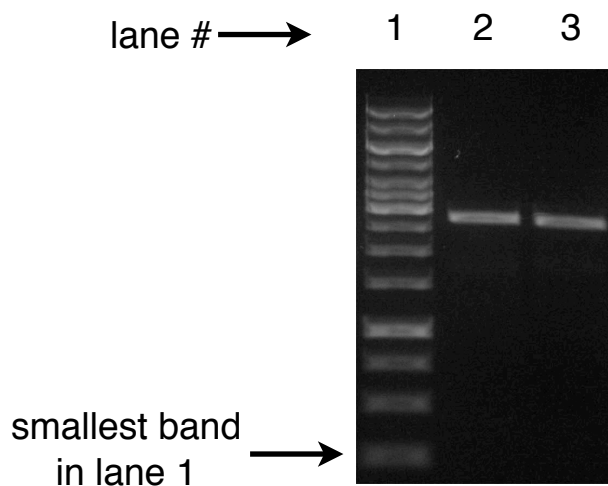


Figure 1. How to read label a gel photo. Gels are orientated with the wells at the top. Thus, the largest band is at the top and the smallest at the bottom. Gel lanes are numbered from left to right. In lane 2 there are two distinct bands, suggesting at least two distinct fragments of DNA were in the sample.

For details on how to estimate sizes of bands see "Estimating the sizes of DNA molecules".

Estimating DNA fragment sizes on an agarose gel

DNA molecules separate on agarose gels in a semi-logarithmic manner. That is, there is an inverse linear relationship between the distance migrated by the DNA molecule in the gel and the log of its molecular weight. We can exploit this relationship to determine the size of the unknown DNA molecules in the gel. To do this we need a standard curve made by plotting the log molecular weight of standards against the distance migrated (from the well to the front edge of the band). Regression analysis will generate an equation that can be used to estimate unknown DNA molecule sizes based on the distance they have travelled.

In order to estimate band sizes, there must be a lane containing known sized DNA fragments.

When we know exactly what sizes we will see we usually do not need to make a standard curve.

Estimating DNA concentration

UV spectrophotometer method

This method can be used to estimate relatively pure DNA concentrations. At 260 nm, 1 optical density (OD) unit equals 50 µg/ml of double stranded DNA. Samples contaminated by RNA, phenol or EDTA cannot be quantified with this method because these substances also absorb at 260 nm. This method is only reliable over a fairly narrow concentrations range, from 5 µg/ml to 90 µg/ml. These concentrations correspond with OD readings from 0.1 to 2.

To quantify DNA

1. Dilute the DNA with H₂O (start with 5 µl of plasmid in a total volume of 200 µl).
2. Read the OD at 260 nm. If it falls outside the allowable range, adjust the dilution accordingly.
3. Convert the OD reading back to µg/ml (remember to take your dilution factor into account).

For example, if you diluted 5 µl of your plasmid prep with 195 µl of H₂O (final volume is 200 µl) and your OD reading was 0.3; you would calculate the concentration of your plasmid prep like this:

$$0.3 \text{ OD units} \times 50 \text{ OD/unit} \times 40 \text{ (dilution factor)} = 300 \text{ µg/ml}$$

Agarose gel method

This method works better than the spec method for plasmid DNA, PCR products and dirtier samples

When DNA stained with ethidium bromide is exposed to UV light at 590 nm, the fluorescence intensity is proportional to the mass of DNA in the samples. DNA concentration can be estimated visually by comparing fluorescence intensity of unknown and known DNA masses.

For example, take a look at the GeneRuler™ 1 kb DNA ladder (p. 30). The table next to the marker shows the mass of each band if 0.5 µg of DNA is loaded. A band matching the intensity of the 6th fragment of the ladder would have a mass of 30 ng. If 2 µl of DNA were loaded onto the gel, the DNA concentration would be 30±2 ng/µl.

It is important to compare to a fragment of similar molecular weight to the one you are quantifying (i.e. to quantify a 300 bp band, don't compare it to a 6 kb band).

Spot method

If you need to quantify DNA and can't use the spectrophotometer method, and don't want to take the time to run a gel, you can use the spot method. In this method a series of known concentrations of DNA is made up. The standards, along with the DNA you want to quantify, are spotted on an agarose plate containing ethidium bromide (0.5 µg/ml) and photographed under 590nm light. The unknown's concentration is the same as the standard whose spot has the same fluorescence intensity.

Preparation and transformation of competent bacteria: calcium chloride method

One way to produce large quantities (μg amounts) of recombinant plasmid DNA is to put the plasmid back into a bacterium (usually *E. coli*) and then grow up large quantities of the plasmid containing bacteria. *E. coli* will not normally take up naked DNA (i.e. they are not naturally competent for transformation). However, we can make the *E. coli* chemically competent by treatment with divalent cations. We are not sure exactly how the treatments make the bacteria competent. It is thought that the cations serve two purposes. First the cations make the bacteria more permeable to DNA and secondly the cations coat the negatively charged bacteria, giving them a positive charge, thereby attracting the negatively charged DNA to the surface. Once the DNA is attached to the cell surface, the heat-shock step is thought to stimulate transfer of the DNA into the cell via Bayer's junction.

Materials

Note: See Appendix for solution preparation.

0.1 M CaCl_2 – pre-chilled
 MgCl_2 - CaCl_2 solution (80 mM MgCl_2 , 20 mM CaCl_2) – pre-chilled
SOB plates with appropriate antibiotic
SOB plates with no antibiotic
SOB medium (50 ml in a 300 ml flask)
SOC media (1 ml per transformation reaction)
plasmid DNA
Sorvall GSA rotor or equivalent
50 ml polypropylene tubes
1.5 ml tubes
water bath or heat block set at 42 °C

Important notes:

All steps in this protocol should be carried out aseptically. All components are biohazard and must be autoclaved before disposal. Dispose of in biohazardous waste buckets.



Protocol

Preparation of competent cells

1. Transfer a single colony from a fresh plate of bacteria to 5 ml of SOB in a culture tube. Incubate the culture overnight (16 – 18 h) at 37 °C with vigorous shaking.
2. Transfer 200 μl of overnight culture to 50 ml of SOB in a 300 ml flask. Incubate the culture at 37 °C with vigorous shaking. After the 1st hour check the culture's OD₆₀₀ regularly until it reaches 0.35 (the closer it is to 0.35, the more frequently you need to check it).

It is essential that the culture be in early log phase for the production of competent cells, this stage is typically reached at an OD₆₀₀ of 0.35 to 0.45.

3. Transfer the bacterial cells to a sterile disposable, 50 ml polypropylene tube. Cool the cells on ice for 10 min.

Keep the cells chilled at all times from this point on. Work with the tube sitting on ice as much as possible.

4. Centrifuge the tubes at 2700 g for 10 min at 4 °C. Decant the supernatant. Stand the tube upside down for 10-30 s on a paper towel to drain away all media.
5. Resuspend the cell pellet in 30 ml of ice cold MgCl_2 - CaCl_2 solution by gentle swirling and pipetting up and down with a 10 ml pipette.

Start by adding only 5 ml of solution and pipetting up and down until the pellet is resuspended – then add the rest of the solution. Avoid introducing air bubbles.

6. Pellet cells and decant supernatant as in step 3.
7. Resuspend the cells in 1 ml of ice-cold 0.1 M CaCl₂ by gently pipetting up and down with a P1000 set to 1 ml.
8. Cells can be used immediately for transformation (continued below), or stored at -70 °C for later use as noted below.

Storage of competent cells for later use:

- a. Add 35 µl of DMSO per ml of resuspended cells. Mix by gently pipetting up and down. Incubate on ice for 15 min.
- b. Add an additional 35 µl of DMSO for each ml of resuspended cells. Mix by gently pipetting up and down.
- c. Working quickly aliquot cells to **pre-labelled** and **pre-chilled** microfuge tubes in 100 µl aliquots. Immediately snap-freeze the cells by immersing in liquid nitrogen. Store tubes at -70 °C until needed.

To use frozen competent cells:

- a. Remove the number of tubes required from the -70 °C, thaw by warming with your hand. When cells are just thawed, place on ice.
- b. Incubate cells on ice for 10 min.
- c. Do not use glass tubes; this will lower transformation efficiency.

Transformation

1. Add DNA to cells (for 100 µl of cells add no more than 25 ng in a volume no more than 5 µl). Mix the contents by pipetting gently up and down once. Incubate the tubes on ice for 30 min.

Include a negative control (cells to which no DNA is added). Treat the cells exactly the same way as the cells to which you added DNA. This control is plated on selective (expect no growth) and non-selective (expect growth) media.

Include a positive control (one aliquot of cells transformed with 5 – 25 ng of purified supercoiled plasmid DNA carrying a selectable marker). This control is plated on selective media and used to calculate TE.

2. Place the tubes at 42 °C for exactly 90 s. Immediately put the tubes back on ice. Chill tubes for 1-2 min.
3. Add 900 µl of SOC medium to each tube of cells, and transfer the entire contents to a sterile 17x100 mm polypropylene culture tube. Incubate the cells at 37 °C for 30 - 45 min with gentle agitation (less than 50 rpm).

Plating

The volume of cells plated depends on the DNA source and on the antibiotic being used for selection. For the positive control (using supercoiled plasmid) we usually plate 100 µl each of undiluted, 1/10 and 1/100 dilutions. Transformations using ligated DNA, are at least 10 fold less efficient than transformations using supercoiled plasmid (supercoiled plasmid DNA is used to determine transformation efficiency), so the number of cells plated is adjusted accordingly (determined empirically).

1. For each transformation reaction prepare 3 plates of SOB medium with appropriate selection antibiotic and indicators as required.

Generally any required antibiotics are added to the media before the plates are poured. If blue-white screening is being used, add X-gal to solidified plates by spreading 40 µl of 20% X-gal per 20 ml plate.

2. For the positive control make a 1/10 and a 1/100 dilution of the cells in SOC. Remember, you need at least 100 µl of each for the plating step.

3. Transfer 100 μl of undiluted positive cells to one plate and spread with a sterile spreader (hockey stick).

Flame a hockey stick (bent glass rod) by dipping in ethanol and passing it through a flame. Allow the alcohol to burn off and let the hockey stick cool for 20 s. Check that the hockey is cool by touching it to a cell free area of the agar. Then spread the cells across the plate.

4. Repeat step 3 with the 1/10 and 1/100 dilutions of the positive control.
5. Repeat steps 2 through 4 with the appropriate amount of experimental cells.
6. Plate 100 μl of the negative control on a non-selective plate and on a plate with selection.
7. Incubate plates appropriately. For *E. coli* strains we generally incubate at 37 °C overnight (~ 18 h).

Save the rest of the cells at 4 °C until you are sure you have enough colonies to count.

Note: calcium chloride mediated transformation is only one of several methods used to get foreign DNA into bacterial cells.

Calculating transformation efficiency

Count the colonies on a positive control plate that has between 50 and 200 colonies on it.

Transformation efficiency is the number of transformed colonies per μg of DNA. I find it easiest to calculate the amount of DNA that was added to the cells on the plate I am counting (usually less than a ng) and then use the following formula

$$\frac{\text{avg \# of colonies on the plate}}{\text{amount of DNA in } \mu\text{g on the plate}}$$

For example:

- Let's say I added 1 ng of DNA to 100 μl of cells.
- The plate I ended up counting was the 1/10 dilution, and it had 150 colonies.
- I initially had 1 ng of DNA/ml of cells, after dilution I had 0.1 ng of DNA/ml of cells.
- I plated 100 μl of this dilution so the cells I plated had a total of 0.01 ng of DNA associated with them; 0.01 ng is 0.000 01 μg so I calculate:
- $\text{TE} = 150/0.00001 = 1.5\text{e}7$ (1.5×10^7).

Transformation efficiencies up to 1×10^{10} are possible; however, using the calcium chloride method we rarely get efficiencies higher than 1×10^7 . Efficiencies as low as 1×10^6 are good enough for subcloning.

QIAEX II Gel Extraction Kit

The protocol for this kit is available from the Qiagen Website.

DNA molecular weight markers

Lambda digested with *BstE* II

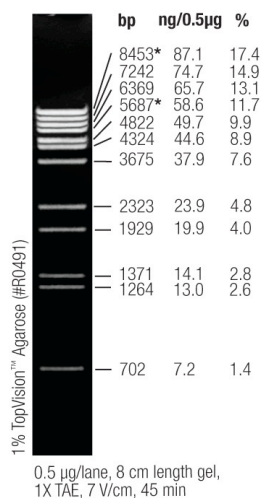
RECOMMENDATIONS FOR USE

- Vortex gently just prior to use.
- Prepare the DNA marker before loading:
1 µl (0.5 µg) of the DNA marker;
1 µl of 6X DNA Loading Dye;
4 µl of deionized water.
- Heat for 5 min at 65°C and then cool on ice for 3 min (see Note).
- Apply the prepared amount (6 µl) of the DNA marker on a 5 mm lane of agarose gel.
- Following electrophoretic separation on gel, visualize the DNA bands by ethidium bromide staining.

Note

- One vial (50 µg) is sufficient for ~100 applications.
- Use 0.1 µg (0.2 µl) of the DNA marker (before dilution) per 1 mm of an agarose gel lane width.

Lambda DNA/Eco91I (BstEII) Marker, 15



PRODUCT USE LIMITATION.

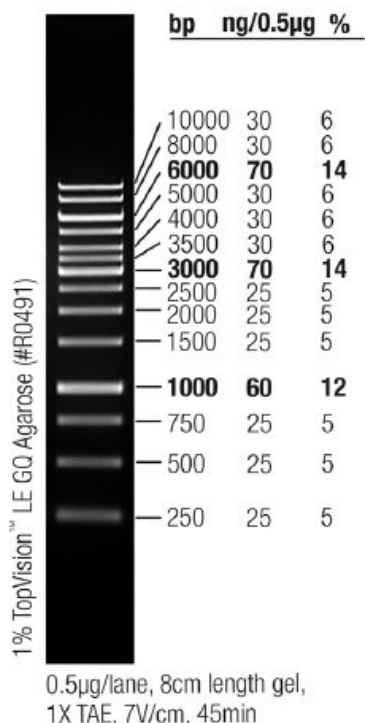
This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

* The cohesive ends (the 12 nt *cos* site of bacteriophage lambda) of fragments 8453 bp and 5687 bp may anneal and form an additional band of 14140 bp. These fragments can be separated by heating at 65°C for 5 min and then cooling on ice for 3 min. 224 and 117 bp fragments are not visible and they comprise 0.7%.

GeneRuler™ 1 kb DNA ladder (Fermentas)

IMPROVEMENTS

1. Three reference bands: 6000, 3000 and 1000 bp.
2. Amounts of DNA per band equalized for easier quantification.



Protocol for Loading

Loading mixture for the 5mm agarose gel lane*:

DNA Ladder	1µl
6X Loading Dye Solution	1µl
Deionized water	4µl
	<hr/>
	6µl

Step 1: Mix gently

Step 2: Load on the gel

*For gels with other lane widths, the components of the mixture should be scaled either up or down. Use 0.2µl (0.1µg) of DNA Ladder per 1mm of lane.

Recommendations

- Do not heat before loading.
- Dilute your DNA sample with the 6X Loading Dye Solution (#R0611, supplied with the ladder): mix 1 volume of the dye solution with 5 volumes of the DNA sample;
- Load the same volumes of the DNA sample and the DNA ladder;
- For quantification, adjust the concentration of the sample to equalize it approximately with the amount of DNA in the nearest band of the ladder.
- Visualize DNA by staining with ethidium bromide or with SYBR® Green I.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.