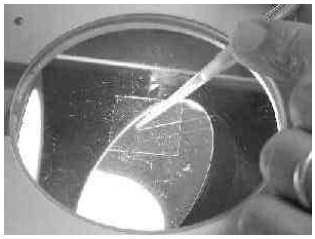
Molecular Identification of Nematodes Manual



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This manual provides information on the techniques used for the molecular identification of nematodes used at the Nematology Laboratory, Department of Plant Pathology, University of Nebraska-Lincoln. The techniques presented are intended as a general guide, and are the ones best suited to our own needs.

Chapter

Check List

DATE _____

1. Single juvenile squash

Pick individual onto a 15 ul drop of water on a coverslip.
Smash nematode on the stage of a dissecting microscope using the end of a pipetman tip.
Pipet solution to a labeled 500 µl PCR tube and place on ice until use.

2. PCR reagents

Master mix for N amplifications (mix well after all reagents have been added):

N* 5.0 ul 10x Taq buffer	Total _____ ul
N* 4.0 ul dNTP mix	Total _____ ul
N*0.8 ul primer 1	Total _____ ul
N*0.8 ul primer 2	Total _____ ul
N* 0.4 ul Taq total	Total _____ ul
N*29.5 ul ddH_2O	Total _____ ul

Pipet 40.5 ul PCR mix into each PCR tube and mix

3. PCR Run

Start thermocycler program
Once it reaches 94 °C insert PCR tubes
Place tubes in fridge after PCR run

4. Post-PCR Check Gel

Agarose Gel
- Microwave 1% agarose and allow to cool
- Pour 60 ul into gel box with comb
- Let cool 10 min on bench, and 20 min in fridge

PCR Products
- Pipet "N" 1 ul drops of gel dye onto parafilm
- Mix 5 ul of each PCR product in drop of gel dye

Loading gel
- Add gel to gel box (wells at anode "black" end) and fill with 0.5X TBE
- Pipet 10 ul of ladder in first and middle wells
- Pipet PCR products into individual lanes
- Add 6 ul EtBr to the TBE buffer at the cathode "red" end (if using EtBr infiltrated gel)

Running the gel
- Place cover on gel box and plug in terminals into power supply
- Turn on power supply and set at 120 V
- Turn off power supply once dye marker is approx. 5 cm from end of gel (54-60 min)

Taking a picture of the gel
- Put on gloves and place gel into glass tray
- Put on a UV eye shield, place gel on UV box
- Focus camera, and turn on UV box
- Set exposure and photograph gel

5. Restriction Digest (RFLP)

Pipet 8.0 ul of each PCR sample into a labeled individual 500 ul tube (1 tube for each restriction enzyme)

Master mix for "N" samples(one for each restriction enzyme)
- N* 2.0 ul ddH_2O
- N* 1.2 ul 10X RE buffer
- N* 1.0 ul restriction enzyme

Pipet 4.2 ul of each RE's master mix into each sample tube and mix

Maintain digests at 37 °C for 1 hour

Place tubes in fridge

6. Post RFLP Agarose Gel

Same as #4

1. Nematode Smash/DNA Isolation

A. Nematode smash

You will require:

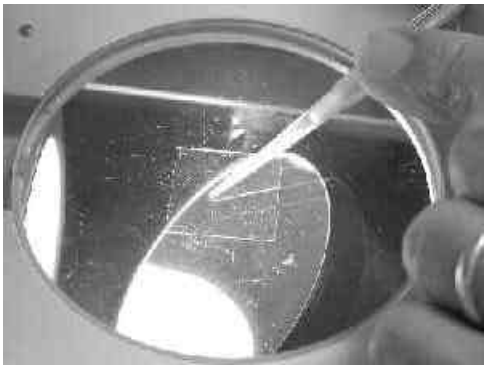
1)Watchglass
2)Dissecting microscope
3)Glass cover slips
4)Sterile ddH_2O
5)Pick with very fine tip
6)Yellow, flat-tipped micropipet tips
7)Designated pre-PCR P-20 pipetman
8)Sterile PCR tubes
9)Bench chiller or ice bucket with ice

Set up equipment:

1)Pour collected nematodes in a watchglass and place on dissecting microscope.
2)Set out 1-3 glass cover slips.
3)Place 1-4 15ul drops of sterile ddH2O on each of the glass cover slips. *The number of cover slips and drops of water on each slip that you will use will depend on the number of nematodes you find, how adept you are at picking them out of the watchglass, and how fast you are at smashing them. You don't want the drops of water to evaporate too quickly (try to to be able to transfer about 10 ul of the smash solution to the tube each time).

Disrupting the nematode:

1)Pick the nematode out of the watchglass and place in one of the drops of water. *If you're interested in obtaining an egg to smash, I find it easiest to draw one up into a pipet tip with a P-20 with as little of the surrounding solution as possible, and place it in a drop of water. Note: we have found that smashed eggs don't amplify as well as smashed juveniles.
2)Check the pick to see if the nematode isn't still wrapped around the tip before going on to pick out any more nematodes.
3)Bring the nematode in the drop of water into focus under the microscope.
4)With a pipet tip on the pipetter, carefully bring the end of the tip over the nematode.
5) As you gently bring the tip down over the nematode, make sure you can see it through the tip.
6) Continue to gently lower the tip until you see the nematode rupture. *If you don't see it rupture, don't use it.



7) One can pump the nematode-smash solution a few times before transferring it to a sterile PCR tube. *To "pump" a solution means to draw it up into the pipet tip and expel it several times—this helps to mix the solution quickly.
8)Place the PCR tube in ice or in a bench chiller as quickly as possible. *DNases released from the ruptured nematode will rapidly chew up the DNA at room temperature.
9) Store the tubes at -20°C (minimum). They should remain usable for several years.

B. Phenol/Chloroform DNA extraction

Note: Phenol and choloroform are toxic, wear appropriate protective gear (goggles, gloves, lab coat) for this procedure. Handle phenol and choloroform in a fume hood.

1.Label tubes & aliquot 100 ul DNA extraction buffer into each tube.

2.Put cysts into tube, & swirl thoroughly with sterile plastic pestle to homogenate. Incubate at 55 °C for 3 hours to overnight.

3.Centrifuge for 20 sec. and add 1 µl 10 mg/ml RNase A.. Pipet to mix and maintain tubes at 37 °C for 15 min.

4.Add 50 µl buffer satuated phenol and flick tubes to mix solution. Place tubes in a 55 °C water bath for 10 - 15 min (flick tubes every 2-3 min).

6.Add 50 µl chloroform / isoprophyl alcohol [CHCl/IAA] (24:1) to each tube. Vigorous mix tubes for 5-6 times.

7. Centrifuge tubes for 5 min, during this, label a new set of tubes. Transfer all of the aqueous phase to the new tubes.

8.Add 100 ul CHCl3/IAA to each tube, flick to mix as in step 6.

9. Centrifuge tubes for 5 min, during this, labeling another set of tubes. Transfer aqueous phase to new tubes.

10.Add 4 µl of 5 M NaCl to facilitate DNA precipitation.

11. Add 200 µl of 100% ethanol (stored at -20 °C), and invert tube to mix. Store tubes at -20 °C for at least 1 hour.

12. Centrifuge tubes for 10 min. Remove ethanol with pipettor.

13. Add 250 µl 70% ethanol (stored at -20 °C), into each tube. Centrifuge tubes for 7-10 min.

14. Remove the ethanol with a pipeter. Place open tubes in a vacuum for 15 min.

15. Resuspend DNA pellet in 100 µl Tris:EDTA (TE) pH 7.5, tap the tube repeatedly so the TE washes completely the side walls. Briefly centrifuge tubes and store at -20 °C until use.

2. Pre-PCR Setup

You will need:

1)Gloves
2)Clean tube rack (i.e., washed & stored in designated "Pre-PCR" area)
3)Sterile ddH2O
4)10X *Taq* buffer
5)dNTP mix
6)20uM working stocks of the two primers needed
7)*Taq* enzyme
8)Microcentrifuge
9)DNA template tubes, including nematode-smash tubes and tube of control DNA template
10)Sterile1.6ml microfuge tube(s)
11)1 "Pre-PCR" designated P-20 and 1 P-200 pipetmen
12)Racks of aerosol-barrier tips to fit both the P-20 and P-200 pipetmen
13)Lid opener for microcentrifuge (µfuge) & PCR tubes

Set up equipment:

1)Put on a clean pair of gloves and wipe down working area with tap water. This helps remove any "post-PCR" products which might end up in your "pre-PCR" master mix or reaction tubes.
2) In a clean tube tray, set out one tube of each reagent: sterile ddH2O, 10X *Taq* buffer, dNTP mix, and primers #1 and #2.
3) While your reagent tubes thaw at room temperature (RT), write out the recipe you will use for your PCR master mix.

I use the following recipe for one 50µl PCR reaction:

29.5µl sterile ddH2O

5.0ul 10X *Taq* buffer—gives 1.5mM Mg+2 final concentration

4.0ul dNTP mix -- 100uM of each dNTP final concentration
0.8ul 20uM primer #1 -- 0.32uM final concentration
0.8ul 20uM primer #2 -- 0.32uM final concentration
0.4ul *Taq* -- 2.0 units final amount
40.5ul total volume

*Remember, this will be added to the approximate 10.0ul of smashed-nema solution.

*Aliquot these reagents to the master mix in the order given (i.e., first ddH2O, next buffer, etc.). A general rule is to add the most dilute reagents first and adding the most concentrated reagents at the end. Another rule is to add the enzyme to a solution last.

*I have found the PCR process to be fairly forgiving when it has been optimized for a particular DNA template/primer pair—amounts/ concentrations used do not have to be exact.

*If there is only a small volume (i.e., less than about 15ul) left in any of your reagent tubes (except *Taq*), especially if they have sat around unused for a long time, toss it and use a fresh tube. For some reason, under these circumstances, I have had reagent tubes "go bad" on me.

*Multiply these volumes by the number of PCR reaction tubes you want to use. (Remember to include enough for a positive and negative control.)

***Trick**—I set the P-200 pipetman to aliquot out only 40.0ul of this mix to the PCR tube. This is because each time you stick the pipet tip into the master mix, about 0.5ul sticks to the outside of the tip. If you made the volume any less, you would risk running out of master mix by the time you were ready to aliquot it to the last PCR tube.

*Remember, when adjusting a P-20 pipetman, do not allow it to go below 0.0ul or above 20.0ul. Likewise, when adjusting a P-200, do not allow it to go below 20.0ul or above 200.0ul. Doing so compromises its calibration.

4) "Blip" all of the reagent tubes, except *Taq*. (If it is a brand new tube of *Taq*, or it is almost empty and you want to get the last bit of it, then blip the *Taq* tube as well.)
*To "blip" a tube of solution is to spin it in a microcentrifuge for only a second. (If you are spinning 0.5ml PCR tubes, make sure they are properly stabilized in the rotor. They can easily become bullets if not set in there correctly.) This brings down any condensation that may have built up on the inside of the cap or side walls of the tube.
*A good habit to get into is to keep the "tabs" (i.e., the plastic piece connecting the tube and its cap) of the tubes you are about to spin "up" (i.e., toward the outside of the rotor). This trick comes in especially handy when extracting or cleaning DNA by pelleting the DNA and/or precipitate in a uniform fashion.
5) Aliquot the needed volume of sterile ddH2O to a sterile 1.6ml ufuge tube, which will be the "master mix" tube.
*Keep in mind, this is all "pre-PCR" stuff. So use aerosol-barrier pipet tips, designated pre-PCR pipetmen, etc.
***Trick**—Set the tubes (in this case, reagent tubes) in a different well of the rack to help you remember which reagents you have already used.
***Trick**—Pump all the other reagent tube contents at least 10 times before aliquoting the needed volumes. This is especially important for the 10X *Taq* buffer (and any other buffer) which separates out while stored away. You want to make sure each is mixed thoroughly before aliquoting it to the master mix. Results support this. Also, I find it a lot easier to use my index finger on the plunger button to pump the solution rather than my thumb.
6) Aliquot the needed volumes of 10X *Taq* buffer, dNTP mix, and primers #1 and #2 to the master mix, remembering to pump each 10 times before aliquoting.
7) *Taq* enzyme also tends to separate out over time. However, only pump it enough times to get the proper volume of solution in the pipet tip. And don't stick the pipet tip into the solution very far. If you do, you will be adding more *Taq* to your master mix than you originally intended. (It is very viscous in its glycerol environment and will stick to the outside of the tip.)
*Ensuring accurate *Taq* amounts is one of the main reasons for using this particular protocol, rather than aliquoting everything separately. Another is increased uniformity across reaction tubes.
8) Once you have aliquoted the *Taq* to the master mix, pump it at least 10 times to make sure it is thoroughly mixed as well. And then store it on ice, in a +4oC benchtop chiller, or in a +4oC fridge until it is ready to be used. (Do not freeze it.) Use it soon, however. *Taq*'s efficiency begins to drop once it is removed from its high glycerol environment.
9) "Blip" your frozen nema-smash PCR reaction tubes before aliquoting the master mix. (Also, keep them at +4oC while aliquoting the master mix.)
10) With the P-200 set at 40.0ul and sticking the pipet tip into the master mix only a little bit, aliquot the master mix to each of the nema-smash PCR tubes, and pump the solution 5-6 times. Set the PCR tubes back at +4oC till you are all ready to place them all in the thermocycler. (No mineral oil is placed on the top of the PCR reaction mix, if you are using a thermocycler with a heated lid. If you are not, place a couple drops of mineral oil on top of the completed reaction mix before capping the tube. A quality mineral oil can be obtained from a chemical supply company or even a hardware store. There is no reason to buy over-priced mineral oil from some molecular biology company. The mineral oil does not need to be autoclaved to sterilize, but a negative control needs to be run on it.)
*A negative control is the 40.0ul of master mix with 10.0ul of sterile ddH2O mixed in. No amplification reactions should occur during the PCR run, since there is, theoretically, no DNA template. If amplification products are found on the check gel after the PCR run, then one or more of your reagent tubes has contaminating DNA. It is usually most time efficient to toss the whole set and start again with new. This is the most important control and needs to be done with each set of PCR reactions.
*A positive control is the 40.0ul of master mix with 10.0ul of a DNA template that is known to amplify consistently. (For our lab, this is 1.0ul of extracted nematode DNA plus 9.0ul of sterile ddH2O.) If no amplification occurs in any tube, including the positive control, then you may have left a reagent out of your master mix, your PCR parameters may have been altered, or the thermocycler is not functioning properly. When you are getting consistently good PCR results, this control may be left out.
11) You are now ready to prepare the thermocycler for the PCR run.

***"Re-amps"**

- I use a variation of this protocol sometimes to re-amplify DNA fragments. I do this to increase the amount of DNA in PCR reaction samples: 1) which originally gave very low-intensity ("faint") products, 2) on which I want to later do several restriction digests, &/or 3) which I want to later clean (probably via GeneClean) and then sequence.
- The only differences in the pre-PCR setup are: 1) use 39.5ul sterile ddH2O per reaction instead of 29.5ul, and 2) use about 0.5ul of the original

PCR reaction as the template for the reamplification. (No cleaning of this original PCR reaction is usually required for good reamplification.)

- Jumping ahead a bit, the only change in the PCR parameters to do a re-amp is to reduce the number of cycles from 40 to 20.

*The number of desired amplification products is in high excess to the original template DNA's still in solution. Therefore, very little of this product is required and very few cycles are needed to further increase their numbers. Too much DNA template &/or more than 20 cycles will result in a "smear" (i.e., no real product band, but there will be a long blur in the gel lane).

3. Guide to the PCR Run

You will need:

1) Your particular set of parameters for this PCR run

2) Your set of completed PCR reaction tubes

Set up equipment:

1) If you have not designed the PCR parameters to be used for run you are about to do, you must do this now.

Things to keep in mind:

A) PCR runs are usually done in three stages.

a) The first is a single-cycle file where the tubes are brought to 94°C as quickly as possible (using a "modified hot-start") and kept there for two minutes to ensure the denaturation of all the double-stranded template DNA into its single-stranded components.

*A "modified hot-start" involves starting the thermocycler on this first file so that the heat block ramps up to 94°C. Once this target temperature is reached, the tubes are quickly placed in the heat block and the heated lid closed. Once inside, the tubes should remain at 94°C for two minutes before the main PCR file begins. What you are trying to have happen is to get the contents of each tube from +4°C to 94°C as quickly as possible. All the required components of the polymerase reaction are there and functioning as the heat block ramps through *Taq*'s prime operating temperature of 72°C, and any primers already annealed to the template (correctly matched or not) can be extended by the *Taq* enzyme. The intent is to minimize the possible extension of any mismatches. (A true "hot-start" involves leaving out a critical reagent until the tube contents reach 94°C.)

b) The second (main) file incorporates the three sub-stages: denaturation, annealing, and extension.

*Denaturation was explained above. Its very important to get all the double-stranded DNA templates into single-stranded form, especially during the first several cycles of the main file. (What gets amplified and what does not during these first several cycles greatly influences the final outcome of the PCR run.) Normally, one minute at 94oC is sufficient.

*Annealing involves the matching of the two primers to their specific "binding sites." We use this particular form of PCR where two primers are used in the reaction. They were specifically designed from a DNA template sequence (previously obtained from the literature or personal DNA sequencing) to be complimentary to that specific sequence, they are paired (i.e., their 3' ends are pointed toward each other with the DNA sequence to be amplified located in between), and they each have optimum melting temperatures ™.

*A Tm for a primer can be found by using this "quick 'n' dirty" formula: Tm is approximately equal to 2°C (A + T) + 4°C (G + C). For example, if your 20-mer primer has 5 A's, 7 C's, 6 G's, and 2 T's, its Tm is about 66°C. (There are other, more accurate formulas you could use, but this one has worked well for our purposes.) This is the temperature at which, theoretically, one-half of all the primers having that Tm will be annealed to their specific binding sites. Above that temperature, fewer of those primers will be annealed. Below that temperature, more than half will be annealed. Further below that temperature, some will anneal to sites that are not their specific binding sites (in other words, mismatched). Once you have found both of your primers' Tm's (and ideally, they should be within a few oC of each other), take the lower of the two numbers and subtract 5°C from it. (Using the above example, the annealing temperature used would be 61oC.) This is a good starting point when optimizing your particular DNA template/primer pair PCR reaction.

*Note: We have found that the ramping rates (i.e., the time it takes for the heat block to be raised or lowered to its next target temperature) are usually not significant to the success or failure of the PCR reaction, except in the case where the heat block ramps from its annealing temperature to the usual 72oC extension temperature. If the ramping rate is too fast, the thought is that too many of the annealed primers are knocked off their binding sites before a *Taq* enzyme finds the empty 3' end and is able to extend it. The result is a very little product. In our parameters, we keep all the ramping rates at maximum, except in the case of one template/primer pair combination, where we have had to slow the rate down. Other researchers have found this to be the case as well.

*Extension involves a *Taq* enzyme finding a primer annealed to its binding site, attaching itself to the primer's 3' end and moving "downstream" along the DNA template, incorporating the free dNTP's in solution as it goes, thereby forming the second strand of the newly made double-stranded DNA template. This process is usually done at 72oC (*Taq*'s optimum extension temperature) and continues until the heat block temperature ramps back up to 94oC for the beginning of the next cycle.

*We usually use 35-40 cycles in our main file. 40 cycles is considered to be the maximum—any more usually is disadvantageous to the process.

c) The third is a single-cycle file in which the heat block is kept at 72oC for about 5-10 minutes in order for *Taq* enzymes to finish extending any partially completed products. This is not so important when your amplified product is only a few hundred base pairs (bp's) in length, but becomes significant for products that are about one kilobase (kb) in length or longer.

B) So now, program your parameters into the thermocycler, or, if you have already done so, check through them to make sure they are correct. For example, the set of parameters we use when doing PCR with the primers rDNA2/rDNA1.58S are:

File #1:94oC for 2 min., 45 sec.*
File #2:94oC for 1 min.
55oC for 45 sec.
72oC for 2 min.
for 40 cycles
File #3:72oC for 10 min.**

(All ramping rates are at maximum in this case.)

*When the heat block temperature reaches the initial 94oC after starting File #1, I have 45 seconds to place all my reaction tubes into the block and close the lid. The tubes then remain at 94oC for two minutes till the beginning of the main file (#2).

**The tubes can remain at RT following a PCR run for quite some time (overnight if need be). The general rule is to keep DNA cold to keep it from degrading. In this case, the contents of the reaction tubes have been at 94oC long enough to denature any DNases that were originally in the PCR tubes. They are considered DNase-free until the moment you open the caps.

****Important**—You will now deal with post-PCR products—keep in mind how you are going to minimize the chance of cross-contamination from these reactions to any subsequent reactions you do.

4. Post-PCR Check Gel

You will need:

1) Gloves
2) Gel electrophoretic cell with tray, comb(s), lid, and power source
3) Melted 1.0% agarose, low EEO
4) 0.5X TBE
5) Hot Hand Protector
6) Your set of completed PCR reaction tubes
7) DNA ladder(s)
8) Tube of gel dye
9) Microcentrifuge
10) 1 "Post-PCR" designated P-20 pipetman
11) "Regular" pipet tips
12) Lid opener for ufuge & PCR tubes
13) Designated "Post-PCR" trash receptacle

14) 2.5mg/ml EtBr

Set up equipment:

1) Melt the 1.0% agarose, low EEO you are going to use. We do this in a microwave.

*Unused portions of melted agarose are allowed to resolidify and can be remelted and used at a later date.

***Caution—**Loosen the cap on the bottle before placing it in the microwave to melt the agarose. The

bottle may burst if the gases from the melted agarose cannot escape.

a) If the agarose crystals have not been melted yet, microwave the bottle for a couple of minutes, take it out and swirl it vigorously. You will see small bubbles as well as translucent crystals of unmelted agarose floating around. Microwave the bottle an additional 30-60 seconds, take it out and again swirl it vigorously. Continue to do this till all the crystals of agarose are melted.

*If unmelted agarose crystals remain in the solution when it is poured, they will adversely affect the DNA bands as they travel through the gel.

b) If you are using resolidified agarose, loosen the cap and remelt it in the microwave. Again, make sure it is completely melted. Pieces of unmelted agarose will also adversely affect the DNA bands as they travel through the gel.

c) Allow the hot, melted agarose to cool a bit before pouring the gel.

2) While the agarose is cooling, pick out an appropriate gel electrophoretic cell with its tray and comb(s), given the number of samples you have. Wear gloves when you are working with the pieces of the cell, because they are probably contaminated with EtBr. Also keep in mind that the purposes for running this gel are to: 1) see if there is any product or not in each of the PCR reaction tubes and 2) see what the products look like—intensity (i.e., amount of amplification product), size of band, presence/absence of multiple bands, and presence/absence of smears. The gel does not have to be run very long in order to get this information. Therefore, you can use two combs per gel if need be. (Place them in the tray such that both the top and bottom sets of DNA samples have about the same distance to travel.) Make sure each piece is clean—free of any old pieces of agarose, dust, etc.

3) Place each comb(s) temporarily in the tray to see how much clearance there is between the bottom of the teeth of the comb and the surface of the tray. There should be 0.5-1.0mm, and this should apply across the length of the comb. Any less clearance means you run the risk of breaking the bottom of the "well" formed when you remove the comb from the solidified agarose.

4) The trays we use require taping at both ends before pouring the agarose. Tear off a piece that will cover the entire end of the tray, and place it over the end so that there is almost a centimeter of tape up from the surface of the tray. What you want is enough tape to cover the end so that the agarose will not spill out over the tray. Also, make sure to press it tightly all the way around the end of the tray so that no leaks occur when you pour in the agarose. Do the same with the other end.

***Trick**—I orient the empty tray so that the end that will hold the comb is nearest me on the bench, and the "bottom" of the tray is furthest from me. This is because the benches in our lab slant slightly downward from the interior portion to the part nearest me. Therefore, slightly more agarose collects at the "top" end (with the comb) than at the bottom end. This allows more volume per well when the comb is removed. Usually well volume is not a problem, especially for check gels. But it often comes into play when setting up good MetaPhor agarose gels for running out DNA digested with a restriction enzyme.

5) Pour the agarose into the tray. You want to pour in enough to cover the bottom and then a little bit more. (I do not quantify the amount of agarose I use for check gels.) Keep in mind: 1) that larger teeth on some combs will create more volume than the smaller teeth on others, 2) the volumes of the DNA samples you will be loading, and 3) that agarose is extremely expensive. Gels more than about ¼" thick are usually too thick. Also, do not pour the agarose too hot, because this tends to warp the trays over time. If buying new trays is not a problem, then you do not have to worry about this. Do not pour the agarose too cool either, because portions of the solution may have begun to resolidify already.

6) Set the comb into place. Some trays have notches where the comb is to be placed. For these, I set the comb in such that the teeth are oriented closest to the end. (It just gives that little more room for the DNA to travel.) Other trays do not have these notches, and their associated combs are fastened to a holder. Place the holder/comb onto the tray such that the teeth are no less than 5/16" from the end of the tray. I found that if the teeth are placed closer to the end, then the bands of the DNA that was later aliquoted into those wells ran slanted as seen from the side of the gel. When looking at the same bands from overhead, they appeared large and diffuse, and accurate measurements of the size of the amplified product band were impossible. Also, make sure that the teeth are all at a uniform distance from the end of the tray (i.e., make sure it is straight).

7) Allow the agarose to solidify completely which usually takes almost 30 minutes. It will turn opaque in color. To speed solidification, the gel can be poured or, once poured and partially solidified, placed at +4oC.

8) While the gel is solidifying, pour 0.5X TBE into the electrophoretic cell ("gel box") -- the "running buffer." Pour in enough buffer so that it fills both end reservoirs and also covers the place in the middle where the tray sits by about 1cm. (You will adjust the volume later after you put the gel/tray in place.) Also, blip all of your PCR reaction tubes and the

DNA ladders you will use and set up all the tubes in the order you want. (I have a form on which I fill in all the various information on the gel, the order of the tubes, and the running conditions which I fill out at this time.)

***Important**—Make sure that the type of buffer you use in making the agarose solution is the same buffer you use to fill the gel box. For example, if you happen to forget and make the agarose solution with ddH2O, the agarose melts and solidifies the same as with 0.5X TBE. But when the gel is placed in the gel box, the DNA samples are loaded into the wells, and an electric current is applied, the voltage differentials causes the running buffer to heat up and melt your gel.

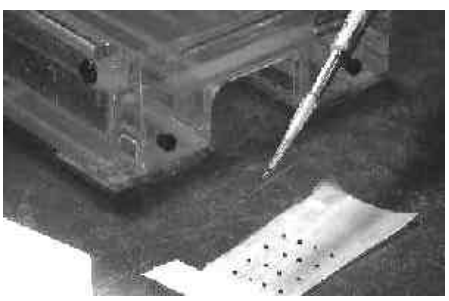
***Trick**—If you cannot use your gel for awhile (i.e., a couple hours or more), pour a layer of 0.5X TBE over it, so that it will not dry out. Also, gels left overnight do not seem to give as good results as fresh gels.

***Trick**—Get into the habit of orienting you gel box with the black (negative) electrode on the left-hand side and the red (positive) electrode on the right. To help you remember, you can think of the colors in terms of emotions.

9) When the agarose has completely solidified, slowly remove the comb by holding the tray down with your thumbs while pulling up on the ends of the comb with your other fingers (or vice versa, depending on the comb) with a gentle rocking motion. You do not want to rip the bottoms of any of the wells. Rinse off the comb. Then remove the tape from both ends of the tray. (The tape can be saved and reused several more times.) Do this very carefully—the gel can easily slide off the tray now. Keep this in mind whenever you need to move the gel/tray.

10) Gently lower the gel/tray into the running buffer in the middle of the gel box, oriented so that the line of wells is to your left. Do not allow any bubbles to get trapped under the tray. Make sure there is enough buffer to completely cover the gel. (I usually pour in an excessive amount, lower the gel/tray into the buffer, and then remove buffer until the tops of all the wells are showing above the surface.)

*Remember, electric current takes the path of least resistance (in this case, the running buffer). The less running buffer there is above your gel, the more current goes through the gel allowing greater resistance.



Loading the gel:

1) Cut off a piece of parafilm, remove its paper cover, lay it down with the newly uncovered side up on the bench in front of the gel box, and stretch it flat by pressing down the corners. The reason I use parafilm at this point is that it saves on time and ufuge tubes.

***Trick**—Originally, I used it to soak up mineral oil. During the time we used a thermocycler with no heated lid, we had to use mineral oil to keep the PCR reaction solution from condensing on the tube cap. But it was a mess to deal with following the amplification. We found we could eliminate nearly all traces of it by pulling the entire PCR amplification out of the tube and dragging the droplet around on parafilm.

2) Aliquot 1ul drops of 10X gel dye to the parafilm equal to the number of PCR amplifications you are checking. Using the "post-PCR" designated P-20, pump the PCR amplification solution about 5-6 times and pull 5.0ul into the regular pipet tip. While holding the pipetman with one hand and guiding the pipet tip with the other, aliquot the solution into the first drop of gel dye and pump about 5-6 times to mix well. Then draw up the entire amount back into the pipet tip. (You can do this by first depressing the plunger button slightly past the normal stopping point and then drawing the solution into the tip.) Again, while holding the pipetman with one hand and guiding the pipet tip with the other, place the tip of the tip about a millimeter within the top of the well. Depress the plunger button slowly so the blue solution dribbles out of the tip. Bubbles formed on the inside of the pipet tip are usually not a problem, unless they are right at the very end of the tip. If you try to pipet the solution in this case, you will not be able to control it. So work the solution back on the parafilm till you get rid of the bubble.

*If the well volumes are relatively large, you can pipet faster. If you are pipeting into a smaller well, however, pipeting faster will tend to make the solution spill out over the top of the well.

*Also, do not allow the pipet tip to go into the well too deeply. You may puncture the bottom of the well. The solution will also tend to spill out of the well, since the pipet tip mostly takes up the well's volume.

*Remember, dispose of your post-PCR-contaminated pipet tips and parafilm in a designated trash receptacle. Ordinary zip-lock bags work well for this. If thrown away in regular trash, post-PCR solutions can get on trash can liners, dry, flake, and become airborne, thus becoming a good source of contamination for later PCR runs.

*Remember, you have now opened this reaction tube thus exposing it to the possible contamination of DNases. So, from now on, it must be kept cold.
3) Do this for each of the PCR amplification solutions. Also aliquot the appropriate volumes of the chosen DNA ladders to their designated wells.
***Trick**—I use DNA ladders not only as size markers but also as "boundary" markers for the different groups of PCR amplifications I have done. It helps make the gel look nicer.
*By convention, the well closest to you is considered well #1, and so on. The area of agarose through which the DNA travels is referred to as the "lane." They are numbered the same way as the wells. Also by convention, the "top" of the gel is the side with the wells nearest it. Likewise, the "bottom" of the gel is the opposite side. The DNA travels through the agarose matrix from top to bottom -- the shorter fragments traveling faster and farther than the larger fragments.
4) Once you are finished loading all your samples and ladders, you need to add some EtBr to the lower buffer chamber. (This is the reservoir at the "bottom" of the gel.) I pipet a certain amount of EtBr solution into the buffer on that side, swirling and pumping the pipet tip through the buffer to mix. When using the mini-sub cell, I aliquot 2ul EtBr to the lower buffer chamber and 4ul when using the wide mini-sub cell.
*Remember, the DNA is negatively charged and, when an electric current is applied, is drawn toward the positive electrode. EtBr, on the other hand, is positively charged and is drawn toward the negative electrode. The EtBr already in the agarose gel moves through the matrix and falls off the "top" end of the gel, leaving an area at the bottom of the gel nearly void of any EtBr. Any DNA in that void will not be stained and, subsequently, will not be seen by fluorescence when the gel is placed on a UV box. Therefore, additional EtBr must be added to the lower buffer chamber to be drawn up into the agarose during the run.

Running the gel:
1) Place the lid on the gel box, making sure to line up the color-coded electrodes properly. Plug the other ends of the electrodes into their proper plug-ins on a power source, and turn on the power source. I run my gels at constant voltages anywhere from 20V to 150V, but usually around 100V. The higher the voltage, the faster the DNA moves and the greater the heat buildup. Also, it is permissible to change the voltage setting during the run.
***Trick**—Try using the "volt-hour" to help you figure out how long you will need to run your gel. For example, if you ran a 1% gel at 100V for 2 hours, multiply the voltage by the number of hours to get 200 V.Hrs. Suppose the next time you run a similar gel, you need to let it run for 5 hrs. Set the voltage to 40V. Keep in mind 1) you are again using a 1% agarose gel and 2) the relationship is not a straight line since the DNA's rate of movement depends on the gel's temperature.
2) It is a good idea to 1) check to see if air bubbles are coming off the electrode, telling you the electric circuit is complete and 2) watch the blue dye to see if it is running the correct direction. If it is moving toward the top of the gel, you need to correct the electrode plug-ins. The DNA will have to run back through the wells and will therefore be blurrier but will probably still be discernible.
3) It is good to have an idea how large your PCR fragments will be, so you know how long you will need to run your gel.

Take a picture of the gel:

- Different labs will have different camera setups, so I will not go into the specifics of our own system. A couple things you will want to keep in mind:

*By this time, the agarose gel will easily slide off the tray, so be very careful when you lift it out of the gel box/running buffer and carry it anywhere.
*If, after you look at your gel on the UV box, you figure out you forgot to add the EtBr to the lower buffer chamber, put a layer of ddH2O in glass dish, add about 5-8ul EtBr, mix, and place your gel in the solution. Leave it there about 15-20 minutes to allow the EtBr to soak in. Rinse the gel off several times with distilled H2O or ddH2O and look at it again on the UV box. You will notice a lot more background flourescence using this staining method.
*Your lab may have different rules & regs. on how to properly dispose of your gel and running buffer. Our lab collects the gels in a waste container that is later picked up by our Hazardous Materials Program. The EtBr in the running buffer is considered dilute enough to be safely put down the sink. Rinse off your gel box and tray being careful of the platinum wires in both ends of the gel box.

General notes:

*It is a good idea to organize your PCR amplification tubes from the start. They tend to accumulate very rapidly. I use a spreadsheet and note information about each amplification such as tube label, template type and source, primer pair used, amplification results, where the tube is located, how much solution is left, and what digestions have utilized it.

5. Prep for DNA sequencing

You will need:

1) Gloves
2) Your set of completed PCR reaction tubes
3) Microcentrifuge
4) Lid opener for ufuge & PCR tubes
5) 1 "Post-PCR" designated P-20 and 1 P-200 pipetmen
6) Micron Micron 50 microconcentrator kit
7) 1.5 ml Eppendorf tubes
8) Nanopure water
9) Low DNA mass ladder

General information:

This procedure is used to prepare the PCR amplification product for DNA sequencing. We have had good experience using the Iowa State University DNA Sequence and Synthesis Facility, Ames, Iowa. http://www.biotech.iastate.edu/facilities/DSSF/. This facility uses a Applied Biosystems Inc. Prism DNA sequencer. They are able to reliably sequence 800 bp in each direction at a cost of $28 per direction. PCR amplification primers are used for the sequencing and the turn around time is less than 48 hrs once the samples are received The PCR product is first centrifuged through the Micron Microcon 50 microconcentrator to remove the PCR primers and concentration the PCR product. The concentration of the purified DNA is then quantified by running a small amount on an agarose gel which contains a DNA mass ladder. Samples to be sequenced are then mailed off to the DNA sequencing facility and sequences are obtained from the internet via FTP.

1) Set up in a rack a Micron Microcon 50 filter inserted into a Microcon 50 1.5 ml tube labeled for each PCR sample (per Centricon 50 instructions). Pipet the entire PCR product into the Microcon 50 tube. Note: if you used an oil overlay for the PCR amplification, first pipet the PCR product onto parafilm, then repipet it into the Microcon 50 tube (this will remove most of the oil).
2) Centrifuge the tubes at 2500 rpm for 15 minutes using a microcentrifuge.
3) Remove the tubes from the centrifuge and place in a rack. Label new Microcon 50 tubes for each sample on the side of the tube and cut off the cap of the tube (we have found that the cap will often hit the top of the centrifuge when the filter is inverted). Next remove the filters from each sample, invert the filter and place into the new tubes. Place the tubes in a microcentrifuge and centrifuge at 3500 rpm for 5 minutes.
4) Remove the tubes from the centrifuge and place in a rack. Label regular 1.5ml Eppendorf tubes for each sample. Remove the filter from each Microcon 50 tube and discard. Pipet the filtered PCR product from each Microcon 50 tube into the new tube (usually around 4-6 ul), and KEEP the Microcon 50 tube. Next, pipet 5-7 ul of nanopure water to each of the Microcon 50 tubes, and pipet the solution to the tube containing the purified DNA.This is done so that you will end up with approx. 11 ul of solution for each sample.
5) Pour a 1% agarose gel. Pipet 2 ul of gel loading dye onto a piece of parafilm for each sample. Pipet 1 ul of the purified DNA, mix with the gel dye and load onto the gel. Load a 100 bp marker into one lane of the gel and 2.5 ul of low DNA mass ladder (GibcoBRL, 2 ul ladder plus 0.5 ul gel dye) into another lane. Run the gel at 120 v for approx. 30 min.
6) Place the gel on a UV box and photograph with a 1 sec exposure at F5.6. Then, based on the DNA mass ladder, estimate the amount of purified DNA for each sample. Ie. if the DNA sample has the same staining intensity as the 40 ng band of the ladder then the concentration of the sample is 40 ng/ul. The DNA sequencing facility that we use recommends a DNA concentration of 10 ng/ul per 100 bp to be sequenced. So for a amplicon that is 500 bp in size, the purified DNA concentation should be at least 50 ng/ul.
7) Your PCR primers need to be sent along with the purified DNA. If your PCR primers are at the same concentration that we use, 20 uM, pipet 5 ul of each 20 uM PCR primer to 15 ul of nanopure water in an individual labeled 1.5 ml Eppendorf tube. Next, seal the tops of the primer and DNA sample tubes with parafilm.
8) Fill out the sequencing form for your sequencing facility. This usually includes billing information, the name of each sample, sample size, sample concentration, primer names, and primer concentration. Send the tubes and form to the sequencing facility using first class mail or FedEx. Sequences are usually downloaded from the facility using FTP.
9) Once the sequences are obtained, they can be aligned and edited using Genetics Computer Group (GCG) software.
10) After the sequences have been aligned and consensus have been obtained, RFLP sites can be determined using webcutter 2.0. http://firstmarket.com/firstmarket/cutter/cut2.html.

6. Restriction Digests (RFLP)

You will need:

1) Gloves
2) Your set of completed PCR reaction tubes
3) Microcentrifuge
4) Lid opener for ufuge & PCR tubes
5) 1 "Post-PCR" designated P-20 and 1 P-200 pipetmen
6) 1 or more trays—the bottoms of the

styrofoam storage boxes work well for this
7) "Regular" pipet tips
8) Sterile ddH2O
9) Sterile1.6ml ufuge tube(s)
10) Designated "Post-PCR" trash receptacle
11) Access to a (Savant) SpeedVac Concentrator
12) 37oC Incubator

General information:

- In this procedure, you are digesting a volume of your PCR amplification with a restriction enzyme. You will then run the digested products out on a MetaPhor agarose gel, and take a good picture of it that may well be used later in a publication. So you want to make it look as nice as possible.

1) The thing to do right now that will greatly influence how your gel turns out is to use as uniform an amount of DNA in each digestion as possible. This is where the use of the Low DNA Mass Ladder comes in really handy. For a digest, I try to aliquot a volume of a post-PCR reaction that contains about 100ng DNA. This entails a lot of "eye-balling." For example, suppose I ran 5.0ul of a PCR amplification on a check gel, and then, comparing the product band seen to the Low DNA Mass Ladder, I conclude that the intensity of that band represents approximately 40ng of PCR product. I would therefore use about 12ul of that product in a digestion.

*About 100ng of DNA provides enough PCR product that, when it is digested, there is enough DNA per each small fragment that they show up well on the MetaPhor agarose gel.
*Digestions seem to work the best when the total volume is about 10-15ul. This total includes your DNA volume, 10X buffer, enzyme, and maybe some ddH2O. They also seem to work better if done in 1.6ml ufuge tubes rather than PCR tubes. (People have found that certain plastics contain substances that inhibit some types of enzymatic activity.)
*About 60ng of DNA seems to work well if you are just running undigested PCR amplification product out on a nice MetaPhor agarose gel.

- In some cases, you may need to digest DNA from a PCR amplification that gave a rather faint product band. You cannot use all of the remaining 45ul in the digestion, mainly because there would not be enough space in the well of the MetaPhor gel. So first, figure out how much volume of that PCR amplification you would need to have about 100ng, aliquot that amount to a 1.6ml ufuge tube, and dry down in a speed vac—takes about 15-30 minutes. Then resuspend the pellet in 10ul sterile ddH2O. (Do this for volumes of about 17ul or more. The wells of the typical MetaPhor gel I set up hold no more than about 19ul.)

***Trick**—Setting the bottom of one of the Styrofoam storage boxes broadside in front of you, I use the first row of slots nearest me to place PCR amplification tubes from which I will aliquot 6ul of the product solution. In the next row of slots further away, I place tubes from which I will aliquot 8ul, the third (middle) row 10ul, the fourth row 12ul, and the fifth (furthest) row 15ul. (This is all very arbitrary, and there may be volumes you will want to use which fall between these.) Similarly, I set up another box bottom to hold tubes in rows corresponding the volumes I need to aliquot out and dry down.
2) On the back of one of my gel sheet forms, I write down the type and volume of each ladder I will use in the MetoPhor gel, the restriction enzyme and its source, the digestion recipes for each PCR amplification I will use, and the digestion conditions.

A typical digestion recipe would be:
2.0ul sterile ddH2O*
8.0ul volume of PCR amplification
1.2ul 10X buffer**
1.0ul enzyme***
12.2ul total volume

*The volume of ddH2O varies by how much amp. volume you need. There are times when you will not add any ddH2O at all.
**A restriction enzyme always comes with its corresponding tube of 10X buffer. Whatever the total

volume is, the volume of 10X buffer must be 1/10th of it (i.e., diluted down to 1X concentration). Also, BSA (bovine serum albumin) is needed to be used in conjuction with certain restriction enzymes and will be supplied along with the enzyme and buffer by the company. It may need to be diluted from 100X to 10X first and then is figured into the recipe just like the buffer.

***The volume of 1.0ul of restriction enzyme is standard for most restriction digestions of this volume.

3) Place a 1.6ml ufuge tube beside each of the PCR amplification tubes that you have set in designated slots in the tray. Blip all the amplification tubes, and set them back in their designated slots.

4) Aliquot the required volumes of sterile ddH2O to the respective ufuge tubes, and then pump each PCR amplification 5-6 times before aliquoting them to their respective ufuge tubes.

*There is no need to pump the digestion solution after each reagent is added. After you aliquot the 1.0ul of enzyme, you will pump the solution several times to mix well.

5) As with the 10X *Taq* buffer, pump the enzyme's 10X buffer at least 10 times to make sure it is mixed well before aliquoting.

6) If the enzyme solution is new or has been sitting idle for a long time, you may want to pump it several times before aliquoting. When you add it to the other reagents, pump the now complete digestion solution several times to mix well.

*Keep the enzyme tube cold, preferably at -20oC, during the time you are using it. They tend to lose their potency, if they are allowed to warm up too much. A -20oC benchtop chiller works well for this. (They also work well for storing your enzymes in frost-free freezers.)

7) Place your tubes into a ufuge rack, and place the rack in the proper incubation conditions. I usually allow them to incubate overnight.

*Most restriction enzymes are obtained from E. coli which lives comfortably at body temperature (37oC), and their enzymes function best at this temperature. There are a few restriction enzymes that are derived from other bacteria (e.g., *Taq*I from *Thermus aquaticus*) which function best at temperatures other than 37oC.

*Our lab used to use heat blocks for incubation of restriction digestions, but solutions tended to condense on the cap over time. I found that placing the whole rack of tubes in an incubation oven works much better. The proper temperature surrounds the whole tube.

7. MetaPhor Agarose Gels

You will need:

1) Gloves
2) Gel electrophoretic cell with tray, comb, lid, and power source
3) 250ml Erlenmeyer flask
4) 0.5X TBE
5) MetaPhor agarose
6) Aluminum foil
7) 2.5mg/ml EtBr
8) Heavy (autoclave) gloves
9) Bunsen burner (or microwave)
10) Your set of completed PCR reaction tubes
11) DNA ladder(s)
12) Tube of gel dye
13) Microcentrifuge
14) 1 "Post-PCR" designated P-20 pipetman
15) "Regular" pipet tips
16) Lid opener for ufuge & PCR tubes
17) Designated "Post-PCR" trash bag

Set up equipment:

*For a good (i.e, potentially publishable) gel picture, we use the Model H5 gel apparatus from BRL. Its gel tray is long enough to be able to separate out most digestion products nicely. We also use MetaPhor agarose. It separates DNA fragments almost as cleanly and clearly as polyacrylamide without the hassel of dealing with PA's hazardous aspects.

1) Make sure the gel tray and comb are clean—free of dust, old agarose gel particles, and water stains. Tape the ends (with new pieces of tape) as you had when doing a check gel. The reason I set this up now is I like to pour the melted MetaPhor agarose solution as hot as possible—it begins to set up very quickly.

2) Rinse out a 250ml Erlenmeyer flask with dH2O a few times and drain, aliquot 100ml 0.5X TBE to the flask, add 2.5g MetaPhor agarose, and cover the flask with a piece of aluminum foil about 3-4" square.

*Add these reagents to the flask in this order. If the MetaPhor is added before the 0.5X TBE, the agarose will stick to the bottom of the flask and then burn when heated over a Bunsen burner.

*100ml of this solution works well with this particular gel tray. The gel thickness gives enough volume for each well while not being too thick and wasteful of the agarose.

*This gives a 2.5% solution—a concentration that separates out our particular sized digestion products well. Each bottle of MetaPhor is shipped with an instruction booklet which contains information on

what percentage of agarose you should use with what sized DNA fragments.
3) Melt the agarose. I do this by holding the flask directly over the biggest flame of a Bunsen burner—constantly and vigorously swirling the solution. Agarose stuck to the bottom of the flask will burn, while clumps of agarose floating around in solution or sticking to the sides of the flask will eventually melt. If you hold the solution still over the burner or swirl it only gently, it will burn some on the bottom of the flask. After a couple minutes, the solution will begin to boil. (You will be able to feel it.) Continue to swirl the solution over the flame, but keep a very close watch on the bubbles forming. **The solution can bubble over extremely quickly.** When the bubbles begin to rise up in the flask, remove it from the flame and continue to swirl vigorously for 5-10 seconds. Swirl the flask over the flame again till the bubbles begin to rise. Repeat this process 3-4 times—you want to make sure all of the agarose crystals are melted.
*You can melt the agarose in a microwave, if you keep a very close watch on it so it does not boil over. The reason I like using a Bunsen burner is that you can remove the flask from the heat source very quickly. You do not have quite as much control using a microwave.
*My experience is that the bubbles can be very small and foam or they can be large and break up easily. If you poured the agarose with the foam still present, the surface of the solidified gel would be pocked which can be seen later on the resulting picture. Rinsing the flask before adding anything is an attempt to reduce the chances of getting foam.
4) When all the crystals are melted, add 4.0ul 2.5mg/ml EtBr to the solution, swirl to mix, and continue to swirl over the flame. Bring the solution to boiling 2-3 more times as before.
*For some reason, something happens during the time between heating the solution over the Bunsen burner—the chance the solution still boils into a foam is reduced.
5) Pour the agarose into the tray as soon as possible after taking the flask off the flame for the last time. Moving right along insert the comb and remove any bubbles that may have formed anywhere in the gel.
6) Allow the agarose to solidify for 30 minutes at RT. Then place it into a cold room or fridge at +4oC for an additional 30 minutes.
*This is one of the key differences between using "regular" versus MetaPhor agarose.
7) While the gel is setting up, take your tubes out of their incubation conditions, blip them along with the ladders you will use, and set them in a tray/styrofoam box bottom in the order you want to load them on the gel. Also, pour 0.5X TBE running buffer into the gel box as you did with the check gel earlier.
8) When the gel/tray is ready to be taken out of +4oC, set it at RT and pour a layer of 0.5X TBE over it. Let it sit for about 10-15 minutes.
*MetaPhor agarose gels seem to dry out rather quickly, especially in a cold room where there is constant air circulation. Pouring a layer of 0.5X TBE over the gel after, or even during, its +4oC incubation keeps it well hydrated. Before you take the tape off, simply pour the liquid into your gel box, keeping your gel well supported.
*Allowing the gel to warm up to RT seems to aid in the removal of the comb from the gel. The colder the gel is, the more tightly the comb seems to be held in place.
9) When you are ready to take the comb out, first slide a finger on the gel through the 0.5X TBE in the area between the comb and the tape. Then as you are gently pulling up on one side of the comb, gently press the gel down away from the teeth that are being pulled up. Do this a couple times, and then do the same thing on the other side. Switch back and forth till the comb is pulled free.
10) Remove the tape carefully—the gel will easily slide off the tray at this point. Then gently set the gel/tray into place in the gel box and remove or add 0.5X TBE as needed as you did with the check gel earlier.

Loading the gel:

1) Place the tray of tubes between you and the gel box and pop open the caps of all your digestion and ladder tubes.
***Trick**—Set your "post-PCR" P-20 on about 1.4ul. (Remember, the gel dye is at a 10X concentration, so aliquot a volume of dye to the digestion that is about $1/10^{th}$ of the digestion volume. This does not have to be exact however. Also, keep in mind not every molecule of PCR product was digested by the restriction enzyme—dispose of tips and tubes properly.) Pipet the gel dye up into the pipet tip and then eject the tip itself into the digestion tube. Do the same for all your digestion tubes, but not your ladders. This saves a little time and a lot of pipet tips.
2) Set the "post-PCR" P-20 at 20.0ul. Pick up your first reaction tube, hold it so its bottom sits on the bench, and insert the P-20 shaft back onto the pipet tip. Pump the solution 5-6 times and then pull as much it into the pipet tip as possible. Load the gel as you did earlier with the check gel.
3) Once you are finished loading the gel, aliquot 5-6ul 2.5mg/ml EtBr to the lower buffer

chamber/reservoir as you did earlier with the check gel.
4) I usually run this type of gel at about 96V for about 3.5 hours. This separates the digestion products out nicely, and the 50bp fragment of the ladder is still on the gel (so you know you have not lost any small fragments off the bottom end).
*There are times when you might want to run your MetaPhor gel in cold room. An example would be when you want to run out undigested PCR amplification products on a MetaPhor gel to show size differences between the different products for a good picture. Since they are large molecules, they need more time to travel through the gel. You would probably want to use a 2.0% MetaPhor gel run at about 100V for about 6-7 hours using the 100bp ladder. I have usually run these types of gels using 0.5X TBE buffer, but you may want to use 1.0X TBE, considering the length of time. Running the gel in the cold room dissipates the heat built up during electrophoresis and reduces the diffusion that occurs over that length of time.

Running the gel:
1) Run the gel using the suggestions as you did earlier with the check gel.

Take a picture of the gel:
1) Take a picture of the gel using the suggestions as you did earlier with the check gel.
2) Dispose of your gel properly and rinse off the gel box and tray as with the check gel.

8. Appendix A —Solutions Needed

**If at all possible, obtain a copy of *Molecular Cloning: A Laboratory Manual*, 2nd Edition, by J. Sambrook, E.F. Fritsch, & T. Maniatis, published by Cold Spring Harbor Laboratory Press, 1989. It is usually referred to as "Maniatis" and is the "Bible" of molecular biology.

**For future reference:

"T" stands for Tris (or Tris-HCl) -- stabilizes DNA by buffering solutions very well
"B" stands for Boric Acid
"E" stands for EDTA (Disodium Ethylenediaminetetraacetate . 2H2O) -- chelates Mg+2 ions (i.e., takes them out of solution thereby preventing them from interacting with enzymes such as DNases and *Taq* Polymerase.

**Molarity (M) is a concentration (i.e., moles per liter), while moles (m) is an amount.

****ddH2O** refers to distilled, dionized water and sometimes to double distilled water, which are two different things, but both appear to work equally well in the various protocols/recipes. Store at room temperature (RT).

DNA extraction buffer

(100 mM EDTA, 100 mM NaCl, 100 mM Tris pH 7.5, 0.5% SDS, 200 ug proteinase K).

RNase A.

buffer satuated phenol

chloroform / isoprophyl alcohol [CHCl/IAA] (24:1)

5 M NaCl

10X Taq Buffer

- Enough always comes with commercial *Taq*, but you might want to make your own sometime.
- Make these stock solutions first:
- **1M Tris**

  Dissolve 121.1g of Tris base in 800ml of ddH2O. (*Can make smaller volumes than this—ex. 200ml.)

  Adjust the pH to the desired value by adding concentrated HCl. (For example, if you want pH 8.0, add about 42ml conc. HCl.) The temperature will increase. Allow the solution to cool to RT before making final adjustments to the pH.

  Adjust the volume of the solution to 1L with ddH2O.

  Dispense into 200ml aliquots and sterilize by autoclaving.

  Store at RT.

*Tris is a very good buffer (i.e., it soaks up H+ ions really well). So keep adding conc. HCl till pH meter reads 8.0 continuously.
*When the solution has been equilibrated with concentrated HCl, it is sometimes refered to as Tris-HCl. You can even buy crystalized Tris-HCl which, when put into solution, has an initial pH much lower than Tris, but it's actually too low for our needs.
*If the 1M solution has a yellow color, discard it and obtain better quality Tris.
*Although many types of electrodes do not accurately measure the pH of Tris solutions, suitable electrodes can be obtained from most manufacturers.
*The pH of Tris solutions is temperature-dependent and decreases approximately 0.03 pH units for each 1oC increase in temperature. For example, a 0.05M solution has pH values of 9.5, 8.9, and 8.6 at 5oC, 25oC, and 37oC, respectively.

1M MgCl2

Dissolve 203.2g of magnesium chloride, hexahydrate in 800ml of ddH2O. (*Can make smaller volumes than this—ex. 200ml.) -- Adjust the volume to 1L with ddH2O.

Dispense into 200ml aliquots and sterilize by autoclaving.

Store at RT.

MgCl2 is extremely hygroscopic (i.e., it loves to soak up water which adds to its weight; this can lead to inaccurate measurements). Buy small bottles (e.g., 100g) and keep your stock in a dehydrating system.

3M KCl

Dissolve 223.7g KCl in 800ml of ddH2O. (*Can make smaller volumes than this—ex. 200ml.)

Adjust the volume to 1L with ddH2O.

Dispense into 200ml aliquots and sterilize by autoclaving.

Store at RT.

- Now make the 10X *Taq* Buffer:

  10.0ml 1.0M Tris-HCl, pH 8.0 (100.0mM)
  1.5ml 1.0M MgCl2 (15.0mM)
  16.7ml 3.0M KCl (500.0mM)
  71.8ml ddH2O
  100.0ml total

- Bring pH to 8.3 (20oC)
- Aliquot 1.0ml to each of 100 screw-cap tubes and autoclave
- Store at constant -20oC.

dNTP mix

- If you buy your dNTP's from Pharmacia Biotechnology, you will receive four tubes—dATP, dCTP, dGTP, & dTTP. Each comes at an amount of 25 micromoles (umol) and at a 100 millimolar (mM) concentration, pH 7.5. I make up dNTP mix tubes which include all four of the aforementioned dNTP's each at the same concentration of 1.25mM. I can usually make about 20-21 stock tubes from one shipment.
- This is "pre-PCR," so use aerosol-barrier tips.
- Calculations (-- starting simply and then expanding):

1) For one 1.0ml stock tube using only one dNTP:

HAVENEED
(X ml)(100mM) = (1.0ml)(1.25mM)
X mL = (1.0ml)(1.25mM)
100mM
X mL = 0.0125ml or 12.5ul

2) 12.5ul (only 1 dNTP) X 4 dNTP's/1.0ml stock tube = 50.0ul (subtotal)

50.0ul (subtotal volume)

950.0ul st. ddH2O
1,000.0ul total for 1 stock tube

3) For one 1.0ml stock tube using all four dNTP's:

1st, find out what volume you have for each dNTP and find total:

ex.,dATP --- 259.0ul
dCTP --- 261.0ul
dGTP --- 258.0ul
dTTP --- 254.0ul
1,032.0ul total volume for all dNTP's

2nd, find the average:In this case, the average is 258.0ul

3rd, plug that average into the HAVE/NEED equation as seen in 1) :

HAVENEED
(258.0ul)(100mM) = (X ml)(1.25mM)
(0.258ml)(100mM) = (X ml)(1.25mM)
X mL = (0.258ml)(100mM)
1.25mM
X mL = 20.64ml**This 20.64ml volume is the final volume you will get when you have mixed all the dNTP volumes with the needed amount of sterile ddH2O (which you will calculate next).

4) 50.0 ul (volume of all 4 dNTP's in one stock tube) X 20.64

= 1,032.0ul (This is total volume of all dNTP's sent in shipment)

950.0ul (volume of sterile ddH2O in 1 stock tube) X 20.64 = 19,608.0ul

1,032.0ul

19,608.0ul 20,640.0ul (= 20.64ml final volume)

- Mix each dNTP well individually by pumping about 10 times. (Remember, this is "pre-PCR," so use aerosol-barrier tips.)
- Aliquot all of each of the four dNTP's into a sterile 50 ml tube, and then aliquot sterile ddH2O with a sterile 10ml pipet.
- Mix well by pumping and aliquot 1.0ml to as many sterilized 1.6ml ufuge tubes as needed.
- Store at constant -20oC.

1mM storage stocks of your primers

- I recommend that when you get your newly synthesized primer(s) in lyophilized/pellet form*, you resuspend it in enough 1X TE that you end up with a 1mM concentration.
- You should be given the optical density (O.D.260) and the molecular weight of the primer(s) you had synthesized, and 1 O.D. corresponds to approximately 33ug/ml (Maniatis, p. 11.30). From this, you can do the needed calculations. As an example,

rDNA1.58S --- 5'-ACG-AGC-CGA-GTG-ATC-CAC-CG-3'
46.5 O.D.; Info. sheet said M.W. --- 6,107.0
46.5 O.D. X 33ug X 1 g X 1 mole X 1 L X 106ul = 251.3ul
1 O.D. 106ug 6,107.0g 10-3 mole 1 L
Therefore, I resuspended this primer pellet in 251.3ul 1X TE, pH 8.0 to obtain a 1mM storage stock.

- This is "pre-PCR," so use aerosol-barrier tips.
- Allow the primers to fully resuspend by gentle shaking about 20 minutes and sitting at -20oC overnight if possible.
- Store at constant -20oC.
- 1mM storage stocks should only be thawed when you need to make more 20uM working stocks—cuts down on chances of contamination and primer degredation.

*This presumes that you'll receive your primers ready to be resuspended. Some other synthesizing methods/machines require additional chemical

processing before the primers are ready to be resuspended.

20uM working stocks of your primers

- This is "pre-PCR," so use aerosol-barrier tips.
- Mix the 1mM storage stock of the primer by "pumping" it 10-20 times. ("Pumping" refers to drawing the solution into the pipet tip and then expelling it. This is done enough times to ensure the solution is thoroughly mixed before it is aliquoted to another solution.)
- In a 1.6ml ufuge tube, aliquot 98.0ul sterile ddH2O, 98.0ul 1X TE, pH 8.0, and 4.0ul of 1mM primer storage stock (already mixed well by pumping). This gives 200.0ul of a 20uM working stock of your primer with a 0.5X TE concentration—an amount quickly used up with little chance of becoming degraded. And if it does become contaminated, just toss it, and make a fresh batch.
- Store at constant -20oC.
- By the time you aliquot the appropriate amount of the 20uM primer working stock to your PCR master mix, the EDTA in the solution will be diluted out enough so as not to adversely affect *Taq*'s polymerase functions.

0.5M EDTA, pH 8.0

- Add 186.1g of disodium ethylenediaminetetraacetate . 2H2O to about 600ml ddH2O. Stir vigorously on a magnetic stirrer. Adjust the pH to 8.0 with NaOH (about 20g of NaOH pellets).

*The disodium salt of EDTA will not go into solution until the pH of the solution is adjusted to approximately 8.0 by the addition of NaOH.
*Be very careful while adding NaOH to the solution when approaching pH 8.0 -- solution tends to change pH much faster the nearer it gets to 8.0.

- Pour the solution into a 1L graduate cylinder and bring volume up to 1L. (*Can make smaller volumes than this—ex. 200ml.)
- Dispense into aliquots (ex., 2 500ml, etc.) and sterilize by autoclaving.
- Store at RT.

1X TE, pH 8.0

- Make up your needed volume of solution so that you have 10mM Tris . HCl, pH 8.0 & 1mM EDTA, pH 8.0 as your final concentrations.

*For TE at a certain pH (ex., 8.0), use Tris-HCl at that certain pH. No matter at what pH you want the TE, use EDTA at pH 8.0.

*We usually use TE at pH 8.0.

- Dispense into aliquots and sterilize by autoclaving.
- Store at RT.

10X TBE (stock electrophoretic buffer)

108.0 g Tris --- 0.89 M
55.0 g Boric Acid --- 0.89 M
40.0 ml 0.5 M EDTA, pH8.0 --- 0.02 M

- Dissolve the above reagents in 500-600ml ddH2O and then bring solution to 1L.

*A precipitate forms when concentrated solutions of TBE are stored for long periods of time. To avoid problems, store the solutions at RT and discard any batches that develop a precipitate.

0.5X TBE (electrophoretic running buffer)

- To make this involves a 1 to 20 dilution of a 1L stock of 10X buffer. I usually make a large batch of it in a 20L carboy. We go through it quickly.
- Dump in 1L 10X TBE buffer and add 19L of ddH2O.
- Mix well before using.
- Store at RT.

*A working solution of 0.5X TBE provides enough buffering power for nearly all applications. However, if you intend to electrophorese your gels at a high voltage (thereby creating significant heat enough to melt your agarose gel) or for a long period of time (e.g., 5 or more hours), then you may want to consider using a 1.0X TBE concentration—a 1 to 10 dilution of a 1L stock of 10X buffer.

1.0% Agarose, Low EEO (used for check gels)

- In a 250ml glass bottle, place 2.0g agarose and 200.0ml 0.5X TBE buffer (order in not important), and then aliquot 4.0ul 2.5mg/ml EtBr (see below).
- Cap tightly and store at RT till ready to use.

*Remember, 1.0g of solute in 100.0ml of liquid makes a 1.0% solution.

10X Gel Dye <u>ReagentFinal Concentration in 10X Sol'n</u>
500ul glycerol 50%

400ul 0.5M EDTA 0.2M
2.5mg Bromophenol Blue (dye)0.25%
About 5ul 1M Tris (i.e., however much you need to change the pH so that the color turns from green to blue)

About 100ul sterile ddH2O

Approximately 1,000ul total volume

- Mix well by pumping and aliquot 1.0ml to as many sterilized 1.6ml ufuge tubes as needed.
- Store at constant -20oC.

*This gel-loading buffer serves three purposes:

(1) Glycerol increases the density of the sample, ensuring the DNA drops evenly to the bottom of the well
*A recent article in Gibco/BRL "Focus" (1997) Vol. 19, #1 stated that "...on PAGE gels, glycerol may accentuate smiling bands...Sucrose is superior to Ficoll and glycerol (as a density agent), since it is more dense and less viscous." We usually make our loading buffer with glycerol, but we have also used loading buffer with sucrose with no problems.
(2) EDTA stops any enzymatic reactions that may be occurring, such as DNases chewing up your amplification products, by chelating Mg+2 ions out of solution so they cannot be used for any other enzymatic activity
(3) Bromophenol blue adds color to the sample, simplifying the loading process, and it co-migrates with DNA fragments of predicted sizes relative to the agarose concentration of the gel. (e.g., Bromophenol blue migrates through agarose gels run in 0.5X TBE at approximately the same rate as linear double-stranded DNA 300 bp in length, independent of the agarose concentration in the gel over the range of 0.5% to 1.4%.)

1Kb DNA Ladder

- This comes at a 1ug/ul concentration—extremely concentrated. I dilute 8.0ul of this stock in 392.0ul sterile ddH2O (1:50 dilution), aliquot 40.0ul gel dye, and mix by pumping several times.
- 10.0ul of this mix (or about 0.2ug DNA) is used per lane on a check gel.

100bp DNA Ladder

- This comes at a 1ug/ul concentration—extremely concentrated. I dilute 8.0ul of this stock in 392.0ul sterile ddH2O (1:50 dilution), aliquot 40.0ul gel dye, and mix by pumping several times.
- 10.0ul of this mix (or about 0.2ug DNA) is used per lane on a check gel.

50bp DNA Ladder

- This comes at a 1ug/ul concentration—extremely concentrated. I dilute 8.0ul of this stock in 392.0ul sterile ddH2O (1:50 dilution), aliquot 40.0ul gel dye, and mix by pumping several times.
- 20.0ul of this mix (or about 0.4ug DNA) is used per lane on a MetaPhor gel.

Low DNA Mass Ladder

- The recommendation is to mix four parts ladder with one part gel dye. I aliquot 50ul gel dye to the entire 200ul ladder and mix well by pumping.
- I recommend using 2.5ul of this mix per lane on a check or MetaPhor gel rather than their suggested 5.0ul. It uses less DNA, and the range of the post-PCR DNA concentrations fall well within the range shown by the ladder.

2.5mg/ml Ethidium Bromide (EtBr)

- Add 0.25g (= 250mg) of ethidium bromide to 100ml of ddH2O.

*This is the concentration we use in the lab. ("Maniatis" suggests making a 10mg/ml solution—i.e., add 1g of EtBr to 100ml of ddH2O, which also happens to be a 1% solution).

- Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved.
- Wrap the container in aluminum foil or transfer the solution to a dark (amber) bottle and store at +4oC. (EtBr is sensitive to light.)

***Caution**—Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye, and a mask should be worn when weighing it out. Check to see what regulations your lab may have regarding its disposal. (For example, can solutions be dumped down the sink and do you have to dispose of your gels in a dedicated container?)

*Pre-made solutions may be bought, but they cost more.

*I use about 1.0ul of this solution per 50.0ml of an agarose solution.

9. Appendix B — Supplies

*The University of Nebraska - Lincoln has a contract with Baxter Scientific Products. Therefore, our prices for supplies obtained from them are lower than their list prices.

Glass Cover Slips—S/P No. 1 Rectangular Cover Glasses
Cat.#M6045-2; 0.13 to 0.17 mm, 22 X 22 mm, 10 oz/cs, No. 1
Last purchase price: $18.98/cs
Company: Baxter Scientific Products
Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787
800-723-1390

Yellow Pipette Tips

Cat.#1035; yellow, bagged, flat tipped, 1-200 ul, 1,000/unit, 10,000/cs

Last purchase price: $112.00/cs
Company: Out Patient Services, Inc.
1320 Scott Street
Petaluma, CA 94954
800-648-1666
*Great for smashing nematodes

*May need to write: "**No substitutions**" on order

Gloves:

N-Dex Nitrile Disposable Gloves

Cat.#G7200-22, Mfr. No. 7005L, Large, 100/bx, 20 bx/cs
Last purchase price: $10.96/bx
Company: Baxter Scientific Products
Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787
800-723-1390
*My personal favorite—they are tough, form to the hand well, and have almost no powder on them to dry out your skin. They are expensive, but you can wash them as you wash your hands, and so you use them longer than other gloves.

N-Dex Nitrile Disposable Gloves
Cat.#G7200-21, Mfr. No. 7005M, Medium, 100/bx
Last purchase price: $11.14/bx
Company: Baxter Scientific Products
Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787
800-723-1390
*Additional sizes are available.

Pipetman (adjustable) -- P-20, P-200, & P-1000

Cat.#Models P-20, P-200, & P-1000
Last purchase price: $189.50 (might be gotten elsewhere somewhat more cheaply)
Company: Rainin Instrument Co., Inc.
Mack Road
P. O. Box 4026 Woburn, MA 01888-4026
617-935-3050

Screw Cap Microcentrifuge Tubes
Cat.#2055; 1.5 ml, cap with o-ring, natural, 500/unit
Last purchase price: $40.20/unit
Company: Out Patient Services, Inc.
1320 Scott Street
Petaluma, CA 94954
800-648-1666
*Used to make up 1.0ml sterile aliquots of ddH2O—very handy for small, quick usages, & then tossed

0.5ml Microcentrifuge ("PCR") Tubes
Cat.#UP 2060; 0.6 ml, clear polypropylene, natural color, ungraduated, flat caps, 1,000/bag
Last purchase price: $18.00/bag
Company: United Scientific Lab Plastics (a.k.a. United Laboratory Plastics)
P. O. Box 8585
St. Louis, MO 63126-8585
800-722-2499
*Used for PCR reactions

*May need to write: "**No substitutions**" on order

Benchtop Chiller Flipper Rack/Tray

Cat.#BC-2020; -20oC chiller—maintains samples at temperature on benchtop, ultra-clear polycarbonate lid, 24 X 1.5 microtubes on 1 side & 24 X 500ul microtubes on the other
Cat.#BC-2000; 0oC chiller—maintains samples at temperature on benchtop, ultra-clear polycarbonate lid, 24 X 1.5 microtubes on 1 side & 24 X 500ul microtubes on the other
Last purchase price: $54.90 for either chiller
Company: Phenix Research Products
3540 Arden Road
Hayward, CA 94545
800-767-0665

80-Well Microfuge Tube Rack/Tray

Cat.#RF-RACK-AD; 8/pkg, "AD" = Assorted Dayglo colors
Last purchase price: $35.00/pkg
Company: Owl Scientific Plastics, Inc.
P. O. Box 566
Cambridge, MA 02139
800-242-5560

1.6ml Microcentrifuge Tubes

Cat.#UP 2050; Polypropylene, 1.6 ml, 500/bag

Last purchase price: $9.00/bag
Company: United Scientific Lab Plastics (a.k.a. United Laboratory Plastics)
P. O. Box 8585
St. Louis, MO 63126-8585
800-722-2499

*Used in PCR reactions, PCR product digestions, and GeneClean's

*May need to write: "**No substitutions**" on order

dNTP Set
Cat. #27-2035-01; 100mM Ultrapure Solutions, 4 X 25umol
Last purchase price: $174.00
Company: Pharmacia Biotechnology, Inc.
800 Centennial Ave.
P. O. Box 1327
Piscataway, NJ 08855-1327
800-526-3593

*Avoid multiple freeze-thaw cycles or exposure to frequent temp. changes. Long-term: -80 C; daily/weekly: -20 C.

Filtered tips for a 20ul, 200ul and 1000ul pipetteman:

20ul—Aeroseal (Aerosol-Barrier) Advantage Filter Pipet Tips

Cat. #1010-1810; 2-20ul, 10 racks/cs
Last purchase price: $59.00/cs
Company: USA Scientific Plastics
P. O. Box 3565
Ocala, FL 32678
800-LAB-TIPS (522-8477)

*Used in all "pre-PCR" protocols.

200ul—Aeroseal (Aerosol-Barrier) Advantage Filter Pipet Tips

Cat. #1024-5810; 0-160ul, 10 racks/cs
Last purchase price: $59.00/cs
Company: USA Scientific Plastics
P. O. Box 3565
Ocala, FL 32678
800-LAB-TIPS (522-8477)

*Used in all "pre-PCR" protocols.

1,000ul—Aeroseal (Aerosol-Barrier) Advantage Filter Pipet Tips

Cat. #1026-7810; 101-1,000ul, 10 racks/cs
Last purchase price: $79.00/cs
Company: USA Scientific Plastics
P. O. Box 3565
Ocala, FL 32678
800-LAB-TIPS (522-8477)

*Used in "pre-PCR" reaction protocols—but not often.

*You may find a better price at VWR/Baxter.

Nonfiltered ("regular") tips that fit a 20ul or 200ul pipetteman

Cat. #21-197-2K; 1-200ul, natural, 1,000/pkg
Last purchase price: $10.00/pkg**
Company: Fisher Scientific
St. Louis Branch
1241 Ambassador Blvd.
P. O. Box 14989
St. Louis, MO 63178-4989
800-766-7000

*Used in "post-PCR" reaction protocols (e.g., check gels, digestions, etc.).
**This is a special price we get thru our salesman. I don't know what their regular price is. You may find a better price elsewhere.

Nonfiltered ("regular") tips that fit a 1,000ul pipetteman

Cat. #21-197-8F; 201-1,000ul, blue, 1,000/pkg
Last purchase price: $12.00/pkg**
Company: Fisher Scientific
St. Louis Branch
1241 Ambassador Blvd.
P. O. Box 14989
St. Louis, MO 63178-4989
800-766-7000

*Used in "post-PCR" reaction protocols—but not often.

**This is a special price we get thru our salesman. I don't know what their regular price is. You may find a better price elsewhere.

Lid opener for Microcentrifuge tubes

Cat. #1012-20-0 (for 10/pk) or #1012-21-0 (for 25/pk); blue, polypropylene, autoclavable, virtually indestructable, and the design reduces

possible cross-contamination from post-PCR products

Last purchase price: $20.00 (for 10/pk) or $40.00 (for 25/pk)**
Company: Robbins Scientific
814 San Aleso Avenue
Sunnyvale, CA 94086-1411
800-752-8585
**Information may need to be updated.

Thermal Cycler—with heated lid
Cat. #Gene E, model #FGENEEHP; and lid model #FHLID
Last purchase price: $ --??
Company: Techne, Inc.
3700 Brunswick Pike
Princeton, NJ 08540-6192
800-225-9243

Styrofoam Microcentrifuge Tube Storage Boxes
Cat. #145746; 10/cs
Last purchase price: $36.00
Company: Research Products Int'l Corp.
410 North Business Center Drive
Mount Prospect, IL 60056
800-323-9814
*Very useful to store post-PCR products/tubes.

Agarose, Low EEO

Cat. #BP160-500; 500g
Last purchase price: $362.65
Company: Fisher Scientific
St. Louis Branch
1241 Ambassador Blvd.
P. O. Box 14989
St. Louis, MO 63178-4989
800-766-7000
*You may find a better price elsewhere.

MetaPhor Agarose

Cat. #50180; 125g/bottle
Last purchase price: $288.00/1 bottle or $266.00/2 or moreCompany: FMC BioProducts
FMC Marine Colloids Bioproducts Dept.
5 Maple Street
Rockland, ME 04841
800-341-1574

Submerged Gel Electrophoresis Cells:
Wide Mini-Sub Cell*
Cat. #170-4343
Last purchase price: $315.00
Company: Bio-Rad Labs
19 Blackstone St.
Cambridge, MA 02139
800-274-4246

Mini-Sub Cell*
Cat. #170-4307
Last purchase price: $230.00
Company: Bio-Rad Labs
19 Blackstone St.
Cambridge, MA 02139
800-274-4246
*Both of the above units are no longer available from Bio-Rad, but similar cells with accessories may be purchased from Bio-Rad or elsewhere.

Model H5 Horizontal Gel Apparatus
Cat. #21087-010
Last purchase price: $325.00
Company: Gibco/BRL
Bethesda Research Laboratories
P. O. Box 630760
Baltimore, MD 21263-0760
800-828-6686
*Our lab has several of the older gel boxes of this model.
*This model utilizes a 11cm X 14cm gel.
*We use this model for our good/MetaPhor/digestion gels.

1Kb DNA Ladder
Cat. #15615-016; 250ug
Last purchase price: $102.00
Company: Gibco/BRL
Bethesda Research Laboratories
P. O. Box 630760
Baltimore, MD 21263-0760
800-828-6686
*This is a good general purpose size marker with a large range.

100bp DNA Ladder
Cat. #15628-050; 250ug
Last purchase price: $288.00
Company: Gibco/BRL
Bethesda Research Laboratories
P. O. Box 630760
Baltimore, MD 21263-0760
800-828-6686
*This is a really good size marker for generally smaller DNA bands, like the ones we get using the rDNA2/rDNA1.58S primer set.

50bp DNA Ladder
Cat. #10416-014; 50ug
Last purchase price: $79.00
Company: Gibco/BRL
Bethesda Research Laboratories

P. O. Box 630760
Baltimore, MD 21263-0760
800-828-6686

*This is a really good size marker when running DNA restriction digests out on a MetaPhor agarose gel.

Low DNA Mass Ladder
Cat. #10068-013; 200ul
Last purchase price: $78.00
Company: Gibco/BRL
Bethesda Research Laboratories
P. O. Box 630760
Baltimore, MD 21263-0760
800-828-6686

*This is a good size marker as well, but its important novelty is that each specific-sized band also comes at a different set DNA concentration. Using this ladder, you can not only figure out the approximate size of your PCR band of interest, but you can also give an accurate guess as to how much DNA is there.

Hot Hand Protector
Cat. #17-000-000A
Last purchase price: $21.80
Company: Lux Scientific Instruments Corp.
2460 North Dodge Blvd.
Tucson, AZ 85716
602-327-4848

*Probably can get this somewhere else cheaper.

*These really work well for holding hot flasks, etc.

S/P Pressure-Sensitive Labeling Tape
Cat. #L1600-75; 0.75” wide, white
Last purchase price: $2.55/rl
Company: Baxter Scientific Products
Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787
800-723-1390

*Used to tape up the ends of gel electrophoretic trays

Parafilm M Laboratory Films
Cat. #P1150-4; 4” X 250 ft, ~0.005” thick
Last purchase price: $16.17/rl
Company: Baxter Scientific Products
Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787
800-723-1390

Micron Microcon 50 Microconcentrators
http://www.millipore.com/analytical/amicon/td-185.html
Cat # 42416
Millipore Corporation
80 Ashby Road • Bedford, MA 01730
800-645-5476

*Used for purification of PCR product for DNA sequencing