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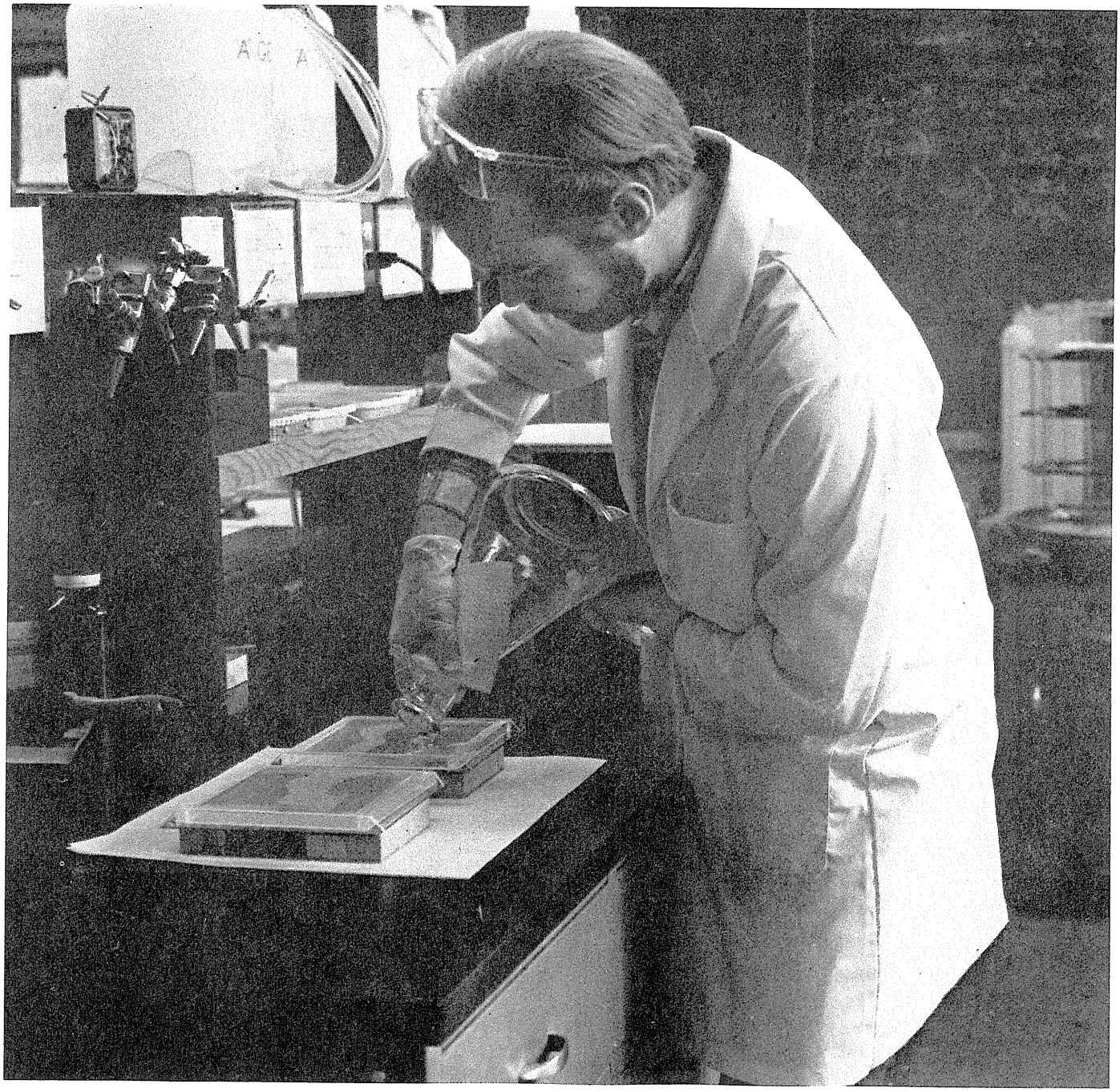
Starch Gel Electrophoresis of Conifer Seeds: a laboratory manual

M. Thompson Conkle Paul D. Hodgskiss Lucy B. Nunnally Serena C. Hunter



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Foreword

The procedures described in this manual were adapted from starch gel techniques in use at the University of California, Davis. Alex Kahler, now plant geneticist, Northern Grain Insects Research Laboratory, Agriculture Research Service, U.S. Department of Agriculture, Brookings, S.D., provided information on electrophoresis that led to the establishment of an isozyme laboratory in 1970 at the Pacific Southwest Forest and Range Experiment Station under the direction of M. Thompson Conkle and to the development of a user's manual. The first draft of the manual was prepared by Lucy B. Nunnally and Serena C. Hunter. Recent modifications of techniques and additions of stain systems prompted Paul D. Hodgskiss to revise and enlarge the manual. Demand for copies of the first draft and requests for details about the procedures encouraged this publication. Because these procedures are constantly being modified, the authors would appreciate receiving suggestions for the improvement of this manual.

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CONTENTS

The identification of different forms of enzymes by the process of electrophoresis is a powerful research tool for the genetic analysis of forest trees (Feret and Bergmann 1976). Electrophoresis is the movement of enzymes in a gel, under the influence of an electric current. Dissimilar enzymes and the alternative forms of similar enzymes migrate at different rates when direct current is applied to macerated seed samples in a buffered starch gel. Enzyme migration in gels and the separation of enzymes with different charges results from the interaction of the electric current, the pH in the gel, and the pH in the electrode tray (gel system). The direction—anodal or cathodal—and rate of enzyme migration depend on the kind of electric charge—plus or minus—and the charge strength.

Several gel systems are needed to analyze maximum numbers of enzymes. After gels have been held for a sufficient time in the electric field, gel slabs are sliced and thin gel slices are stained for different enzymes. Stain solutions contain specific substrates, and enzymes from the seed samples act on these substances to produce reaction products. Reaction products are stained or are the bases of stain processes. The locations of visible bands on the gels mark the migration distances of specific enzymes. Different bands on a gel that has been stained for one reaction denote functionally related molecules that differ in electrical charge—isozymes. Isozymes, tested by determining Mendelian segregation ratios and found to be phenotypic expressions of alleles of a single genetic locus, are allozymes.

The electrophoresis of conifer seeds has many useful applications. Conifer seeds are excellent for genetic studies because gene products are present in haploid and diploid tissues. The nutrient material surrounding the embryo is haploid. Electrophoresis of this tissue resolves enzyme alleles identical with the female gamete of the seed. Analysis of gametophytes from several seeds of one tree provides an accurate evaluation of that tree's genotype. Electrophoresis of embryos resolves diploid banding patterns. The pollen contribution to individual embryos can be deduced by subtracting the allele carried by the gametophyte from the pair of alleles carried by the embryo.

This manual provides detailed information on seed preparation techniques, equipment, and chemical formulas, including how to prepare tissue and gel, supply electric current, and stain gel slices to produce visible bands marking the location of enzymes. It outlines proven procedures for obtaining isozyme phenotypes in 23 enzyme systems, for resolving genotypes at about 45 loci. The procedures for conifer seed electrophoresis are presented in sections in the order they are performed during day-to-day laboratory operation. This manual, which updates and supersedes an earlier publication (Conkle 1972), does not cover genetic interpretation of the bands on the gels.

SEED PREPARATION

Seed processing—collection, extraction, storage, stratification, and germination—varies by species (consult Agriculture Handbook 450 [U.S. Dep. Agric., Forest Serv. 1974] for species information). The methods we describe provide satisfactory results for a wide range of conifers and are suited for small quantities of seed.

Seeds, once collected and dried, should be frozen for storage. In this state they remain viable for many years and retain high levels of enzyme activity. The efficient scheduling of studies using stored seed tissues permits a constant output from the laboratory and support staff, without breaks caused by seasonal lack of research material.

The seeds should be germinated under uniform conditions and sampled at uniform growth stages. This procedure guarantees that a specific set of enzymes will be active in all seed of the test, and bypasses problems encountered with tissues sampled from field trees—differences arising from microsites, seasons and years, and difficulties of collection, transportation, storage, and processing.

It is advisable to analyze seed at the stage of germination when the emerging radicle of the embryo extends 2 to 5 mm beyond the seed coat. Enzymes are present from dry seed stage through the shedding of the seed coat (Conkle 1971), but female gametophytes and embryos in the early stages of germination are superior to dry seed for most species. Additionally, germination simplifies removal of the seed coat and separation of the embryo from the gametophyte.

Collection, Extraction, and Storage

Collect mature cones, while still closed, from the tree. Air dry or warm the cones in a circulating air oven or kiln (32° C for 24 to 48 hours). Shake or tumble the open cones to release the seeds.

Remove the seed wings by gently rubbing a small quantity of seed within a folded cloth. Separate the seed from the wing particles by

a. Shaking the seed on a wire mesh. Small wing pieces will sift through the mesh; larger pieces can be removed from the seeds by a quick downward motion of the mesh.

b. Placing the seed material in a variable-flow air column. Place the seeds and wing fragments on a wire mesh inside an air tube and increase the air flow until the wing pieces are blown into the collector. This procedure is also useful for separating filled from hollow seed.

Store dry seeds in labeled envelopes in a freezer (−15° C).

Stratification

Withdraw from cold storage the seed samples to be included in a study. Place seeds of each sample in plastic bags labeled with identifying codes. Pour enough Captan fungicide solution (2.5 g Captan 50-W/liter of water) into each bag to cover the seeds.[1] Soak seeds for 24 hours in a refrigerator (throughout this manual, refrigeration implies a temperature of 4° C). Drain the Captan solution and refrigerate the moist seed 90 days for white pines and 45 days for all other species.

Prepare petri dishes by adding a cellulose pad or sterile sand. Overlay the pad or sand with a filter paper labeled indelibly with identifying codes. Wet the pad and filter paper with Captan solution. The filter paper should be saturated but the dish should not have freestanding solution. Transfer the seeds into the petri dishes and place in a germinator. Throughout the subsequent germination period add distilled water as needed to maintain moisture on the seeds and paper.

Germination

Germinate seeds at 20° to 22° C with 12 hours of light. Most seed lots begin to germinate in 3 to 7 days. When the radicle of a seed extends beyond the seed coat 2 to 5 mm, place the germinant in a second petri dish (prepared with moist pad or sand and filter paper) and refrigerate. Refrigeration halts the growth of seeds while maintaining enzyme activity 6 to 8 weeks and longer, and allows you to accumulate seeds at the same stage of development.

Sample Preparation

Prepare a record sheet (*fig. 1*) listing in order the identifying codes and tissue types for samples to be analyzed. Recommendation: In addition to the seeds that are being tested, stratify, germinate, and include seeds of alternative species or particular families as standards for comparing the mobility of enzyme bands on different gels. Label grinding plates (*fig. 1*) with sample identities corresponding with the record sheet.[2]

[1]Trade names and commercial enterprises or products are mentioned solely for information. No endorsement by the U.S. Department of Agriculture is implied.

[2]Figures are found on pages 8 to 11 of this manual.

Each sample consists of a paper wick (12 × 3.5 mm, Whatman chromatography paper, no. 3MM) saturated with liquid derived from macerated tissue combined with extraction buffer solution. To obtain the liquid from the germinated seeds, proceed as follows:

a. For each seed, split and remove the seed coat and peel off the brown papery tissue surrounding the gametophyte. Split the gametophyte on one side and remove the embryo.

b. Place the gametophyte tissue and embryo in separate labeled wells of a frozen grinding block (*fig. 1*). The amount of tissue to process depends on seed size and enzyme activity. Use the entire gametophyte or embryo of species with small seeds but subsample the tissues from species with large seeds.

c. Process the samples loaded in the grinding block immediately if electrophoresis run will be made that day. Otherwise, store them frozen overnight so as to reduce the workload on the day of the run.

d. On the day of the run, remove the samples from the freezer. Add one or two drops of extraction buffer (*app. A*) to the tissue in each well. Use the minimum quantity necessary to just saturate paper wicks; too much extraction buffer dilutes the enzyme solution and diminishes the resolution of isozyme bands. Note that in *appendix A* there are suggestions for additives that improve resolution of specific enzymes, and of enzymes of seeds with large amounts of resins.

e. Grind thawed, cold tissues in the extraction buffer with a glass rod or blunt-tipped electric engraver. Macerate each sample thoroughly—the macerate in each well should be opaque and contain no sample chunks. After grinding each sample, clean the grinding tip with absorbent tissue paper. Once a sample is homogenized, enzymes begin to break down. Work rapidly once you begin the grinding step.

f. Insert paper wicks in the wells containing the macerated tissue. Use one wick per well for each gel system to be run; if, for example, each sample is to be used in four different gels, use four wicks per well. Keep the saturated wicks cold. You can also include on a gel one or more wicks with dye marker (0.1% solution bromophenyl blue, 0.1 mg/100 ml of water) to monitor freely migrating molecules during the electrophoresis run.

GEL PREPARATION

Four gel systems, with pH values from 6.2 to 8.8, are useful for resolving different enzymes. The specific chemical formulations for gel and tray buffers are given in *appendix B*. Instructions call for preparing two gels of each gel system. Two gels provide analyses for numerous tissue samples each day and make efficient use of space and materials by allowing staining of two slices in each stain

tray. You may wish to adjust the formulations to meet different experimental design goals.

Starch, when cooked with a buffer solution and cooled, forms a gel. The proportion of starch to buffer determines the consistency of the gel. Gels that are 12.5 percent starch appear to work well, but the proportions may need adjusting to produce gels with good characteristics. A major variable is the brand of starch. Other factors—the quality of distilled or deionized water, starch lot, laboratory and refrigerator temperatures, and the length of time that gels are vacuum degassed—contribute to gel quality. There is an art to producing consistently good quality gels; a visit to an electrophoresis laboratory is highly recommended to anyone planning to establish a new laboratory.

Molding the Starch

Form the gel in a mold consisting of plastic bars secured to single-strength glass with rubber bands (*fig. 2*). This mold forms a gel slab, 18.5 × 15.5 × 1.2 cm, capable of yielding up to 10 gel slices. Lines on the side bars serve as guides for cutting the gel where the wicks will be inserted—a location on the gel called the origin because enzyme migration starts from this point. Lines on side bars also mark locations for folding the plastic wrap cover, placing electrode sponges, and determining the 8-cm migration distance for gels that develop a visible front as electrophoresis proceeds.

Starch Preparation

Prepare and level two molds for each batch of starch. Weigh out 94 g of starch. If necessary, remove any small, hard-to-suspend aggregates by sifting or screening the dry starch. Measure out 750 ml of gel buffer solution from laboratory stock bottles (*app. B*). Of the 750 ml of solution, reserve 160 ml for suspending the starch, and heat the remaining 590 ml to boiling in a 1000-ml long-necked volumetric flask (Pyrex no. 5600). While the solution is heating, use a 400-ml beaker to combine 150 ml of the reserved buffer solution with the starch. Mix the starch and buffer thoroughly with a stirring rod to eliminate all lumps. Pour the starch suspension into a 2000-ml thick-walled vacuum flask (Pyrex no. 5340). Wash the remaining starch from the beaker into the vacuum flask with the remaining 10 ml of buffer. Swirl the mixture to keep the starch well suspended. Here and elsewhere in the text, the word *swirl* is used to describe the required motion for the flask containing the starch suspension. Hold your forearm stationary and use wrist action to produce a circular motion of the base of the vacuum flask. The starch suspension should circle the bottom of the flask; it should not break up and splash against the wall.

Put on insulated gloves to handle hot glassware and wear eye protection. Shortly after the 590 ml of solution comes to a rapid boil, grasp the vacuum flask in one hand and the volumetric flask in the other. Swirl the starch in the vacuum flask until the starch is again thoroughly suspended. Then carefully invert the volumetric flask containing the boiling buffer, sliding its neck deep into the vacuum flask (*fig. 3*). Point the neck of the vacuum flask away from you. *CAUTION: Failure to insert the flask with boiling buffer deep into the vacuum flask may result in a violent kickback of steam and boiling buffer.* Swirl the vacuum flask as the buffer solution empties into the starch suspension. Remove the volumetric flask, when empty, and continue swirling the starch and buffer for 10 to 15 seconds.

If gels are consistently weak and difficult to work with, you may need to heat briefly the suspended starch after the boiling buffer is added to further cook the mixture. Such further cooking may be required to produce gels sufficiently strong to withstand slicing and handling.

Stopper the flask and apply vacuum to degas the hot starch solution (*fig. 4*). A rapid effervescent boiling will occur within the starch solution as gases are removed, but will quickly subside. Slowly swirl the flask while degassing to maintain an even consistency of the starch. Continue degassing until large bubbles boil through the solution. About 4 seconds after the large bubbles appear, release the vacuum and immediately pour the starch into the molds. The starch solution will be clear and will pour freely. The process of degassing should last only 15 to 20 seconds; the starch will congeal inside the flask if degassing is prolonged.

Rapidly pour the starch into the molds, filling each mold with one continuous pour, as the starch cools quickly. Start pouring midway between the upper left corner and the center of the mold. When the starch reaches the edge of the mold and mounds slightly above the top of the plastic bars, continue to pour, moving to the right, then down, then left. Continue pouring until the mold fills and starch bulges about 1 mm above the top of the forms. Pour the second gel in the same manner. With practice, you can produce two gels with equal quantities of starch, and thus equal thickness, from one starch preparation.

The starch mixture should be free of opaque streaks and small opaque pellets. These opaque areas are usually due to incomplete mixing and cooking when the boiling buffer is added. If you see inclusions, discard the contents of the flask and start over.

After pouring, gently shake or lightly tap each mold to distribute the starch evenly. Burst any bubbles on the gel surface with a warm needle. Cool the gels at room temperature until they turn opaque (about 10 to 15 minutes), then cover them with plastic wrap. If gels are prepared the day before they are used, store them at room temperature.

Gel Loading

Refrigerate prepared gels for 1 hour before inserting wicks. Never freeze gels. Use a scalpel (no. 11 blades are useful) to cut and trim the gels. Cut between the gel and all

four sides of the gel mold. Remove any excess starch from the top of the bars. With the scalpel held vertically, cut along a straight-edge held in line with the origin marks on the right and left sides of the mold (*fig. 2*).

Remove the rubber bands holding the mold bars and slide the smaller cut portion of the gel toward the edge of the glass, using light pressure from both hands on the surface of the gel. The gel slice should be moved until the opening at the origin is about 1 cm. Place a wick spacing guide on the upper gel slice just at the origin (*fig. 5*). Repeat the process to trim, open, and place wick spacers on those gels required for the number of wicks prepared.

Use tweezers to remove the cold wicks from the wells of the grinding block, working with one well at a time. Lightly blot the wicks on a clean section of absorbent tissue. Place the wicks, one per gel system, on the fresh-cut gel surface of the larger gel portion. The bottom edge of each wick should touch the glass of the gel mold. The order of wicks along the gel should correspond to the sample order on the record sheet. Each gel accommodates 36 wicks.

When all the wicks are in place, push the smaller gel portion back against the wicks on the larger gel portion. Replace the gel mold bar and rubber bands. Press lightly on the gel near the wicks to remove air gaps. Cover the gel with plastic wrap that extends about 5 cm beyond the ends of the mold.

Gel and Tray Setup

Place the loaded gel on the electrode tray (see *fig. 6* for details of tray construction). Position the origin of the gel toward the cathodal connection of the electrode tray. Forming a sharp line across the gel, fold the plastic wrap back to the fold lines (*fig. 2*) marked on the gel bars. This procedure exposes an 18-mm strip of gel surface.

Place sponges in contact with gel surface, in each electrode tray. The sponges are nylon reinforced cellulose kitchen sponges. Because new sponges increase the time required for an electrophoresis run, condition new sponges by rinsing and using them in anodal trays with previously conditioned sponges in cathodal trays. Do not interchange the sponges between systems. Store sponges in plastic bags in a refrigerator.

Position sponges to cover 1 cm of plastic and all the exposed gel surface, and extend down into the electrode tray. Saturate sponges with electrode tray buffer and press sponges against the gel to make thorough contact. Fold the plastic wrap back over the sponge and attach the plastic wrap to the glass on both sides with binder clips (see *fig. 7* for views of the tray and gel setup).

Pour 250 ml of refrigerated electrode buffer into each electrode tray. Place the tray and gel unit in a refrigerator equipped to circulate cold air. The refrigerator should have the capacity to maintain the temperature at 4° C throughout the run.

ELECTROPHORESIS

Enzyme separation is achieved by applying direct current to the buffered gel. Ions from the buffer in the cathodal tray enter the gel. In the A and B gel systems (see *app. B*) the ions cause a clearing and a slight depression of the gel at the location of the fastest migrating ions. This area is the front. We allow the front to migrate 8 cm beyond the origin before turning off the electric current. Gels of the C and D systems do not develop fronts; the runs are timed so as to give consistent migration distances between gel runs on different days.

Electrical Requirements

Caution: Power sources for electrophoresis produce direct current at voltages high enough to cause severe injury. The possibility of electric shock must be eliminated by constant awareness of the electrical hazard, clear communication between workers, and safe work procedures to minimize shock hazards. All electrical equipment should be examined on a regular basis to verify that wires and connections are in safe condition. Special care should be taken to protect visitors from electrical hazards. New employees should be thoroughly trained in safe procedures.

Electrical current can be supplied to a gel with a Heathkit-Shumberger high-voltage power source (models IP-17 and SP-2717) or similar power sources. The power sources produce 400 volts and 100 milliamps at maximum settings. These models, once set, hold constant voltage. Adjust the amperage manually to the desired output until the voltage rises to 320 volts. Power adjustment is not required after the voltage reaches 320.

Each gel is attached to a power source; the current on each gel is monitored and adjusted separately. Monitoring each gel allows detection of problems such as poor sponge contact or separation of the gels at the origin; these conditions may lead to irregularities in enzyme migration.

To begin the run, connect wires from the power sources to the electrode terminals of the buffer trays in the refrigerator: the cathode (color-coded black) connects to the electrode on the origin side of the gel, the anode (color-coded red) connects to the opposite electrode. Apply current at amperages specified for the individual gel buffer systems as follows:

A buffer system 75 mA
B buffer system 70 mA
C buffer system 60 mA
D buffer system 60 mA

Periodically check the amperage and voltage gauges on the power sources and adjust the amperages to maintain the recommended values. Amperages tend to decrease during

the run. Adjustments that return amperages to the recommended levels cause an increase in voltage.

Gain experience with the normal range of voltages for different buffer systems, gel thicknesses, and laboratory conditions. Abnormal voltages may signal problems such as poor contact between the electrode sponges and the gel or the use of the wrong solution as tray buffer.

Dewicking and Running Gels

After the current has been on for 15 minutes, turn off the power, disconnect a gel unit, remove it from the refrigerator, remove the clips holding the plastic wrap over the cathodal sponge, and fold back the plastic wrap and the sponge to expose the origin. Use slight pressure with the fingers of one hand to separate the gel slices at the origin. Lift the wicks out of the gel with tweezers. When all wicks are removed from a gel, rejoin the gel slices and verify that there is good contact between the surfaces. Eliminate bubbles between the gel and the glass plate. Reassemble the gel unit with the plastic wrap and the cathodal sponge in their original positions. Return the unit to the refrigerator and reconnect the electrical wires.

Place refrigerated waterbags on the dewicked gels. Waterbags are 20.3 × 30.5 cm heat sealable pouches of plastic 4.5 mils thick (Kapak/Scotchpak, stock no. 504). Replace waterbags on the gels with a second set of cold bags if there is any tendency for the gels to warm.

Reapply the electrical current at the recommended amperages for each gel system. Adjust the amperages throughout the run until voltages reach 320. Thereafter, further adjustments are unnecessary (amperages will continue to decrease). A sudden drop in amperage signals poor current flow and may be caused by poor contact between the electrode sponge and the gel, or separation of the cut gel surfaces at the origin. Correct poor sponge contact by resetting the sponge. Gel separation at the origin may be due to excessive tension on the covering plastic wrap or unusual heating and cooling conditions within the gel. Correct gel separations by pressing the surfaces back together and resetting the plastic wrap and sponges.

When the fronts on gels of the A and B buffer systems reach 8 cm beyond the origin, disconnect the current. The C and D system gels do not develop visible fronts and we have standardized the length of time for the C and D gel runs at 4.5 hours.

SLICING AND STAINING

Many stain solutions are perishable and should be prepared as close as possible to the time the gels are ready. Some chemicals used in stains are hazardous; several are carcinogenic. Handle all chemicals with extreme care and an awareness of OSHA guidelines for laboratory safety (U.S. Dep. Labor 1976). Follow the label precautions on all chemicals.

Slicing the Gel

Remove a gel setup from the refrigerator, unclip the plastic wrap, and fold the electrode sponges into the electrode trays. Remove the plastic wrap and the gel mold forms. Enzymes will be in the anodal section of the gel between the origin and the 8-cm front. The gel beyond the anodal side of the 8-cm front will not contain enzymes and can be trimmed away and discarded. The cathodal section should be sliced and stained to locate all bands. Cut one notch in the upper left hand corner to identify gel 1 and two notches to identify gel 2.

Attach plastic strips (200 × 25 × 1.0 mm) to the glass on either side of the gel slab using large spring-loaded clips (*fig. 8*). Wrap monofilament nylon sewing thread around the index fingers on both hands and use thumbnails to stretch the line taut. Adjust the length of the thread so that thumbnails ride on top of the plastic strips as the cut is made. Start with the sewing thread on the far side of the gel and pull the thread toward you, cutting one slice through the gel. Add a plastic strip at both sides of the gel and cut the next slice. Use a new section of sewing thread for each cut. Continue adding spacer strips to slice the entire slab. Do not separate slices at this time; return the sliced gel slab to the refrigerator. Trim and slice the second gel of the same buffer system.

Mixing the Stains

Consult *appendix C* for stain recipes. Stain buffer solutions and some stain components can be prepared in quantity for use over a period of time. The stain solutions (with some exceptions) are made up in 75-ml volumes for one-time use. Stain trays are glass baking dishes 20.5 cm square (2 qt Pyrex) that accommodate two gel slices, one from each of the two gels of a buffer system. Prewarm the stain solutions and staining trays to 37° C to maximize stain activity when the gel slices are placed into the solutions.

For each enzyme to be identified, write the abbreviation for it and the date of the run on a 5-cm strip of masking tape, and attach it to a 250-ml Erlenmeyer flask. Measure out and add the stain buffer to the flask, then add and mix the stain components in the same order they are given in the recipe (*app. C*). Each batch of stain is enough for a pair of gels.

Set out warm trays and place a flask of stain in each tray. Transfer the masking tape from the flask onto a stain tray and pour the stain solution into the tray. Prepare all the stain trays for one gel system.

Remove the sliced gels from the refrigerator; peel off and discard the top slice of the cut gel slab. Use fingertips to peel off the second slice and place it in the first stain tray (*fig. 7*). Proceed to put one slice from the first gel into each stain solution. Repeat with slices from the second gel. Agitate each tray to cover the gel slices with the stain solution. Cover each tray with plastic wrap and follow the incubation recommendations for specific stains. Warming ovens will maintain stains at 37° C and shield light-sensitive stains. If ovens are not available, shield light-sensitive stains with a cover of aluminum foil. Examine the gels frequently to follow the development of bands. When the bands on both gels in a stain tray are well resolved, draw off the stain solution and add just enough tap water to cover the gels. The resolution of bands is the clearest at this stage and data should be collected while the gels are in water.

Storing the Gels

To store the gels for 6 to 12 months, replace the water in a tray with a fixing solution consisting of a 5:5:1 mixture of methanol:water:glacial acetic acid. If the gels are not examined immediately after staining, skip the tap water step and replace the stain solution with fixing solution. The fixing solution toughens and shrinks the gels and the gels become opaque. Dark bands are distinct after fixing but faint bands may have to be viewed over a light table. *Caution: Keep stain trays with fixing solution covered with plastic wrap. Do not inhale the fumes from the fixing solution.* Methanol is a poison that is only slowly degraded by the body. Repeated exposure, sometimes over a warm light table, may lead to early signs of poisoning: headache, loss of energy, and fatigue. If possible, work with the fixing solution only in a vented hood.

Gels should remain in the fixing solution for a minimum of 2 hours. We place the gels in fixing solution overnight, then blot and wrap the fixed gels in plastic on the following day. Wrapped gels will remain readable for long periods of time if they are protected from desiccation by additional wraps of plastic and stored in a refrigerator.

SAFETY AND PROCEDURAL CAUTIONS

Safety

Gel Pouring

When combining the starch slurry and boiling buffer in the vacuum flask, wear gloves and safety glasses, and point the vacuum flask away from face and body to avoid the possible kickback of steam and hot starch.

Electrophoresis

Never touch electrodes or the tray solutions while current is supplied to the gels; a severe electric shock is possible.

Staining the Gels

Many of the compounds used for staining are hazardous to health. Wear protective clothing, particularly gloves and a dust mask, when handling. Avoid contaminating other items with these compounds. Follow prescribed safety measures. Mix the stains in a vented hood. Use similar care in examining stained gels treated with fixing solution.

Procedural Cautions

Seed Dissection

Work rapidly and keep the sample tissue as cool as possible to maintain enzymatic activity.

Make sure that accurate seed identity is maintained on the record sheet, in the seed tray, and in the gel.

Gel Pouring

Degas the hot starch solution no more than 20 seconds to avoid congealing of the starch while pouring.

Do not allow the gels to freeze during cooling.

Loading the Gels

Blot each wick to remove excess moisture.

Make sure the bottom edge of the wick touches the glass plate.

Eliminate bubbles between the gel and the glass at the origin, after loading and after dewicking.

Electrophoresis

Keep the gels as cool as possible, without freezing; refrigerate and place chilled water bags on the gels after the dewicking step.

Make sure that the wires to the electrode trays are not reversed; determine that the polarity is correct.

Hold amperage constant at the prescribed level until a gel system reaches 320 volts, then hold the system at 320 volts and allow the amperage to decrease for the rest of the run.

Slicing the Gel

Keep the monofilament thread taut between the thumbs.

Be sure to put the same number of plastic spacers on each side of the gel.

Notch the gels for identification.

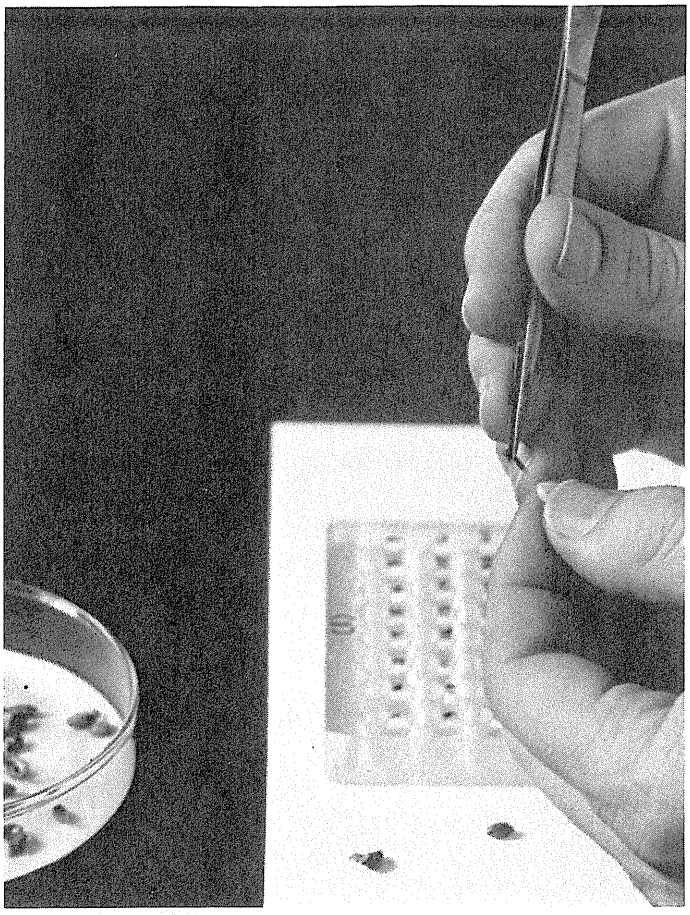
Staining the Gels

Warm the staining solutions to 37° C.

Because compounds are light-sensitive and perishable, cover trays with aluminum foil and handle chemicals as directed to preserve their activity.

TROUBLESHOOTING

Problem	Probable cause	Solution
Lumps or white specks appear in hot gel solution	Buffer solution may not have boiled rapidly or the starch may not have been well mixed with cold buffer to form the slurry	Discard lumpy starch and start over. Allow buffer to come to a rolling boil and thoroughly mix starch slurry
Starch is too thick or lumpy when poured from vacuum flask	Starch may have been degassed too long and is too cool	Start over; degas for a shorter time and quickly pour starch into mold
Voltage is abnormally high on a gel	Separation at origin or poor sponge contact with gel	After disconnecting power, open plastic wrap to inspect origin. Press sponges to improve gel contact
Front on gel is irregular:	Waterbag unevenly placed	
Straight but higher on one side than on the other	Worn electrode on anodal side. (Cathodal electrode is long-lasting)	Replace anodal electrode
	Gel is thicker on one side than on the other	Level mold before pouring gel for next run
	Gel separates in region of origin	Push gel surfaces together at origin and place a glass or plastic rod between gel and bar on cathodal end
Wavy	Uneven cooling of gel, separation at origin or poor sponge contact with gel	Check air flow in refrigerator or change waterbags more often. Push gel surfaces together at origin and place a glass or plastic rod between gel and bar on cathodal end
Gel front migrates slower in one gel than other gel of same system	New sponge may be on cathodal side of electrode tray	Place old sponge in well of cathode tray and new sponge in well of anode tray
	Electrode may be corroded	Replace with new electrode
	Unequal gel thickness	Decrease amperage on gel with faster migration so that electrophoresis proceeds to same point in same time
Stain does not work	Possible error in mixing stains or buffers	Check recipe. Some missed items can be added late and stain may still work
	Possible deterioration of buffers	Make new buffers. Prepare stocks of buffers in quantities that will last for about 1 month
	Possible deterioration of chemicals	Purchase fresh supplies. Date each bottle and use older supplies first. Follow storage instructions on bottles



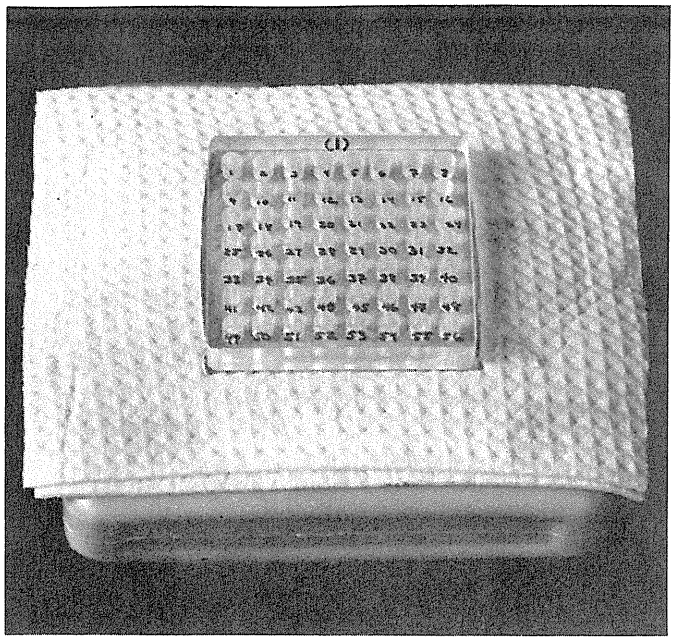
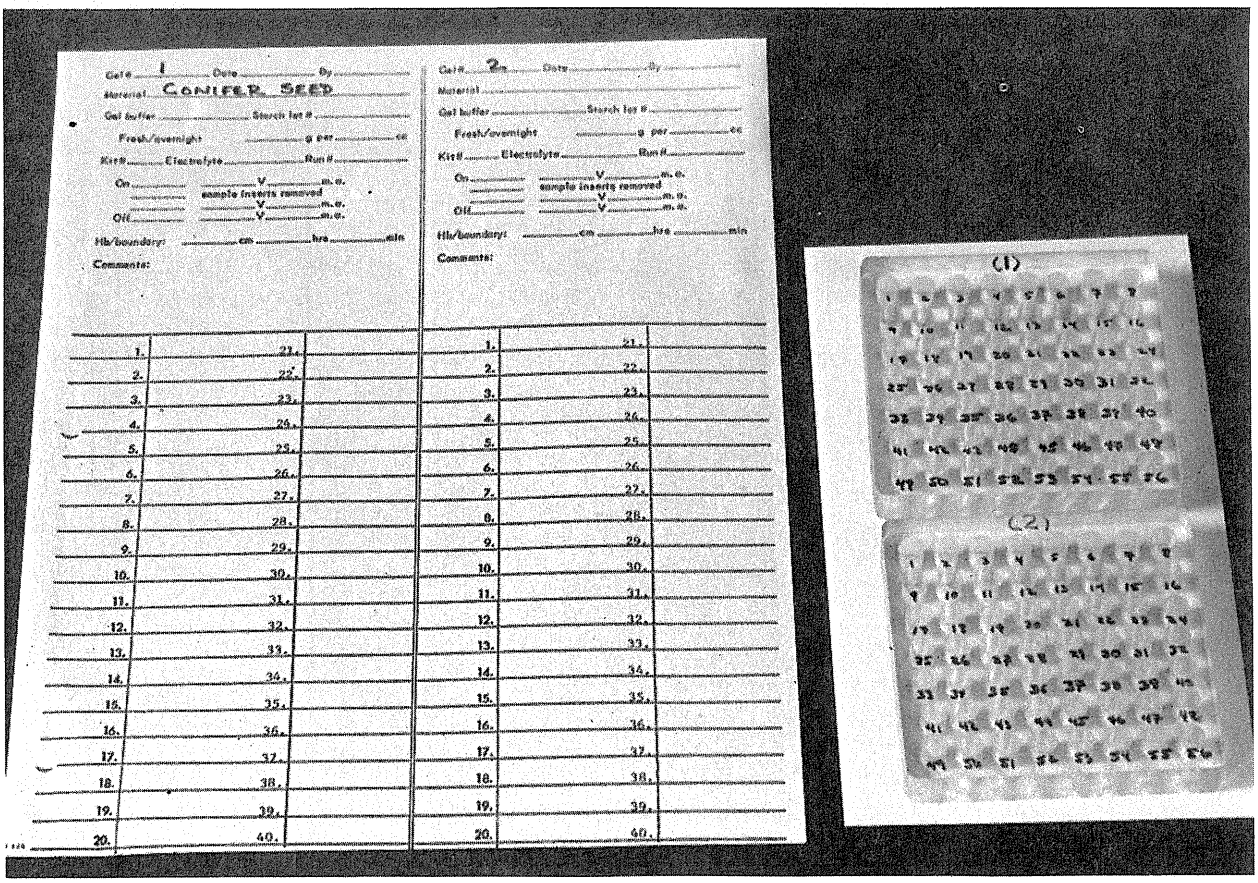
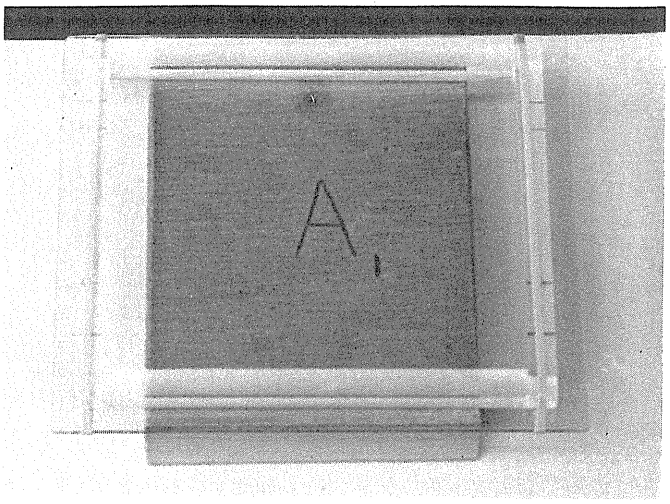


Figure 1—Sample preparation materials. *Left:* germinated seed in the petri dish used for germination, and a dissected seed with gametophyte and embryo. *Above:* grinding block, 8.5×8.5×1.3 cm clear plastic with 0.7-cm holes (¼ inch) drilled 1.0 cm deep (flat bottom holes were drilled with a bit squared off on a grinding wheel), placed on a frozen pad of Blue Ice for cooling (grinding block and pad were frozen together before samples were loaded into the holes). *Below:* data sheet with the identity of the samples coded to the numbered holes in the grinding blocks.





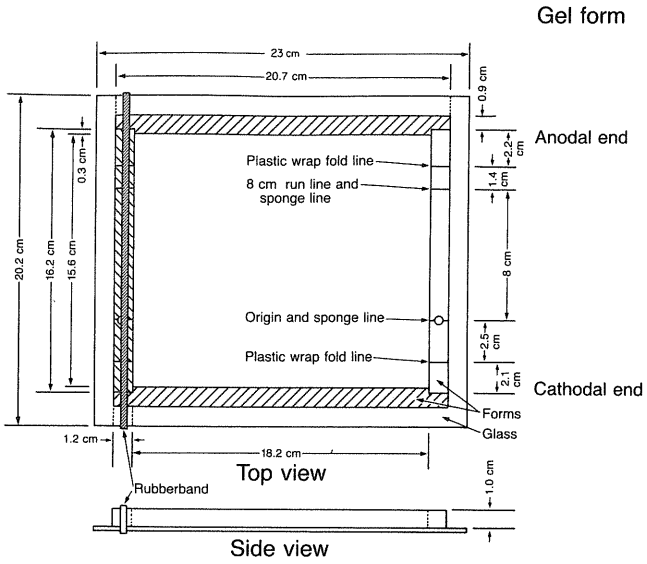


Figure 2—Gel mold consisting of plastic bars secured with rubber bands to single strength window glass.

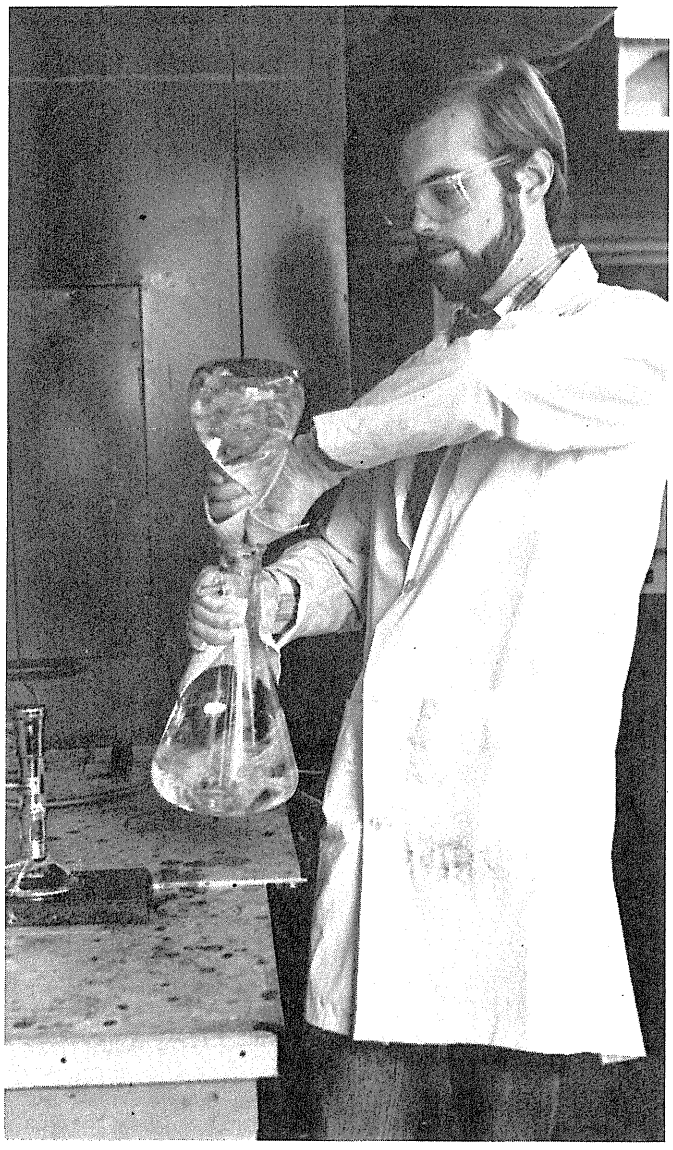


Figure 3—Pouring boiling buffer into starch suspension.

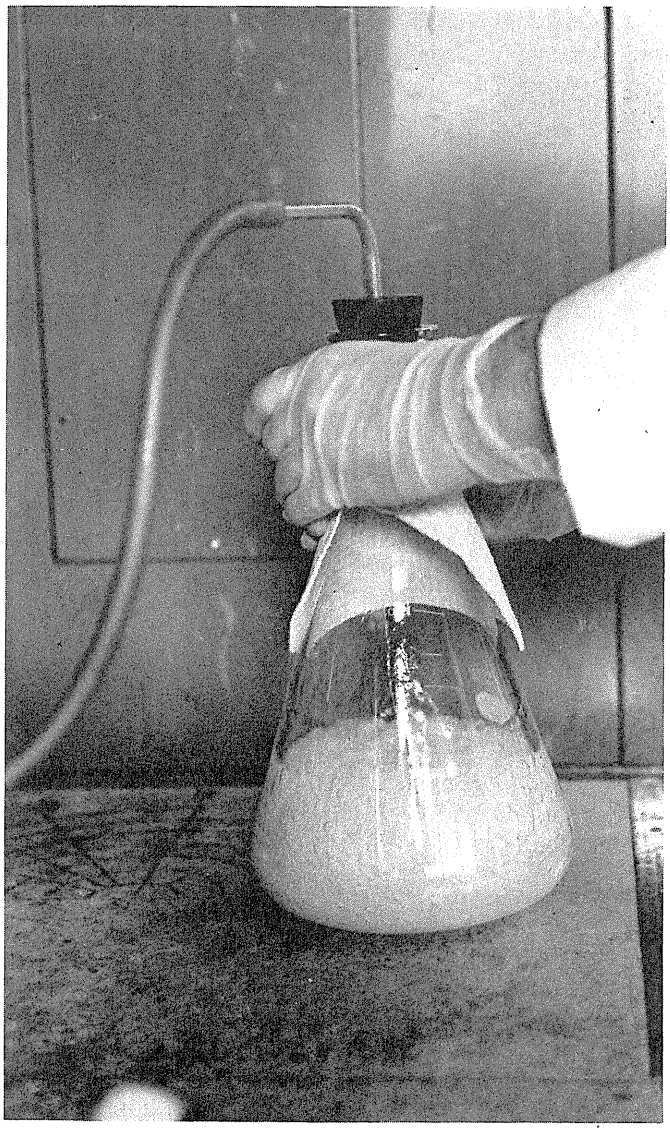
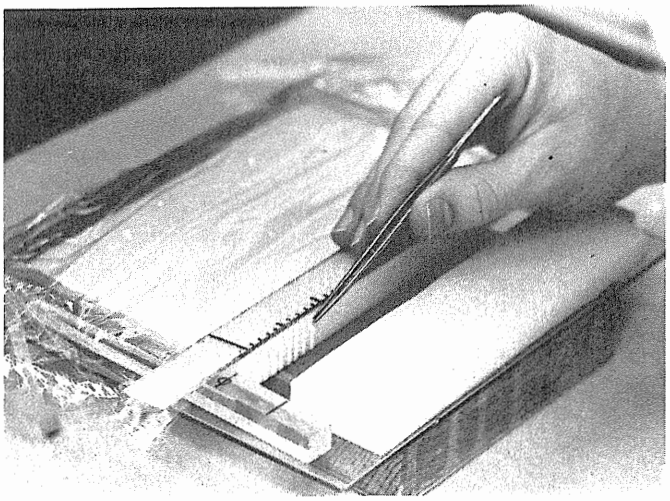
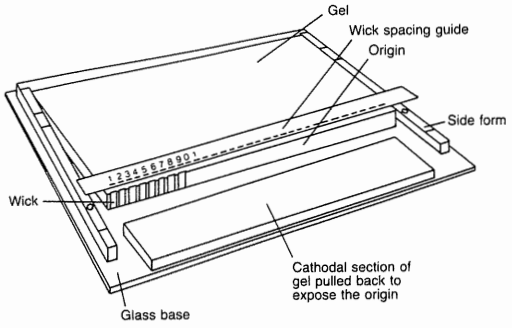


Figure 4—Degassing hot starch using vacuum line attached to a cork in the top of the vacuum flask while blocking side port of the flask with a finger.

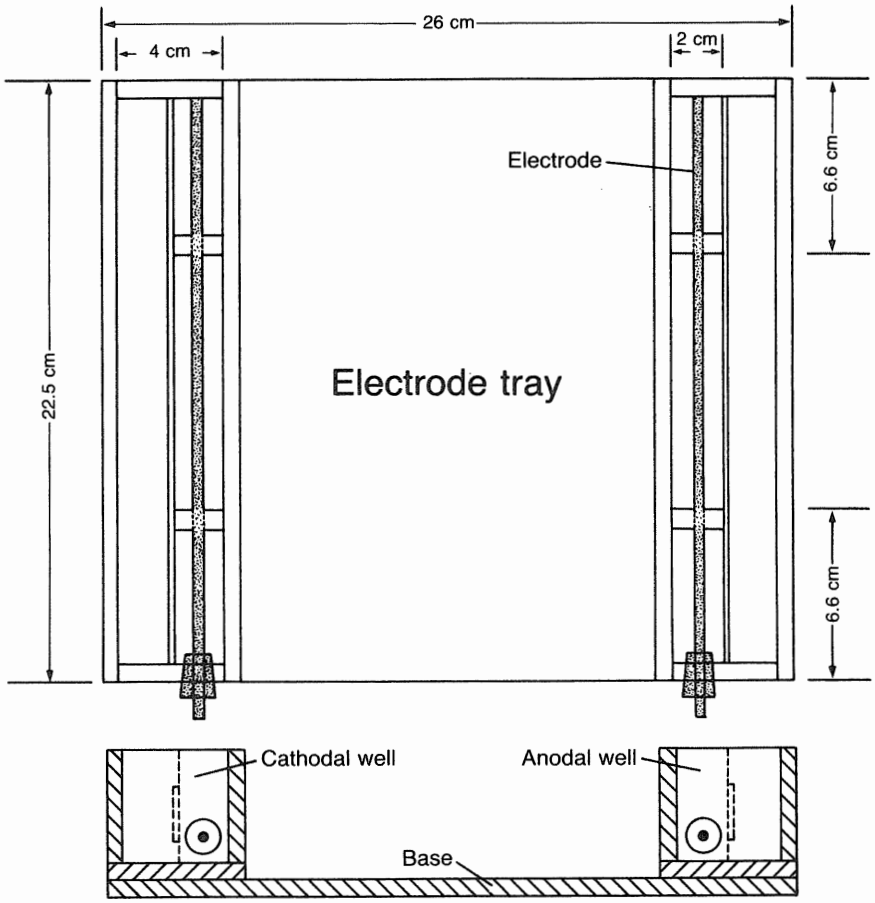




Wick spacing guide

1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3
1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6

Figure 5—Cut gel surface open at the origin. Wicks containing the absorbed liquid fraction of samples are placed on the vertical surface in order corresponding to their listing on the data sheet.



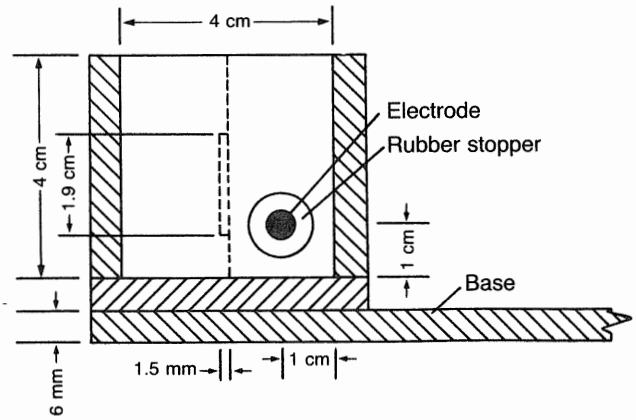


Figure 6—Exact dimensions of electrode tray. The $^{3}/_{16}$-inch pure carbon electrodes are secured into the tray with no. 00 rubber stoppers. The tray is constructed of $^{1}/_{4}$-inch lucite.

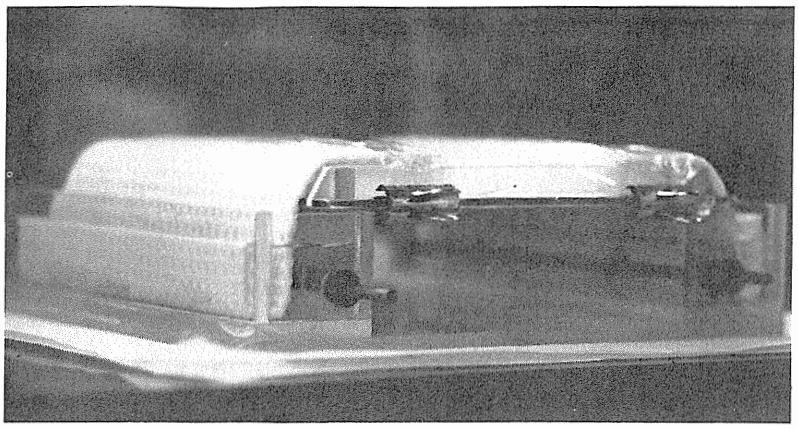
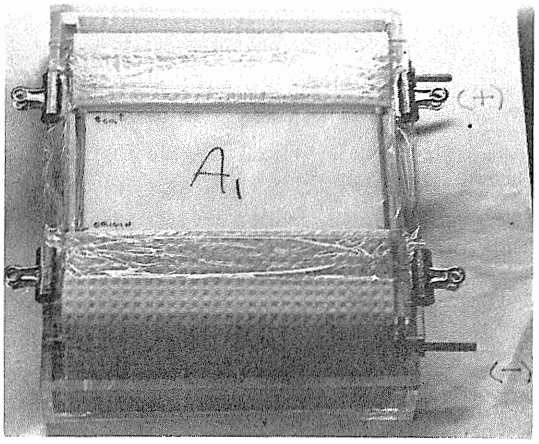
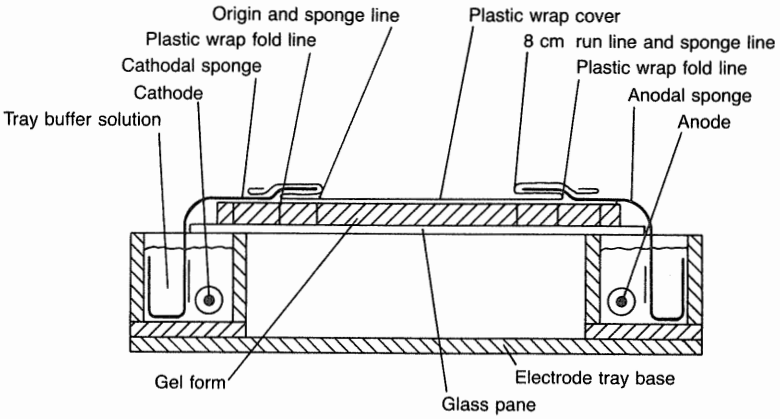
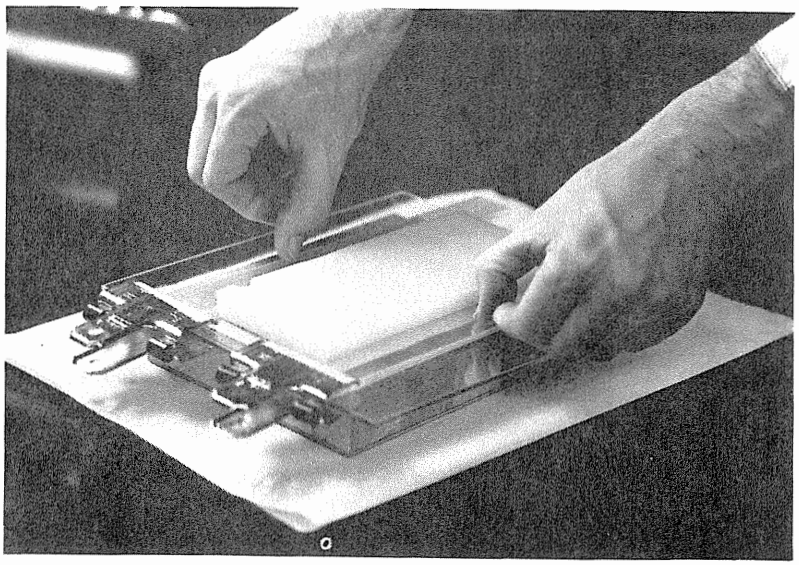


Figure 7—Side and top views of an electrode tray with gel and wicks, sponges, and plastic wrap in place.







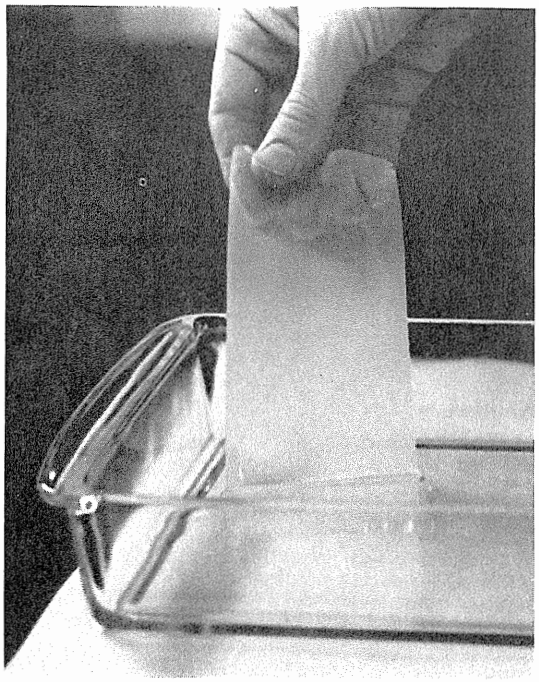


Figure 8—Gel is prepared for slicing by using plastic spacers attached to the glass on either side of the gel slab with spring loaded clips. *Above:* taut monofilament nylon sewing thread cuts horizontal slices through the gel slab. *Right:* gel slice is placed in an appropriate stain solution.

APPENDIX

A. Extraction Buffer

Use pH 7.5, 0.2 M phosphate (*app. C*, Stain buffer formulations) as the extraction buffer. Certain compounds added to the extraction buffer can improve poorly resolved sets of enzyme bands, and in the initial study of a new species, various such compounds should be tested. Our general rule is to keep the buffer as simple as possible by including only additives that improve resolution.

The addition of a small quantity (20 mg/ 100 ml buffer) of substrate to the extraction buffer may improve the resolution of specific enzymes. Several substrates can be added to the same extraction buffer (L-glutamic acid for GDH, D-glucose-6-phosphate for G6PD, α-D-glucose 1-phosphate for PGM, and others). From our experience, adding substrates to the extraction buffer is better than adding them to the gel or the cathodal electrode buffer as suggested in some literature. The substrate in the extraction buffer probably protects the active site of the enzyme while it is in the mixture of cell components. After a short period of electrophoresis, an enzyme is likely to be isolated from compounds that degrade it.

The resolution of some enzymes is greatly improved by the addition of bovine albumin (40 mg/ 100 ml) to the extraction buffer. The albumin binds phenolics and free fatty acids (Anderson 1968).

Seed resins and phenols can decrease resolution. The addition of a small quantity of 2-mercaptoethanol (1 drop/ 100 ml extraction buffer) helps to bind and reduce the effect of resins and phenols. Mercaptoethanol improves resolution in true firs, incense-cedar, and redwood, but we do not add mercaptoethanol to the extraction buffer for pines, cypresses, and Douglas-fir.

Complex extraction buffers are available for difficult tissues (Kelley and Adams 1977, Mitton and others 1979, Soltis and others 1980); but most conifer seed analyses do not require these complex buffers.

B. Gel and Tray Buffer Formulations

System	Gel Buffer	Tray Buffer
A[1]	**Tris citrate (pH 8.3)**	**Lithium borate (pH 8.3)**
Formulation	Trizma base ..... 62.0 g Citric acid ..... 14.6 g Distilled water ..... 10.0 liters	Lithium hydroxide ..... 12.0 g Boric acid ..... 118.9 g Distilled water ..... 10.0 liters
	Dissolve the chemicals and check the pH. Store at room temperature	Dissolve the chemicals and check the pH. Store at room temperature
Procedure	To use, add 75 ml of the lithium borate tray buffer to 675 ml of the tris citrate gel buffer to make the required 750 ml	Use as is
B[2]	**Tris citrate (pH 8.8)**	**Sodium borate (pH 8.0)**
Formulation	Trizma base ..... 121.1 g Distilled water ..... 10.0 liters	Sodium hydroxide ..... 20.0 g Boric acid ..... 185.5 g Distilled water ..... 10.0 liters
	Dissolve the trizma base and titrate to pH 8.8 with 0.2 M citric acid solution. Store at room temperature	Dissolve the chemicals and titrate to pH 8.0 with 4N NaOH. Store at room temperature
Procedure	Use as is	Use as is
C[3]	**Tris citrate (pH 6.2)**	**Same as gel buffer**
Formulation	Trizma base ..... 162.0 g Citric acid ..... 108.9 g Distilled water ..... 3.0 liters	Same as gel buffer
	Dissolve chemicals and titrate to pH 6.2 with 4N NaOH. Refrigerate	
Procedure	To use, mix 16 ml of the buffer with 734 ml of distilled water	Mix 250 ml of the buffer with 750 ml distilled water
D[4]	**Morpholine citrate (pH 6.1)**	**Same as gel buffer**
Formulation	Citric acid ..... 15.4 g Distilled water ..... 2.0 liters	Same as gel buffer
	Dissolve citric acid and titrate to pH 6.1 with N-(3-aminopropyl)morpholine (20 ml). Refrigerate	
Procedure	To use, mix 37.5 ml of buffer with 712.5 ml of distilled water	Use full strength

[1]Scandalious 1969
[2]Fowler and Morris 1977
[3]Nichols and Ruddle 1973
[4]Clayton and Tretiak 1972, Yeh and O'Malley 1980

C. Stains

Enzyme stains used on gel slices from specific gel systems are listed in *table 1*. Stock solutions for chemicals required in several stain recipes are listed in *table 2*. Stain buffer solutions are listed in *table 3*. Recipes for preparing stains are described in *table 4*. The stains listed in *table 4* are used in the analysis of conifer seeds. Additional stains can be found elsewhere in the literature (Harris and Hopkinson 1976, O'Malley and others 1980).

Prepare stock solutions for stains in quantities that will be used up in 4 weeks. Several of the components of these solutions are light-sensitive and perishable. Store them refrigerated (do not freeze) in dark containers; foil-wrap light-colored containers. Add these components to the stain solutions just before adding the gel slices.

Table 2—*Stock solutions for stain components*

Abbreviation	Name	Standard concentration
G6PDH	Glucose-6-phosphate dehydrogenase[1]	5 units/ml buffer
NAD	β-Nicotinamide adenine dinucleotide	10 mg/ml water
NADP	β-NAD phosphate	10 mg/ml water
NBT	Nitro blue tetrazolium	10 mg/ml water
PMS	Phenazine methosulfate	5 mg/ml water

[1]G6PDH is subject to sulfate ion inhibition. This enzyme is supplied in a concentrated solution, which should be diluted using 1 percent bovine albumin in 0.05 M phosphate buffer (pH 7.5). The albumin binds sulfate ions to enhance the activity of the enzyme.

Prepare stain buffers in quantities sufficient to last up to 2 months by anticipating laboratory schedules. These buffers can be stored at room temperature.

Table 1—*Stains by gel buffer system*

Gel system	Stain		EC reference
	Abbreviation	Name	
A	ADH	Alcohol dehydrogenase	1.1.1.1
	AAP	Alanine aminopeptidase	3.4.11.1
	EST	Alpha esterase	3.1.1.1
	EST	Beta esterase	3.1.1.1
	FLEST	Fluorescent esterase	3.1.1.1
	LAP	Leucine aminopeptidase	3.4.11.1
	MNR	Menadione reductase	
	PEP	Peptidase	3.4.13.1
	PER	Peroxidase	1.11.1.7
	PGM	Phosphoglucomutase	2.7.5.1
	PGI	Phosphoglucose isomerase[1]	5.3.1.9
B	ACP	Acid phosphatase	3.1.3.2
	CAT	Catalase	1.11.1.6
	G6PD	Glucose-6-phosphate dehydrogenase	1.1.1.49
	GDH	Glutamate dehydrogenase	1.4.1.3
	GOT	Glutamate-oxaloacetate transaminase[2]	2.6.1.1
	SOD	Superoxide dismutase[3]	1.15.1.1
C, D[4]	ACON	Aconitase	4.2.1.3
	ALD	Aldolase	4.1.2.13
	IDH[5]	Isocitric dehydrogenase	1.1.1.42
	MDH	Malic dehyrogenase	1.1.1.37
	MNR	Menadione reductase	
	PGD	Phosphogluconate dehydrogenase[6]	1.1.1.44
	SKDH	Shikimate dehydrogenase	

[1]Also called glucose phosphate isomerase (GPI) in the literature.

[2]Also called aspartate aminotransferase (AAT) in the literature.

[3]Also called tetrazolium oxidase (TO) in the literature.

[4]All of the stains listed for the C system resolve well with the D buffer system, but both systems are necessary to detect variants within different loci.

[5]Also abbreviated ICD in the literature.

[6]Also called 6-phosphogluconic dehydrogenase (6PGD) in the literature.

Table 3—*Stain buffer formulations*

Buffer	pH	Formulation
ACP acetate buffer	4.0	Sodium acetate, trihydrate ..... 2.43 g Acetic acid, glacial ..... 4.7 ml 1.0 M magnesium chloride ..... 5.0 ml Distilled water..... 1.0 liter
Aminopeptidase buffer	4.5	Trizma base ..... 12.1 g Maleic anhydride ..... 9.8 g Sodium hydroxide ..... 1.6 g Distilled water..... 1.0 liter
Catalase buffer	6.5	Sodium phosphate, monobasic ..... 18.5 g Sodium phosphate, dibasic .... 17.9 g Distilled water..... 1.0 liter
Esterase buffer	6.4	Sodium phosphate, monobasic ..... 13.9 g Sodium phosphate, dibasic ..... 5.3 g Distilled water..... 1.0 liter
Peroxidase buffer	5.6	Arsenic acid, sodium salt..... 5.74 g Acetic acid, glacial ..... 1.2 ml Distilled water..... 1.0 liter
0.2 M phosphate buffer	7.5	Sodium phosphate, monobasic ..... 3.84 g Sodium phosphate, dibasic .... 23.86 g Distilled water..... 1.0 liter
1.0 M tris hydrochloride[1]	8.0	Trizma base ..... 74.0 g Trizma hydrochloride..... 61.4 g Distilled water..... 1.0 liter
1.0 M tris hydrochloride[1]	7.0	Trizma base ..... 16.0 g Trizma hydrochloride..... 137.4 g Distilled water..... 1.0 liter

[1]Dilute these solutions to the concentrations specified in the stain charts.

Table 4—*Stain recipes*

Enzyme	Gel buffer	Stain buffer	Stain components	Procedure
ACON Aconitase[1]	C,D	75 ml 0.2 M tris HCl pH 8.0	*Cis*-aconitic acid ..... 150 mg NADP ..... 1 ml NBT ..... 1 ml 1% $MgCl_2$ solution ..... 1 ml PMS ..... 0.5 ml Isocitrate dehydrogenase ..... 20 units (0.3 ml)	Add stain components to warm (37° C) stain buffer; incubate gels at 37° C in the dark
ACP Acid phosphatase[2]	B	80 ml ACP acetate buffer	α-naphthyl acid phosphate ..... 100 mg Fast Garnet GBC Salt[2] ..... 100 mg	Allow stain components to mix well in stain buffer. Develop at room temperature. Include cathodal slice of gel in the stain.
ADH Alcohol dehydrogenase[2]	A	75 ml 0.05 M tris HCl pH 8.0	NAD ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml 95% ethyl alcohol ..... 1 ml	Add components to warm buffer and incubate gels in the dark
AAP Alanine aminopeptidase[4]	A	75 ml Aminopeptidase buffer	L-alanine β-naphthylamide[3] ..... 30 mg dissolved in dimethylsulfoxide ..... 2 ml Fast Black K Salt ..... 20 mg	Add components to warm buffer and incubate gels in the dark
ALD Aldolase[1]	C,D	75 ml 0.05 M tris HCl pH 8.0	D-fructose-1,6-diphosphate ..... 250 mg Arsenic acid, sodium salt ..... 75 mg NAD ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml Glyceraldehyde-3-phosphate dehydrogenase ..... 300 units (0.25 ml)	Add components to warm buffer and incubate gels
CAT Catalase[2]	B	100 ml Catalase buffer	2% potassium iodide solution ..... 100 ml 0.03% H_2O_2 solution, (3% H_2O_2, 1 ml/100 ml distilled water) ..... 100 ml	Refrigerate gels in catalase buffer for 30 min. Drain off buffer and soak in 2% KI for 2 min. Drain off KI and wash twice with tap water. Add H_2O_2 solution and score when resolved
EST Alpha esterase[2]	A	75 ml Esterase buffer	Fast Blue RR Salt ..... 80 mg 1% α-naphthyl acetate solution (dissolve 1 g in 50 ml acetone, add 50 ml distilled water, store refrigerated) ..... 1 ml	Add components to warm buffer. Add the naphthyl acetate solution late in mixing. Incubate the gels. Include the cathodal slice of gel
EST Beta esterase	A	75 ml Esterase buffer	Fast Garnet GBC Salt[3] ..... 100 mg 1% 2-naphthyl acetate solution (dissolve 1 g in 50 ml acetone, add 50 ml distilled water, store refrigerated) ..... 1 ml	Add components to warm buffer; add naphthyl acetate to solution just before adding gels. Incubate gels
FLEST Fluorescent esterase[5]	A	10 ml Peroxidase buffer	4-methylumbelliferyl acetate solution (1 mg dissolved in 3 ml acetone) ..... 3 ml	Add solution to buffer and paint gels. Score bands on gels within 5 minutes under longwave UV light
GDH/SOD Glutamate dehydrogenase, superoxide dismutase[6]	B	75 ml 0.10 M tris HCl pH 8.0	L-glutamic acid ..... 2 g NAD ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml	Add components to warm buffer. Incubate in the dark. (SOD bands are white against the blue background.)
GOT Glutamate-oxaloacetate transaminase[7]	B	25 ml 0.2 M phosphate buffer pH 7.5	0.5% pyridoxal 5-phosphate solution (0.5 g/100 ml of distilled water) ..... 0.8 ml 3.0% bovine albumin solution (3 g/100 ml of distilled water) ..... 1.6 ml 0.2 M L-aspartic acid (adjusted to pH 7.5 with 2N KOH) ..... 6.8 ml	Combine first four components with buffer. When ready for staining add the Fast Blue BB solution to component solution. Add gels and develop at room temperature. Include a cathodal slice of gel

Table 4—*Stain recipes* (continued)

Enzyme	Gel buffer	Stain buffer	Stain components	Procedure
			α-ketoglutarate solution ..... 2.0 ml Fast Blue BB Salt ..... 120 mg dissolved in distilled water ..... 8 ml	
G6PD Glucose-6-phosphate dehydrogenase[8]	B	75 ml 0.05 M tris HCl pH 7.0	D-glucose-6-phosphate ..... 20 mg 3.0% bovine albumin solution ..... 1 ml NADP ..... 1 ml NBT ..... 1 ml 1% $MgCl_2$ ..... 1 ml PMS ..... 0.5 ml	Add components to the warm buffer and incubate gels in the dark
IDH Isocitrate dehydrogenase[9]	C,D	75 ml 0.05 M tris HCl pH 8.0	DL isocitric acid ..... 60 mg NADP ..... 1 ml 0.25 M $MnCl_2$ ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml	Add components to warm buffer and incubate gels in the dark
LAP Leucine aminopeptidase[2]	A,D	75 ml Aminopeptidase buffer	0.4% L-leucine β-napthylamide solution (400 mg/100 ml distilled water) ..... 5 ml Black K Salt ..... 20 mg	Add components to warm buffer and incubate gels
MDH Malic dehydrogenase[9]	C,D	75 ml 0.05 M tris HCl pH 8.0	Malic acid solution ..... 5 ml (134.1 g DL-malic acid, 80 g NaOH, 1.0 liter H_2O, adjust to pH 7.0 with about 18 ml of 4 N NaOH) NAD ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml	Add components to warm buffer and incubate gels in the dark
MNR Menadione reductase	A,C,D	75 ml 0.05 M tris HCl pH 7.0	NADH ..... 25 mg Menadione ..... 20 mg NBT ..... 1 ml	Add components to warm buffer and incubate gels in the dark
PEP Peptidase[9]	A	75 ml 0.2 M tris HCl pH 8.0	Glycyl-L-leucine ..... 10 mg L-leucyl-L-tyrosine ..... 10 mg L-valyl-L-leucine ..... 10 mg Peroxidase, crude ..... 10 mg Snake venom ..... 10 mg 50 mg of 3-amino-9-ethyl carbazole in 5 ml of N,N-dimethylformamide ..... 5 ml	Combine first five components with buffer and mix well. When ready to stain, add amino-ethyl-carbazole solution and incubate gels
PER Peroxidase[8]	A	75 ml Peroxidase buffer	0.1 M $CaCl_2$ solution ..... 2 ml 3-amino-9-ethyl carbazole ..... 50 mg dissolved in N,N-dimethylformamide ..... 5 ml 3% H_2O_2 ..... 1 ml	Add components to buffer just before staining. Add gels and develop at room temperature. Use anodal and cathodal slices. Note: origin of gel could be moved 1 cm toward anode at beginning of the run for this system
6PGD 6-phosphogluconate dehydrogenase[10]	C,D	75 ml 0.05 M tris HCl pH 8.0	6-phosphogluconic acid ..... 20 mg NADP ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml	Add components to warm stain buffer, add gels and incubate in the dark
PGI Phosphoglucose isomerase[10]	A	75 ml 0.05 M tris HCl pH 8.0	D-fructose-6-phosphate ..... 25 mg 1% $MgCl_2$ solution ..... 1 ml NADP ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml G6PDH ..... 20 units	Add all components to warm buffer. Add gels and incubate in the dark
PGM Phosphoglucomutase[10]	A	75 ml 0.05 M tris HCl pH 8.0	α-D-glucose 1,6-diphosphate solution (10 mg/100 ml distilled water) ..... 1 ml α-D-glucose 1-phosphate ..... 140 mg	Add all components to warm buffer. Add gels and incubate in the dark

Table 4—*Stain recipes* (continued)

Enzyme	Gel buffer	Stain buffer	Stain components	Procedure
			1% $MgCl_2$ solution ..... 1 ml NADP ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml G6PDH ..... 20 units	
SKDH Shikimate dehydrogenase[8]	C,D	75 ml 0.05 M tris HCl pH 8.0	Shikimic acid ..... 75 to 50 mg NADP ..... 1 ml NBT ..... 1 ml PMS ..... 0.5 ml	Add all components to warm buffer and incubate in the dark

[1]Yeh and O'Malley 1980
[2]Scandalious 1969
[3]Carcinogenic, handle with care
[4]Ott and Scandalious 1978
[5]Mitton and others 1979
[6]Shaw and Koehn 1968
[7]Brewbaker and others 1968
[8]Shaw and Prasad 1970
[9]Nichols and Ruddle 1973
[10]Brewer 1970

D. Chemicals Needed for Processes Described[1]

Catalog number[2]	Chemical
	Acetic acid, glacial[3]
A7251	*Cis*-aconitic acid
A2628	L-alanine β-napthylamide[3]
A4503	Albumin, bovine, fraction V powder
A5754	3-amino-9-ethylcarbazole
12,390-9[4]	N-(3-aminopropyl)morpholine[3]
A6756	Arsenic acid, sodium salt[3]
A9256	L-aspartic acid, free acid
B0252	Boric acid
C0759	Citric acid, free acid, anhydrous
D4254	N,N-dimethylformamide[3]
D5879	Dimethyl sulfoxide
	Ethanol, 95%
F7253	Fast Black K Salt
F3378	Fast Blue BB Salt[3]
F0500	Fast Blue RR Salt[3]
F0875	Fast Garnet GBC Salt[3]
752-1	D-fructose 1,6-diphosphate, trisodium salt
F3627	D-fructose-6-phosphate, sodium salt, grade I
G5875	α-D-glucose 1,6-diphosphate, tetra(cyclohexylammonium) salt
G7000	α-D glucose 1-phosphate, disodium salt: tetrahydrate
G7879	D-glucose-6-phosphate, monosodium salt
G8878	Glucose-6-phosphate dehydrogenase, Type XI
G1626	L-glutamic acid, monosodium salt
G5126	Glyceraldehyde-3-phosphate dehydrogenase
G2002	Glycyl-L-leucine
	Hydrogen peroxide (3%)
I1252	DL-isocitric acid, trisodium salt
I2002	Isocitric dehydrogenase, Type IV
410-2	α-ketoglutarate solution, 0.1 M, pH 7.5 in 0.1 M phosphate buffer
L0376	L-leucine β-naphthylamide, hydrochloride[3]
L0501	L-leucyl-L-tyrosine
L4256	Lithium hydroxide, monohydrate[3]
M0250	Magnesium chloride, hexahydrate
M0625	Maleic anhydride[3]
M0875	DL-malic acid
M5626	Menadione
M6250	2-mercaptoethanol
	Methanol, 95%[3]
M0883	4-methylumbelliferyl acetate
N8505	α-naphthyl acetate[3]
N6875	2-naphthyl acetate[3]
N7000	α-naphthyl acid phosphate, monosodium salt[3]
N7004	β-nicotinamide adenine dinucleotide (NAD)
N8129	β-nicotinamide adenine dinucleotide, reduced form (NADH), disodium salt
N0505	β-nicotinamide adenine dinucleotide phosphate (NADP), sodium salt
N6876	Nitro blue tetrazolium (NBT)[3]
P8250	Peroxidase, type II
P9625	Phenazine methosulfate (PMS)[3]
P7877	6-phosphogluconic acid, trisodium salt
P1767	Potassium hydroxide[3]
P8256	Potassium iodide
P9255	Pyridoxal 5-phosphate
S5375	(−)shikimic acid
S8625	Sodium acetate, trihydrate
S5881	Sodium hydroxide, anhydrous pellets[3]
S0751	Sodium phosphate, monobasic
S0876	Sodium phosphate, dibasic
S4501[5]	Starch, hydrolyzed for electrophoresis
T1503	Trizma base
T3253	Trizma hydrochloride
V1625	L-valyl-L-leucine
V7000[3]	Venom, snake (*Crotalus atrox*)

[1]Many chemicals are available in different forms, with different salts and hydration levels. Sigma and Aldrich Chemical Company catalog numbers and chemical names are supplied to avoid confusion for all but the most common chemicals—glacial acetic acid, ethanol, hydrogen peroxide, and methanol.

[2]Sigma Chemical Co., P.O. Box 14508, St. Louis, Mo. 63178.

[3]Hazardous chemical, handle with care.

[4]Aldrich Chemical Co., 940 West Saint Paul Avenue, Milwaukee, Wis. 53233.

[5]Additional brands of starch are Fisher, Connaught, and Electro-starch.

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The Forest Service, U.S. Department of Agriculture, is responsible for Federal leadership in forestry. It carries out this role through four main activities:

- Protection and management of resources on 191 million acres of National Forest System lands.
- Cooperation with State and local governments, forest industries, and private landowners to help protect and manage non-Federal forest and associated range and watershed lands.
- Participation with other agencies in human resource and community assistance programs to improve living conditions in rural areas.
- Research on all aspects of forestry, rangeland management, and forest resources utilization.

The Pacific Southwest Forest and Range Experiment Station

- Represents the research branch of the Forest Service in California, Hawaii, and the western Pacific.

Conkle, M. Thompson; Hodgskiss, Paul D.; Nunnally, Lucy B.; Hunter, Serena C. **Starch gel electrophoresis of conifer seeds: a laboratory manual.** Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture; 1982. 18 p.

This manual describes fast, low-cost biochemical procedures for separating enzymes representing numerous genes of forest trees. During electrophoresis the mixture of enzymes from a megagametophyte or embryo of a germinated seed separates in a gel. Specific stains applied to gel slices locate each enzyme. These procedures expand on those developed for crops research. They provide a means for forest geneticists to get urgently needed information on the amount and geographic distribution of genetic variation in conifers for evaluating species relationships, for protecting rare natural populations, and for deciding on breeding programs for commercial species. Electrophoresis of conifers is an alternative to long-term, high-cost field trials.

Retrieval Terms: technique, gametophytes, embryos, allozymes, electrophoresis