FLINN SCIENTIFIC

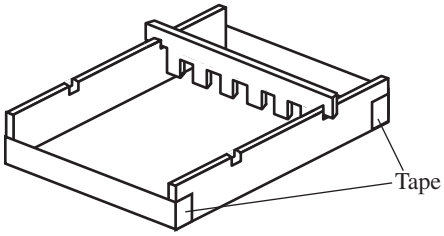
Preparation of 0.8% Agarose Gel

Materials Needed, Casting Agarose Gel

Agarose, 3 g
Balance, 0.01-g readability
Casting trays with well combs
Cotton, non-absorbent or foam plug
Erlenmeyer flasks, borosilicate, 250-mL, 6
Gloves, heat-protective
Marker or wax pencil
Microwave, hot water bath or stirring hot plate
Stirring rod
TAE electrophoresis buffer, 360 mL
Weighing dishes, small or weighing paper

Preparation of one 0.8% agarose gel (equivalent to 1 minigel)

1. Stir 0.48 g of agarose into 60 mL of the electrophoresis buffer in a borosilicate Erlenmeyer flask. Stopper with a non-absorbent cotton, or foam plug.
2. Mark the height of the solution on the Erlenmeyer flask.
3. Dissolve agarose by heating in a microwave, hot water bath, or on a hot plate. *Caution:* Be careful not to superheat the solution because it will NOT boil until you disturb or disrupt it, whereupon it may spontaneously boil out.
   a. Microwave — 30–40 seconds, stir, repeat.
   b. Hot water bath — do not boil the water
   c. Hot plate — do not boil or scorch the agarose solution
4. Heat until the solution is clear and agarose appears to be fully dissolved.
5. Stir frequently and do not allow solution to boil for more than a few seconds.
6. Use heat protective gloves to remove the bottle.
7. Check the level of the solution. Add distilled water, if needed.
8. To prevent damage to the casting trays, allow the agarose to cool to 55 °C before pouring.



Prepare the casting trays while waiting for the agarose to cool.

1. Attach the rubber dams to the ends of the casting tray or use tape to create the end walls.
2. Place the well-forming comb in the groove toward the end of the gel box.
3. Ensure the casting tray is on a level surface.
4. Slowly pour the melted agarose into the assembled casting tray being careful not to create bubbles in the gel. Use a stirring rod or pipet tip to push any bubbles to the edge of the casting tray. Only add enough agarose to equal the height of the indentations in the well-forming comb—do not fill the tray to the top.
5. Thoroughly rinse out the Erlenmeyer flask immediately.
6. Allow the gel to sit undisturbed for at least 20 minutes until the gel is firm to the touch. The set gel will appear opaque and somewhat white. 60 minutes is optimal.
7. Once the gel is thoroughly set, carefully remove the well-forming comb by rocking it gently from side to side and then pulling it upward. Remove the end dams and carefully slip the gel out of the form.
8. Slide each gel into a separate resealable bag, add 5 mL of buffer, and refrigerate. *Note:* A solidified gel can be stored under buffer in a laboratory refrigerator for up to two weeks.

Safety Precautions

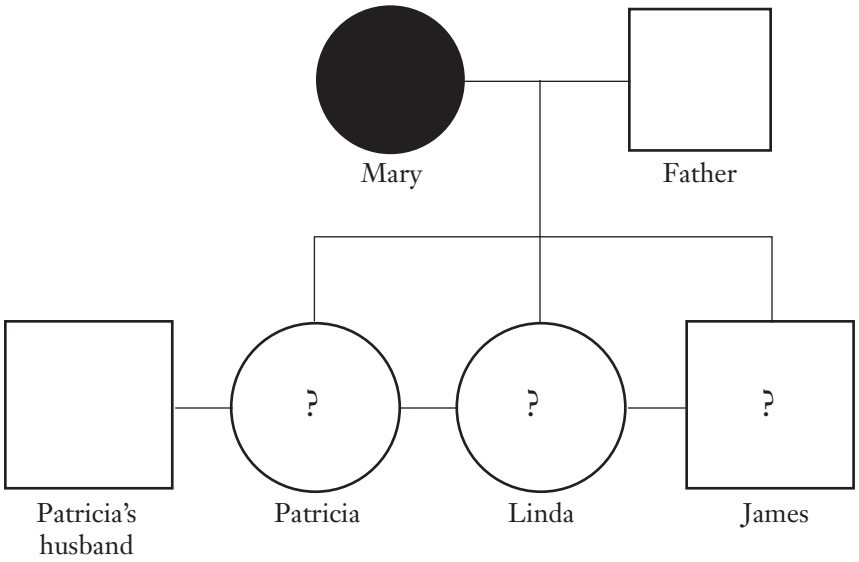
Wear chemical splash goggles and heat protective gloves when handling hot liquids. Be careful not to superheat the solution because it will NOT boil until stirred, whereupon it will boil over. Wash hands thoroughly with soap and water before leaving the laboratory. Please consult current Material Safety Data Sheets for additional safety, handling and disposal information.

The Genetics of Cancer Scenario

Three siblings have been contacted by their mother's geneticist concerning genetic testing for the tumor suppressor genes *BRCA1* and *BARD1*. Their mother, Mary, had recently been diagnosed with an aggressive form of breast cancer called hereditary breast-ovarian cancer (HBOC) syndrome. HBOC has been linked to germline mutations in the *BRCA1* region of chromosome 17 and in the *BARD1* region of chromosome 2.

Patricia, Linda, and James are concerned because of the aggressive nature of this familial cancer. The family has a high risk of breast cancer because their grandmother and maternal aunt died of breast cancer at relatively young ages (52 and 40, respectively). The siblings are concerned that they may be carriers of the mutated *BRCA1* and *BARD1* genes.

A *BRCA1* mutation is an autosomal dominant disorder, meaning that one copy of the mutated gene increases the probability of inherited breast cancer by 50–85%. A *BARD1* mutation is autosomal recessive, meaning that two copies of the defective gene are necessary to cause breast cancer but when found in conjunction with *BRCA1* the risk of developing early onset breast cancer rises to nearly 100%. Additionally, Patricia is newly married and she is concerned about the potential risk of passing these germline mutations on to her future children. Their family tree is as follows:



As a genetic laboratory technician, you have been provided with DNA samples from the three siblings and Mary to determine their cancer gene status. DNA Sample 1 contains buccal cells collected from Patricia. DNA Sample 2 contains buccal cells collected from Linda. DNA Sample 3 contained buccal cells collected from James. DNA Sample 4 contains buccal cells collected from Mary.

Note: The above scenario is ficticious. The DNA samples included in this laboratory are not human. They are Lambda phage DNA fragments.