Low-Power Vacuum Apparatus for Blotting

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Low-power vacuum pumps are commonly used for Southern and Northern transfer from agarose gels and for slot and dot blotting. Unfortunately, commercially available vacuum pumps are expensive and susceptible to contamination with transfer reagents. The following describes the use and construction of a very simple and cheap apparatus, which generates and measures a partial vacuum as effectively as commercially available pumps (Figure 1). It functions on the principle that water attempting to move out of the drum will generate a partial vacuum proportional to the difference in height between the upper water level and the outlet (e.g., 40 cm). See Table 1 for use.

At this stage, approximately 100 mL of water escape from “straight connector-a” for about 5–10 s, but should then essentially stop. Continued loss of water indicates a leak. The water levels in the two arms of the vacuum gauge should now have moved to the points marked “v”, where the distance between them gives the vacuum obtained (e.g., 40 cm). Thus, the vacuum is determined by adjusting the height of “straight connector-a”.

During transfer of 2 h, 100–200 mL escape from “straight connector-a”. Thus, the use of a large-diameter drum results in only a small drop in water level (200 mL result in about a 3–4-mm drop).

Table 1. Use of Vacuum Pump

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tr>
<td>1</td>
<td>Fill drum to 2–3 cm from top.</td>
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<tr>
<td>2</td>
<td>Fill vacuum gauge tubing to “water level” mark.</td>
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<tr>
<td>3</td>
<td>Attach to gel transfer manifold or slot/dot blot apparatus.</td>
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<td>4</td>
<td>Lower “straight connector-a” to bottom of retort (emptying into sink).</td>
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For construction of the vacuum pump, the Plasticware must consist of: (i) an aspirator bottle (25 L), (ii) a T un-tapered connector (3.3-mm valley, 3.6-mm crest), (iii) a straight connector tapered (a) (10–12-mm outside diameter), (iv) a straight connector tapered (b) (4–6-mm outside diameter) and (v) tubing measurements for inside diameters. To put “straight connector-b” into the drum, just drill a hole and wrap Parafilm® around the connector. It is handy to have “straight connector-a” pointed slightly upwards, to stop air bubbles from entering the tubing.

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Increased $^{32}$P-SSCP Sensitivity by Combining RE Digestion and Extended X-ray Film Exposures


Radioactive polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis is currently the most frequently used mutation scanning method, applied for detecting both disease-causing mutations and DNA polymorphisms. The technique was first described by Orita et al. (11,12) and relies on polyacrylamide gel electrophoresis (PAGE) fractionation of distinctive conformational isomers (conformers) of single-stranded (ss) DNAs generated by denaturation of short PCR products. Theoretically, single-base changes may alter the mobility of one or both of the ssDNA strands. However, in practice, a mobility shift in SSCP is dependent on several different complex electrophoretic conditions. Optimum conditions that detect all mutations have been difficult to find, but several examples of different conditions that can detect different base substitutions have been shown. Variation in PAGE temperature, ionic buffer strength, PCR fragment length and gel matrix are factors found to influence SSCP sensitivity (1–3,5–7,10,13,14).

The sensitivity has been reported to vary from 50%–100% in detection rate. The highest radioactive $^{32}$P-SSCP sensitivity is found when performed with 100–350-bp PCR fragments in a 6% gel at 4°C electrophoretic temperature (4,6).

Here we report how it may be possible to increase the sensitivity of the SSCP analysis by simple modifications of the SSCP protocol and, at the same time, increase PCR fragment length from about 200 to 1600 bp. Our suggestions to improve the SSCP protocol include digests of the PCR product with several different restriction enzymes before PAGE, as well as both brief and long X-ray film exposures afterwards.

We used two different fragments of