Variable-Size Injectable Dialysis Chambers

BioTechniques 23:665-666 (October 1997)

We required a disposable dialysis chamber of approximately 100 µL internal volume to fit within a small reaction vial. Since our application demanded the highest possible rate of diffusion into and out of the chamber, the surface area-to-volume ratio had to be as high as was practical. Injectable dialysis chambers are commercially available, e.g., the Slide-A-Lyzer® Cassette™ (Pierce, Rockford, IL, USA). However, they are relatively expensive and the outside dimensions too bulky (7 × 40 × 40 mm) for our application. The device described here uses washers cut from silicone rubber, with dialysis membrane glued to both faces. While the devices are more complicated to construct than other small dialysis chambers described recently (1), they afford an increased flexibility in size.

Washers were cut from 1.6-mm-thick translucent silicone rubber, of high tensile strength (1100 psi) and medium hardness (McMaster-Carr, Chicago, IL, USA) with handheld punches. No smoothing of cut edges was required. The washers had an external diameter of 15.9 mm and an internal diameter of 9.5 mm. A small amount of Super Glue was spread over one side of a washer, which was then glued onto a sheet of Spectra/Por® Dialysis Membrane (MWCO: 12 000–14000; Spectrum, Laguna Hills, CA, USA) (Figure 1, left panel). Even pressure applied over the entire washer (with, in our case, the base of a handheld 7-mL plastic scintillation vial) for 10–15 s results in a good seal. The resulting half-finished devices were cut from the dialysis membrane, and the gluing process was repeated on the other side to create a chamber with an internal volume of approximately 110 µL. Construction was completed by cutting each chamber from the sheet. Dialysis chambers can be constructed to any size, using a variety of thicknesses of silicone rubber and diameters of the punches. With the punches available to us and 1.6-mm silicone rubber, we can easily make dialysis chambers with up to 900 µL internal volume. This could be increased by cementing two half-finished dialysis chambers together or by using thicker silicone rubber. The width of the glued surface of the rubber needs to be sufficient to provide satisfactory bonding. We found that a width of 3 mm was sufficient to ensure a good seal. Flat sheets of dialysis membrane were used not only for ease but also to avoid the creases in the membrane found in tubular dialysis membranes, which are difficult, if not impossible, to glue across sufficiently tightly to prevent failure of the seal. The glue was not affected by the Tris buffers that were used, and the chambers maintained their integrity overnight in small vials in a shaking water bath.

Samples (100 µL) were injected into the chambers using a 1-cc insulin syringe with micro-fine IV needle (Becton Dickinson Labware, Bedford, MA, USA). The fine-gauge needles were required to penetrate the very thin chamber walls (1.6-mm thickness). Some stretching of the membrane results if the sample is introduced prior to evacuating the air in the chamber. To avoid this stretching, it is possible to evacuate air from the chamber before injection of the sample by carefully drawing back on the syringe plunger. To completely exclude air bubbles from the chamber, it is necessary to remove the needle from the chamber after evacuation, expel the air from the syringe needle,
fill the syringe with the sample and then reintroduce the needle into the chamber to inject the sample. However, no loss of structural integrity of the membrane was noted when air was not evacuated before introducing the sample.

The thin walls of the dialysis chamber place some demands on the dexterity of the experimenter. It is possible to increase the thickness of the silicone rubber if maintaining a very high surface area-to-volume ratio is not critical. To aid in the injection of the thin-walled dialysis chambers, a jig was constructed from 6-mm-thick Plexiglas® (plan available on request). The jig holds the dialysis chamber steady and has a 0.45-mm-diameter hole to guide the needle through the sidewall of the chamber (Figure 1, right panel). This prevents the rupture of the dialysis membrane by an improperly placed needle. A small groove on the top of the jig facilitates removing the chamber with the aid of the injection needle.

The dialysis membrane sheets were used unwashed because holes form in the membrane if the cellulose-based dialysis membrane is allowed to dry after wetting. The membrane we used contains glycerol as a humectant, 0.1% sulfur as sulfides and trace quantities of some heavy metals. None of these contaminants interfered with our assay. Rinsing the completed dialysis chambers to remove these materials should be possible if they pose a problem for a particular application. Silicones and glues can leak material that absorbs at 280 nm. When the dialysis chambers were incubated overnight (16 h), submerged in a small volume of buffer (2 mL), a significant absorbance at 280 nm resulted from leaching from the glue and/or silicone. However, such leachate should not unduly affect protein determinations at 280 nm because not only would an actual dialysis run be complete within approximately one hour, but also the sample would be dialyzed against excess buffer that would dilute the leachates that absorb at 280 nm.

Recovery of the sample after dialysis was best achieved by gently puncturing one dialysis membrane with a hypodermic needle and drawing off the sample. Recovery of the sample was essentially 100%. Removing the sample through the sidewall of the chamber allows only about 80% of the sample to be recovered because of the vacuum generated inside the dialysis chamber.

The surface area-to-volume ratio of the device described here (approximately 1.3 cm²/mL) compares very favorably to the Slide-A-Lyzer cassette (approximately 0.3 cm²/mL) or 10-mm flat width dialysis tubing (approximately 0.6 cm²/mL). The higher surface area-to-volume ratio results in much faster dialysis. For example, while a tyrosine (M₀ 181.2) solution within a Slide-A-Lyzer cassette comes to equilibrium with the surrounding medium in approximately 120 min (2), the device described here takes approximately 60 min for a glucose (M₀ 180.2) solution to come to equilibrium. It should, however, be remembered that loss of proteins due to adsorption onto the membrane will increase as the surface area-to-volume ratio increases. Applications requiring dialysis of dilute solutions of proteins likely to bind to cellulose-based membranes should avoid the use of the thinnest configurations of this device.

REFERENCES


This work was supported by NSF Grant No. MCB-9407122 to D.H.K. P.M.D. was supported in part by a Monsanto Postdoctoral Fellowship in Plant Biology. The authors would like to thank the reviewers for their helpful suggestions. Address correspondence to Daniel H. Kohl, Biology Department, Campus Box 1137, Washington University, 1 Brookings Drive, St. Louis, MO 63130, USA. Internet: kohl@biodec.wustl.edu

Received 23 December 1996; accepted 14 April 1997.

Phillip M. Debnam and Daniel H. Kohl
Washington University
St. Louis, MO, USA

Ethidium Bromide Enhances Transformation of E. coli with Homopurine/Pyrimidine-Rich DNA

BioTechniques 23:666-670 (October 1997)

Studies regarding structure and function of eukaryotic genes rely on the availability of efficient recombinant DNA techniques. Bacterial transformation with recombinant plasmid represents a very important step to obtain large numbers of bacterial clones carrying the plasmid of interest. The cloning of eukaryotic DNA sequences in plasmid vectors could present certain difficulties because of the presence of poison sequences in the foreign gene fragment. Such sequences may introduce constraints in the DNA topology that prevent subsequent replication and/or transcription in the bacterial host cells. To circumvent this problem, we have developed a simple device that is quick, inexpensive and particularly useful when fragments of DNA containing oligonucleotide purine/pyrimidine tracts have to be inserted in plasmid vectors. This technique involves ethidium bromide (EtBr) incubation in the ligation mixture before bacterial transformation.

We have previously established the exon-intron structure of the human melanin-concentrating hormone (MCH) gene following polymerase chain reaction (PCR) amplification and subcloning of a 1.28-kb fragment that yields to a clone named phMCH-G34 (Figure 1A and Reference 2). However, we failed to isolate clones corresponding to the authentic MCH gene by screening several commercial genomic libraries and one homemade lambda phage library (2). Since we have succeeded in characterizing yeast artificial chromosomes (YACs) bearing this MCH gene, we hypothesized that difficulties in subcloning were possibly due to the existence of particular sequences preventing efficient transformation and/or growth of recombinant MCH plasmid in E. coli cells. Hence, the method described here was originally designed for subcloning of large fragments of the human MCH gene (5).