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 leach 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 (M_r 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 (M_r 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.

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

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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).

Benchmarks

DNA fragments of the human or mouse MCH gene were prepared as described elsewhere (6). pUC-hMCH(I) and pUC-hMCH(II) plasmids were constructed by inserting a *Bam*HI-*Xho*I fragment (8.90 kb) and an *Xba*I fragment (3.36 kb), respectively, in pUC19 vector (Boehringer Mannheim GmbH, Mannheim, Germany) after cleavage with *Xba*I (Figure 1A). Similarly, mouse MCH gene fragments of different lengths were subcloned in pUC19 vector and generated three clones named pUC-mMCH(I), pUC-mMCH(II) and pUC-mMCH(III) (Figure 1B and Reference 6). Ligation was performed as described by Zhixing and Nahon (6), except that *M. luteus* DNA gyrase was omitted, and EtdBr was added before transformation. After ligation, 6 μ L of the reaction mixture were transferred into a sterile microcentrifuge tube, and 2 μ L of EtdBr (0.1–100 μ g/mL solution) were added. The mixture was incubated at 80°C for 5 min, then chilled to 0°C. The whole reaction mixture was used to transform competent *E. coli* cells according to the conventional calcium method (3). When

Table 1. Comparison of the Efficiency of Transformation Using Different Protocols

Method	Calcium Chloride			High-Voltage Electroporation		
	Standard	DNA Gyrase	EtdBr	Standard	DNA Gyrase	EtdBr
pUC-hMCH(I)	-	-	+	-	-	+
pUC-hMCH(II)	+	n.d.	n.d.	n.d.	n.d.	n.d.
phMCH-G34	+	n.d.	n.d.	+	n.d.	n.d.
pUC-mMCH(I)	-	+	n.d.	-	+	n.d.
pUC-mMCH(II)	-	+	n.d.	-	+	n.d.
pUC-mMCH(III)	-	+	n.d.	-	+	n.d.

Finding of colonies with MCH gene transformants is indicated by + and absence by - ; n.d. = not done.

transformation by electroporation (3) was performed, 2 μ L of the reaction mixture were added to 30 μ L of competent cell suspension. *E. coli* strains used for transformation were XL1-Blue (Stratagene, La Jolla, CA, USA) and DH5 α TM (Life Technologies, Gaithersburg, MD, USA). The cells were diluted appropriately and plated on LB agar containing ampicillin, isopropyl- β -D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) as described elsewhere (6). Isolated white colonies

from each plate were resuspended individually in 15 μ L of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 1.5 μ L of 10% sodium dodecyl sulfate (SDS) solution were added, and the mixture was incubated at 95°C for 2 min. After centrifugation at 10000 rpm, 10–15 μ L of supernatant were loaded onto slab agarose gel, and Southern blot analysis was carried out with a ³²P-labeled MCH PCR subclone (phMCH-G34) encompassing the whole exonic-intronic region of the human MCH gene (2). Transformation efficiency was calculated as the percentage of MCH-positive colonies among the white colonies. Finally, DNA sequencing of part of the pUC-hMCH(I) clone was performed with specific oligonucleotides and a Model 373A Automated DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA) (5).

Table 1 shows that addition of EtdBr before using either of the transformation methods is necessary to yield MCH gene transformants with pUC-hMCH(I), whereas a standard transformation method can be used with pUC-hMCH(II). Since the main difference between the two plasmid MCH clones resides within the presence of homopurine/pyrimidine-rich tracts in the 5' flanking region of the MCH gene, it is possible that these DNA elements were preventing subcloning during usual procedures. Oligopurine tracts differ markedly from the canonical B form of DNA and have been involved in triple helix formation or hairpin structures (1,4). These tracts are abundant in chromosomal DNA of eukaryotic cells but rather rare in prokaryotes, and this could reflect the usefulness of these versatile structural elements in the

Figure 1. Schematic representation of the human MCH gene (A) and mouse MCH gene (B). Exons I, II and III of the MCH gene are indicated with black boxes. Location of purine/pyrimidine tracts upstream from the human MCH gene is indicated. Fragments used for subcloning are noted as open boxes. The restriction enzyme sites used to generate the fragments subcloned in pUC19 (or pKS) and locations and lengths of these MCH gene parts are indicated—phMCH-G34 is an MCH genomic clone obtained through PCR amplification (2).

Benchmarks

mammalian genome (1). By intercalating between the bases, EtdBr causes the triple helix to unwind and leads to an increase in superhelical turns. However, supercoiling by itself was not sufficient to allow efficient transformation, because successful recovery of MCH-positive clones has not been reached after addition of DNA gyrase (which introduces negative supercoils into DNA) to the ligation mixture following a method we described elsewhere (Table 1 and Reference 6). On the contrary, incubation of mouse MCH gene plasmid with DNA gyrase yields transformants (Table 1 and Reference 6).

Further analysis using DNA isolated from human MCH-positive clones demonstrated that the maximum effect was noted with 0.25 µg/mL EtdBr (Figure 2A). Transformation of *E. coli* with plasmid treated with DNA gyrase de-

creased by 50%, while transformation with the same plasmid treated with EtdBr increased compared to the controls (Figure 2A). As DNA gyrase removes supercoiling and introduces negative supercoils into DNA, we can assume that faithful replication or segregation of MCH plasmid requires that specific hairpin structures have to be relieved or that positive superhelicity has to be introduced before transformation. We observed also a length-dependent effect (Figure 2C) similar to that reported following gyrase treatment of plasmid DNA (6). As controls, no significant difference in *E. coli* transformation efficiency was observed when only pUC19 vector was used after EtdBr treatment and when cells were incubated with EtdBr in a range of 2×10^{-5} to 2 µg/mL in the growth medium (not shown).

Our technique has turned out to be convenient, rapid and cost-effective and should serve to facilitate subcloning of mammalian genes, encompassing secondary structures that prevent subcloning according to standard transformation protocols.

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Figure 2. Comparison of different parameters on *E. coli* transformation efficiency. (A) Effect of EtdBr concentration on cell transformation with pUC-hMCH(I). (B) pUC-hMCH(I) plasmid was treated with DNA gyrase (4 U; 1 h at 37°C) or EtdBr (0.25 µg/mL) before transformation with *E. coli* cells. (C) Effect of the length of inserted DNA (as indicated in parentheses) on transformation efficiency of pUC19 carrying either human or mouse MCH gene after EtdBr treatment (0.25 µg/mL). The relative efficiency of transformation is expressed by taking 1 as the mean percentage of MCH-positive colonies among the white colonies in the control samples.