Increased Yield of Plasmid DNA During Removal of CsCl by Ethanol Precipitation

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Our laboratory is interested in intramuscular injection of plasmid DNA as a method of eliciting immune responses. This requires large-scale preparation of plasmid DNA because 4–8 mg of plasmid DNA can be required for a single experiment. Several techniques are available for large-scale preparation of plasmid DNA, including ion-exchange resin and CsCl gradient centrifugation (1,2). We have been purifying plasmid DNA using CsCl gradient centrifugation and have found that an additional centrifugation step, during removal of CsCl, increases the DNA yield at least twofold.

Following the protocol established by Maniatis et al. (2), we purified the plasmid DNA on CsCl gradients, extracted the ethidium bromide (EtdBr) with isoamyl alcohol and removed the CsCl by adding 3 vol of H₂O (to dilute the CsCl) and 2 vol of 100% ethanol (to precipitate the DNA) as described. While this will precipitate some of the plasmid DNA, we have found that a significant proportion of it is retained in the supernatant. We added 2 vol of 70% ethanol to the decanted supernatant to determine whether we could recover any of the residual plasmid DNA. By spectrophotometric analysis, we observed that we had isolated an additional 17 mg of plasmid DNA with a high level of purity (Table 1). To determine if the material was similar to the plasmid DNA isolated in the first precipitation, we subjected both plasmid DNAs to restriction enzyme digestion and analysis by gel electrophoresis. As shown in Figure 1, cutting the vector pcDNA-IL-12p40 with BglII and XhoI (Promega, Madison, WI, USA) generated an expected band of approximately 1.9 kb from both the first and second precipitations of plasmid DNA (lanes 2 and 4, respectively). In addition, uncut plasmid DNA from both precipitations generated similar bands after gel electrophoresis (lanes 1 and 3, respectively). We have also performed this procedure with another vector (pcDNA3; Invitrogen, San Diego, CA, USA) and obtained similar results; 5 mg were recovered in the first precipitation and 11 mg were recovered in the second precipitation. We next determined whether
the isolated DNA contained significant amounts of CsCl by measuring the refractive index. The refractive index of the DNA solution was 1.3354, which is comparable to that obtained using a microconcentrator (3).

Removal of CsCl is essential in obtaining purified plasmid DNA. A common method used to remove CsCl is dialysis against large volumes of TE buffer, which is more costly and time-consuming than the method we describe. Alternatively, spin dialysis through a microconcentrator removes CsCl quickly, but at the cost of the microconcentrator. Finally, we have shown that using two ethanol precipitations is both inexpensive and fast. Therefore, this simple centrifugation step can at least double the yield of plasmid DNA when using ethanol precipitation to remove CsCl during large-scale preparations of plasmid DNA.

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Determining the Potentiative State of a Chromatin Domain

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Gene potentiation represents the obligate first step toward the expression of a given gene or genes within a locus (1). It is the process by which chromatin is remodeled so that the gene(s) and its corresponding regulatory elements become accessible to transacting factors. Cellular phenotype is determined by those genes that exist in an open chromatin conformation in that cell. This includes the constitutively expressed genes like those of the glycolytic pathway and those that are tissue-specific like the genes of the \( \beta \)-globin locus (4).

DNase I-sensitivity provides an effective means to assess the potentiative state of chromatin domains. Potentiated genes exhibit an approximately 10-fold enhanced rate of digestion by DNase I compared to regions of closed chro-