Práctico N° 1

Extracción de DNA de mucosa bucal

Objetivo: Extracción de DNA genómico a partir de células epiteliales de la mucosa bucal. Se realizará la toma de muestra de todos los alumnos y de algunos familiares por vía materna.

Procedimiento:

1- Frotar con cepillo pequeño las paredes de la boca, los fondos de saco contra las encías y debajo de la lengua, durante al menos 1 minuto.

2- Desprender las células del cepillo agitándolo en 1 ml de buffer TE en un tubo eppendorf. Centrifugar a 14.000 rpm. durante 5 minutos y descartar por inversión el buffer sin remover el pellet de células.

3- Resuspender las células en 300 µl de buffer de digestión con agregado de proteinasa K (150 µg/ml conc. final). Incubar 2 horas a 50ºC y luego *overnight* a 37ºC.

4- Agregar un volumen igual (300 µl) de LiCl 5 M (puede sustituirse con NaCl 5 M o $NaClO_4$ 2.5 M aunque la eficiencia de obtención de DNA es menor). Mezclar por inversión el tubo durante 1 minuto, agregar 600 µl de mezcla SEVAG y colocar en agitador rotatorio durante 30 minutos. Centrifugar a 14.000 rpm

durante 15 minutos y pasar el sobrenadante con el DNA en solución a otro tubo.

5- Agregar 2 volúmenes de Etanol absoluto (aprox. 600-800 µl), agitar por inversión hasta que el DNA precipite. Centrifugar a 14.000 rpm, tirar el alcohol sobrenadante y lavar el pellet de DNA con Etanol 70%. Repetir la centrifugación, descartar el sobrenadante, tapar con Parafilm perforado y secar al aire en bloque térmico (temperatura aproximada: 50ºC).

6- Resuspender el DNA en 30 µl de buffer TE y guardar a 4ºC hasta usar. Después del primer uso guardar a - 20ºC.

Soluciones

- Proteinasa K 10mg/ml. conservar a -20ºC.
- Buffer TE: 10 mM Tris
  1 mM EDTA pH 8.0
- Buffer de digestión: 100 mM NaCl
  50 mM Tris-HCl
  1% SDS
  50 mM EDTA pH 8.0
- Cloruro de Litio 5 M
- Etanol absoluto.
- Etanol 70%

- Mezcla SEVAG: Cloroformo / alcohol iso-amílico (24:1)

An efficient method for the extraction of DNA from vertebrate tissues

With the advent of PCR (Ref. 1), the extraction of DNA for genetic analysis has become one of the most time-consuming procedures in the laboratory, particularly when a large number of samples must be processed. Numerous DNA extraction procedures have been published. While some of these are undoubtedly rapid, most require either the use of hazardous chemicals or the purchase of exotic, often expensive reagents and equipment. We have adapted two existing methods[2,3] to produce a simple and efficient DNA extraction procedure that uses common laboratory reagents. This method has been used to obtain pure, high molecular mass DNA from whole blood, skin and liver biopsies obtained from a variety of vertebrate species. Amplifiable DNA has also been obtained from plucked hair samples using this method.

Each sample (100 mg of tissue or 100 µl of whole blood) was suspended in 1.5 ml microfuge tubes with 300 µl of digestion buffer (100 mM NaCl, 50 mM Tris–HCl, 1% SDS, 50 mM EDTA, pH 8.0). Proteinase K (10 mg ml^{-1}) was added to a final concentration of 100 µg ml^{-1} and the samples incubated for 2 h at 50°C, then overnight at 37°C. After digestion was complete, an equal volume (300 µl) of 5 M LiCl was added to each tube (5 M NaCl or 2.5 M $NaClO_4$ can be substituted, but the DNA quality and yield decreases slightly, see Table 1). The sample was mixed thoroughly by inversion for 1 min, then 600 µl of chloroform added and the samples placed on a rotating wheel for 30 min. The sample was spun for 15 min at high speed in a benchtop microfuge and the supernatant carefully removed to a new microfuge tube. Exactly 2 volumes of room temperature absolute ethanol were added and the tube inverted several times until the DNA precipitated. The DNA was recovered by high speed centrifugation, the supernatant decanted, and the DNA pellet washed briefly with 70% ethanol. Excess ethanol was removed and the sample allowed to air dry for 10 min. The sample was then resuspended in 100–200 µl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5) and allowed to dissolve overnight at 4°C.

TABLE 1. Spectrophotometric comparison of DNA extraction procedures[a]

	Phenol	NaCl	$NaClO_4$	LiCl
No. of samples	4	4	4	34
Mean spectrophotometric absorbance ratio (260/280 nm)	1.79	1.61	1.75	1.79
Mean DNA yield (µg) from 100 mg sample[b]	227	105	260	318

[a] Tissue samples were extracted as described in the text and the spectrophotometric absorbances determined at 260 and 280 nm.
[b] DNA yields were extrapolated from the absorbance at 260 nm.