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DNA extraction by zinc

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ABSTRACT

A fast, very simple and efficient method of DNA extraction is described which takes advantage of DNA sedimentation induced by millimolar concentrations of $ZnCl_2$. The zinc-induced sedimentation is furthermore strongly promoted by submillimolar phosphate anion concentrations. Within <30 min, the method recovers >90% of DNA irrespective of whether a plasmid DNA or short oligonucleotides are the extracted material. The method works with plasmid DNA and oligonucleotide concentrations as low as 100 ng/ml and 10 μg/ml, respectively, without using any expensive facilities or toxic chemicals.

Millimolar concentrations of Zn^{2+} and other transition metal cations (Mn^{2+}, Ni^{2+} and Co^{2+}), but not the earth metal ions (Mg^{2+} and Ca^{2+}), induce a strong DNA sedimentation under favourable conditions (E.K. and J.K., unpublished results). We studied the zinc-induced DNA sedimentation in detail because of the biological significance of zinc and its interesting effects on DNA structure, including induction of Z-DNA (1,2), a DNA triplex (3) and DNA bending (4,5).

The zinc-induced DNA sedimentation is so strong that it does not require centrifugation. Mere gravity causes almost all DNA to sediment after a few hours incubation with $ZnCl_2$. The concentration of $ZnCl_2$ causing the sedimentation depends on the sample volume and pH, being lower for larger volumes and alkaline pH values. The sedimentation is furthermore strongly promoted by submillimolar concentrations of the phosphate anions whose high concentrations (>5 mM), however, inhibit the sedimentation. In addition, the phosphate makes the DNA extraction efficiency insensitive to pH in the range of 6.0–8.5. Several minutes of incubation with millimolar zinc and submillimolar phosphate ions, followed by a brief centrifugation, results in a complete sedimentation of DNA. The sedimented DNA is easily resuspended in a buffer containing EDTA (e.g. TE buffer) because EDTA concentrations >0.1 mM eliminate the DNA sedimentation. After the extraction, the zinc can be removed by the common ethanol precipitation (6), if necessary, and the recovered DNA is suitable for further manipulations, e.g. cleavage by restriction endonucleases (we tested *Eco*RI, *Hin*dIII, *Pst*I and *Ssp*I), ligation with T4 DNA ligase or PCR. The zinc-induced DNA sedimentation can be used for extraction of plasmid DNA at concentrations as low as 100 ng/ml and the method is also efficient with short oligonucleotides (20 bp), both GC rich and AT rich, where, however, at least a 10 μg/ml concentration is necessary for good recovery. Zinc also precipitates bacterial RNA and bovine serum albumin (BSA). Both types of biomacromolecules were quantitatively extracted using the approach described here. Zinc chloride also precipitates bacteriophages (7,8).

The efficiency, speed, universality and requirement of no expensive facilities or toxic chemicals makes the present approach an attractive alternative to the existing methods of DNA precipitation; none of which is ideal or universal (9). In contrast to ethanol and isopropanol precipitation of DNA, where 2–3 or 1 vol of alcohol should be added to the DNA samples, respectively, we only add several microliters of zinc chloride and Na-phosphate to extract DNA. This is advantageous especially when the sample volume must not much increase. The phenomenon of DNA sedimentation by zinc will be described in detail elsewhere. Additional information can be obtained at the e-mail address kejnovsk@ibp.cz.

Protocol: first check that your sample (volumes of 0.02–8 ml were tested) contains at least 5 mM Tris-chloride, Tris-borate or Tris-acetate buffer (pH 7.0–8.5) and that it does not contain higher than 0.1 mM EDTA or 1 mM phosphate concentrations. If necessary, dilute the sample with 10 mM Tris–HCl buffer (pH 7.0) below these limiting concentrations of EDTA and phosphate. Add Na-phosphate buffer (pH 7.0) to get a 0.1–1.0 mM phosphate concentration in the sample. We recommend its concentration is minimized because the phosphate coprecipitates with DNA and can influence enzyme activities. On the other hand, NaCl concentrations <300 mM only weakly diminish the method efficiency. Then add 1/20 or 1/100 vol of 0.1 M $ZnCl_2$ depending on whether the sample volume is, respectively, smaller or larger than 0.5 ml. Mix the solution and incubate for 15 min at room temperature. Centrifuge for 5 min at 10 000 r.p.m. and carefully remove the supernatant. Resuspend the pellet in the desired volume of buffer containing at least 1 mM EDTA (e.g. TE buffer). The pellet dissolved poorly if the pH value of the sample, from which the DNA was extracted, exceeded 8.0. If necessary, remove the zinc by common ethanol precipitation.

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REFERENCES

1 Fazakerley,G.V. (1984) *Nucleic Acids Res.*, **12**, 3643–3648.
2 Woisard,A., Fazakerley,G.V. and Guschlbauer,W. (1985) *J. Biomol. Struct. Dyn.*, **2**, 1205–1220.
3 Bernués,J., Beltrán,R., Casasnovas,J.M. and Azorín,F. (1989) *EMBO J.*, **8**, 2087–2094.
4 Laundon,C.H. and Griffith,J.D. (1987) *Biochemistry*, **26**, 3759–3762.
5 Nickol,J. and Rau,D.C. (1992) *J. Mol. Biol.*, **228**, 1115–1123.
6 Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
7 Santos,M.A. (1991) *Nucleic Acids Res.*, **19**, 5442.
8 Chang,P.K. and Natori,M. (1994) *J. Biotechnol.*, **36**, 247–251.
9 Hengen,P.N. (1996) *Trends Biochem. Sci.*, **21**, 224–225.