Room Temperature Method for Increasing the Rate of DNA Reassociation by Many Thousandfold: The Phenol Emulsion Reassociation Technique†

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ABSTRACT: A phenol aqueous emulsion allows the reassociation of DNA at temperatures from 6 to 68 °C. This phenol emulsion reassociation technique (PERT) also promotes the very rapid reassociation of DNA. *E. coli* and human DNAs at a concentration of 4 μg/mL reacted at room temperature with the PERT reassociate many thousand times faster than under the standard conditions of 0.18 M Na^+, 60 °C. Solutions of DNA ranging in concentration from 6×10^{-5} to 6400 μg/mL have been successfully reassociated using the emulsion method. The greatest rate increases are seen at low DNA concentration. The half-time of reassociation does not decrease proportionally with an increase in DNA concentration when using the PERT. At 6400 μg/mL the phenol emulsion rate of reassociation is only about 10 times faster than under the standard aqueous reference conditions of 0.12 M phosphate buffer (PB), 60 °C. The rate of DNA reassociation observed with the emulsion technique is at least dependent on: (a) the presence of an emulsion; (b) the type and concentration of ion present; (c) an appropriate temperature of incubation; (d) the proper pH; (e) the rate and manner of agitating the emulsion; (f) the amount of phenol present; (g) the fragment size of the DNA; (h) the complexity of the DNA; (i) the concentration of DNA. The presence of salt is necessary for the emulsion reassociation, and the effect of a variety of salts ($NaHPO_4$, NaCl, $NaClO_4$, NaSCN, CsCl, RbCl, KCl, LiCl, and others) has been examined. As a general rule the more chaotropic the anion or cation, the faster the DNA reassociation in the emulsion system. DNA will reassociate over a wide range of pH (5-9) and phenol concentration (5-95%). Optimum emulsion reassociation rates are obtained by vigorous shaking of the emulsion. At high DNA concentrations the rate depends on the violence of shaking. The fragment size of the DNA used in the emulsion reaction does not greatly influence the rate of reassociation. DNAs from viruses, bacteria, and mammals ranging in G + C content from 30 to 70% have successfully and rapidly reassociated with the PERT. The criterion of those PERT mixtures examined is comparable to about 56 °C, 0.21 M NaCl in the aqueous system. RNA:RNA and RNA:DNA reactions also occur in the emulsion system. However, the greatest rate increase observed as yet is only 50-100 times the reference aqueous rate.

Nucleic acid reassociation has proved to be a powerful tool for analyzing the genetic material of a wide variety of organisms, from virus to man. The various applications of the technique are too numerous to list. An understanding of the rate of nucleic acid reassociation (Britten and Kohne, 1968; Wetmur and Davidson, 1968), the development of quick, efficient nucleic acid reassociation assays (Kohne and Britten, 1971; Britten et al., 1974) and the elucidation of the nucleotide sequence composition of the mammalian genome (Britten and Kohne, 1968) have greatly increased the versatility of this tool for genetic analysis. While nucleic acid reassociation has been used to answer many important questions, a major limitation on the feasibility of using this technique in many significant biological experiments is the basic rate of reassociation seen for the standard one-phase aqueous systems. We have discovered a technique which increases the rate of reassociation of DNA many thousandfold. The technique also allows reassociation to occur at or below room temperature. Most laboratories working with nucleic acids already possess the reagents and equipment needed for this technique which is simple and inexpensive.

Phenol is a common laboratory chemical which is frequently used to purify DNA and RNA. When phenol and water are mixed in the right proportions and shaken, an emulsion forms. When the shaking stops, the emulsion breaks and the two phases separate. Addition of single-strand DNA and salt to the water phase, and then shaking the mixture at room temperature results in the extremely rapid formation of double-stranded DNA. This two-phase system, water and phenol, is the basis for a new, rapid nucleic acid reassociation technique. We have named this method the phenol emulsion reassociation technique (PERT).[1]

Materials and Methods

DNA Preparations. DNAs used in these experiments were purified by the urea-phosphate-hydroxylapatite technique (Britten et al., 1974; Meinke et al., 1974) or by the standard phenol-chloroform technique. Iodinated DNA was prepared by a modification of the Commerford technique (Commerford, 1971). No nonradioactive carrier iodine was used and mercaptoethanol was utilized to terminate the iodination reaction

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[1] Abbreviations used: PERT, phenol emulsion reassociation technique; Tris, tris(hydroxymethyl)aminomethane; PB, phosphate buffers; $NaDodSO_4$, sodium dodecyl sulfate; poly(a), poly(adenylic acid); poly(U), poly(uridylic acid); EDTA, ethylenediaminetetraacetic acid.