HITACHI

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Subject Collection of DNA by ethanol precipitation using a swing rotor capable of being used with microplates

Model R6S swing rotor for high-speed refrigerated centrifuges

1. Introduction

Ethanol precipitation has been noticed as a convenient DNA-collecting method to effectively determine DNA alignment from multiple samples by cycle sequence. The newly developed R6S swing rotor for high-speed refrigerated centrifuges can accommodate 96-hole microplates to handle multiple samples at a time. Following are the result of our examination about the centrifugal conditions and DNA-collecting rate for the ethanol precipitation method, and application of the R6S swing rotor to the sequencing operation.

2. Procedure of experiment

Sample DNA
↓ Add 3M Ac0Na by one-tenth of the sample amount.
Add EtOH by 2.5 times of the sample amount and mix them well.
↓ Keep the sample at -20 ˚C for 20 minutes.
↓ Perform centrifugation (at designated RCF and run time).
↓ Remove the supernatant.
↓ Dissolve the collected DNA in sterilized distilled water.
↓ Measure with the spectrophotometer at 260 nm.

Procedure 1. Ethanol precipitation of DNA

Ethanol precipitation was performed using the following instruments.
Centrifuge: CR-F/G series high-speed refrigerated centrifuge
Rotor: R6S swing rotor
Adapter: Adapter for 0.5-ml microtubes
Tube: Siliconized 0.5-ml microtubes

The samples were the bacteriophage λDNA (48.5 Kbp, produced by NIPPON GENE CO., LTD., hereinafter called "λ-DNA") and the λ-DNA that was cut by NIPPON GENE's restriction endonuclease Hind Ⅲ (up to 23 Kbp, hereinafter called "λ/Hind Ⅲ"). Ethanol precipitation was performed 5 times respectively for λ-DNA and λ/Hind Ⅲ, by putting about 5 µg of sample in each microtube and performing operation according to the procedure 1. The mean values are graphed in the following pages. The concentration of the collected DNA was obtained from the equation (1) below after the absorbance measurement at 260 nm.

DNA concentration (µg/µl) = OD_{260} x 0.05 ··· (1)

The samples were sequenced with ABI's Model 373 DNA sequencer and electrophoresed at 1,300 V, 40 W and 16 hours. Long Ranger Gel (6.75%) was used for electrophoresis.

3. Specifications of R6S swing rotor

(1) Max. speed: 5,700 rpm
(2) Max. RCF: 5,010 x g
(3) Dimensions: 398 mm in diameter and 207 mm in height
(4) Weight: 10.6 kg

Fig. 1 R6S swing rotor

4. Result and explanation

4-1. Collecting rate of λ-DNA (48.5 Kbp) in ethanol precipitation

We examined the effect of RCF on the collecting rate of λ-DNA in ethanol precipitation. λ-DNA collecting rate was measured at 620 x g (2,000 rpm), 1,390 x g (3,000 rpm) and 5,010 x g (5,700 rpm) for 10 minutes respectively. Fig. 2 shows the result of measurement. As shown in Fig. 2, λ-DNA is relatively large DNA (48.5 Kbp) and the collecting rate was high about 80% even though the centrifugation time was only 10 minutes and the RCF was 620 x g (this is the RCF of the conventional microplate rotors whose maximum speed is 2,000 rpm).

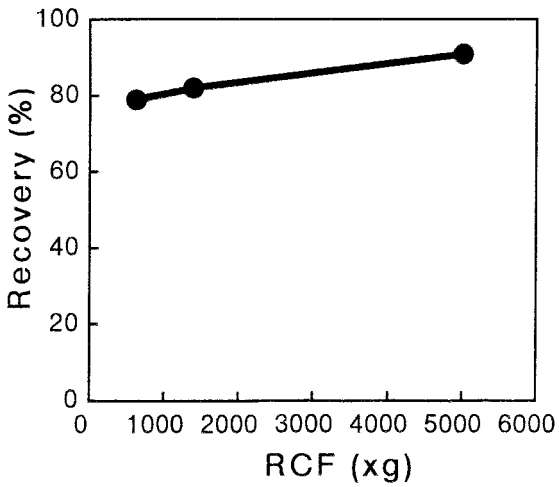


Fig. 2 Collecting rafe of λ-DNA in ethanol precipitation

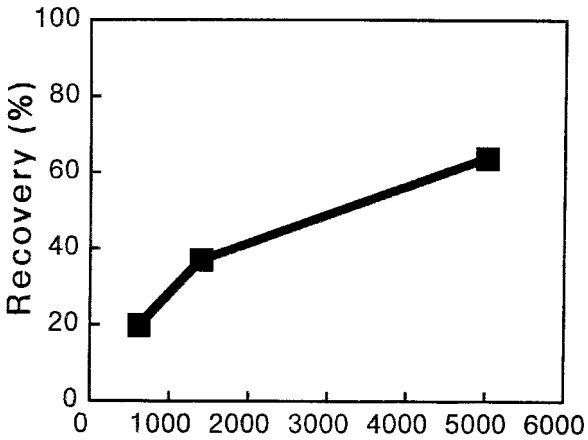
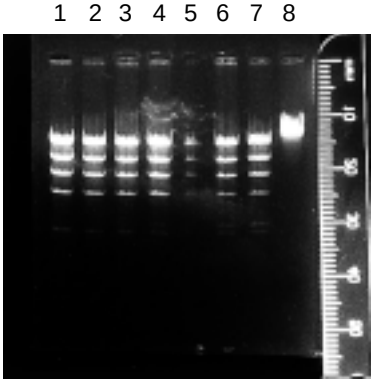


Fig. 3 Collecting rate of λ/Hind Ⅲ in ethanol precipitation

4-2. Collecting rate of λ/Hind Ⅲ (up to 23 Kbp) in ethanol precipitation

We performed the same examination as the case of λ-DNA using λ/Hind Ⅲ contained small DNA as shown in Fig. 3. The collecting rate of λ/Hind Ⅲ was low about 60% even though the RCF was 5,010 x g and the centrifugation time was 10 minutes. Difference in size of DNA exerted significant influence on ethanol precipitation. Fig. 4 shows the electrophoresed λ/Hind Ⅲ that were collected at each centrifugal condition. The lanes from 1 to 7 are equal (lane 5: improper sample loading) and there is no significant difference in the composition of the collected DNA (2 to 23 Kbp).



1, 2: 5,010 x g, 10 minutes
3, 4: 1,390 x g, 10 minutes
5, 6: 620 x g, 10 minutes
7: λ/Hind Ⅲ
8: λ-DNA

Fig. 4 Electrophoresed λ/Hind Ⅲ collected at each centrifugal condition

4-3. Relationship between centrifugation time and λ/Hind Ⅲ collecting rate at each RCF

We examined the relationship between the centrifugation time and the collecting rate at each RCF using λ/Hind Ⅲ (about 5 µg). For the examination at 620 x g and 5,010 x g, centrifugation was performed for 10 minutes, 20 minutes and 30 minutes respectively. For the examination at 1,390 x g, centrifugation was performed for 10 minutes. Fig. 5 shows the result of examination. It is evident that centrifugation at 5,010 x g for more than 20 minutes makes it possible to collect almost 100% of DNA contained in λ /Hind Ⅲ. For centrifugation at 620 x g for 20 minutes, the collecting rate is about 60%, and about 70% for 30 minutes. Even though the centrifugation time was elongated, high collecting rate could not be obtained (reached a plateau). As a consequence of the examination, proper centrifugal conditions are as follows:

RCF: about 5,010 x g

Centrifugation time: more than 20 minutes

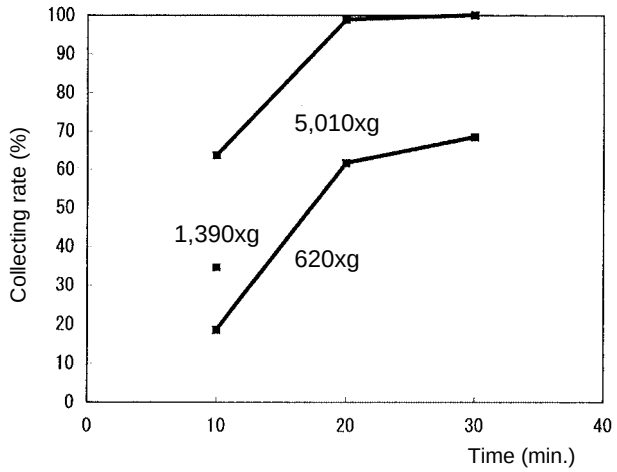


Fig. 5 Centrifugation time and collecting rate at each RCF

4-4. Result of sequencing operation

(1) Comparison between RCF and sequence result

As is evident from the result of examination described in 4-3, the collecting rate reached a plateau in about 20 minutes of centrifugation. We compared the result of sequencing operation using four kinds of templates (A: 4,096 bp, B: 6,776 bp, C: 4,271 bp, D: 3,883 bp). These templates were collected by ethanol precipitation at 5,010 x g, 1,390 x g and 620 x g for 20 minutes respectively and the number of base was about 3.9 to 6.8 Kbp. As an example, Figs. 6 through 8 show the results of sequencing the template A at 5,010 x g, 1,390 x g and 620 x g for 20 minutes respectively. There is no remarkable difference in the results of each sequencing operation, and the other templates show the same results. It means that a large amount of DNA was used in this experiment and the difference among the collecting rates did not give remarkable difference in the results of sequencing operation. The reading accuracy is 99.5 % in Fig. 6, 99.2 % in Fig. 7 and 99.0 % in Fig. 8. However, we noticed the number of base that could not be read in the sequencing operation for easier comparison. Fig. 9 shows the result. The number of base that could not be decoded in the sequencing operation tends to decrease with increasing RCF.

(2) Comparison between the centrifugation time and sequence result

We examined the influence of centrifugation time upon the result of sequencing operation by performing ethanol precipitation at 5,010 x g for 10, 20 and 30 minutes respectively. Four kinds of templates (A: 4,096 bp, D: 3,883 bp, E: 8,906 bp, F: 3,591 bp) having number of base between 3.6 and 8.9 Kbp were used. Because there is no remarkable difference in the results as in the case of the above (1), we noticed the number of base that could not be encoded in the sequencing operation. Fig. 10 shows the result.

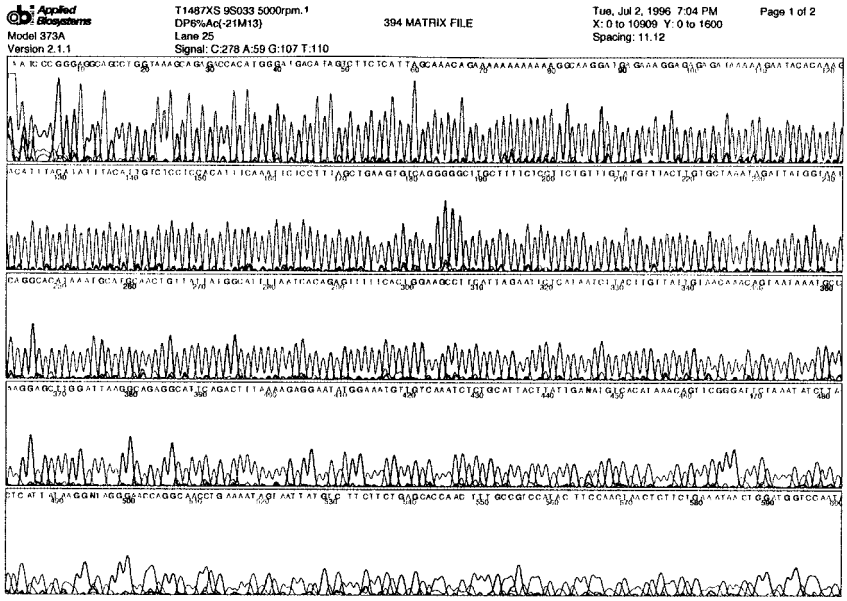


Fig. 6 Template A: Result of sequencing operation at 5,010 x g, 20 minutes

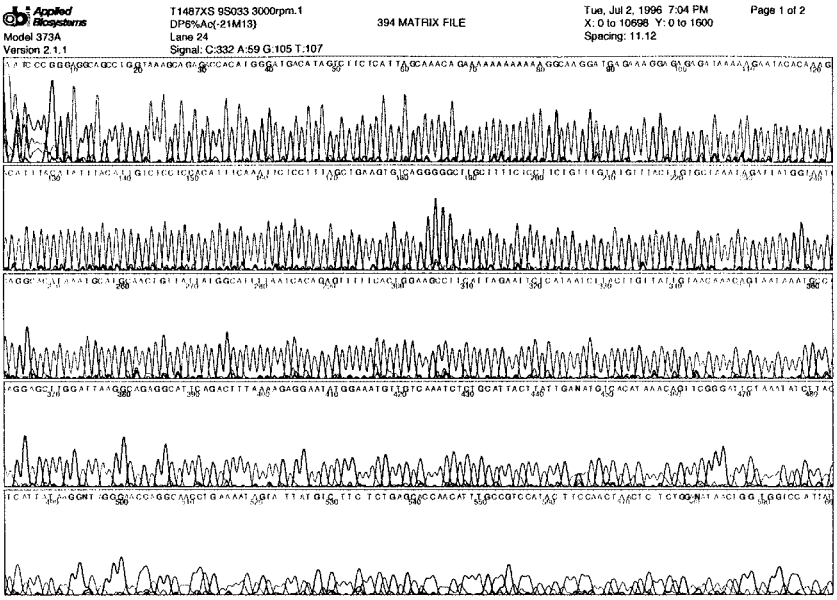


Fig. 7 Template A: Result of sequencing operation at 1,390 x g, 20 minutes

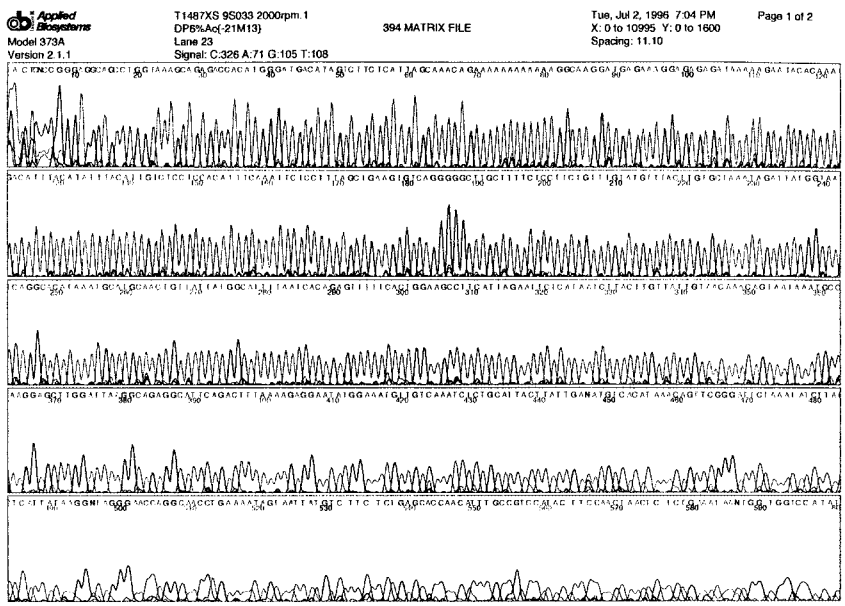


Fig. 8 Template A: Result of sequencing operation at 620 x g, 20 minutes

As is evident from Fig. 10, the number of base that could not be decoded in the sequencing operation tends to decrease with increasing centrifugation time. It means that 30-minute centrifugation is reliable even though the RCF is 5,010 x g. Especially, it is recommended to perform centrifugation at least 30 minutes for collection of small DNA.

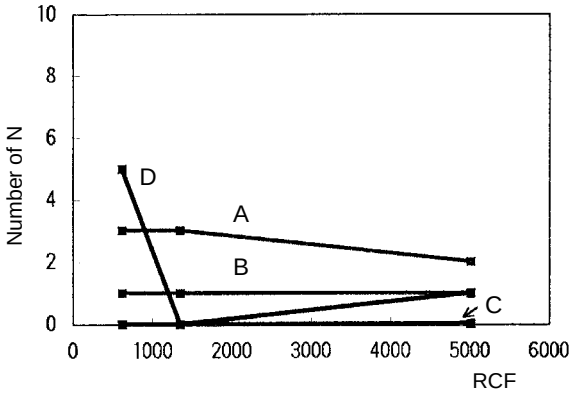


Fig. 9 Influence of RCF

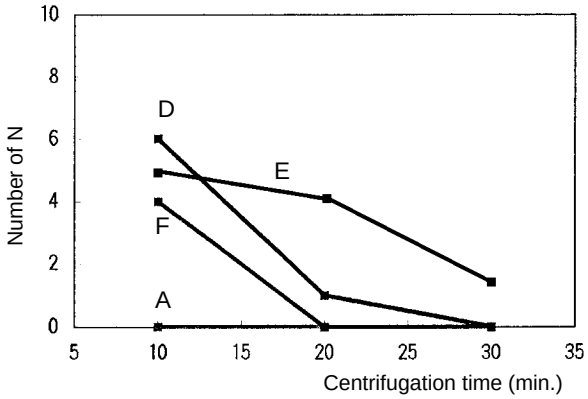


Fig. 10 Influence of centrifugation time

4-5. Relationship between the DNA content and collecting rate

We examined the relationship between the amount of DNA contained in the sample and the collecting rate using λ/Hind Ⅲ.

Both collecting rates in the case of using about 0.1 µg of λ/Hind Ⅲ and about 0.5 µg of λ/Hind Ⅲ were compared with the above-mentioned collecting rate in the case of using about 5 µg of λ/Hind Ⅲ. For the case of about 0.1 µg of λ/Hind Ⅲ, another experiment using Ethachinmate (Wako Pure Chemical Industries, Ltd.) as a coprecipitating agent was performed in parallel with the above experiment. Centrifugation was performed at 5,010 x g for 20 minutes. Fig. 11 shows the results. As is evident from Fig. 11, the collecting rate when the content of DNA is 0.1 µg is about 20 %, and about 30 % when the content of DNA is 0.5 µg. However, the collecting rate when the content of DNA is 0.1 µg is increased up to about 70 % thanks to the use of a coprecipitating agent. It means that the use of a coprecipitating agent is effective in the case of a low content of DNA about 1 to 2 µg or less.

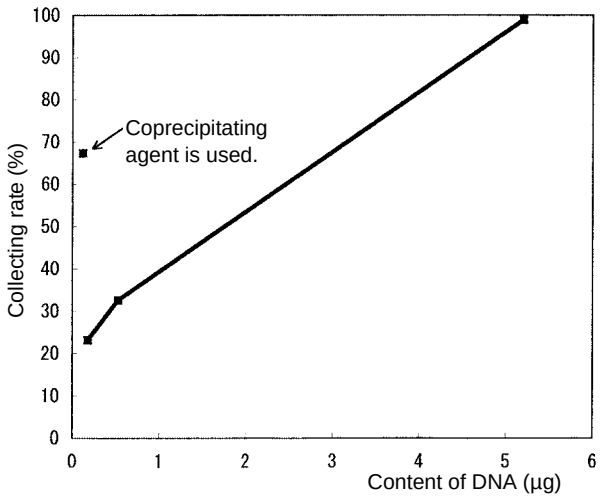


Fig. 11 Influence of content of DNA

5. Conclusion

Following are found from the above experiment.

(1) The R6S swing rotor that can accommodate the 96-hole microplates can be used for ethanol precipitation (DNA sequencing operation).

(2) Ethanol precipitation for DNA sequencing operation requires an RCF at least 1,390 x g (about 3,000 rpm).

(3) For reliable sequencing operation, centrifugation at 5,010 x g (5,700 rpm) for 20 minutes or more is required.

(4) A coprecipitating agent such as glycogen or Ethachinmate (Wako Pure Chemical Industries, Ltd.) should be used in the case of a low content of DNA about 2 µg or less.

(5) A coprecipitating agent is not necessarily required in the case of a content of DNA about 5 µg or more.

(6) Although the 0.5-ml microtubes were used in this experiment, the other microplates such as the ones made of ethanol-resistant polypropylene (PP), PCR plates and 0.2-ml microtubes for PCR may be usable.

This experiment was performed in cooperation with Dr. Katsuji Murakawa, Medical Systems Research Department, Hitachi Ltd. Central Research Laboratory. The result of the experiment was based on the report that was put out at the annual joint meeting of the Molecular Biology Society of Japan, the Japanese Biochemical Society in August 1996.

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