HITACHI

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Subject Purification of DNA sequencing samples after the cycle sequence operation using the R10H microplate rotor for high-speed refrigerated centrifuges

Model CR-F/G series high-speed refrigerated centrifuges

Purification of DNA sequencing samples using a 96-hole PCR plate and a microplate rotor having a maximum speed of 10,000 rpm

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The R6S swinging bucket rotor (maximum speed 5,700 rpm, maximum RCF 5,010 x g) was the highest-specification model among the existing microplate rotors that could be used in the high-speed refrigerated centrifuges until the R10H horizontal rotor (maximum speed 10,000 rpm, maximum RCF 13,000 x g) came along. The newly developed R10H horizontal rotor can process up to four microplates at a time and provide about 2.6 times greater RCF than that of the R6S swinging bucket rotor. DNA sequencing samples can be reliably purified after the cycle sequence operation by using the plates for small amounts of samples in the R10H horizontal rotor.

Operating procedure

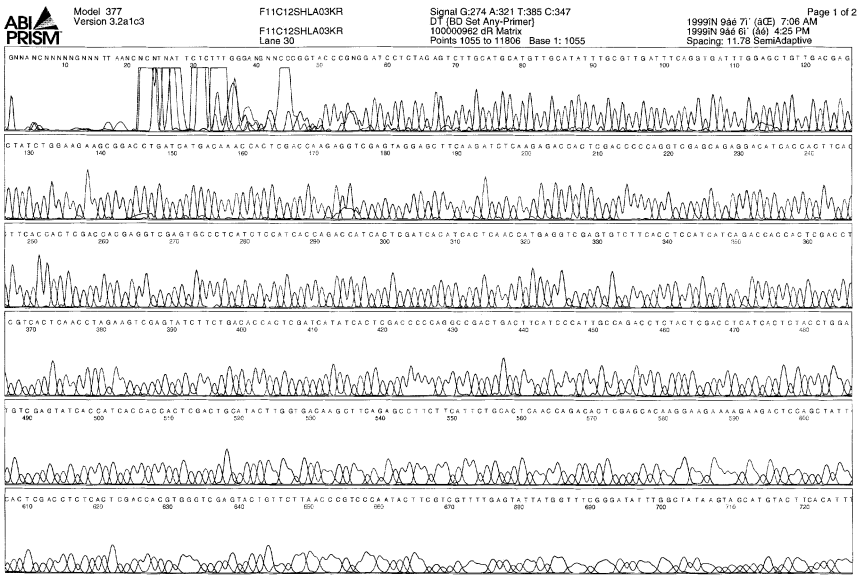
Perkin Elmer's 96-hole plate for PCR devices is used.
Adapter: "0.2 ml PCR Tube Rack" made by FUNAKOSHI
(Code number LP-0351-0x: The rack color is different depending on the last digit of "x" from 0 to 5.)

1. Put 20 µl of the sample after cycle sequence operation and 80 µl of 0.5 mM $MgCl_2$ in 80% ethanol in each well.
2. Adhere the PCR sheet.
3. Mix the solutions well by using a shaker.
4. Load the plate in the R10H rotor together with the adapter.
5. Perform centrifugation at 10,000 rpm, 10 minutes, 4˚C, ACCEL "9" and DECEL "9".
6. After centrifugation, remove the plate from the rotor together with the adapter.
7. Peel the PCR sheet off the plate and remove the supernatant as much as possible by decantation.
8. Put a paper (KIMTOWELS or equivalent) on the interior wall of the R10H rotor.
9. Load only the plate in the R10H rotor turning upside down.
10. Reset only the speed to 1,000 rpm and start centrifugation again under the same conditions as the above step 5 except the speed. Press STOP button as soon as the speed display indicates 300 rpm.
11. Remove the plate from the rotor.

Explanation

Since the centrifugal operation using 0.2 ml microplates handles small amounts of samples, a great loss may be caused if the RCF is insufficient. To cope with this problem, the new R10H rotor having the maximum speed 10,000 rpm and the maximum RCF 13,000 x g has been developed. After centrifugation, the supernatant can be easily removed just by loading the plate in the R10H rotor turned upside down and rotating the rotor at a low speed. Although a string of 12 or 8 pipets is required for pipeting reagents, supernatant can be easily removed by centrifugation. Washing with a 70% ethanol solution can also be performed in the same manner. A quick and inexpensive multi-sample processing method is essential to use a capillary-type sequencer featuring high throughput, and the R10H rotor can satisfy the demand. In addition, the R10H rotor is capable of using 384-hole plates whose capacity is smaller than the above plate. More effective operation is anticipated in this case.

The samples after cycle sequence operation were purified by using the R10H rotor and sequenced by using the model 377 DNA sequencer (made by Perkin Elmer). The data below shows the experiment results. (This data was provided by Dr. Hirokazu Kotani, Head of the Second Laboratory for Chromosome Gene Research, Kazusa DNA Research Institute.)



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