

# himac APPLICATION

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**Subject** Purification of a PCR product using a microplate with the R10H horizontal rotor for high-speed refrigerated centrifuges

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

Purification of a PCR product using a 384-hole microplate with the R10H horizontal rotor having the maximum speed 10,000 rpm

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 accommodate up to four microplates at a time and provide about 2.6 times greater RCF than that of the R6S swinging bucket rotor. Purification of a PCR product can be performed by centrifugal operation using a 384-hole microplate in the R10H horizontal rotor. The R10H horizontal rotor has high RCF and is suitable for operations using polyethylene glycol (PEG) having high purification capability as a precipitant because polyethylene glycol (PEG) makes the viscosity of the solution high. Polypropylene 96-hole microplates can be used with the R10H horizontal rotor in the same manner as the 384-hole microplates provided that FUNAKOSHI's 0.2 ml PCR Tube Rack is used as the adapter (Code No. LP-0351-0x: The rack color is different depending on the last digit of "x" from 0 to 5).

## Operating procedure

Perkin Elmer's 384-hole microplate for PCR devices (type 9700) is used.

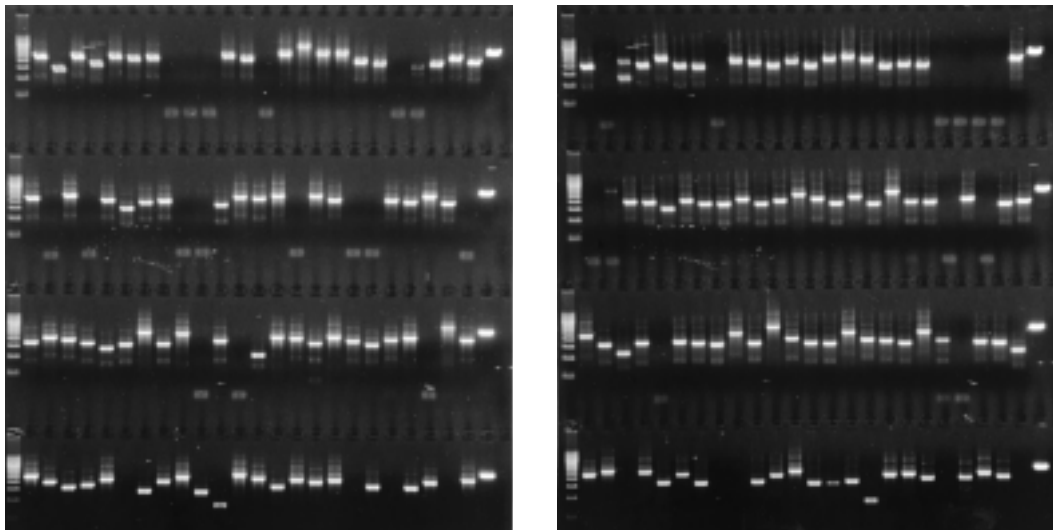
1. Put 10  $\mu$ l of PCR product and 10  $\mu$ l of precipitant \*1 in each well.
2. Adhere the PCR sheet.
3. Mix the solutions well by using a shaker.
4. Load the microplate in the R10H horizontal rotor.
5. Perform centrifugation at 10,000 rpm, 10 minutes, 4°C, ACCEL "9" and DECEL "9".
6. After centrifugation, remove the microplate from the rotor.
7. Put a paper (KIMTOWELS or equivalent) on the interior wall of the R10H horizontal rotor.
8. Peel the PCR sheet off the microplate and load the microplate in the R10H horizontal rotor again turning upside down.
9. 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.
10. Remove the microplate from the rotor.

\*1: Precipitant: 20% PEG 6000, 2.5M NaCl, isopropanol, etc.

## Explanation

Since the centrifugal operation using 384-hole microplates handles trace amounts of samples, a great loss may be caused if the RCF was insufficient. To cope with this problem, the new R10H horizontal rotor having the maximum speed 10,000 rpm and the maximum RCF 13,000 x g has been developed. The supernatant after centrifugation can be easily removed just by loading the microplate in the R10H horizontal 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 be performed in the same manner. A quick and inexpensive multi-sample processing method is essential to high-throughput operations using 384-hole microplates, and the R10H horizontal rotor can satisfy the demand.

The data below shows the results of PCR product purification using the R10H horizontal rotor and electrophoresis. (This data was provided by Dr. Hirokazu Kotani, Head of the Second Laboratory for Chromosome Gene Research, Kazusa DNA Research Institute.)



For further information, please contact Hitachi Koki Scientific Instruments Group.

# HITACHI

### (Export office)

Nissei Sangyo Co., Ltd.

Head Office :

1 - 24 - 14, Nishishinbashi, Minato-ku, Tokyo, 105 Japan

Tel : 81 - 3 - 3504 - 7281

Fax : 81 - 3 - 3504 - 7302

### (Manufacturer)

Hitachi Koki Co., Ltd.

Scientific Instruments Div.

1060, Takeda, Hitachinaka - city, Ibaraki - pref., 312 Japan

Tel : 81 - 29 - 276 - 7384

Fax : 81 - 29 - 276 - 7475