Application data sheet #04

Constant temperature incubator shaker DWMax M·BR-034P

DNA extraction from cultivated yeast by shaking with deep well plates

M·BR-034P is used for DNA extraction from cultivated yeast by shaking with deep well plates.

Overview

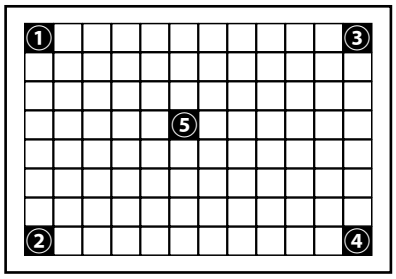
Thanks to the newly developed NewMax drive mechanism, the DWMax M·BR-034P has become a constant temperature incubator shaker equipped with a mixing capability by shaking, as well as uniformity and stability that exceeds the Maximizer M·BR-022UP, a similar product that is also currently sold. This Product can uniformly and strongly mix micro tubes and a 96-hole deep well. It is capable of exerting culture efficiency that is sufficient for microorganisms having a large cell body that tend to sink like yeast in a deep well which is generally unsuitable for shake-cultivating.
This document reports on the results of the following experiment. M·BR-034P was used to cultivate yeast (Saccharomyces cerevisiae S288C) in a deep well. A DNA extraction kit (Nippon Gene) was then used to extract the DNA from cultivated yeast. As a result, we were able to sufficiently collect the DNA as compared to a culture using a test-tube type culture tube.

Equipment used, reagents, etc.

- Constant temperature incubator shaker: DWMax M·BR-034P (TAITEC), BioShaker BR-23FP·MR (TAITEC)
- Deep well: 96-well deep well plate 2.0 mL, rectangular hole (AxyGen Scientific)
- Reagent kit that can extract DNA from yeast: ISOPLANT (Nippon Gene)
- Yeast: *S. cerevisiae* S288C
- Culture medium: YPD
- Centrifugal machine: Can support 12,000g (for a micro tube)
- Test tube mixer (Vortex): Delta mixer Se-04 (TAITEC)
- Reagent and equipment for agarose gel electrophoresis; ultraviolet visible spectrophotometric

Method

1) Shake-cultivation of yeast using a deep well

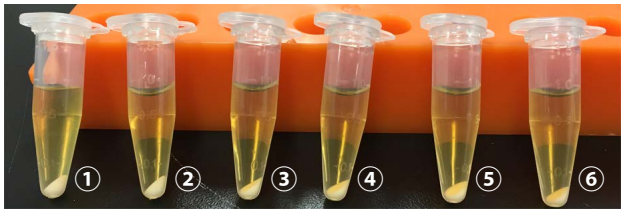


2mL of a culture medium inoculated with yeast was dispensed to positions 1 to 5 in the deep well as shown in the illustration on the left, and then shake-cultivated with the DWMax M·BR-034P (1,500 r/min., +30° C, 24 hours).

As a subject for comparison, 2 mL of an inoculated culture medium was dispensed in a 12 mL disposable culture tube and was then shake-cultivated with a BioShaker BR-23FP·MR (reciprocal 180 r/min., +30° C, 24 hours). This is referred to as (6).

2) DNA was extracted in accordance with the ISOPLANT protocol.

900 μL was taken from the above culture solutions (1) to (6), each of which was put into a micro tube and centrifuged, with supernatant removed.



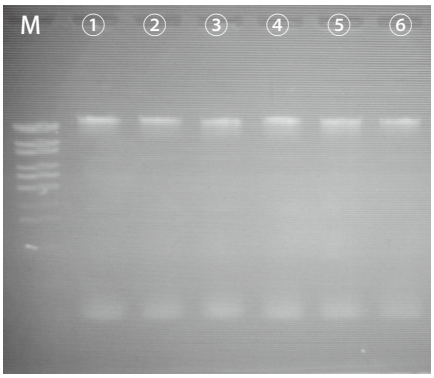
Centrifuging (1) to (6). With visual inspection, the amounts of precipitated fungi were almost the same.

↓
Each was added with Solution I, and then vortexed.
↓
Each was added with Solution II, and then vortexed.
↓
Microtubes (1) to (6) were statically placed in the M·BR-034P and incubated at 50° C for 25 minutes.
(Protocol specifies 15 minutes for incubation, but as the M·BR-034P in a gas phase was used, incubation was performed for a little longer. Light inverted agitation by hand every five minutes.)
↓
Each was added with Solution III, and then vortexed.
↓
Each was incubated in ice for 15 minutes.
↓
Each was centrifuged at 12,000g for 10 minutes.
↓
The water phase of each was taken, added with two times that amount of ethanol, and properly mixed and centrifuged at 12,000g for 10 minutes.
↓
After removing supernatant, sediments were cleansed with 70% ethanol and air-dried.
↓
The sediment was dissolved into 30 μL TE as a DNA solution, while purity and yield were checked by spectrophotometry and electrophoresis.

Results and discussion

The yield, purity, and results of the electrophoresis of the extracted DNA are shown below. Compared to yeast cultivated in a culture tube (6), we were able to favorably collect DNA from yeast cultivated in a deep well (1) to (5). In each case, there was a good yield of 1.3 to 2 μg/mg.

	Yield (μg/mg)	A260/280
① (Cultivated in a deep well with M·BR-034P)	1.345	1.855
② (〃)	2.020	1.906
③ (〃)	1.475	1.916
④ (〃)	1.735	1.896
⑤ (〃)	1.470	1.909
⑥ (Cultivated in a culture tube with BR-23FP)	1.515	1.930



1% Agarose S electrophoresis ▶
M : Marker 6 (λ /*Sty* I digest, Nippon Gene)

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Publication: 2017
For inquiries about the constant incubator shaker DWMax M·BR-034P and the contents of this leaflet, please contact us as provided on the left.