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Magnetic Bead Based Isolation of DNA from Microbial Cultures using the ep*Motion*® and SwiftMag™ Technology

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Abstract

There is a growing need for rapid, high-throughput methods that enable isolation of high quality, pure DNA from microbial cultures as well as samples containing PCR inhibitors, such as swabs and cultured foods. While filter plate-based, high-throughput purification of DNA from microbial cultures has been previously automated on Eppendorf ep*Motion* 5075 VAC workstation, magnetic bead technology brings higher level of automation. This Application Note describes the successful automation of DNA isolation from these sample types with magnetic bead-based technology from MO BIO Laboratories.

Introduction

MO BIO® Laboratories has developed a magnetic bead-based purification method that enables walk-away purification on the ep*Motion* 5075 TMX automated pipetting system. The new PowerMag™ Microbial DNA Isolation kit employs SwiftMag magnetic bead technology, which does not require chaotropic salts for binding nucleic acids to beads, thus providing a higher purity of DNA than comparative methods. Using mechanical force in the presence of an enhanced microbial lysis buffer, cells are efficiently broken using a 96 Well Plate Shaker and then centrifuged to remove debris. Inhibitor Removal Technology® (IRT) is provided to remove PCR inhibitors, including lipids and polysaccharides. The addition of IRT ensures isolation of pure DNA even from difficult bacterial samples such as skin, fecal or vaginal swabs, and food cultures of red pepper, chocolate, or coffee.

The Eppendorf ep*Motion* 5075 TMX is an open system that is designed to automate many procedures, including the magnetic bead-based nucleic acid purification. Every system comes with 12 deck spaces for virtually unlimited combination of consumables. Six pipetting tools are available to cover the range of 1-1000 µL with high accuracy and precision. In addition, a thermomixer is integrated on the TMX position, combining high-speed mixing and incubating in one spot. All these features make optimization of the MO BIO PowerMag Microbial DNA Isolation Kit on the epMotion an easy task.

Here we describe the successful purification and analysis of DNA from microbial and food cultures purified using the PowerMag Microbial DNA Isolation Kit on the ep*Motion* 5075 TMX.

Materials and Methods

Pure Microbial Cultures

E. faecalis cultures were grown over night and 1.8 mL aliquots were dispensed into a 2 mL Collection Plate. Following centrifugation and removal of supernatant, cells were resuspended in 350 µL of MicroBead Lysis Solution/ RNase A and were transferred into the PowerMag Bead Plate. Cell lysis was performed in a 96-well plate shaker. The optional Inhibitor Removal Technology step was omitted. The remaining purification steps were performed on the Eppendorf ep*Motion* 5075 TMX. Sample quality and yield were evaluated using a NanoDrop® 2000 Spectrophotometer and by running 5 µL of each sample on a 1.2 % TAE agarose gel.

Food Cultures

Listeria monocytogenes was grown overnight in a 10 % ground beef (22 % fat) or in dark chocolate (86 % cacoa) culture. 1.8 mL of culture was used for DNA isolation. Samples were purified using either the PowerFood™ Microbial DNA Isolation Kit (silica spin filter method) or the PowerMag Microbial DNA Isolation Kit with the optional Inhibitor Removal Technology step included. RNase A was used in all samples.

Automated Procedures

Lysed samples were stored in a 2 mL deep well plate then transferred to the worktable of the epMotion, which is prepared as shown in Figure 1 and Table 1. The following DNA binding, bead washing and elution steps were performed automatically on the workstation. Microbial DNA was eluted in 100 µL of elution buffer and subjected to subsequent analyses by electrophoresis and spectrophotometry.

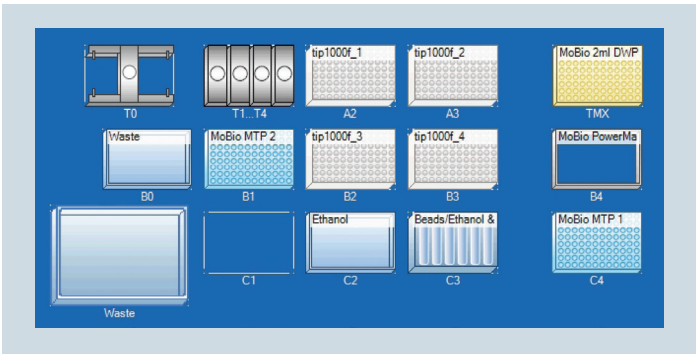

Figure 1: Screenshot of the epBlue™ software showing the setup of the ep*Motion* 5075 TMX worktable for use with MO BIO PowerMag Microbial DNA Isolation Kit.

Table 1: ep*Motion* 5075 TMX worktable details for the PowerMag Microbial DNA Isolation Kit.

Position	Labware	Comment
A2	epT.I.P.S.® *Motion* 1,000 µL, filtered	
A3	epT.I.P.S.® *Motion* 1,000 µL, filtered	
TMX	MO BIO® 2 mL Deep Well Plate (MoBio_DWP)	Sample lysate plate
B0	Reservoir 400 mL	Waste collector
B1	MO BIO® MTP 2 (MoBio_MTP)	Elute collection plate
B2	epT.I.P.S.® *Motion* 1,000 µL, filtered	
B3	epT.I.P.S.® *Motion* 1,000 µL, filtered	
B4	MO BIO® PowerMag Magnetic Separator (MOBIO_POWERMAG_Magnet)	
C2	Reservoir 400 mL	Wash buffer
C3	Beads/Bind & Elution Reservoir Rack (MoBio_PowerMag) Position 1: Beads/Bind Position 3: Elution	 100 mL tub 30 mL tub
C4	MO BIO® MTP 1 (MoBio_MTP)	
T0	Gripper	
T1	TM1000-8	

Results

Pure Microbial Cultures

When performing high-throughput purification, it is essential to achieve consistent, reproducible results with respect to DNA quality and yield. Here, we examined DNA isolated from eight replicates of overnight *E. faecalis* culture using the PowerMag Microbial DNA Isolation Kit on the Eppendorf ep*Motion* 5075 TMX. Gel electrophoresis analysis demonstrated high quality, high molecular weight DNA in all samples (Figure 2).

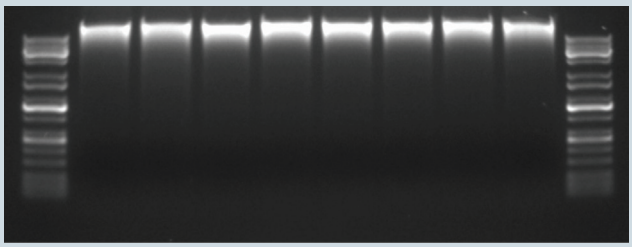

Figure 2: DNA isolated from 1.8 mL of *E. faecalis* culture using the PowerMag Microbial DNA Isolation Kit on the ep*Motion* 5075 TMX. High quality, high molecular weight DNA was observed in eight replicate samples examined on a 1.2% agarose gel. No differences in yield or quality were observed between the replicate samples.

DNA yield and purity were examined using a spectrophotometer, revealing consistent yields ranging from 50.3 – 54.4 µg, with $A_{260/280}$ ratios ranging from 1.83 – 1.86 (Table 2).

Table 2: Consistent yields of pure DNA isolated from *E. faecalis* culture using the PowerMag Microbial DNA Isolation Kit on the Eppendorf ep*Motion* 5075 TMX.

Sample	Concentration (ng/µL)	$A_{260/280}$	Yield (µg)
1	512.90	1.86	51.3
2	517.25	1.86	51.7
3	502.84	1.86	50.3
4	513.44	1.85	51.3
5	506.53	1.86	50.7
6	529.14	1.86	52.9
7	543.52	1.84	54.4
8	528.71	1.83	52.9

Food Cultures

Inhibition of downstream reactions due to co-purification of contaminating substances, such as lipids and polysaccharides, is a major obstacle when isolating DNA from cultured food and swabs. To overcome this problem, we included patented Inhibitor Removal Technology as an optional step in the PowerMag Microbial DNA Isolation Kit. Here, we cultured *Listeria monocytogenes 148* in 10 % ground beef (22 % fat)/TSB and 86 % dark chocolate (10 g/90 mL TSB). Starting with 1.8 mL of each culture, DNA isolation was performed using ether the PowerFood Microbial DNA Isolation Kit (silica spin filter technology) or the PowerMag Microbial DNA Isolation Kit (SwiftMag technology) with the optional Inhibitor Removal Technology step. Samples were analyzed via gel electrophoresis, and no difference in DNA quality or molecular weight was observed between DNA isolated using the silica spin filter and the SwiftMag technology.

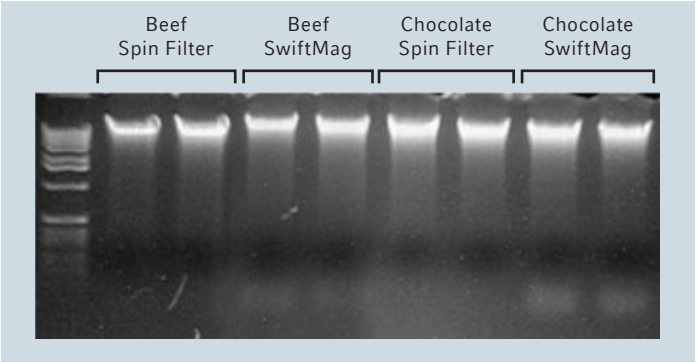

Figure 3: *Listeria monocytogenes* was grown overnight in a 10 % ground beef (22 % fat) or in dark chocolate (86 % cocoa) culture. 1.8 mL of culture was used for DNA isolation with the PowerFood Microbial DNA Isolation Kit (silica spin filter method) or the PowerMag Microbial DNA Isolation Kit. Samples were examined on a 1 % agarose gel and no difference was observed between DNA isolated using either method.

DNA yield and purity were next examined using a NanoDrop 2000 spectrophotometer. Interestingly, we observed increased average yields in samples isolated using SwiftMag technology versus samples isolated using spin filters.

Table 3: Increased average yields of pure DNA isolated *Listeria monocytogenes 148* using the PowerMag Microbial DNA Isolation Kit (SwiftMag technology) compared with the PowerFood Microbial DNA Isolation Kit (silica spin filter technology).

No.	Sample	Isolation Method	Average Conc. (ng/µL)	Average $A_{260/280}$	Average Yield (µg)
1	Beef	Silica spin filter	81.00	1.83	8.1
3	Beef	SwiftMag	126.86	2.02	12.9
5	Chocolate	Silica spin filter	70.18	1.86	7.0
7	Chocolate	SwiftMag	177.74	1.85	17.8

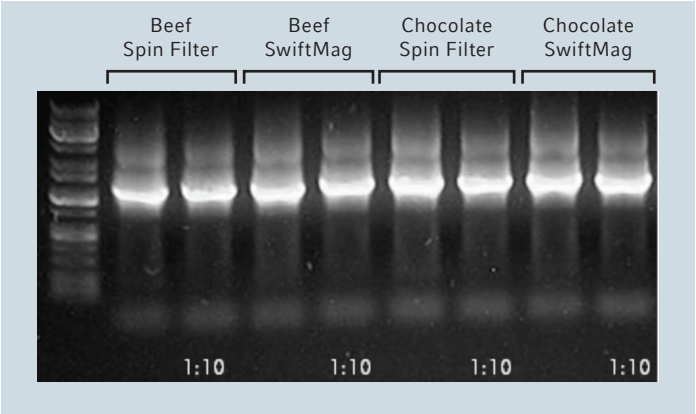

Figure 4: 16S rDNA universal primers were used with the Kapa2G Fast HotStart ReadyMix for endpoint PCR. 1 µL of each sample described in Figure 3 was used along with a 1:10 dilution to check for amplification inhibition. All samples amplified successfully.

Summary

The PowerMag Microbial DNA Isolation Kit is the first successful method of magnetic bead-based automated DNA purification for microbial cultures, food cultures and swabs. A combination of Inhibitor Removal Technology and the SwiftMag magnetic bead technology enables DNA from pure microbial cultures, swabs and food cultures known to be high in PCR inhibitors to be purified on the Eppendorf ep*Motion* 5075 TMX automated pipetting system. Here, we have shown that consistent yields of high quality DNA can be obtained from pure cultures of *E. faecalis*. Additionally, we cultured *Listeria monocytogenes* overnight in a 10% ground beef or in dark chocolate broth and demonstrated that high quality, inhibitor-free DNA can be isolated, followed by successful PCR amplification. Although a limited number of samples were tested, this study demontrated that the Eppendorf ep*Motion* 5075 TMX is fully compatible to the SwiftMag technology. Equipped with 12 deck spaces for additional tips and other consumables as well as 8-channel pipettes for increased throughput, this method can be easily scaled up to process up to 96 samples in a single run, allowing better use of your valuable time while high-quality results can still be ensured.

Ordering information

Description	Order no. international	Order no. North America
Eppendorf		
ep*Motion*® 5075 TMX PC complete with gripper		960020555
Dispensing tool TM 1000-8, 40-1,000 µL	5280 000.258	960001061
Reservoir Rack	5075 754.002	960002148
Reservoirs 30 mL	0030 126.505	960051009
Reservoirs 100 mL	0030 126.513	960051017
400 mL reservoir, set of 10	5075 751364	5075751364
epT.I.P.S.® *Motion* 40–1,000 µL PF	0030 003.667	960050088P
MO BIO®		
PowerMag™ Microbial DNA Isolation Kit		27200-4
PowerMag™ Magnetic Separator		27400

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