Mini centrifuges

Isolating environmental DNA in the field using Thermo Scientific™ mySpin™ 6 Portable Centrifuge Kit

Keywords
mySpin, portable centrifuge, environmental DNA, on-site DNA isolation, field analysis, environmental research

Introduction

Analysis of environmental DNA (eDNA) has many applications in environmental research from the non-invasive study of endangered species to faecal pollution source tracking in rivers (Werner et al., 2022). Environmental researchers are increasingly using field-deployable tools for eDNA analysis, to obtain results at the sampling site and in near real time, via portable next generation sequencing (Acharya et al., 2020) and quantitative polymerase chain reaction (Zan et al., 2022). Field analysis enables on the spot decision making and adaptive sampling strategies. As a first step in the eDNA workflow, genetic material is extracted from environmental samples and purified for subsequent molecular analysis. This application note describes and evaluates the extraction of eDNA from sediment in the boot of a car, using the Thermo Scientific mySPIN 6 portable centrifuge.

Methodology

Table 1. Equipment and consumables

Equipment	
mySPIN 6 Portable Kit Complete	mySpin 6 centrifuge (Thermo Scientific) with a 12V car adaptor and 16 space tube rack (Cat. No. 75004085)
Hand-held bead milling instrument for sample disruption/homogenization	SuperFastPrep-2™ Sample Preparation Instrument (MP Biomedicals)
Pipettes	Adjustable volume, single channel, P200, P1000 (any brand)
Reagents and consumables	
50 mL conical centrifuge tubes	Polypropylene, sterilized (any brand)
DNA extraction kit	DNeasy™ PowerSoil kit 12888-50 (Qiagen)
Pipette tips	Matching P200, P1000
1.5/2 mL microcentrifuge tubes	
Nitrile gloves	

Sample upstream and shaking → eDNA extraction with centrifugation → Freeze DNA samples → DNA extraction and dry samples → Real-time PCR

Workflow

1. Sediment from a shallow river in northeast England was sampled upstream and near the exit of a combined sewer overflow (CSO) and placed into sterilized 50 mL conical centrifuge tubes. The vials were capped, and sediment was homogenized by shaking.
2. eDNA was extracted onsite in the boot of a car from triplicate 0.5 mL subsamples of sediment using the equipment and consumables items listed in **Table 1** according to the manufacturer instructions. First, the hand-held bead milling instrument was used for the sample homogenization and cell shearing (**Figure 1a**). Then, the Thermo Scientific mySpin 6 portable centrifuge kit was connected to the 12V car lighter socket and used for the spin-downs during the eDNA extraction at 2,000 xg (**Figure 1b**).
3. At the end of the fieldwork day, sediment and DNA samples were frozen at -20 °C.
4. For comparison, DNA was extracted in the laboratory from triplicate sub samples of thawed sediment using conventional procedures with a laboratory bead milling instrument and high-speed Thermo Scientific™ Fresco™ 17 Microcentrifuge. The water content of the sediment samples was determined by oven drying.
5. DNA yields were quantified with a fluorometric method, DNA quality was assessed with a spectrometric method, and real-time PCR was conducted to quantify from each DNA extract 16S rRNA and HF183 genetic markers for total bacteria and human-host associated Bacteroides, as previously described (Zan et al., 2022).

Figure 1 a) Sample homogenization and cell shearing onsite with a hand-held bead milling instrument,
Figure 1 b) DNA extraction from sediment with Thermo Scientific mySpin 6 portable centrifuge in the boot of a car.

Results

Table 2.

eDNA yield and quality, 16S rRNA and HF183 genetic marker concentrations, avg±stdev of triplicates, comparing onsite with laboratory eDNA extraction methods. 2-way ANOVA was used to assess the effects of factors sampling site and DNA extraction method on the mean value of results. Significant effects are illustrated by p-values < 0.05 in italics.

		eDNA yield (ug/g dry sediment)	eDNA quality		Log_{10} 16S rRNA (genes copies per g dry sediment)	Log_{10} HF183 (genetic marker copies per g dry sediment)
			(260/280)	(260/230)		
Field	Upstream	16.7±3.0	1.9±0.1	1.3±0.1	9.6±0.0	3.8±0.4
	CSO exit	31.6±8.8	1.9±0.0	1.5±0.1	10.7±0.3	5.1±0.3
Laboratory	Upstream	24.0±1.1	1.9±0.0	2.1±0.0	10.6±0.2	3.8±0.4
	CSO exit	56.0±5.9	1.9±0.0	1.9±0.1	11.1±0.2	5.4±0.0
Two-Way ANOVA p-values	Site	*0.0001*	0.2503	0.4126	*0.0001*	*0.0001*
	Method	*0.0011*	0.4158	*0.0000*	*0.0005*	0.3610
	Interaction	*0.0278*	0.9264	*0.0064*	*0.0277*	0.3709

High amounts of eDNA > 10 ug/g of sediment dry weight were obtained with both field and laboratory extraction methods. Despite the difference between methods noted above, the observed patterns between two sampling sites and two genetic markers were consistent when comparing the field and laboratory eDNA extraction methods **(Figure 2)**.

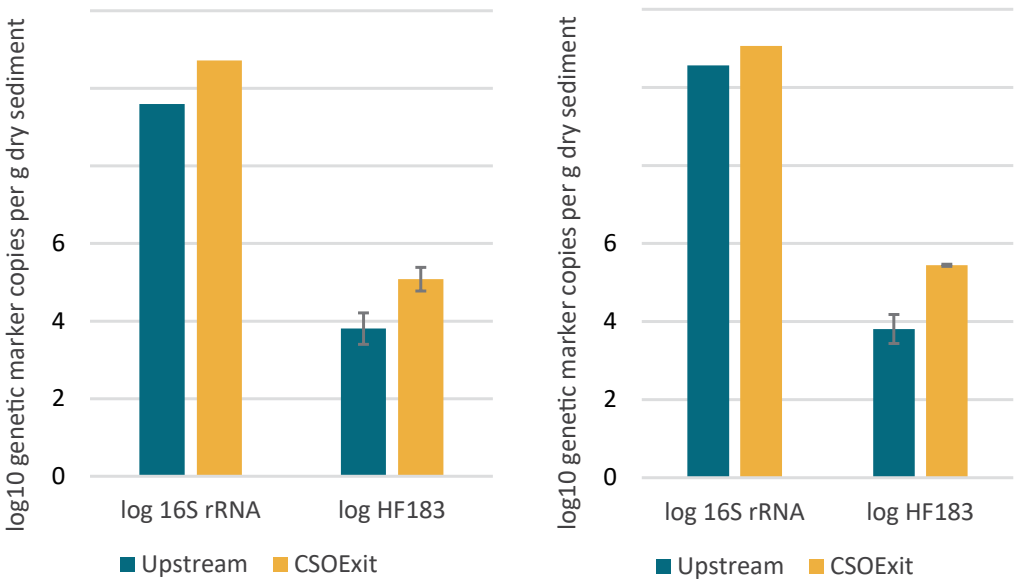

Figure 2 Concentrations of genetic markers in sediment at two sites comparing a) onsite and b) laboratory DNA extraction methods.

Discussion

The eDNA yield of the field extraction method proved adequate for subsequent molecular analysis by a previously described qPCR method for microbial source tracking (Zan et al., 2022). The results exhibit a close performance between field extraction and conventional laboratory techniques.

The decreased eDNA yields observed in the field can be attributed to the differences in efficiencies of handheld and bench-top homogenizers between field and traditional laboratory environments. This presents an exciting avenue for innovation and adaptation of field equipment to enhance efficiency and yield.

Notably, the ratios 260/280 for both field and laboratory-extracted eDNA aligned well with the expected value of 1.8, indicating minimal protein contaminants. Although the ratios 260/230 for field-extracted DNA fell below the anticipated range for pure DNA, suggesting the presence of absorbing contaminants at 230 nm, which did not significantly impact subsequent analyses.

Despite the modestly lower eDNA yield and slightly diminished DNA quality obtained with the field method, our findings demonstrate promising consistency when comparing sediment concentrations of two genetic markers for total and human host-associated bacteria at different sampling sites.

Conclusions

The Thermo Scientific mySpin 6 portable centrifuge kit offers invaluable convenience in field operations, facilitated by its transparent transport box featuring protective inserts for both the centrifuge and its accessories. Moreover, equipped with a 12 V car adapter, the mySpin 6 centrifuge can be effortlessly powered in diverse field settings, even in remote locations such as:

- **Large animal and livestock testing**
- **Student field work, remote field stations, marine biology**
- **Forensic work and emergency response**
- **Wastewater checks and water pollution**
- **Soil analysis and milk collect testing**

Our study underscores the remarkable potential of environmental DNA extraction from sediment under field conditions, affirming its viability for subsequent molecular analysis. The ability to extract sufficient quantity and quality of eDNA directly from the field marks a significant advancement in environmental monitoring and analysis techniques. This opens up exciting possibilities for expedited and cost-effective research in diverse ecological settings.

References:

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