Optimisation of 16S rRNA gut microbiota profiling of extremely low birth weight infants

Feces

CASE STUDY

Alcon-Giner, C.; Caim, S.; Mitra, S.; Ketskemety, J.; Wegmann, U.; Wain, J.; Belteki G.; Clarke P.; Hall, L. J. Optimisation of 16S rRNA gut microbiota profiling of extremely low birth weight infants. **2017**, *18*, 841-856.

Introduction

Infants born prematurely, particularly extremely low birth weight infants (ELBW), have altered gut microbial communities.

There is a requirement to optimally characterise microbial profiles in this at-risk cohort, via standardization of methods, particularly for studying the influence of microbiota therapies (e.g. probiotic supplementation) on community profiles and health outcomes. Profiling of faecal samples using the 16S rRNA gene is a cost-efficient method for large-scale clinical studies to gain insights into the gut microbiota and additionally allows characterisation of cohorts where sample quantities are compromised.

To this end, the bacterial DNA extraction protocol from ELBW infant faeces was optimized by testing three different DNA extraction methods.

Overview

- Keywords: ELBW faecal sample, microbiota, 16S rRNA gene sequencing, shotgun sequencing, taxonomic profiles
- Aim of the study: Optimize a 16S rRNA profiling protocol to allow standardization for studying ELBW infant faecal samples, with or without probiotic supplementation.
- Application: Preparation of a 16S rRNA gene library sequenced on the Illumina MiSeq platform.
- Sample name: ELBW infant faeces
- Sample type: Feces
- Material: FastDNA™ SPIN Kit for Soil (method 1), QIAmp® DNA Stool Mini Kit (method 2), enzymatic lysis followed by the use of the QIAmp® DNA Stool Mini Kit (method 3).
- Buffer: Buffers included in the kits for methods 1 and 2. For method 3, enzymatic mix: 50 mM Tris-HCl, pH 8.0, 10 mM $MgSO_4$, 5 mg/mL lysozyme and 50 U/mL mutanolysin

Protocol and Parameters

Faecal samples were collected in a stool container and stored at 4 °C. DNA was extracted within 4 hours of collection. Optimization of bacterial DNA extraction was performed on faecal samples from two ELBW infants (with/without supplementation) and one term infant sample.

Three DNA extraction protocols involving two different kits were compared to determine the most appropriate method for extracting bacterial DNA:

1. FastDNA™ SPIN Kit for Soil (MP Bio) following the manual's instructions and extending the bead-beating step to 3 min
2. QIAmp DNA Stool Mini Kit (Qiagen) following the manual's instructions
3. QIAmp DNA Stool Mini Kit (Qiagen) including an initial enzymatic lysis step of 1 h at 37 °C (enzymatic mix: 50 mM Tris-HCl, pH 8.0, 10 mM $MgSO_4$, 5 mg/mL lysozyme and 50 U/mL mutanolysin).

The DNA recovered from these samples was assessed using a Qubit 2.0 fluorometer and the concentration was normalized to 5 ng/mL prior to being used for preparing 16S rRNA Illumina MiSeq sequencing libraries.

CASE STUDY

Results

The DNA recovery optimization procedure indicates that the FastDNA™ SPIN Kit for Soil was the most effective method for extracting bacterial DNA from ELBW infant faeces.

Importantly, this methodology included a bead-beating step, which has been previously shown to improve the quality and quantity of the isolated DNA, potentially via disruption of cell membrane components (including cells walls and capsules). This finding has now been expanded to include ELBW faecal samples.

Furthermore, this method obtained higher DNA yields from all samples, particularly *Bifidobacterium* supplemented ELBW and *Bifidobacterium*-rich term infants after extension of the bead-beating time to 3 min. Indeed, it was only with the FastDNA™ SPIN Kit for Soil DNA extraction protocol that sufficient yields were obtained from. All DNA samples for subsequent sample sequencing, all other methods provided inadequate quantities and could therefore not be utilized in further downstream experiments.

DNA yields (ng/µL) from different extraction methods

Sample	Extraction Method	Qubit (ng/µL)
ELBW infant no probiotics (AP10B)	FastDNA™ SPIN Kit for Soil (3 min bead beating)	2.25
	FastDNA™ SPIN Kit for Soil (30 s bead beating)	1.97
	QIAamp DNA Stool Mini Kit	<0.0005
	Enzymatic lysis and QIAamp DNA Stool Mini Kit	0.0146
ELBW infant with probiotics (P66F)	FastDNA™ SPIN Kit for Soil (3 min bead beating)	13.8
	FastDNA™ SPIN Kit for Soil (30 s bead beating)	7.38
	QIAamp DNA Stool Mini Kit	<0.0005
	Enzymatic lysis and QIAamp DNA Stool Mini Kit	0.0156
Term Baby (V3ZC)	FastDNA™ SPIN Kit for Soil (3 min bead beating)	21.4
	FastDNA™ SPIN Kit for Soil (30 s bead beating)	7.7
	QIAamp DNA Stool Mini Kit	0.0164
	Enzymatic lysis and QIAamp DNA Stool Mini Kit	0.77

Conclusion

This study highlights that the FastDNA™ SPIN Kit for Soil is an optimal DNA extraction method for 16S rRNA microbiota profiling, which is now considered a gold standard by many research teams.

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