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Application Note

MolYsis™ Host DNA Depletion for Deeper Microbiome and Metagenome Analysis

Keywords: *Sample pretreatment, host DNA depletion, microbial DNA isolation, DNA-free reagents, next generation sequencing, liquid and tissue biopsies, automation*

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Abstract: In many samples from infected humans and animals host DNA greatly outnumbers microbial DNA. During sample preparation, unspecific primer binding to host DNA decreases the power of resolution of microbiomes and metagenomes. The MolYsis™ technology provides a solution to this problem by depletion of host DNA before extraction of microbial DNA. The *MolYsis™ Basic* kits can be combined with commercial or in-house DNA purification procedures to analyze fluid samples. The *MolYsis™ Complete* kits go through the entire process of host DNA depletion and microbial DNA extraction and purification. Tissue samples (and fluid samples) are processed by *Ultra-Deep Microbiome Prep* and automated *MolYsis-SelectNA™plus*, the latter being a fully automated solution. This application note summarizes the experiences with the *MolYsis™* technology in combination with major NGS systems as a means of marked increase of microbial reads and hence deeper analysis of microbiomes.

Introduction

Infected body sites generally contain low concentrations of bacteria and fungi [1]. On the other hand, host DNA can exceed microbial DNA by several orders of magnitude [2]. Therefore, the great majority of sequencing reads comes from host DNA and thus limits the depth of analysis of microbial sequences.

MolYsis™ is a technology by which samples are depleted from host DNA prior to DNA extraction. Host cells of fluids and tissues are subjected to lysis by a chaotropic buffer and the released host DNA is degraded by a DNase. During this treatment, microorganisms stay intact because of their robust cell wall. The following extraction and bind-wash-elute isolation processes provide pure microbial DNA for NGS analysis. Notably, cell-free microbial DNA is degraded together with the host DNA. As a result, the eluted DNA stems only from live microorganisms (see sketch above Fig. 1).

Wide Range of Specimens

The MolYsis™ technology is available for bacterial and fungal DNA isolation from a variety of fluid and tissue specimens. All samples are processed by only one protocol for host DNA depletion, where tissue samples are predigested by a short proteinase K treatment to release microorganisms, e.g. from biofilms. Table 1 summarizes peer-reviewed studies of NGS analyses of samples from human and animal origins. The studies involved problems in connection with the diversity of microbiota in a variety of human diseases and animal models as well as the efficacy of depletion of host DNA and its influence on NGS sequence quality and quantity.

Custom Solutions of Host DNA Depletion

Kits are available for i) fluid samples and ii) tissue biopsies and fluid samples (Fig. 1).

Fluid samples. *MolYsis™ Basic* kits provide protocols for the removal of host DNA from liquid specimens, including whole blood, aspirates, lavages and other samples (Fig. 1, green arrows).The kits are dedicated to be combined with other commercial kits or in-house procedures for DNA extraction established in the laboratory, including manual and

Fig. 1: Kits available for the depletion of host DNA from complex fluid and tissue samples and microbial DNA isolation.

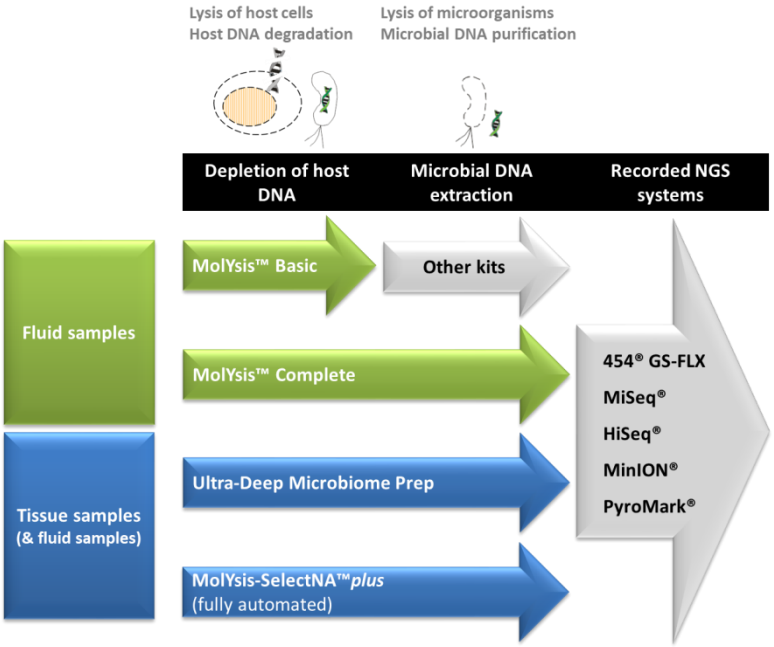

Table 1 Applications of MolYsis™ host DNA depletion for NGS

Applications	Specimen(s)	Molzym host DNA depletion	DNA isolation	NGS platform	Target sequence(s)	Organism(s)	Reference
Human diseases							
Infant nasal microbiome	nasal swabs	MolYsis™ Basic	+ Agencourt Genfind™ (Beckman Coulter)	454® GS-FLX (Roche)	*cpn60*	microbiota	[3]
Oral infection	subgingival plaque	MolYsis™ Basic	+ mirVana® (Life Technologies)	MiSeq® (Illumina)	metagenome	microbiota	[4]
Pneumonia	broncho-alveolar lavage	Ultra-Deep Microbiome Prep		HiSeq® 2500 (Illumina)	metagenome	microbiota	[5]
Bone and joint infection	sonicate fluid	MolYsis™ Basic5	+ Mobio BiOstic® (Qiagen)	HiSeq® (Illumina)	16S rRNA gene, metagenome	*S. aureus*, microbiota	[6]
	sonicate fluid	MolYsis™ Basic5	+ Mobio BiOstic® (Qiagen)	HiSeq® (Illumina)	microbiome	bacteria	[7]
	synovial fluid	MolYsis™ Basic5	+ Mobio BiOstic® (Qiagen)	HiSeq® 2500 (Illumina)	microbiome	bacteria	[8]
	tissue biopsies	Ultra-Deep Microbiome Prep		HiSeq® (Illumina)	microbiome	bacteria	[9]
Urinary tract infection	urine	MolYsis™ Basic5	+ MagNa Pure® (Roche)	MinION® (Oxford Nanopore)	microbiome, antimicrobial resistance	bacteria	[10]
Systemic infection	EDTA blood	MolYsis™ Complete5		454® GS-FLX (Roche)	16S rRNA gene	bacteria	[11]
	EDTA blood	MolYsis™ Complete5		MiSeq® (Illumina)	metagenome	microbiota	[12]
	EDTA blood	MolYsis™ Complete5		HiSeq® 2500 (Illumina)	metagenome	microbiota	[13]
Diabetic foot infection	tissue, culture	MolYsis™ Basic5	+ DNeasy PowerSoil® (Qiagen)	MiSeq® (Illumina); MinION® (Oxford Nanopore)	16S rRNA gene, shotgun cloned metagenome, resistome	bacteria, fungi	[14]
Urinary stent infection	urinary encrustations	MolYsis™ Complete5		MiSeq® (Illumina)	16S rRNA gene	bacteria	[15]
Hepatic brucelloma	necrotized hepatic tissue	Ultra-Deep Microbiome Prep		MiSeq® (Illumina)	16S rRNA gene, β-actin	bacteria, fungi	[16]
Animal models							
Insecticide resistance	whole mosquitos	MolYsis™ Complete5		HiSeq® (Illumina)	metagenome	microbiota	[17]
Intestinal *M. avium* infection (rabbit)	sacculus rotundus	Ultra-Deep Microbiome Prep		MiSeq® (Illumina)	16S rRNA gene	bacteria	[18]
Systemic infection (canine)	EDTA blood	MolYsis™ Complete5		PyroMark® (Qiagen)	16S/23S rRNA genes	*S. aureus*, enteric Gram-negative rods	[19]
Gut microbiome (rabbits)	Sacculus rotundus, vermiform appendix	Ultra-Deep Microbiome Prep		MiSeq® (Illumina)	16S rRNA gene	bacteria	[20]
Methodological							
Host DNA depletion tools	EDTA blood	Ultra-Deep Microbiome Prep, MolYsis-SelectNA™*plus* (automated)		MiSeq® (Illumina)	16S rRNA gene	bacteria	[21]
	CSF	MolYsis™ Basic5	+ MagMax™ Pathogen RNA/DNA (Applied Biosystems)	HiSeq® 2500 (Illumina)	metagenome, transcriptome	bacteria, viruses	[22]

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automated protocols. Recorded systems include kits from Beckman Coulter, Life Technologies, Qiagen, Roche and Applied Biosystems. Small volume liquid samples (0.2 ml) are processed by *MolYsis™ Basic*, higher volumes by *MolYsis™ Basic5* (≤1 ml; 5 ml) and *MolYsis™ Basic10* (5-10 ml).

Another option of fluid processing is given by Molzym's *MolYsis™ Complete5* and *MolYsis Complete10* kits which serve the whole process from host DNA depletion to microbial DNA extraction and purification from ≤1 ml, 5 ml and up to 10 ml samples. An important advantage of this solution is that all buffers, reagents and consumables are supplied free of bacterial and fungal DNA contamination. Besides depletion of host DNA, absence of DNA contamination adds another pillar to reliability and depth of sequencing analysis.

Tissue samples. Colonization of tissues by pathogens takes place by the formation of biofilms. In order to suspend microorganisms, tissue biopsies are subjected to a 10 minutes' partial digestion by proteinase. The suspension is then directed to the protocol of host DNA depletion and extraction and purification of microbial DNA. Two options are provided. The *Ultra-Deep Microbiome Prep and Ultra-Deep Microbiome Prep10* kits follow a manual protocol. The other is a fully automated system comprising the *SelectNA™plus* robot which is run with the *MolYsis-SelectNA™plus* kit (Fig. 2). Handling is limited to the loading of the instrument with cartridges, other consumables and the samples. One to 12 samples can be processed by the instrument at a time. The kits above have been used for NGS analyses of various specimens, including urinary encrustations, necrotized hepatic tissue, rabbit organ biopsies as well as fluid samples like EDTA blood and broncho-alveolar lavage (Table 1).

Validated NGS Systems

Molzym's technology for host DNA depletion has been used with the leading NGS systems from Illumina, Oxford Nanopore, Roche and Qiagen (Fig. 1 and Table 1). Studies focused on the composition of microbiota by determination of microbiome and metagenome structures in relation to disease and function in humans and animals (Table 1). Generally, efficient host DNA depletion as well as reduced background of contaminations by use of Molzym's pure reagents aided much in a deeper view of microbial structures [6, 21, 22].

Fig. 2 The *SelectNA™plus* robot for the automated host DNA depletion and extraction of microbial DNA.

References

[1] Kellogg JA et al. (2000) J Clin Microbiol 38, 2181-2185.
[2] Disqué C (2007) BIOspektrum 06, 627-629. [in German]
[3] Peterson SW et al. (2016) PLoS ONE 11, e0152493. doi:10.1371/journal.pone.0152493.
[4] Duran-Pinedo AE et al. (2014) The ISME Journal 8,1659-1672.
[5] Leo S et al. (2017) Int J Mol Sci 18, 2011, doi:10.3390/ijms18092011.
[6] Thoendel M et al. (2016) J Microbiol Meth 127,141-145.
[7] Thoendel M et al. (2017) J Clin Microbiol 55, 1789-1801.
[8] Ivy MI et al. (2018) J Clin Microbiol 27;56(9). pii: e00402-18. doi: 10.1128/JCM.00402-18.
[9] Ruppé et al. (2017) Sci Rep 7, 7718, doi:10.1038/s41598-017-07546-5.
[10] Schmidt K et al. (2017) J Antimicrob Chemother 72, 104–114.
[11] Benítez-Páez A et al. (2013) PLoS ONE 8, e57782. doi:10.1371/journal.pone.0057782.
[12] Gyarmati P et al. (2015) PLoS ONE 10, e0135756. doi:10.1371/journal.pone.0135756.
[13] Gyarmati P et al. (2016) Sci Rep. 6, 23532. doi: 10.1038/srep23532.
[14] Shurko J et al. (2017) Open Forum Infect Dis. (Suppl 1), S113. doi:10.1093/ofid/ofx163.125.
[15] Buhmann MT et al. (2019) Microbiome 7, 60. https://doi.org/10.1186/s40168-019-0674-x.
[16] Lazarevic V et al. (2018) Front Microbiol 9, 1566. doi:10.3389/fmicb.2018.01566.
[17] Dada N et al. (2018) Sci Rep 8: 2084. doi:10.1038/s41598-018-20367-4.
[18] Arrazuria R et al. (2016) Front Microbiol 7, 446. doi: 10.3389/fmicb.2016.00446.
[19] McCann CD, Jordan JJ (2014) J Microbiol Meth 99, 1-7.
[20] doi:10.1038/s41598-018-32484-1.
[21] Edelmann A et al. (2018) Poster ECCMID 2018 #P0082. www.molzym.com/images/blog/documents/Poster-Edelmann-et-al._ECCMID-2018_NGS.pdf
[22] Miller HB et al. (2019) Poster CPHM-935, ASM Microbe 20-24, San Francisco. www.abstractsonline.com/pp8/#!/7859/presentation/15428.

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