An innovative benchtop platform for automation and miniaturization of a PCR-free WGS library preparation protocol for *Salmonella enterica* NGS typing

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INTRODUCTION

Salmonella enterica is the second most reported bacterial cause of food-borne infections in Europe. Therefore, surveillance activities based on pathogen subtyping are important for controlling Salmonellosis. Whole-genome-based typing is becoming the gold standard due to its improved resolution and reduced cost.

Here, we showcase the advantages of using Magelia's PCR-free library preparation application for blind NGS typing of a relevant foodborne pathogen. **Our multi-OMICs platform challenges the minimum required input for a PCR-free library kit, by reducing reagent volumes 8x and DNA input 50x while yielding high quality sequencing libraries**.

KEY BENEFITS

- High Quality data from low input samples
- Reagent volume reduction
- Turn-around time and hands-on-time reduction
- High Efficiency reactions

PLATFORM FEATURES

- Integrated heating/cooling/thermal cycling
- Full automation of complex workflows
- Compact benchtop platform
- User defined protocol parameters
- Automated reagent dispensing
- Proprietary cartridges prevent inter and intra run cross-contamination

PCR-FREE WGS LIBRARY PREP USING STANDARD HUMAN gDNA

PCR-free WGS library preparation was performed using the Kapa Hyper Prep PCR-free kit (Roche) (Figure 1). Standard human gDNA from Promega was treated at three different input concentrations following the manufacturer's instructions and using in parallel three Magelia prototypes (Table 1) .

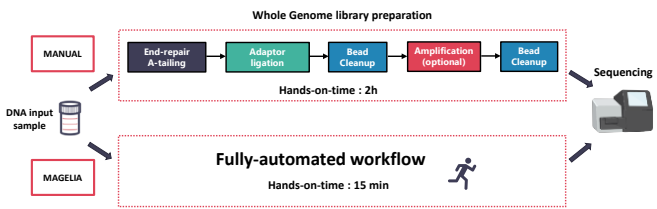


Figure 1. Kapa Hyper Prep® PCR-free workflow showing Manual vs Magelia treatment

Prepared libraries were quality checked using the Bioanalyzer® and Qubit®. As shown in Table 1, Magelia treated samples yielded **3x more ready-to-sequence libraries** when compared to manual treatment.

	Manual			Magelia			Manual			Magelia			Manual			Magelia		
Sheared DNA Input in ng	50ng	50ng	50ng	50ng	50ng	50ng	20ng	20ng	20ng	20ng	20ng	20ng	5 ng	5 ng	5 ng	5 ng	5 ng	5 ng
Magelia prototype				Magelia 1	Magelia 2	Magelia 1				Magelia 2	Magelia 1	Magelia 3				Magelia 2	Magelia 3	Magelia 1
Final Concentration in nM	15,8 nM	12,9 nM	12,2 nM	51,5 nM	52,4 nM	51,9 nM	6,1 nM	6,4 nM	5,6 nM	18,9 nM	25,4 nM	19,7 nM	1,45 nM	1,3 nM	n/a	3,16 nM	5,1 nM	4,4 nM

Table 1. WGS PCR-free Library preparation using standard human gDNA

Importantly, using as little as 5 ng as input (**10x less than the kit's recommended limit**), yielded sufficient material for sequencing.

Furthermore, final library concentrations were comparable between the three prototypes, **highlighting Magelia's robustness**.

Salmonella enterica NGS TYPING APPLICATION

In order to validate our PCR-free application with clinical field samples, eight different *S. enterica* serotypes were used (Table 2). We chose the two most widely prevalent serotypes (Typhimurium and Enteritidis), as well as closely related ones to highlight the resolution of the genomic data generated.

Library preparation was performed as previously described and samples were treated blindly, in parallel in the Magelia and manually (Figure 1). Due to the clinical origin of the samples, an assortment of inputs was treated (Table 2).

Prepared libraries underwent standard Quality Control. Samples were then normalized, pooled then sequenced on a MiSeq™ Sequencing System with a 2x75 bp configuration.

Serovar	Identifier	DSMZ nb	Isolation	Origin	Input qty
Typhimurium	U	101475	Copper fed pigs 2013	Denmark	5 ng
Gallinarum	S	4883	?	?	35 ng
Enteritidis	Y	107306	Patient with diarrhea	Georgia	35 ng
Saintpaul 4,5:e,h:1,2	W	27656	?	?	35 ng
Agona	H	102864	?	?	85 ng
Senftenberg (N)	N	10062	?	?	35 ng
Heidelberg	P	9379	?	Germany	35 ng
Panama (1,9,12:1,v:1,5)	R	9145	Baby 1994	USA	35 ng

Table 2. Salmonella serovars treated blindly for NGS typing

Salmonella NGS TYPING PRE-SEQUENCING METRICS

Magelia treatment of *S. enterica* samples resulted in higher library yield compared to manual preparation for all but one sample, sample U (Figure 3A). This was due to the fact that PCR-free treatment for this manually processed sample did not yield enough material for sequencing, hence, amplification was necessary.

Remarkably, **average adapter dimer percentages were significantly lower (p=0,04) in Magelia treated samples when compared to manual treatment** (Figure 3B).

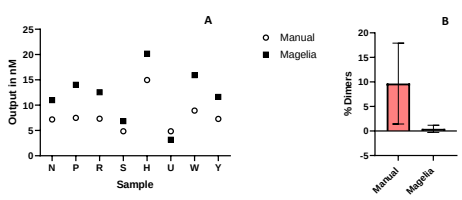
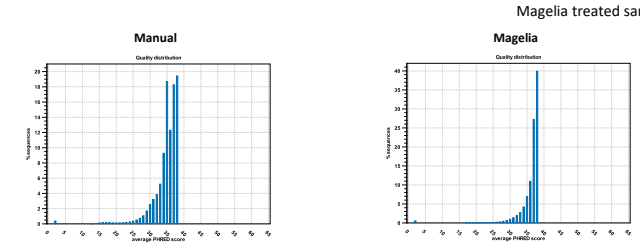


Figure 3. A. Output in nM for *S. enterica* samples treated manually or using Magelia. B. Average adapter dimer percentages in Manual and Magelia treated samples

Figure 4. Average PHRED scores for manual and Magelia treated samples



S. enterica NGS TYPING AND POST-SEQUENCING METRICS

We showed that 90,39 % of reads for the manually treated samples and 95,67 % of the reads for the Magelia treated samples have a PHRED score above 30, **implying a larger proportion of high-quality reads** (Figure 4)**.** Moreover, the manually treated samples display a more spread distribution for reads with scores between 20 and 38, confirming lower overall quality for these samples.

Higher quality sequencing data resulted in improved coverage and assemblies for Magelia treated samples. This in turn, allowed for detection of **more genetic elements of clinical relevance** (Figure 5).

An epidemiologically relevant SNP was detected for the Panama isolate in the Magelia treated sample and not in its manual counterpart. This was due to a lack of coverage in the region of interest for the manually treated sample (Figure 6).

Suitable coverage obtained for Magelia treated samples allowed us to **successfully identify all serotypes**. Conversely, only 5/8 were identified through manual treatment (Table 3).

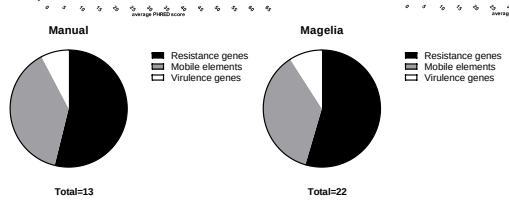


Figure 5. Identification of genetic elements of clinical interest (mobile elements, resistance and virulence genes) in manually versus Magelia treated samples

Serovar	Identifier	Magelia	Manual
Typhimurium	U	+	+
Gallinarum	S	+	+
Enteritidis	Y	+	+/-
Saintpaul 4,5:e,h:1,2	W	+	-
Agona	H	+	+
Senftenberg (N)	N	+	+
Heidelberg	P	+	+
Panama (1,9,12:1,v:1,5)	R	+	-

Table 3. Salmonella serovars treated blindly for NGS typing

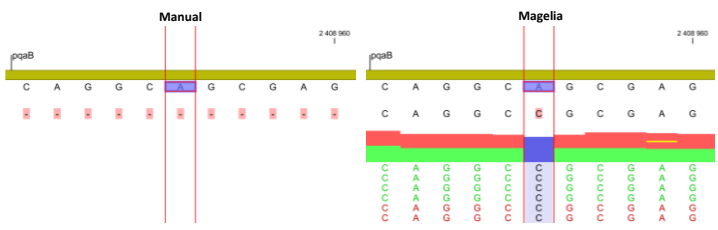


Figure 6. Mapping for the Panama serovar in the manual and Magelia treated samples in the *S. enterica* genomic region containing SNP c.884A>C

CONCLUSIONS

Magelia treatment of low input samples enabled characterization of clinically relevant pathogens to the single nucleotide resolution:

- Blind identification of all *Salmonella* serotypes for Magelia treated samples vs. 5/8 samples for manual treatment underline a **higher success rate** (relevant for regulated environments)
- **Optimal cleanup and negligible adapter-dimer formation** made possible thanks to our patented magnetic tweezer technology
- **Perspectives**: we are continuously developing a variety of miniaturized applications for low input samples:
  - **RNA-seq** library prep with rRNA depletion/mRNA capture
  - **Chromatin Immuno-Precipitation** (ChIP) followed by NGS library prep

MAGELIA, A UNIQUE multi-OMICs PLATFORM:

- Disruptive core technology enabling **high precision magnetic bead handling**
- **No evaporation**, allowing for improved reaction kinetics
- **Higher amplification efficiency**: less PCR cycles needed, reducing bias
- **Faster reactions** (2-hour immunoprecipitation in Magelia vs. 16 hours for manual preparation)