METAGENOME SEQUINS LAB PROTOCOL

INSTRUCTIONS FOR ADDITION OF META-SEQUINS TO DNA SAMPLES FOR NEXT GENERATION SEQUENCING

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Sequins are designed, validated and manufactured at the Garvan Institute of Medical Research, Sydney Australia.

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Material safety data sheets (MSDSs) are available at www.sequin.xyz/downloads/

1 | INTRODUCTION

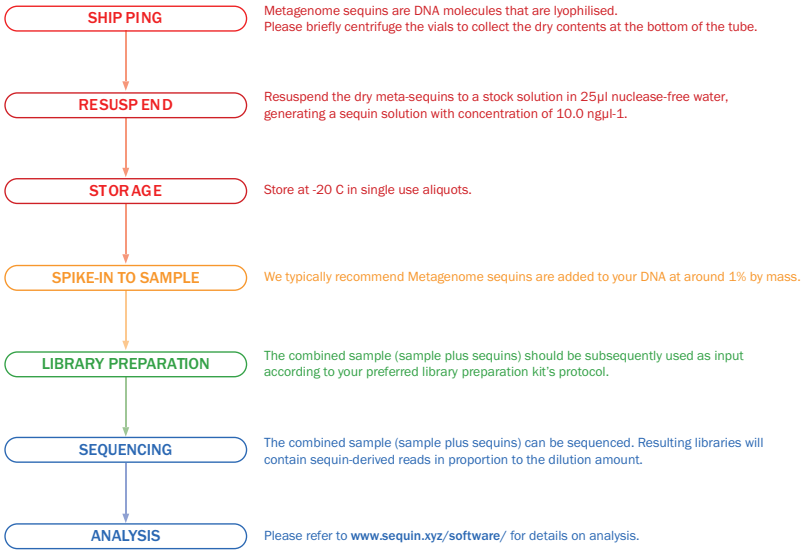
Metagenome sequins constitute a set of synthetic DNA reference standards that reflect the sequence complexity, GC content, phylogenetic diversity and abundance of a natural microbial community. They are added at a fractional concentration to your DNA sample of interest, and undergo concurrent library preparation, sequencing and analysis.

This protocol describes the use of Metagenome sequins in the laboratory, including a step-by-step guide to re-suspending, diluting and adding sequins to your DNA sample of interest. Further detail on the design, software, tutorials and other useful information on how to analyse sequins is available at **www.sequins.xyz**

For each batch of sequins synthesised, we perform a number of quality control procedures, including running Agilent BioAnalyzer analysis and sequencing of a neat mixture randomly selected from each manufactured lot number (**see Figure 1**). These quality control resources (including .FASTQ library files) can be downloaded from *www.sequin.xyz* for troubleshooting or analytical purposes.

We recommend ensuring that you have all necessary consumables and equipment before beginning the protocol below. See Section 5 for a list of related materials and equipment.

An overview of the protocol is as follows:



2 | SHIPPING, RESUSPENSION & STORAGE

2.1 | SHIPPING

Metagenome sequins are DNA molecules that are lyophilised to ensure long-term stability and then shipped at room temperature in specialised thermo-stable low-binding tubes within tamper-proof ziplock bags. Please contact us if there are any concerns with the parcel or the integrity of the tubes. Prior to re-suspension of the meta-sequins, please briefly centrifuge the vials to collect the dry contents at the bottom of tubes. Failure to do so may result in the loss of DNA content and may impact the downstream utility of the sequins.

Each vial contains sufficient Metagenome sequins for approximately 100 library preparations. However, the final number of reactions depends on the amount of input DNA required by the library preparation method used.

2.2 | RESUSPENSION & STORAGE

Re-suspend the dry Metagenome sequins to a stock solution in 25 l nuclease-free water, generating a sequin solution concentration of 10.0 ng µl-1.

Following re-suspension, sequin aliquots should be frozen at -20°C in a frost-free freezer. We would recommend preparing smaller single-use aliquots to minimize the potential effects of subsequent freeze-thaw cycles.

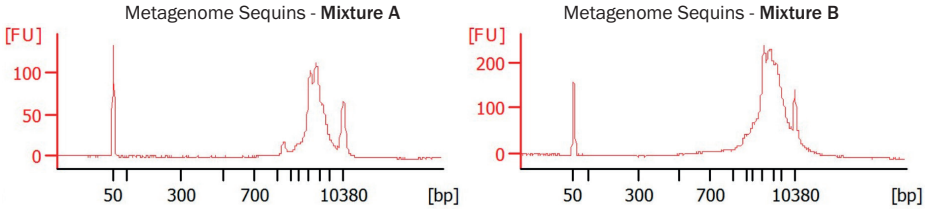


Figure 1. *Example traces of neat Metagenome sequins using a 2100 BioAnalyzer with the 7500 DNA Kit (Agilent Technologies).*

3 | HOW MUCH TO ADD

Metagenome sequins should be added to DNA samples prior to sample processing steps, such as fragmentation or shearing. This ensures that they provide valuable information on all processing stages. The addition and dilution of Metagenome sequins in the user's DNA sample is directly proportional to the fraction of the output library reads that are derived from the Metagenome sequins. Therefore, we recommend Metagenome sequins are added at a fractional amount that is sufficient to confer analytical utility, but does not encompass a large fraction of the final library reads. We typically recommend Metagenome sequins are added to your DNA at around 1.0% by mass. For example:

Sample DNA amount	Input Sequin Amount	Sequin Volume / Dilution
1 ng	0.01 ng	1 µL (1:1000)
10 ng	0.1 ng	1 µL (1:100)
100 ng	1 ng	1 µL (1:10)
250 ng	2.5 ng	1 µL (1:4)
500 ng	5 ng	1 µL (1:2)
1000 ng	10 ng	1 µL (neat)

Table 1. *Guidelines for diluting Metagenome sequins according to sample DNA amounts / library preparation method.*

Sequin Dilution	Sequin	Nuclease-free Water
1 : 2	1 µL undiluted	1 µL
1 : 4	1 µL undiluted	3 µL
1 : 10	1 µL undiluted	9 µL
1 : 100	1 µL 1 : 10	9 µL
1 : 1000	1 µL 1 : 100	9 µL

Table 2. *Guidelines for preparing Metagenome sequin dilutions.*

Once added, the combined sample (i.e. DNA sample and sequins) should then be subsequently used as input according to the protocol of your preferred library preparation kit.

4 | LIBRARY PREPARATION (EXAMPLE WORKFLOW)

Here we describe an example workflow for the library preparation and sequencing of Metagenome sequin mixture added to an natural microbe genome DNA sample. Please note that sequins are compatible with any reagents sourced from alternative vendors, which can be easily substituted and used in the following workflow.

4.1 | 1 µL of a 1:1000 dilution of Metagenome sequins was added to 1 ng of Total DNA extracted from a community of micropbe genomes (i.e. 1.0% by mass).

4.2 | The combined DNA and Metagenome sequins were used as Normalized gDNA in Procedure Step 1 (**page 6**) of the Nextera XT DNA Library Prep Kit Reference Guide (Illumina®, 15031942 v02).

4.3 | Subsequent steps were performed as per manufacturer's protocol, until the Procedure Steps (**page 10**), whereby steps 1 to 16 were repeated for a second time, and transferred to a 1.5 ml Lo-Bind microcentrifuge tube (Eppendorf).

4.4 | The purified libraries were verified and quantified on an Agilent 2100 Bioanalyzer using the Agilent High Sensitivity DNA Kit (Agilent Technologies) (**see Figure 2**).

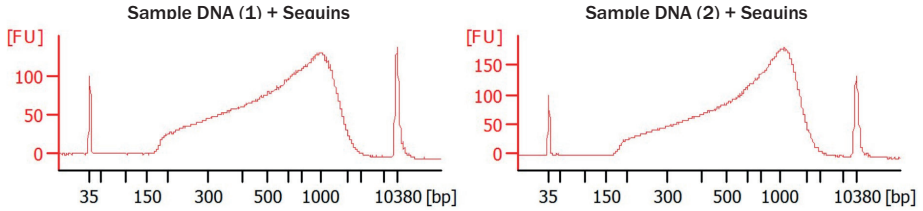


Figure 2. *Successful Metagenome sequin-containing Total DNA Libraries. Two examples of library preparation. Samples analysed by Agilent 2100 BioAnalyzer trace.*

5 | CONSUMABLES AND EQUIPMENT

PRODUCT	PART NUMBER (SOURCE)
Barrier RNase/DNase-free pipette tips	Any vendor
Non-binding nuclease-free Microfuge Tubes	Any vendor
Agilent 7500 DNA Kit	5067-1506 (Agilent Technologies)
Nextera XT DNA Library Preparation Kit	FC-131-1024 (Illumina)
Nextera XT Index Kit (24 indexes, 96 samples)	FC-131-1001 (Illumina)
Agencourt® AMPure® XP beads	A63880 (Beckman Coulter)
Magnetic stand	Any vendor
Agilent High Sensitivity DNA Kit	5067-4626 (Agilent Technologies)

Table 3. *List of equipment and consumables described in this protocol.*