

Effortless DNA library normalization using the ASSIST PLUS pipetting robot equipped with the D-ONE single channel pipetting module

Introduction

Next generation sequencing (NGS) is used for DNA and RNA sequencing in basic, applied and clinical research, as well as diagnostics. NGS was originally called 'massively parallel sequencing', referring to its capacity to analyze millions of sequencing reactions at the same time. At the start of the NGS workflow, libraries of similarly sized DNA fragments are created. Normalization of these DNA libraries is then performed, as differences in the concentrations of fragments affect the sequencing results, leading to under- or over-representation of individual fragments.

Normalization of nucleic acid concentrations – the equalization of sample concentrations – essentially involves dilution of the sample with varying volumes of diluent. Manual normalization is error-prone and tedious and requires cumbersome calculations and constant focus to ensure accurate sample tracking.

The ASSIST PLUS pipetting robot can be paired with the D-ONE single channel pipetting module to provide an easy automated solution for library normalization, ensuring error-free dilution calculations, consistent pipetting steps and precise liquid handling.

Key benefits:

- Automating the D-ONE single channel pipetting module on the ASSIST PLUS pipetting robot provides hands-off, secure processing of library normalization with high accuracy and increased reproducibility.
- The dilution calculator in VIALAB eliminates errors in dilution calculations.
- High precision pipetting results are guaranteed across a wide dynamic volume range, thanks to automatic selection of the correct pipette tip size.
- Optimal pipetting height is achieved by the automatic liquid level detection (LLD). There is no need to specify initial volumes, which makes the program set-up easier and faster.
- No need to input countless values into VIALAB, since worklists can be imported in a .CSV format. The template file – with preprepared column tags – makes data transfer even easier for the user.
- A run report summarizes all diluent and sample transfer volumes, giving full traceability.

Overview: How to perform sample normalization for Illumina 16S metagenomic sequencing

This application note describes the automation of DNA library normalization. The protocol is based on Illumina's [16S Metagenomic Sequencing Library Preparation Guide](#). After quantifying the DNA libraries, the DNA concentrations should be calculated in nM concentration (see a calculation example for 500 bp long amplicons in the DNA concentration calculation Microsoft Excel® sheet). For sequencing, the libraries should be diluted to a concentration of 4 nM. The 'Normalization worklist' programming step allows the user to import a worklist with sample details and concentrations (**Figure 1**). The dilution calculator (**Figure 2**) in VIALAB automatically determines the correct volume of each sample and diluent based on the target concentration (4 nM) and target volume (50 µl). It also automatically selects the best-suited pipette tip size.

ASSIST PLUS



	A	B	C	D	E	F	G	H	I	J
1	SampleID	SourcePlateID	SourceWell	TargetPlateID	TargetWell	InitialConcentration				
2										
3										
4										
5										
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Figure 1: Worklist template for the VIALAB program.

Dilution calculator active

Yes No

Target concentration

4 nM

Target volume

Fixed Variable

50 Target volume (µl)

Sample Volume (µl)

Calculate all samples

Figure 2: Dilution calculator in VIALAB program.

Step by step procedure:

The ASSIST PLUS pipetting robot is used together with the 0.5-300 µl D-ONE single channel pipetting module and 12.5 µl and 300 µl Sterile, Filter GRIPTIPS to automate the pipetting steps of sample normalization for Illumina 16S metagenomic sequencing (**Figure 3**).

The normalization protocol consists of two steps:

- Step 1:** Transfer of the diluent
- Step 2:** Transfer of the samples

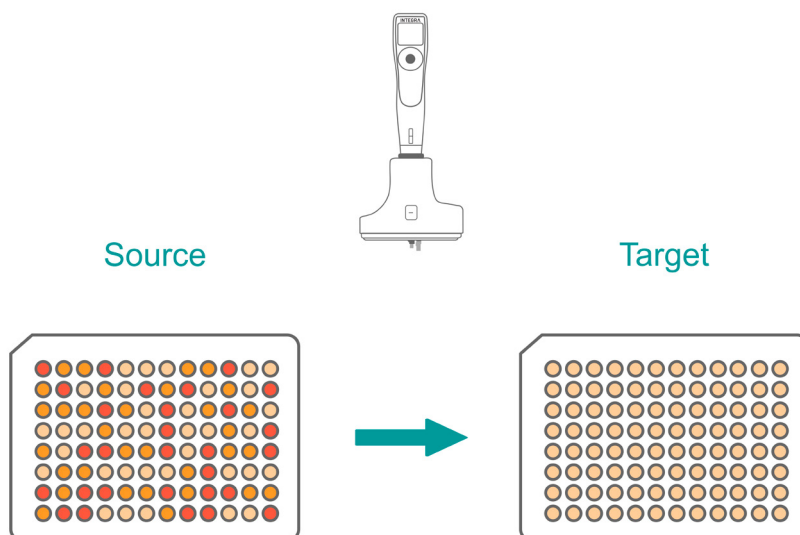


Figure 3: D-ONE single channel pipetting module is used to automate sample normalization.

Experimental set-up

- Deck position A:** Diluent – 10 ml multichannel reagent reservoir
- Deck position B:** Source plate – Sapphire 96 well PCR plate (Greiner Bio-one)
- Deck position C:** Target plate – Sapphire 96 well PCR plate (Greiner Bio-one)

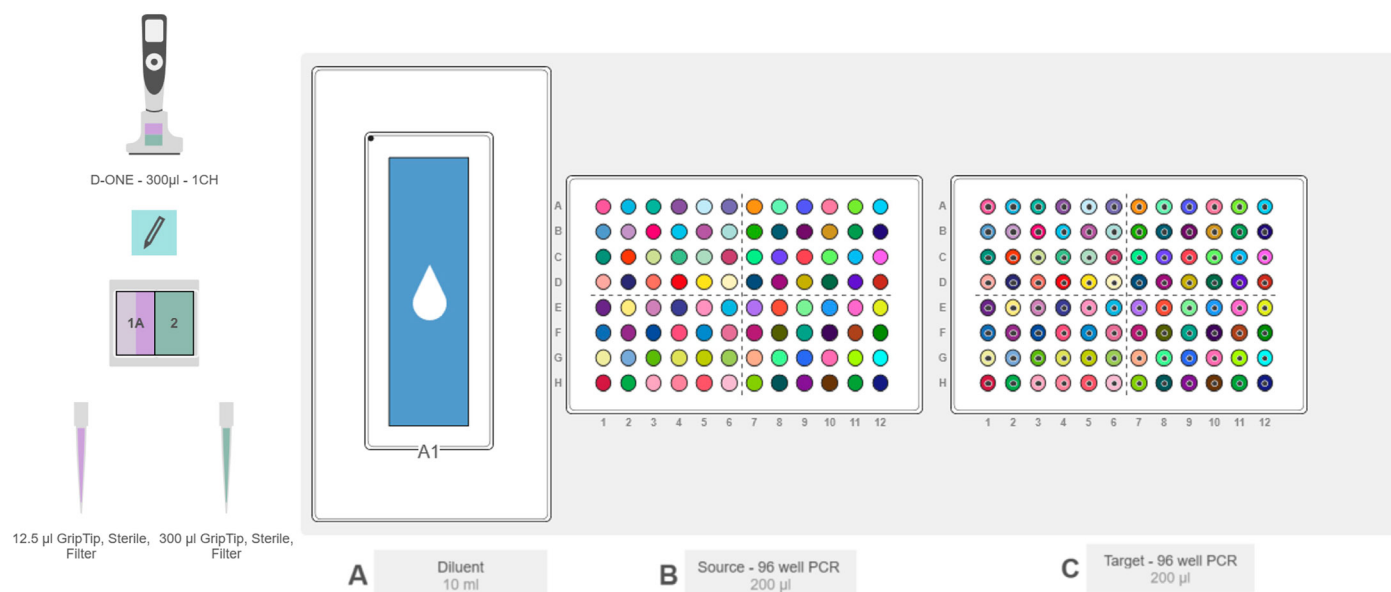


Figure 4: Deck set-up for sample normalization. **Position A:** Diluent – 10 ml multichannel reagent reservoir. **Position B:** Source plate – Sapphire 96 well PCR plate. **Position C:** Target plate – Sapphire 96 well PCR plate.

1. Transfer of diluent

STEP: The diluent is added in repeat dispense steps.

HOW TO: Place the 10 ml multichannel reagent reservoir on deck position A and fill it with 5 ml of 10 mM Tris (pH 8.5) buffer (**Figure 4**, blue with a white drop). Next, place the source plate (Sapphire 96 well PCR plate) with the amplicon samples on position B (**Figure 4**, colored circles) and an empty Sapphire 96 well PCR plate on position C (**Figure 4**, colored circles with dots); this will be the target plate containing the diluted, normalized samples.

Select and run the VIALAB program 'Illumina amplicon normalization'. The system first transfers the calculated diluent volumes to each well in several repeat-dispense steps with tip touch (**Figure 5**). A 15 µl pre- and post-dispense ensures precision while pipetting, and the total volume for each aspiration is calculated automatically. This is the fastest way of dispensing different volumes with a single channel pipette, saving time and money while reducing tip usage.

TIP:

- Pre- and post-dispense settings, together with the tip touch, guarantee precise and accurate pipetting results.



Figure 5: The D-ONE single channel pipetting module transfers the required volume of diluent to each well.

2. Transfer of the samples

STEP: The samples are transferred one by one.

After dispensing the diluent, the ASSIST PLUS pipetting robot starts to add the amplicon samples from the source plate to the target plate in individual transfer steps (**Figure 6**).

The amplicon sample volumes in the source plate may be different. If the liquid level detection (LLD) (**Figure 7**) is activated, the optimal pipetting height is automatically detected and allows the pipette tip to follow the liquid level during aspiration. This constant submerge depth during aspiration contributes to precise pipetting results. At the end of every transfer step, the sample is mixed thoroughly with the diluent in three mixing cycles.

Tip:

- Pipetting parameters (pipetting speed, pre-wetting, tip change) and the mixing conditions (height, speed, cycle and volume) can easily be adjusted in the VIALAB program if needed.

Now the normalized libraries are ready to be aliquoted and pooled for sequencing.



Figure 6: The D-ONE single channel pipetting module transfers the samples individually.

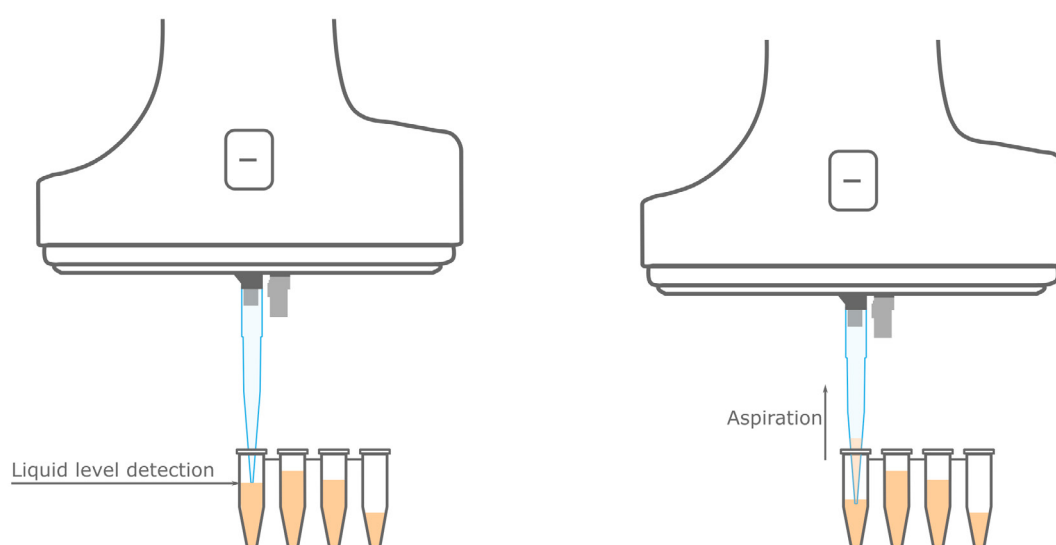


Figure 7: Optimal pipetting height is achieved in every well thanks to the automatic LLD.

Remark

- VIALAB software: VIALAB programs can be easily adapted to the user's specific labware and protocols.
- Run report: If the ASSIST PLUS pipetting robot is connected to the PC with VIALAB, programs can be started directly from the PC. After the run, a run report is automatically generated, documenting details such as the start/end time, user, calculated volumes, and any errors that occurred. This offers a convenient way to fulfill regulatory requirements.

Conclusion

- The ASSIST PLUS pipetting robot equipped with the D-ONE single channel pipetting module is the perfect solution for DNA normalization for NGS. With the push of a button accurate, error-free sample dilutions can be achieved.
- Several VIALAB features help the user to set up normalization quickly and easily. The dilution calculator determines the diluent and sample volumes, and automatically chooses the best pipette tip size to ensure perfect pipetting results. Worklists can be directly imported to VIALAB, saving the user from time-consuming, wearisome and error-prone typing.
- The D-ONE single channel pipetting module performs automatic LLD to overcome the differing liquid levels of samples, ensuring the optimal aspiration height and precise pipetting.
- Each D-ONE single channel pipetting module is compatible with two types of GRIPTIP to provide a broad volume range. This ensures longer walk-away times and eliminates the need to change the pipette between pipetting steps.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4531	D-ONE Single Channel Pipetting Module, 0.5-300 µl	https://www.integra-biosciences.com/global/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	4535	Tip deck for D-ONE on ASSIST PLUS	https://www.integra-biosciences.com/global/en/pipetting-robots/d-one-for-assist-plus
INTEGRA Biosciences	6455	12.5 µl Sterile, Filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	6435	300 µl Sterile, Filter GRIPTIPS	https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide
INTEGRA Biosciences	4335	10 ml Reagent reservoir, Sterile, Polypropylene	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
Greiner Bio-One International	652270	Sapphire microplate, 96 well, PP	https://shop.gbo.com/de/switzerland/products/bioscience/molekularbiologie/pcr-microplatten/652270.html

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