

User Guide



YOUSEQ

16S

Metagenomic Sequencing Library preparation kit

For use on Illumina sequencers

Version 1.0



Simple Library Preparation Workflow

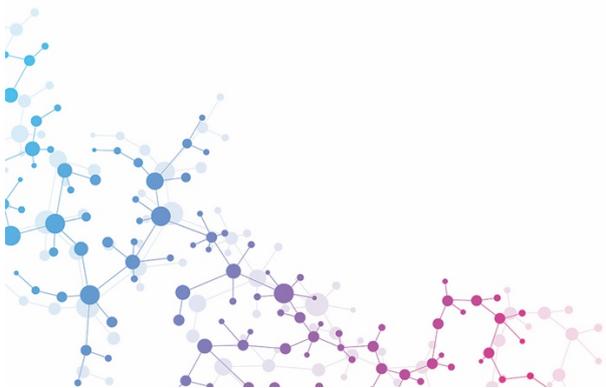
PCR 1

Clean up

PCR 2

Clean up

Finalise



Kit contents

PCR1 Pack

	Cap colour	Size
16S target specific primers		500µl
YouSeq NGS MasterMix		1000µl

PCR2 Pack

	Cap colour	Size
YouSeq NGS MasterMix		2 x 1250µl
YouSeq adapter kit	96-well plate	96 x 10µl

Bead clean pack

	Cap colour	Size
YouSeq beads		6ml
Wash buffer*		80ml*
Elution buffer		10ml

*Add 64 ml of molecular biology grade ethanol to the supplied diluent to make the wash buffer ready to use.

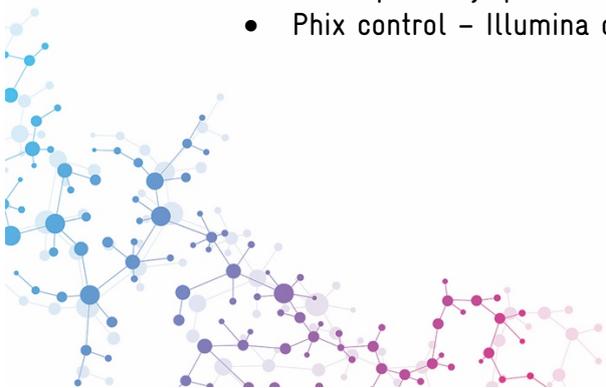
Other items to be supplied by the user

Essential

- Illumina NGS sequencing instrument and reagents
- PCR instrument
- 96-well plates suitable for your PCR instrument
- Magnetic rack
- Pipettes and general laboratory equipment

Optional

- YouSeq Library quantification kit: catalogue No YS-LQK-96
- Phix control – Illumina catalogue no. FC-110-3001



PCR1 Protocol

Load each well of your 96-well PCR plate with the following:

5µl Microbial DNA sample
10µl YouSeq NGS Mastermix
5µl Target specific primers

Seal the plate and load in to your PCR machine.

Run the following PCR protocol:

	Temp	Time
	95°C	3 minutes
25 cycles	95°C	30 seconds
	55°C	30 seconds
	72°C	30 seconds
	72°C	5 minutes
	4°C	HOLD



Clean up protocol 1

Bind

Mix beads well until suspension is homogeneous
Add 16 μ L YouSeq NGS Beads to each PCR1 product and mix well
Incubate for 10 mins at room temp
Place on magnet and wait until liquid is clear
Remove supernatant

1st wash

Leave your tube on the magnet
Add 200 μ L wash buffer
Incubate for 30s
Remove supernatant carefully

2nd wash

Repeat as per 1st wash

Dry beads

Leave for 5min at RT

Elute

Remove your tube from the magnet
Add 60 μ L Elution buffer and mix well
Incubate for 5 min at RT
Place on magnet and wait until liquid is clear

DNA is in the solution. 5 μ L can be moved to a fresh 96-well plate ready for PCR2



PCR2 Protocol

Take a fresh 96-well plate and according to your experimental plan, load each well with the following:

15µl PCR1 product
10µl YouSeq index primer
25µl YouSeq NGS MasterMix

Seal the plate and load in to your PCR machine.

Run the following PCR protocol:

	Temp	Time
	95°C	3 minutes
8 cycles	95°C	30 seconds
	55°C	30 seconds
	72°C	30 seconds
	72°C	5 minutes
	4°C	HOLD

Clean up protocol 2

Bind

Mix beads well until suspension is homogeneous
Add 40 μ L YouSeq NGS Beads to each PCR1 product and mix well
Incubate for 10 mins at room temp
Place on magnet and wait until liquid is clear
Remove supernatant

1st wash

Leave your tube on the magnet
Add 200 μ L Wash buffer
Incubate for 30s
Remove supernatant carefully

2nd wash

Repeat as per 1st wash

Dry beads

Leave for 5 min at RT

Elute

Remove your tube from the magnet
Add 20 μ L elution buffer and mix well
Incubate for 5 min at RT
Place on magnet and wait until liquid is clear

DNA is in the solution.

Your DNA Library is in the solution and ready to be normalised



Finalise

Optional – QC check

If this protocol is new or is not routine you should check the quality of your DNA library with a Bioanalyser/TapeStation (Agilent). Look for a strong peak at around 600bp to indicate a good quality library. Significant peaks at other size points indicate a problem and you should troubleshoot before proceeding.

Quantify

Normalisation is simply a matter of adding the same amount Library for each of your samples to your sequencing run

Either

Check concentration of each sample on a Qubit Fluorometer (Thermo Fisher)

Or run

YouSeq qPCR library quantification kit (Catalogue number: YS-LQK-96)

Dilute

According to your results, dilute each sample in YouSeq Dilution buffer to 4nM

Combine

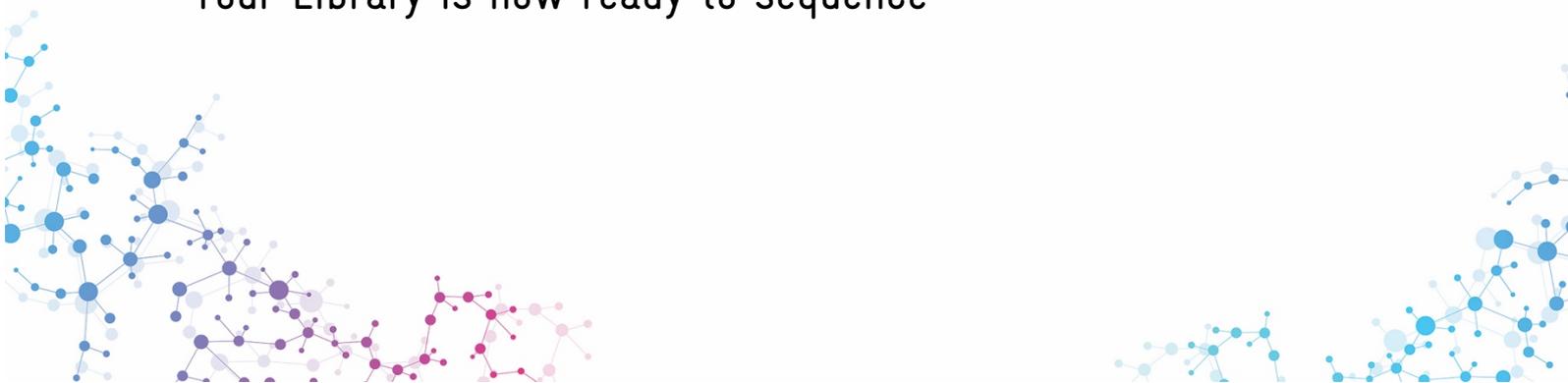
Combine 5µl of all of the libraries you are planning to sequence
Dilute this pool again 1:80 in YouSeq dilution buffer.

1.25µl pooled library mix
100µl YouSeq dilution buffer

Optional – PhiX control

It can help with trouble shooting to spike PhiX control Library (from Illumina). Dilute the Illumina PhiX stock 5µl in 45µl YouSeq dilution buffer. You can store this master stock for up to a month. Dilute 5µl of this master stock in a further 45µl YouSeq dilution buffer. Then add 5µl to your diluted DNA library pool.

Your Library is now ready to sequence



Tips and tricks to make life easy

PCR1

Use a consistent amount of DNA in your reactions for consistent results. We'd suggest an input amount of 5ng. Use a Qubit Fluorometer to quantify your DNA accurately.

It is good practice to set up your PCR plate on ice or on a cold rack. This can improve your results.

Bead clean up

Remove as much ethanol as you can (without disturbing the clump of beads) before the bead drying step. It is sometimes useful to centrifuge your tube, return it to the magnet, and then remove the final few microlitres.

The ideal, precise bead drying time, will depend on the temperature in your lab. Inspect the pellet of beads by eye. As soon as you can see no visible liquid but the bead clump still has a wet sheen to it this is the ideal time to proceed to the elution step. If you over-dry the clump it is difficult to resuspend the pellet in the next step. If the pellet has taken a cracked appearance you will need to increase the elution time as the bead has become too dry.

PCR2

Use a different index/adaptor for each sample and keep a good written record of which is which.

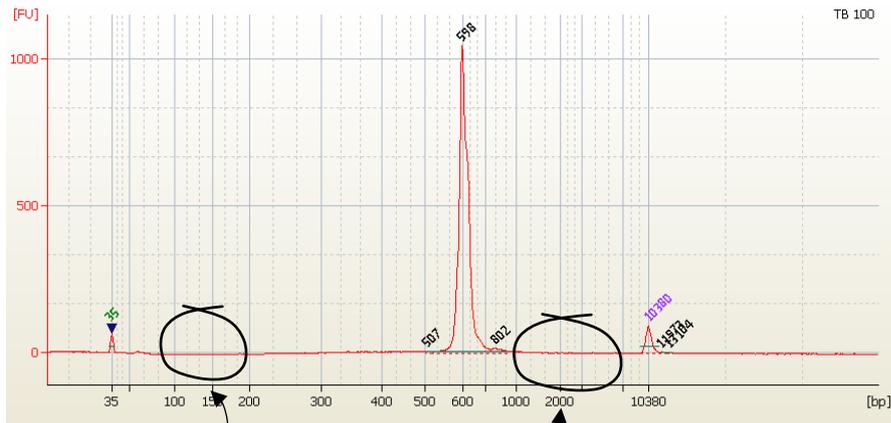
Be extremely careful when opening your plate after PCR 1. The plate contains millions of copies of your library and can easily contaminate other samples if good laboratory practice is not followed.

Finalise

Library QC. If the product is new to you or not in routine use its. Good idea to check the quality of your Library by Bioanalyser/TapeStation (Agilent).

Ideal result look like:





Large peaks here indicate carry over of primers or unincorporated adapters

Peaks here indicate non-specific PCR artefacts

Contact our technical support team if your library looks like it has unincorporated primers, adapters or non-specific PCR artefacts

The PhiX control is highly recommended. It adds complexity to your library which will improve the performance of your sequencing run.

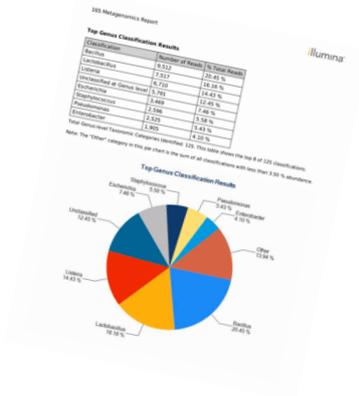


Data Analysis

We recommend the use of the Illumina BaseSpace 16S Metagenomics App for data analysis.



This is a fully automated data analysis tool that delivers an easy to use PDF output:



Follow Illumina's instruction to use this module

http://emea.support.illumina.com/content/dam/illumina-support/documents/documentation/software_documentation/basespace/16s-metagenomics-user-guide-15055860-a.pdf