

菌相研究 全集中攻略



Microbiome Standards



Sample Collection



DNA/RNA Extraction



Library Preparation



NGS Analysis





Standards

Page 6-11

Microbiomics-Grade Quality Control

ZymoBIOMICS® Microbial Community Standard (I/II)

ZymoBIOMICS® Spike-in Control (I/II)

ZymoBIOMICS HMW DNA Standard

Gut Microbiome-Grade Quality Control

ZymoBIOMICS Gut Microbiome Standard

ZymoBIOMICS Fecal Reference with TruMatrix™ Technology

Sample Collection DNA/RNA Shield™

Page 12-13



Fecal Samples

DNA/RNA Shield™ Fecal Collection Tube

Swab Samples

DNA/RNA Shield™ Swab Collection Tube

Microbial Samples

DNA/RNA Shield™ Lysis & Collection Tubes

Saliva Samples

DNA/RNA Shield™ Saliva Collection Kit

Blood Samples

DNA/RNA Shield™ Blood Collection Kit



DNA & RNA Isolation

Page 14-15

DNA Isolation

ZymoBIOMICS® DNA Kits

Quick-DNA HMW MagBead Kit

RNA Isolation

ZymoBIOMICS® RNA Kits

DNA/RNA Isolation

ZymoBIOMICS® DNA/RNA Kits

Microbial DNA Isolation

HostZERO™ Microbial DNA Kit

Next-Gen Sequencing

Page 16-18



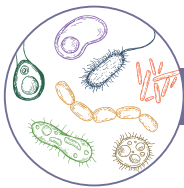
Library Preparation

Quick-16S NGS Library Prep Kit

ZymoBIOMICS® PCR PreMix

Analysis

ZymoBIOMICS® Targeted Sequencing Service



Microbiome Standards

透過已知菌落群，檢視整套實驗，利於優化操作流程

- ZymoBIOMICS Microbial Community Standard
- ZymoBIOMICS Microbial Community Standard II (Log Distribution)
- ZymoBIOMICS Spike-in Control I (High Microbial Load)
- ZymoBIOMICS Spike-in Control II (Low Microbial Load)
- ZymoBIOMICS HMW DNA Standard
- ZymoBIOMICS Gut Microbiome Standard
- ZymoBIOMICS Fecal Reference with TruMatrix™ Technology



Sample Collection

穩定保存樣品中的核酸

- DNA/RNA Shield
- DNA/RNA Shield Fecal Collection Tube
- DNA/RNA Shield Collection Tube w/ Swab
- DNA/RNA Shield Lysis & Collection Tubes
- DNA/RNA Shield Saliva Collection Kit
- DNA/RNA Shield Blood Collection Tube
- Urine Conditioning Buffer



DNA/RNA Extraction

利用 **BashingBead™** 均質化搭配一系列試劑，無偏差萃取樣品中的核酸

- ZymoBIOMICS DNA Miniprep Kit
- ZymoBIOMICS RNA Miniprep Kit
- ZymoBIOMICS DNA/RNA Miniprep Kit

移除 **Host DNA**，保留微生物 **DNA**，不浪費 **NGS reads**

- HostZERO Microbial DNA Kit

長片段 **DNA** 萃取，應用於三代定序

- Quick-DNA HMW MagBead Kit



Library Preparation

獨家優化的 **16S library** 建庫系統，有效提高微生物覆蓋率

- Quick-16S™ NGS Library Prep Kit



NGS Analysis

從收集檢體到結果分析，提供完整專業的服務

- ZymoBIOMICS Targeted Sequencing Service: 16S (V1-V2, V3-V4, V4, or V6-V8)
- ZymoBIOMICS Targeted Sequencing Service: ITS
- ZymoBIOMICS Targeted Sequencing Service: 18S
- ZymoBIOMICS Shotgun Metagenomic Service

ZymoBIOMICS[®] Microbial Community

- 獨家提供標準品 – 混合已知的 3 Gram-negative, 5 Gram-positive and 2 yeast strains
- GC content range 15%-85% 可以監控、優化 NGS 整套流程，使得 data 可信度提升

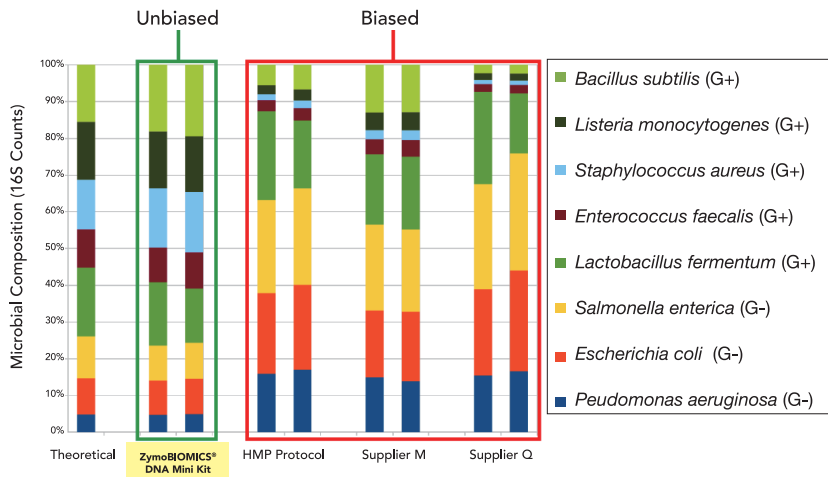
產品形式

純菌：可以確認萃取流程是否無偏差得到所有細菌的核酸

DNA：可以確認 PCR 流程是否無偏差放大所有細菌的核酸

Species	Theoretical Composition (%)				
	Genomic DNA	16S Only	16S & 18S	Genome Copy	Cell Number
<i>Pseudomonas aeruginosa</i>	12	4.2	3.6	6.1	6.1
<i>Escherichia coli</i>	12	10.1	8.9	8.5	8.5
<i>Salmonella enterica</i>	12	10.4	9.1	8.7	8.7
<i>Lactobacillus fermentum</i>	12	18.4	16.1	21.6	21.4
<i>Enterococcus faecalis</i>	12	9.9	8.7	14.6	14.5
<i>Staphylococcus aureus</i>	12	15.5	13.6	15.2	15.1
<i>Listeria monocytogenes</i>	12	14.1	12.4	13.9	13.8
<i>Bacillus subtilis</i>	12	17.4	15.3	10.3	10.2
<i>Saccharomyces cerevisiae</i>	2	NA	9.3	0.57	1.13
<i>Cryptococcus neoformans</i>	2	NA	3.3	0.37	0.73

發現 bias 並消滅他 !!



Catalog No.	Product	Size
D6300	ZymoBIOMICS [™] Microbial Community Standard	0.75 ml
D6305	ZymoBIOMICS [™] Microbial Community DNA Standard	200 ng
D6306	ZymoBIOMICS [™] Microbial Community DNA Standard	2000 ng

ZymoBIOMICS™ Microbial Community Standard II

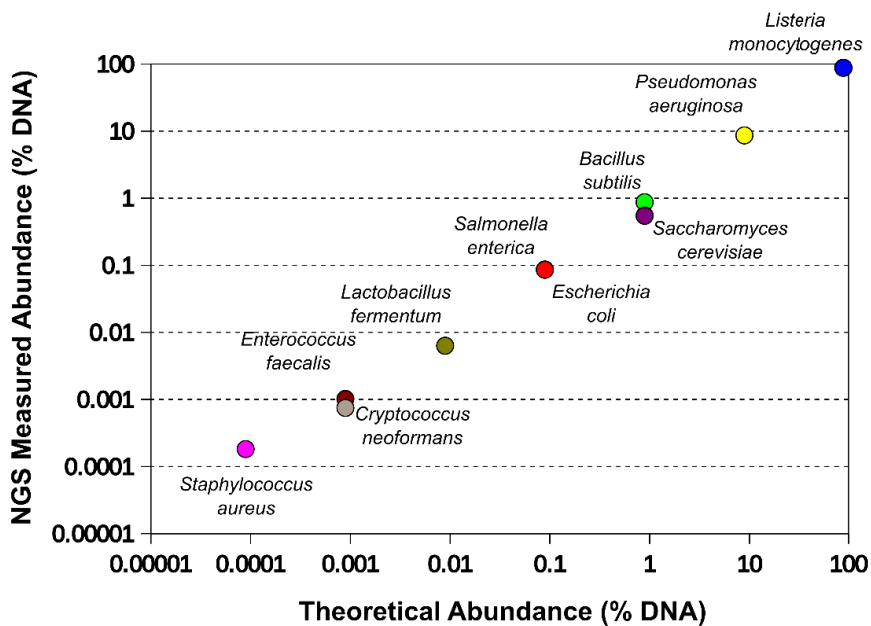
- 標準品中各種菌量以 log 分布， $10^2 \sim 10^8$ cells - 混合已知的 3 Gram-negative, 5 Gram-positive and 2 yeast strains
- 可以監控、優化實驗流程和了解平台的靈敏度，提升 data 可信度

產品形式

純菌：可以確認萃取流程是否無偏差得到所有細菌的核酸

DNA：可以確認 PCR 流程是否無偏差放大所有細菌的核酸

標準品中不同細菌以 log 分布呈現

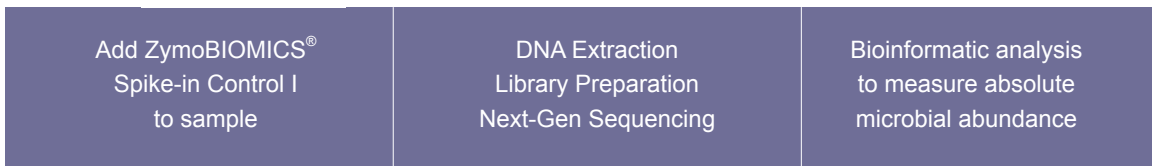


Catalog No.	Product	Size
D6310	ZymoBIOMICS™ Microbial Community Standard II (Log Distribution)	0.75 ml
D6311	ZymoBIOMICS™ Microbial Community DNA Standard II	200 ng

ZymoBIOMICS Spike-in Control (I/II)

- 絕對定量：利用 NGS 來進行細胞數的精確測定
- 品質管理：標準化每一個標準品
- 獨家菌種：內含數種與人類微生物群無關的自然微生物菌種

操作流程



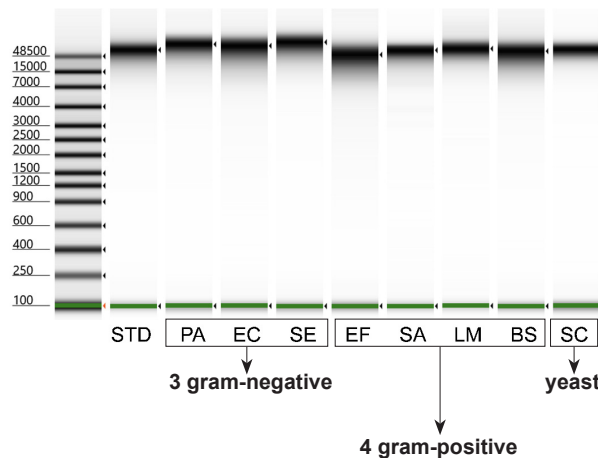
Spike-in Species		20 µl Per Prep (25 preps)					
		Cells (I/II)		16S Copies (I/II)		Total DNA (ng) (I/II)	
<i>Imtechella halotolerans</i>	Gram-negative	2×10^7	1×10^5	6.0×10^7	2.0×10^5	67.2	0.35
<i>Allobacillus halotolerans</i>	Gram-positive	2×10^7	1×10^4	1.4×10^8	3.0×10^4	58.2	0.034
<i>Truepera radiovictrix</i>	Resistant to lysozyme lysis, Very high GC content.	X	1×10^3	X	7×10^3	X	0.0029

Catalog No.	Product	Size
D6320	ZymoBIOMICS Spike-in Control I (High Microbial Load)	25 Preps
D6320-10	ZymoBIOMICS Spike-in Control I (High Microbial Load)	250 Preps
D6321	ZymoBIOMICS Spike-in Control II (Low Microbial Load)	25 Preps
D6321-10	ZymoBIOMICS Spike-in Control II (Low Microbial Load)	250 Preps

ZymoBIOMICS HMW DNA Standard

- 針對長片段定序系統所設計，適合運用於第三世代定序 (如 Oxford Nanopore、PacBio)
- 內含已知的 3 Gram-negative, 4 Gram-positive and 1 yeast strains
- 標準品的 DNA 長度 > 50 kb

標準品中含有三種 **gram-negative**，四種 **gram-positive, yeast**，並且經過原廠測試 所有菌種的基因體皆大於 **50 kb**



提供所有內含菌種的比例及細部數據讓使用者進行對照

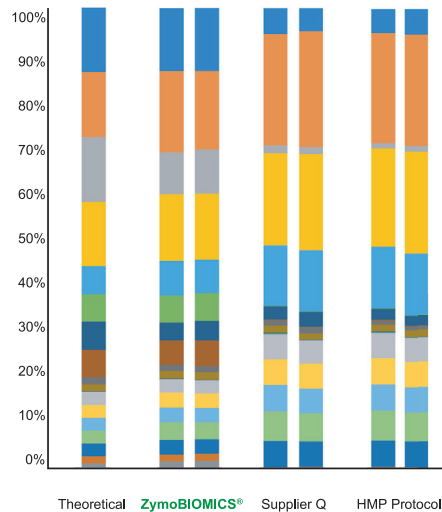
Species	Theoretical Composition (%)	
	Genomic DNA	Genome Copy
<i>Pseudomonas aeruginosa</i>	14	7.8
<i>Escherichia coli</i>	14	10.9
<i>Salmonella enterica</i>	14	11.2
<i>Enterococcus faecalis</i>	14	18.8
<i>Staphylococcus aureus</i>	14	19.6
<i>Listeria monocytogenes</i>	14	17.8
<i>Bacillus subtilis</i>	14	13.2
<i>Saccharomyces cerevisiae</i>	2	0.63

Catalog No.	Product	Size
D6322	ZymoBIOMICS HMW DNA Standard	5000 ng

ZymoBIOMICS Gut Microbiome Standard

- 模擬腸道內菌相，由 21 種不同的腸道菌所組成，包含已知的 12 Gram-negative、6 Gram-positive、2 fungal strains, and 1 archaeal strain
- 可以檢視不同的腸道菌種下的萃取流程與定序是否有無偏差，以及監控實驗的靈敏度與定序的鑑別度

Optimize Microbiome Analysis of Gut Samples



Species	Theoretical Abun. (%)
<i>Faecalibacterium prausnitzii</i>	14
<i>Veillonella rogosae</i>	14
<i>Roseburia hominis</i>	14
<i>Bacteroides fragilis</i>	14
<i>Prevotella corporis</i>	6
<i>Bifidobacterium adolescentis</i>	6
<i>Fusobacterium nucleatum</i>	6
<i>Lactobacillus fermentum</i>	6
<i>Clostridioides difficile</i>	1.5
<i>Akkermansia muciniphila</i>	1.5

Species	Theoretical Abun. (%)
<i>Methanobrevibacter smithii</i>	0.1
<i>Salmonella enterica</i>	0.01
<i>Enterococcus faecalis</i>	0.001
<i>Clostridium perfringens</i>	0.0001
<i>Escherichia coli (JM109)</i>	2.8
<i>Escherichia coli (B-3008)</i>	2.8
<i>Escherichia coli (B-2207)</i>	2.8
<i>Escherichia coli (B-766)</i>	2.8
<i>Escherichia coli (B-1109)</i>	2.8
<i>Candida albicans</i>	1.5
<i>Saccharomyces cerevisiae</i>	1.4

靈敏度
測試

品系鑑別
測試

Catalog No.	Product	Size
D6331	ZymoBIOMICS Gut Microbiome Standard	10 preps

ZymoBIOMICS Fecal Reference with TruMatrix™ Technology



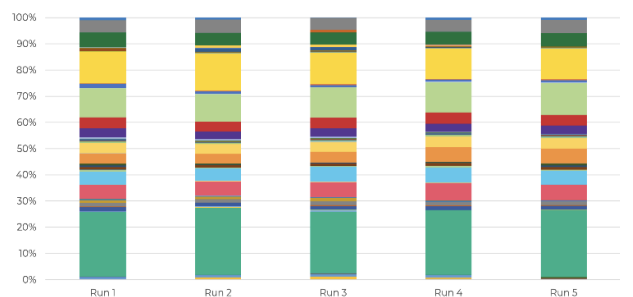
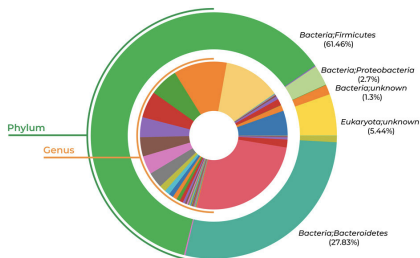
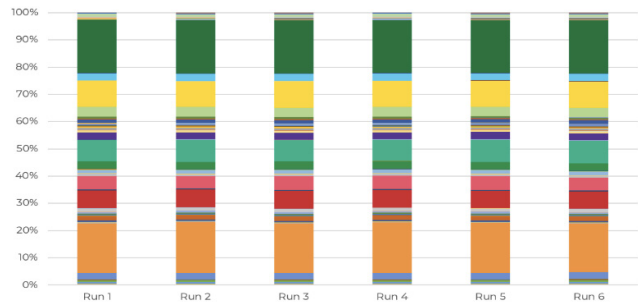
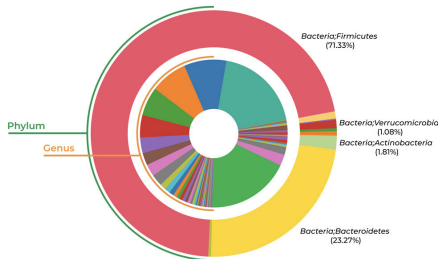
- 最貼近人體來源樣本：由真實人類糞便製作而成
- 標準一致：可以提供 200 萬組相同製程與來源的標準品
- 針對專一研究設計：targeted, metagenomic, and metatranscriptomic sequencing

2 Million Uniform Preps for a Consistent Point of Reference



幫助優化實驗流程與操作，並建立一致的標準

產品最初樣品經過大規模均質處理，並且透過嚴格的品質管理，以確保每一批產品的一致性



Catalog No.	Product	Size
D6323	Fecal Reference with TruMatrix™ Technology	10 preps

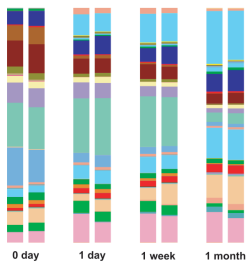
DNA/RNA Shield™ Collection Devices

- 長時間穩定的保存樣本中的 DNA, RNA
- 適用樣本廣，細胞、組織、全血、土壤...等
- 有效抑制病毒與其他感染源的活性
- 可直接搭配 DNA/RNA 純化試劑，不需要額外的移除步驟
- 產品選擇多元，有各種 Collection devices

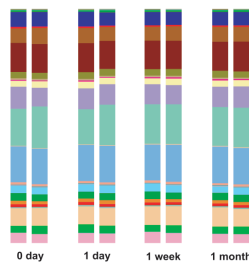
操作流程



Without DNA/RNA Shield™ - Composition Changes



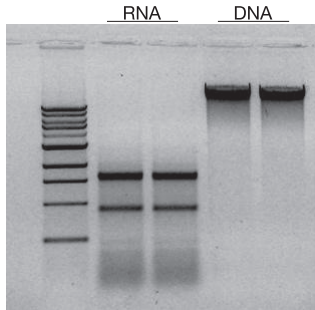
With DNA/RNA Shield™ - Accurate Composition



使用 DNA/RNA shield，
樣品放在室溫都不怕

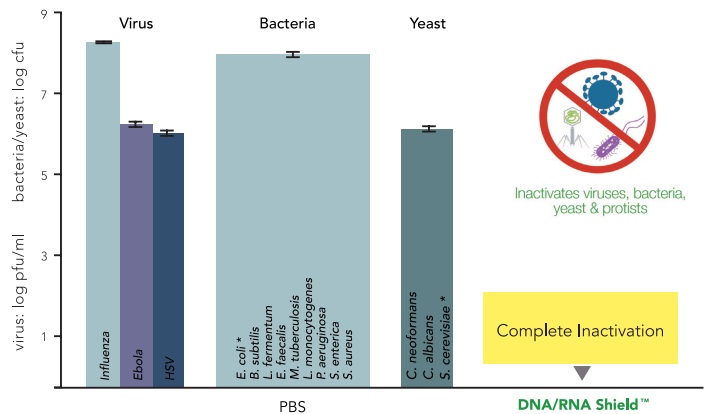
Microbial composition of stool is unchanged after one month at ambient temperature with DNA/RNA Shield™.

DNA/RNA 皆適用



High quality DNA and RNA is effectively purified from blood stored in DNA/RNA Shield™ using the Quick-gDNA™ and Quick-RNA™ kits from Zymo Research respectively.

去除病原體活性，提高安全性



Viruses, bacteria and yeast are effectively inactivated by DNA/RNA Shield™

Catalog No.	Product	Size	Sample Source	format
R1100-50	DNA/RNA Shield	50 ml	Cells, Tissues	
R1100-250		250 ml		
R1102	DNA/RNA Shield™ - Collection Tube (1ml fill)	50 pack		
R1200-25	DNA/RNA Shield (2X Concentrate)	25 ml	Blood, Biological liquids	
R1200-125		125 ml		
R1101	DNA/RNA Shield™ - Fecal Collection Tube	10 pack	Stool	
R1150	DNA/RNA Shield™ - Blood Collection Tube	50 pack	Blood	
D3061-1-140	Urine Conditioning Buffer	140 ml	Urine	
R1106	DNA/RNA Shield™ Collection Tube w/ Swab (1ml fill)	10 pack	Mucosal cells from nose, mouth	
R1107		50 pack		
R1105	DNA/RNA Shield™ Lysis Tube (Tissue)	50 pack	Tissues	
R1104	DNA/RNA Shield™ Lysis Tube w/ Swab (Microbe)	10 pack	Microbe	
R1103	DNA/RNA Shield™ Lysis Tube (Microbe)	50 pack	Microbe	

ZymoBIOMICS® DNA/RNA Kits

- 適用樣本廣，feces, soil, plant, water, biofilms, swabs, saliva, body fluids, etc
- 獨家 lysis system 以及 BashingBead 可將所有微生物徹底裂解
- 貼心的 PCR Inhibitor removal column，移除 PCR 干擾因子
- 後續可應用於 PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing

操作流程



Accurate lysis using ZymoBIOMICS® Lysis Tubes



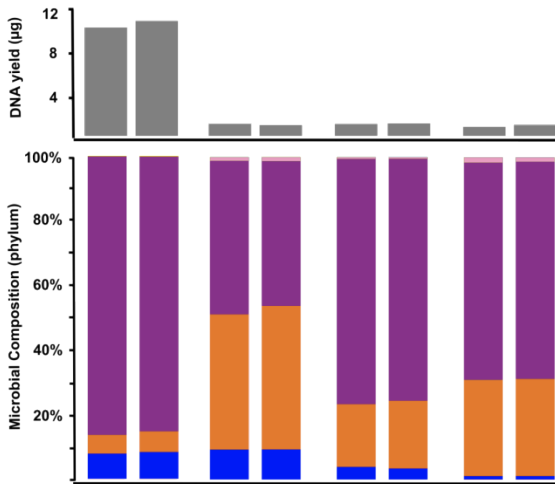
Filter lysate



Superior yields and integrity with Zymo-Spin™ technology

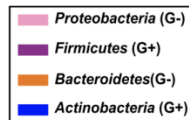


PCR inhibitor removal eliminate polyphenolics, humic/fulvic acid and melanin



高產量 · 無偏差

The ZymoBIOMICS™ DNA Kit reliably isolates DNA from even the toughest to lyse gram positive organisms, enabling unbiased analyses of microbial community compositions.

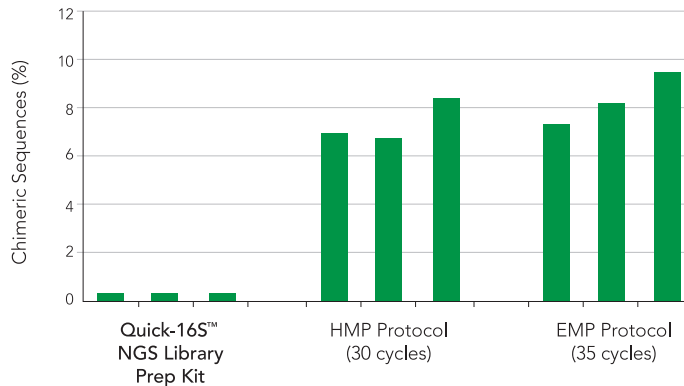


Catalog No.	Product	Size	Format	Binding Capacity	Lysis matrix
D4301	ZymoBIOMICS DNA Microprep Kit	50 preps	Spin-Column	5 µg	BashingBead™
D4300	ZymoBIOMICS DNA Miniprep Kit	50 preps	Spin-Column	25 µg	Lysis Tubes
D4302	ZymoBIOMICS DNA 96 MagBead Kit	2 x 96 preps	Magnetic Beads	10µg	BashingBead™
D4308	ZymoBIOMICS DNA 96 MagBead Kit	2 x 96 preps	Magnetic Beads	10µg	Lysis Rack
R2001	ZymoBIOMICS RNA Miniprep Kit	50 preps	Spin-Column	100 µg	BashingBead™
R2002	ZymoBIOMICS DNA/RNA Miniprep Kit	50 preps	Spin-Column	100 µg	Lysis Tubes

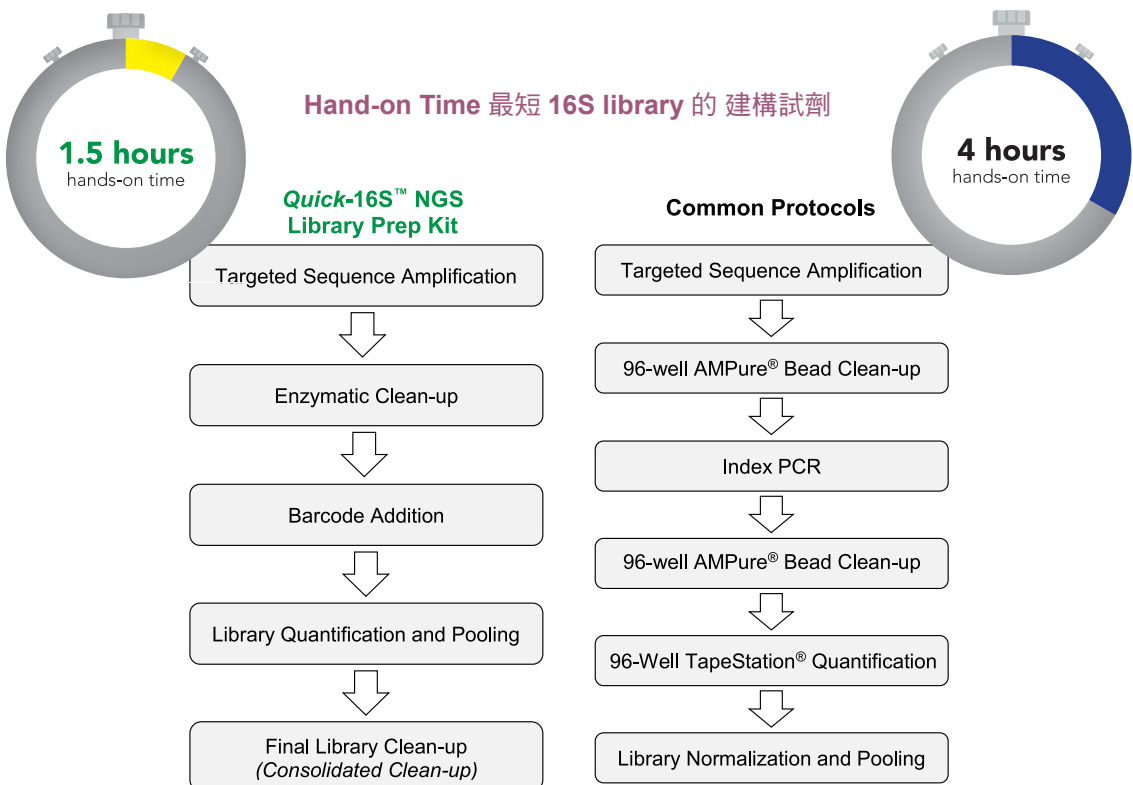
Quick-16S™ NGS Library Prep Kit

- 操作快速簡單：手作時間只要 1.5 小時，不需 TapeStation® analyses 或 AMPure® clean-ups
- 利用 Real-time PCR system 控制建構 library cycles，減少 PCR chimera 產生
- 全新的 primer 設計，分別有 16S V1-V2 set and 16S V3-V4 set，有效提高微生物覆蓋率
- 內含 ZymoBIOMICS® Microbial Community DNA Standard，可直接檢視實驗流程是否有無 bias

有效減少 PCR Chimera 的生成



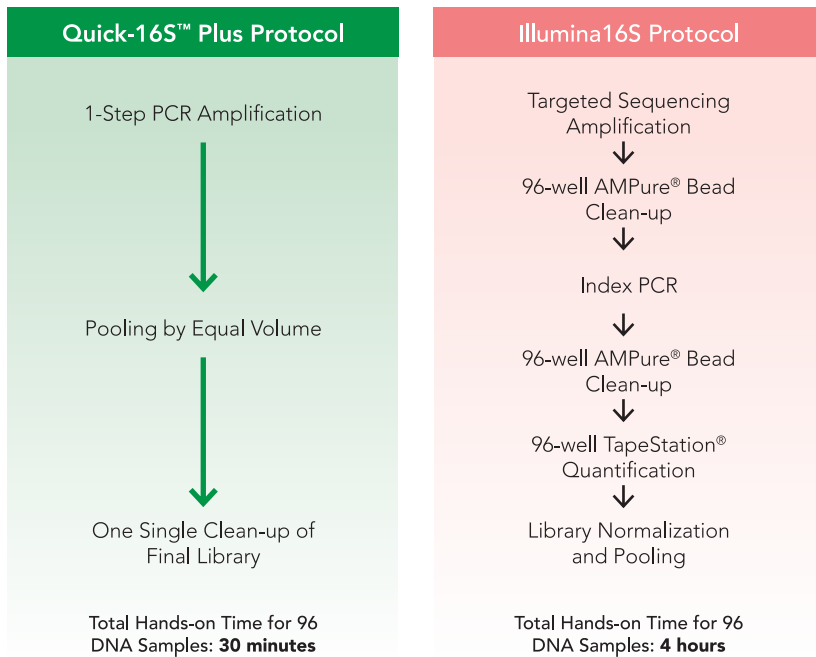
The Quick-16S™ NGS Library Prep Kit produces the lowest percentage of chimeric sequences as compared to two common protocols.



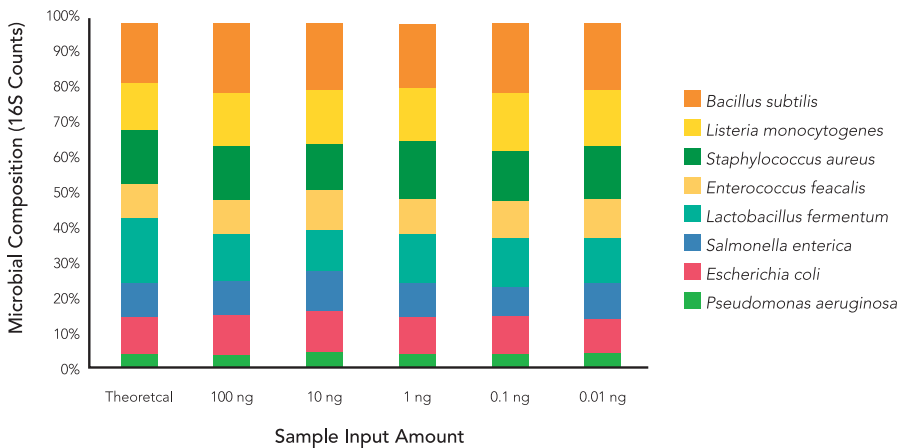
Quick-16S Plus NGS Library Prep Kit (V3-V4)

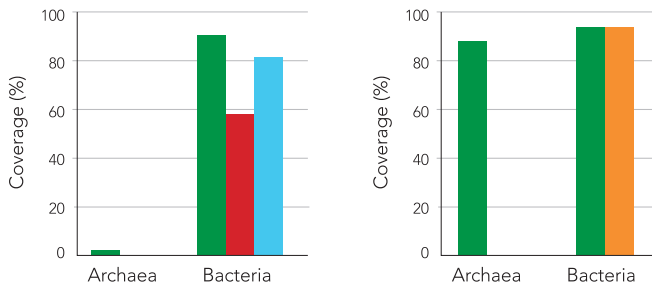
- 操作快速簡單：手作時間只要 30 分鐘，不需 TapeStation® analyses 或 AMPure® clean-ups
- 利用 1-Step PCR 達到快速簡單的自動化操作模式
- 利用 Real-time PCR system 控制建構 library cycles，減少 PCR chimera 產生
- 全新的 primer 設計，針對 16S V3-V4 set，有效提高微生物覆蓋率
- 內含 ZymoBIOMICS® Microbial Community DNA Standard，可直接檢視實驗流程有無 bias

最短時間快速建構 16S library



高準確度的微生物標準品



全新的 **Primer** 設計，提高微生物覆蓋率

The Quick-16S™ Primer Set V1-V2 and the Quick-16S™ Primer Set V3-V4 provide better coverage of bacteria and archaea than commonly used primer sets.

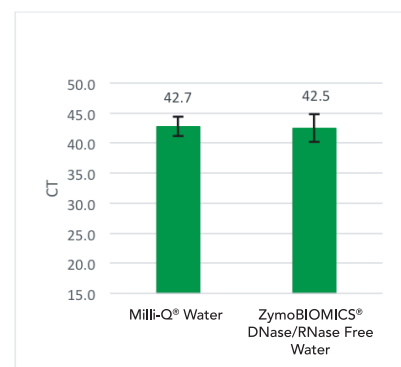
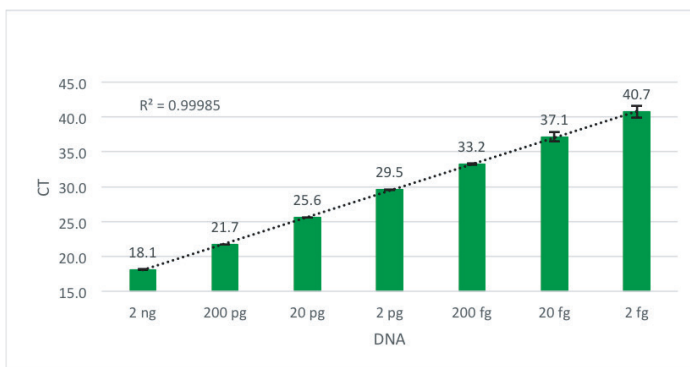
- Quick-16S™ Primer Set V1-V2
- HMP V1-V3 Primers
- Loman Lab V1-V3 Primers
- Quick-16S™ Primer Set V3-V4
- Klindworth V3-V4 Primer Set

Catalog No.	Product	Size
D6400	Quick-16S™ NGS Library Prep Kit	96 Rxns
D6410	Quick-16S™ NGS Library Prep Kit	24 Rxns
D6420	Quick-16S™ Plus NGS Library Prep Kit (V3-V4)	96 Rxns
D6422	Quick-16S™ Plus NGS Library Prep Kit (V3-V4)	24 Rxns

ZymoBIOMICS® PCR PreMix

- 為 2X concentrated "master mix"
- 高產量、高靈敏度的 hot start polymerase
- 亦可作 T/A cloning

可接受的 DNA input: 2 fg-2ng



Quantification of no template controls (NTCs) via real-time PCR was determined by amplification of the 16S rRNA gene, after the addition of 2.5 μ M SYTO® 9 to a 20 μ l reaction volume.

Catalog No.	Product	Size
E2056	ZymoBIOMICS™ PCR Premix	50 Rxns
E2057		200 Rxns



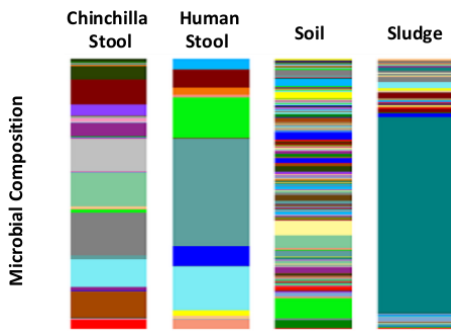
ZymoBIOMICS® Services

Zymo Microbiomics NGS 平台

服務項目：16S rRNA,
Shotgun sequencing

經標準品確效驗證，可提供
publication-quality data

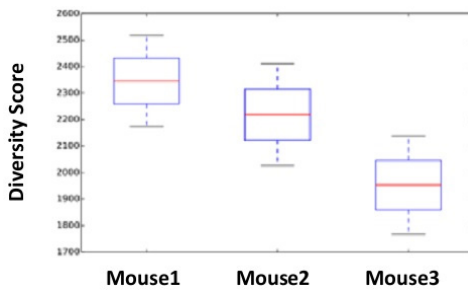
Barchart of Microbial Composition



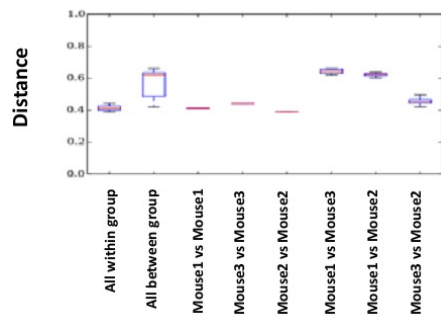
Hierarchical Clustering of Samples



Alpha Diversity (within-sample)



Beta Diversity (between-sample)



ZYMO RESEARCH
The Road of Science is to Make Things Simple

ZymoB:OM:CS[®]
A Comprehensive Solution for Microbiomics and Metagenomics

菌相研究 全集中攻略

