2015. Q3

創世紀季刊 for NGS

DNA/RNA extraction kit

樣品製備

Epicentre (solution based extraction)

- ★ MasterPure™ Complete DNA and RNA Purification Kit
- ★ MasterPure™ DNA Purification Kit
- ★ MasterPure™ RNA Purification Kit
- ★ MasterPure™ Plant Leaf DNA Purification Kit
- ★ MasterPure™ Plant RNA Purification Kit
- ★ MasterPure™ Yeast DNA Purification Kit
- ★ MasterPure™ Yeast RNA Purification Kit
- ★ MasterPure™ Gram-Positive DNA Purification Kit
- ★ MasterPure™ DNA Purification Kit for Blood Version II
- ★ ArrayPure™ Nano-scale RNA Purification Kit

MACHEREY-NAGEL (silica column)

DNA

- ★ Blood and biological fluids
- ★ Plasma
- ★ Tissue and cells
- ★ FFPE samples
- ★ Forensic samples
- ★ Plant and fungi
- ★ Soil, sludge, and sediment
- ★ Food and feed

RNA

- ★ RNA from cells and tissue
- ★ MicroRNA
- ★ RNA, DNA, and protein
- ★ RNA from blood
- ★ RNA and microRNA from FFPE samples
- ★ RNA from plant
- ★ Poly(A) mRNA from total RNA

Virus DNA/RNA

- ★ Cell-free body fluids
- ★ Blood, tissue, feces
- ★ Blood and biological fluids

核酸萃取 (16S Metagenomics)

- ★ SoilMaster DNA Extraction Kit
- ★ Meta-G-Nome DNA Isolation Kit or Water Kit
- ★ Metagenomic DNA Isolation Kit for Water
- ★ ExtractMaster Fecal DNAExtraction Kit

文庫構建

Swift BIOSCIENCES

- ★ Accel-NGS 1S DNA Library Kit for Illumina
- ★ Accel-NGS 2S DNA Library Kit for Illumina
- ★ Accel-NGS Methyl-Seq DNA Library Kit
- ★ Accel-Amplicon comprehensiveTP53 Panel
- ★ Accel-Amplicon 56G Oncology Panel
- ★ Accel-NGS™ DNA library Kit for Ion Torrent

BIOO SCIENTIFIC® THE NGS EXPERTS™

- ★ NEXTflex™ Rapid DNA-Seq kit
- ★ NEXTflex™ Cell Free DNA-Seq kit
- ★ NEXTflex™ mtDNA-Seq kit
- ★ NEXTflex™ Rapid RNA-Seq kit
- ★ NEXTflex™ Small RNA-Seq kitv2
- ★ NEXTflex™ Methyl-Seq Library Kit
- ★ NEXTflex™ Bisulfite-Seq kit
- ★ NEXTflex™ 16S V4 Amplicon-Seq Kit
- ★ NEXTflex™ 16S V1 – V3 Amplicon-Seq Kit
- ★ NEXTflex™ 18S ITS Amplicon-Seq Kit

創世紀生技 Bio-genesis Technology
服務信箱: service@biogenesis.com.tw 服務專線: 0800-211-667
台北: 02-26558877 台中: 04-22602466 高雄: 07-3425707
竹南: 037-680138 花蓮: 03-8463953

Next-Generation sequencing（NGS)

自 1953 年 Watson 與 Crick 解開 DNA 結構開始，人類便開始對於基因遺傳密碼有著無比的興趣。1984 年提出所謂的人類基因體計畫(Human Genome Project)，預計花費約 30 億美元，希望在 1990 到 2005 年間可以完成整個人類基因體的定序工作。然而，在 2000 年，由私人企業團隊花費約 10 億美元，便在短短九個月內完成人類史上第一次的基因圖譜解碼工作。在此之後，隨著各方技術的演進，如定序資料比對的演算、不同基因片段放大與定序的技術，使得所需花費的時間與金錢都大大的縮短。美國國家衛生研究院所設立的"革命性基因組定序技術計畫"，更進一步地號召全世界科學家在 2014 年時挑戰以 1 千美元的價格，完成一個個人的基因組序列讀取。整個基因定序的演進就如下圖所示，越來越便宜、快速、大量 (以 Illumina 定序平台為例)。

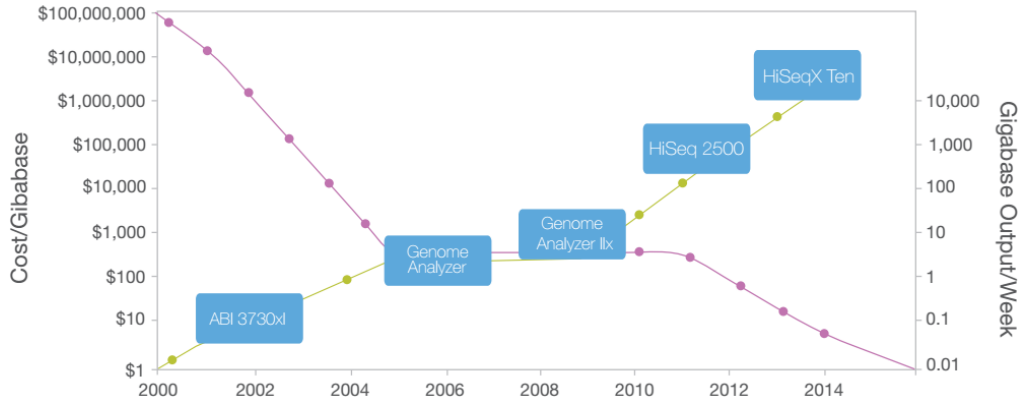


Fig. Sequencing Cost and Data Output Since 2000

The dramatic rise of data output and concurrent falling cost of sequencing since 2000.
(The Y-axes on the both side of the graph are logarithmic.)

然而，原始的定序方式，也就是被稱為第一代定序方式的 Sanger 定序方法已經無法滿足快速、便宜以及大量的需求，於是衍伸出新的定序技術，也就是被稱為 Next-generation DNA sequencing，簡稱 NGS。

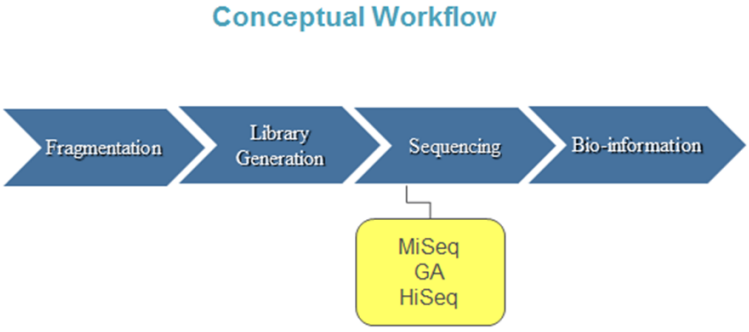
整個 NGS 的精神就建立四個主要的步驟:

1.**Fragmentation** ：先將大片段的 DNA 序列分解成依據不同平台所需長度的小片段 DNA，此步驟稱為核酸片段化(Fragmentation)。

2. **Library generation** ：接著再將各個小片段依據不同的定序平台給予不同的標定方式以方便後續的定序，此一步驟我們稱為建庫(Library construction)。

3. **Sequencing** ：在建立好 library 後，便進入高通量的定序(High-throughput Sequencing)，依據不同的原理而有不同的進行平台。

4. **Bio-information** ：最後在獲取大量資訊後，便對所有的小片段序列作重組，以設法還原成最初一開始的大片段序列，此步驟需要生物資訊軟體的分析。

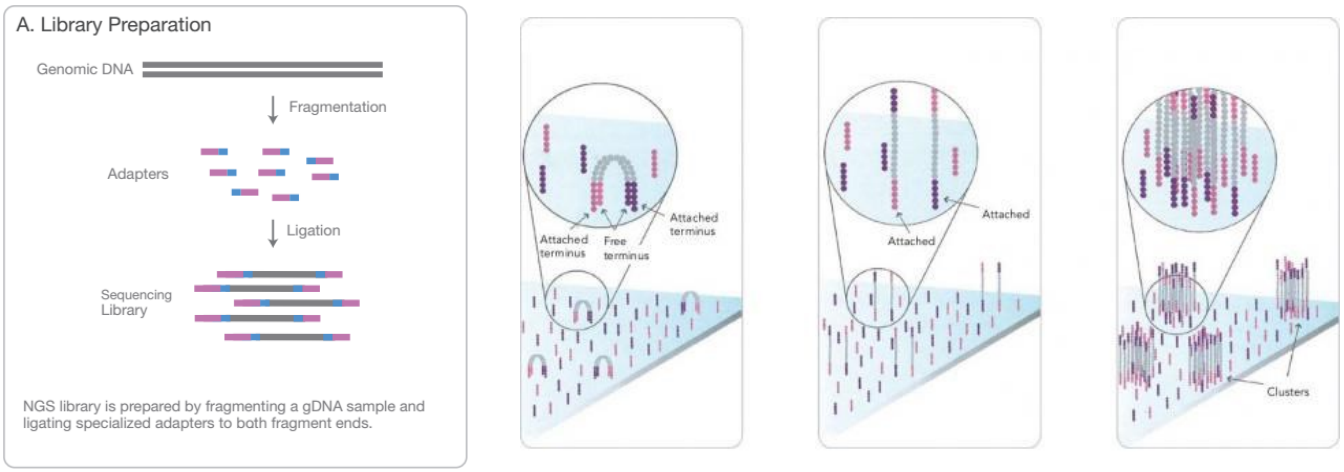


由上述的介紹了解到，平台的選用其實會決定建庫(library construction)的方式，由於篇幅的關係，在此就只對於目前 NGS 的主流平台，Illumina，來作介紹。

2007 年 Illumina 公司併購了 Solexa 公司以整合其 DNA 定序平台，其原理簡述如下，首先，將代測的長段 DNA 打斷成 200-500 bp 的小片段，並在小片段的兩端加上轉接序列(如圖 A)。再將接好的片段貼附到增殖片上(flow cell)進行橋接式增殖(圖 B)。由於可以藉由 Attached terminus (貼附片段) 彼此之間的距離(紫色與粉紅色片段)，搭配最初一開始的 DNA 片段大小，最後可控制每個增殖後的叢聚是來自於單一的 DNA 片段 (如下圖中的最右圖)。而在進行定序的過程中，依序加入不同顏色螢光標示的去氧核苷酸，重複反應、螢光偵測拍照、螢光移除、試劑製換，最後每個叢聚依顏色記錄的順序會得到個別的序列 (圖 C)。而得到的所有 DNA 序列資訊，再利用軟體計算，疊合，由共同的區段拼湊出整個序列，並且可以利用參考序列(reference genome) 比對出變異的位置(圖 D)

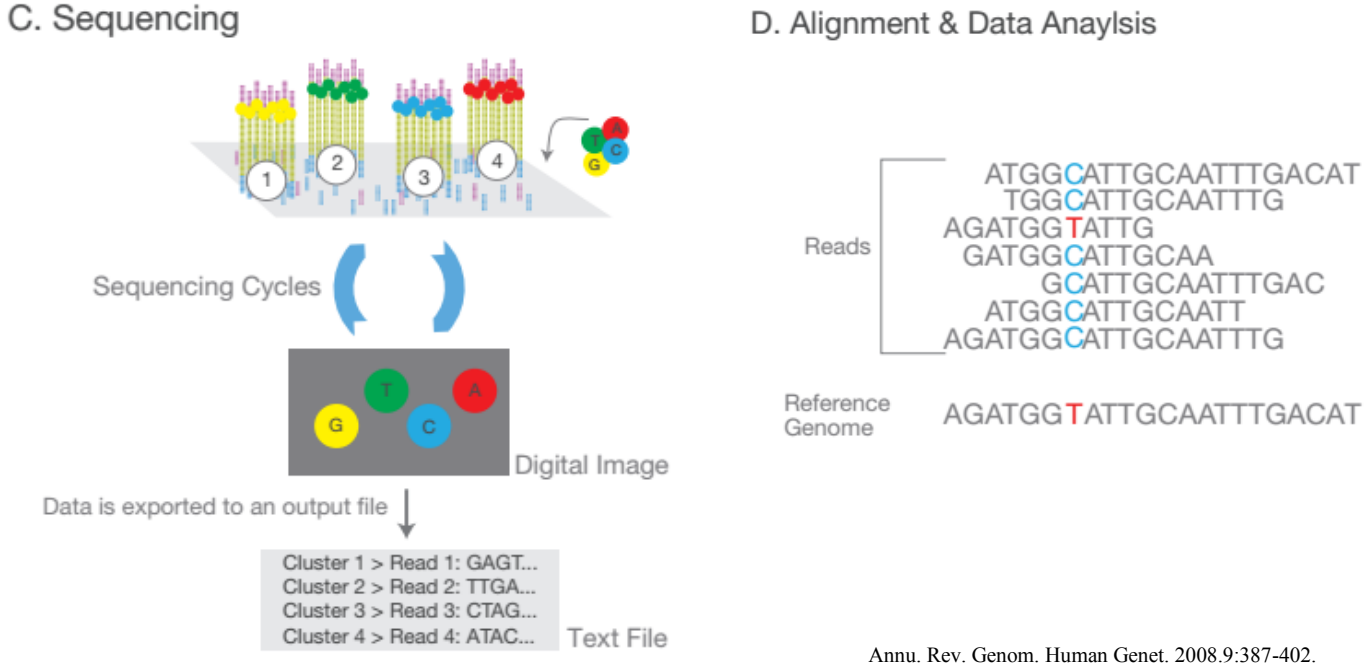
＊ 在小片段的兩端加上轉接序列

B. Bridge Amplification (橋接式增殖)



＊ 依顏色記錄的順序會得到個別的序列

＊ 利用軟體計算，疊合得到完整序列



Annu. Rev. Genom. Human Genet. 2008.9:387-402.

Swift BIOSCIENCES

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DNA library preparation kit

Accel-NGS™ 1S DNA Library Kit

- Single-stranded genomes
- Damaged DNA
- Metagenomic samples

Sequence Difficult Samples ▶

Accel-NGS™ 2S DNA Library Kit

- Broad input range: 10 pg to 1 µg
- PCR-free libraries from 10 ng
- Balanced coverage of AT-rich and GC-rich genomes

Discover More of the Genome ▶

Accel-NGS™ Methyl-Seq Library Kit

- High recovery of input DNA
- Low bias library preparation
- Simple, 90 minute start-to-finish protocol
- 100 pg of DNA input

Sequence the 5th Base ▶

Accel-Amplicon™ Panels for Illumina®

- Fast, single-tube protocol
- 10 ng of input DNA required
- Compatible for FFPE & cfDNA samples

Decrease Input, Not Sensitivity ▶

Soar Above. Discover More.

Accel-NGS™ 1S Plus DNA Library Kit

The Accel-NGS 1S Plus DNA Library Kit for Illumina® and Ion Torrent™ platforms utilizes innovative Swift technology, which allows DNA library construction from single-stranded DNA (ssDNA), as well as double-stranded DNA (dsDNA) which is nicked, damaged, or contains short fragments.

Features

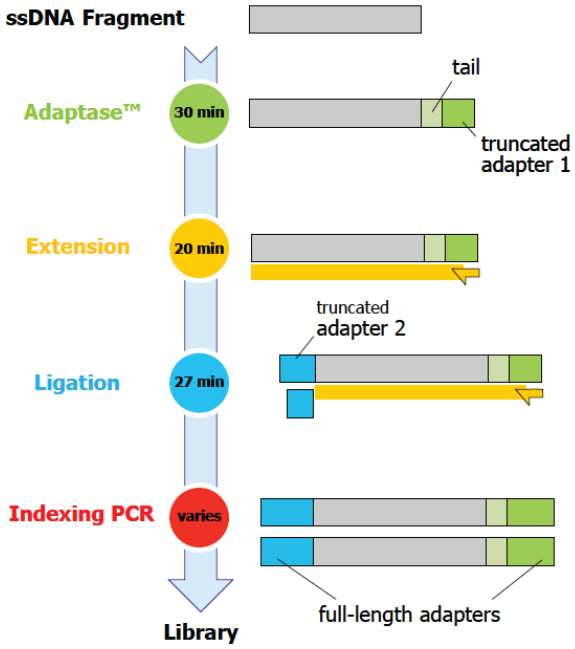
- Does not require intact dsDNA
- Highly efficient adapter ligation
- Inputs as low as 10 pg
- Simple, 2-hour protocol
- High sequence quality and even coverage

Applications

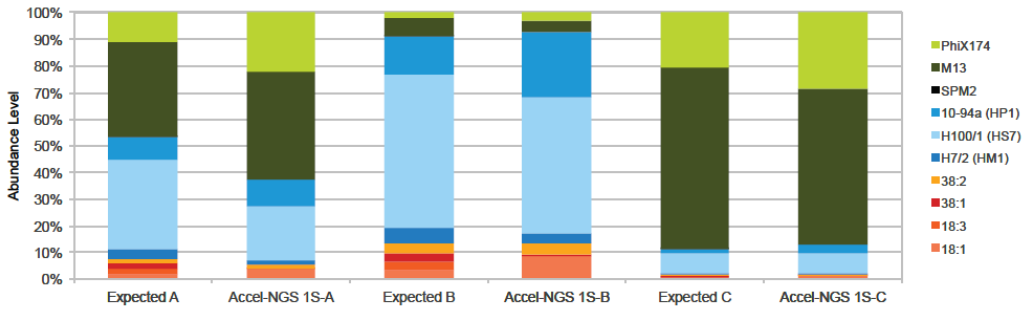
- ssDNA samples
- ChIP-seq
- Damaged samples, including nicked DNA
- Metagenomics
- Viromics
- Difficult-to-extract organisms
- Heat-denatured pathogenic samples
- Synthetic DNA, oligonucleotides
- NimbleGen™ and IDT xGen® Lock-down® captures

Simple Workflow

需先自行將 RNA 轉成 cDNA



Accurate Detection of Both ssDNA and dsDNA Phage

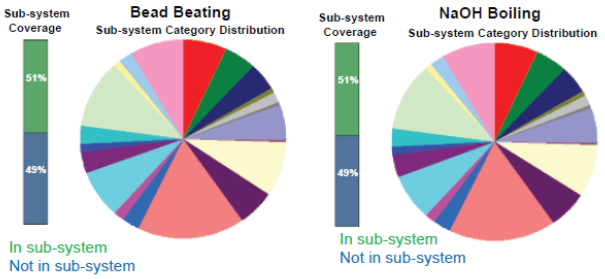


Accel-NGS 1S Plus DNA Library Kit was used to prepare and sequence three artificial viromes containing different proportions of the ssDNA phage PhiX174 and M13 mixed with dsDNA phage. In all cases, the proportions were preserved when sequenced with the Accel-NGS 1S Plus Kit without any prior whole genome amplification for detection of ssDNA phage.

DNA Extraction and Sequencing of the Hard to Extract Microbe, *Facklamia sp.* HGF4

Extraction Method	Qubit® (ng/µl)	Nanodrop™ (ng/µl)
Bead Beating	3.1	5.5
NaOH Boiling	< 2	107.3

Facklamia sp. HGF4	Bead Beating	NaOH Boiling
Fold-Coverage	65.5x	52.9x
Number of Contigs	42	46
Total Consensus	1896447	1892667
Largest Contig	190702	190844
N_{50} Contig Size	85449	86622



Colors in pie charts represent different Facklamia sp. *sub-system categories as annotated by the RAST server.*

- DNA extraction by NaOH boiling produced higher DNA yields from *Facklamia sp*. than bead beating, and in less time.
- Sequencing of the NaOH extracted DNA produced a high quality *de novo* assembled genome sequence that was indistinguishable from that produced from bead beating extracted DNA.

Ordering Information

Product Name	Reactions	Catalog No.
Accel-NGS 1S Plus DNA Library Kit for Illumina	12	DL-IL1SP-12
Accel-NGS 1S Plus DNA Library Kit for Illumina	48	DL-IL1SP-48

Indexing Kits

An Accel-NGS 1S Plus Indexing Adapter Kit is required for complete functionality of the library kit.

Product Name	Reactions	Catalog No.
1S Plus Indexing Kit for Illumina (12 indices, 1 reaction each, Set A)	12	SI-IL1SP-12A
1S Plus Dual Indexing Kit for Illumina (20 indices x 96 combinations)	48	DI-IL1SP-48

Related Products

Product Name	Reactions	Catalog No.
Accel-NGS 2S DNA Library Kit for Illumina	12	DL-ILM2S-12
Accel-NGS 2S DNA Library Kit for Illumina	48	DL-ILM2S-48
Accel-NGS DNA Library Kit for Ion Torrent	10	DL-ION1-10
Accel-NGS DNA Library Kit for Ion Torrent	50	DL-ION1-50

Visit swiftbiosci.com for easy ordering.

This product is for Research Use Only. Not for use in diagnostic procedures.

Accel-NGS™

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Accel-NGS™ 2S DNA Library Kit for Illumina® Platforms

The Accel-NGS 2S DNA Library Kit utilizes a proprietary adapter ligation chemistry which provides complex libraries from low abundance inputs and delivers excellent coverage across a range of inputs.

Features

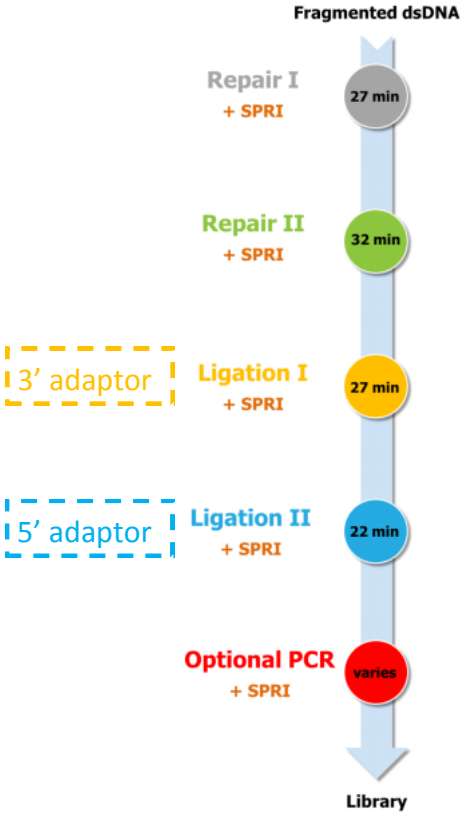
- Broad input range: 10 pg to 1 µg
- PCR-free libraries from 10 ng
- Sequential repair and ligation steps
- Increased library complexity
- Balanced coverage of AT-rich and GC-rich genomes

Applications

- Human WGS
- ChIP-seq
- Metagenomics
- Amplicon sequencing
- Clinical samples such as FFPE and plasma*
- SureSelect^{XT}, IDT xGen Lockdown, and NimbleGen captures

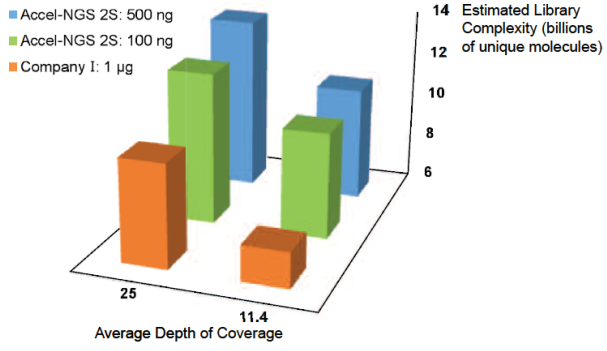
**This product is for Research Use Only. Not for use in diagnostic procedures.*

Protocol Overview



Performance Data

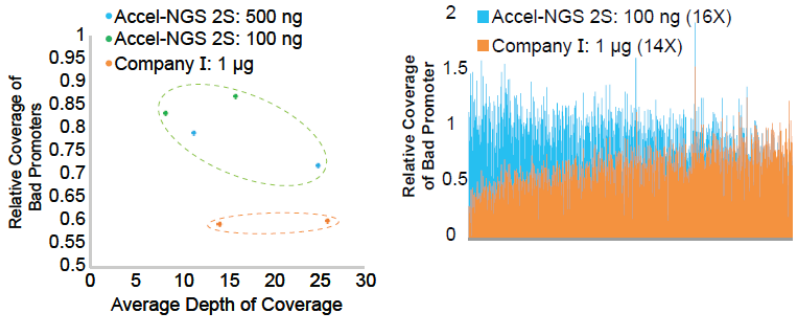
Generate Higher Complexity Libraries than the Leading Kit



Library complexity was obtained at various sequencing depths for Accel-NGS 2S libraries compared to libraries made with the leading kit.

- Using four incubations, this protocol repairs both 5′ and 3′ termini and sequentially attaches Illumina adapter sequences to the ends of fragmented dsDNA.
- Bead-based SPRI clean-ups are used to remove oligonucleotides and small fragments, and to change enzymatic buffer composition between steps. Different SPRIselect bead-to-sample ratios are utilized for different input quantities and insert sizes.
- For PCR-free applications, the resulting functional library is ready for library quantification and sequencing on the Illumina platform.
- Alternatively, an optional PCR step may be used to increase yield of indexed libraries, which then may be quantified and sequenced. Please refer to the table on Page 6 for recommended library sizes and input requirements.

Cover Extreme Base Composition Regions Better



Relative coverage of the GC-rich 1000 bad promoters obtained at various sequencing depths of Accel-NGS 2S libraries compared to libraries made with the leading kit.

Ordering Information

Product Name	Catalog No.
Accel-NGS 2S DNA Library Kit for Illumina - 12 reactions	DL-ILM2S-12
Accel-NGS 2S DNA Library Kit for Illumina - 48 reactions	DL-ILM2S-48

Indexing Kits

An Accel-NGS 2S Indexing Adapter Kit is required for complete functionality of the library kit.

Product Name	Catalog No.
2S Single Index Kit for Illumina (a single index, 12 reactions)	SP-ILM2S-12
XT Compatibility Module (adapter and pre-hyb PCR primers, 12 reactions)	XT-ILM2S-12
XT Compatibility Module (adapter and pre-hyb PCR primers, 48 reactions)	XT-ILM2S-48
2S Indexing Kit for Illumina (12 indices, 4 reactions each, Set A)	SI-ILM2S-48A
2S Indexing Kit for Illumina (12 indices, 4 reactions each, Set B)	SI-ILM2S-48B
2S Indexing Kit for Illumina (24 indices, 4 reactions each, Sets A & B)	SI-ILM2S-96
2S Dual Indexing Kit for Illumina (1 reaction each of 96 unique combinations)	DI-ILM2S-96

Related Products

Product Name	Catalog No.
Accel-NGS 1S DNA Library Kit for Illumina - 12 reactions	DL-ILM1S-12
Accel-NGS 1S DNA Library Kit for Illumina - 48 reactions	DL-ILM1S-48
Accel-NGS DNA Library Kit for Ion Torrent - 10 reactions	DL-ION1-10
Accel-NGS DNA Library Kit for Ion Torrent - 50 reactions	DL-ION1-50

Related Products

Product Name	Catalog No.
Accel-NGS 1S DNA Library Kit for Illumina - 12 reactions	DL-ILM1S-12
Accel-NGS 1S DNA Library Kit for Illumina - 48 reactions	DL-ILM1S-48
Accel-NGS DNA Library Kit for Ion Torrent - 10 reactions	DL-ION1-10
Accel-NGS DNA Library Kit for Ion Torrent - 50 reactions	DL-ION1-50

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Accel-NGS™ Methyl-Seq Library Kit for Illumina® Platforms

The Accel-NGS Methyl-Seq Library Kit maximizes DNA recovery of bisulfite-converted samples and constructs libraries that accurately represent sample composition. The Accel-NGS Methyl-Seq workflow maximizes DNA recovery through a post-bisulfite library preparation, utilizing a highly efficient adapter attachment that is compatible with single-stranded, bisulfite-converted DNA. Library yields from this kit are up to 100x greater than those from methods that bisulfite convert after library construction. Additionally, the template-independent adapter attachment chemistry of the Accel-NGS Methyl-Seq Kit provides a more complete, less biased library as observed from comprehensive methylome coverage by Whole Genome Bisulfite Sequencing (WGBS).

Features

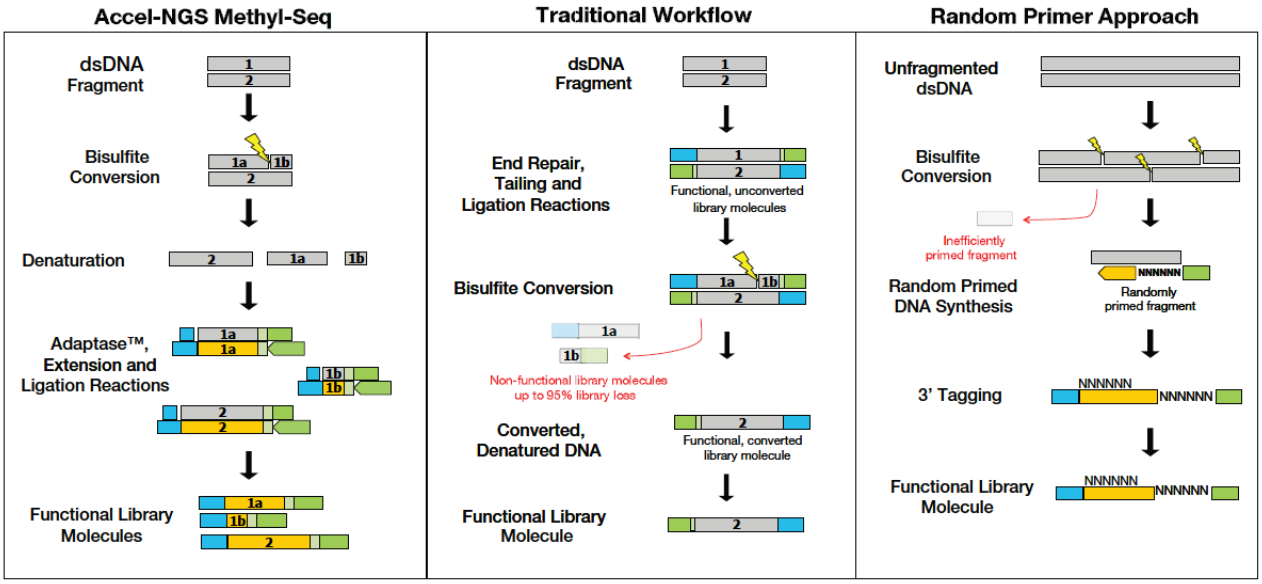
- High recovery of input DNA
- Low bias library preparation
- Simple, 2-hour library prep
- Minimal PCR cycles required
  - 4 cycles for 100 ng
  - 7 cycles for 10 ng
  - 10 cycles for 1 ng
  - 14 cycles for 100 pg

Applications

- WGBS
- Reduced Representation Bisulfite Sequencing (RRBS)
- Circulating, cell-free DNA
- Hybridization capture using NimbleGen™ SeqCap™ Epi Enrichment System
- Bisulfite-converted DNA enriched by MeDIP, ChIP or other methods
- Applications requiring uracil tolerance

不同 cycle 對應不同 input

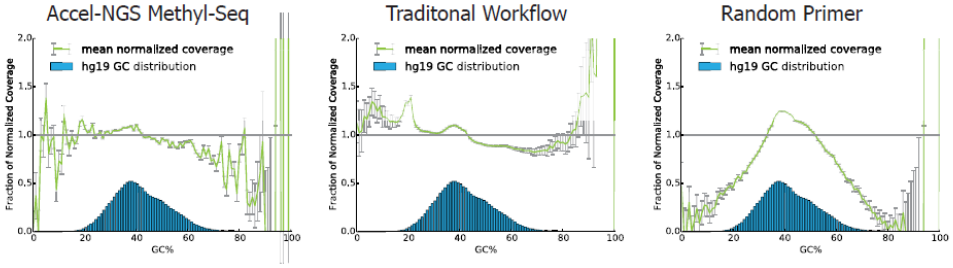
Workflow Superior to the Leading Kits



The Accel-NGS Methyl-Seq workflow utilizes post-bisulfite library construction and template-independent adapter attachment chemistry, which result in high recovery and low bias, respectively. Traditional workflow's pre-bisulfite library construction and random primer DNA synthesis account for their low recovery and high bias, respectively.

A Higher Recovery, Less Biased Library

	Accel-NGS Methyl-Seq	Traditional Workflow	Random Primer
Input (ng)	1	1000	50
# PCR Cycles	10	10	10
Yield (nM)	7	15	20
nM Output per ng Input	7	0.015	0.4
Bias	Low	Low	High



Coverage plots indicate evenness of coverage for unconverted libraries for Accel-NGS Methyl-Seq, traditional workflow, and random primer. Unconverted and bisulfite-converted libraries were quantified by qPCR. Bioanalyzer traces indicate that bisulfite conversion fragments DNA. This enables higher molar yields for libraries created from bisulfite-converted input.

Comprehensive CpG Island Coverage

Input	Unique Mapped	Complexity	Duplicates	Fold Coverage	CGI Coverage	Relative CGI Coverage	% CGI Covered
10 ng	82.5%	1.6 X 10^9	8.8%	11.4x	7.8x	0.68	99.7

WGBS was performed using 10 ng Zymo Gold bisulfite-converted Coriell NA12878 DNA. Sequencing was performed on a HiSeq® with V4 chemistry. Analysis was performed using BSMap and Picard tools. Complexity refers to estimated library size, CGI Coverage refers to CpG Island Coverage, Relative CGI Coverage is the ratio of CGI to total genome coverage.

Ordering Information

Product Name	Reactions	Catalog No.
Accel-NGS Methyl-Seq DNA Library Kit for Illumina	12	DL-ILMMS-12
Accel-NGS Methyl-Seq DNA Library Kit for Illumina	48	DL-ILMMS-48

Indexing Kits

An Accel-NGS Methyl-Seq Indexing Kit is required for complete functionality of the library kit.

Product Name	Reactions	Catalog No.
Methyl-Seq Indexing Kit for Illumina	12	DI-ILMMS-12
Methyl-Seq Indexing Kit for Illumina	48	DI-ILMMS-48

This product is for Research Use Only. Not for use in diagnostic procedures.

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Accel-Amplicon™ Panels for Illumina® Platforms

Accel-Amplicon panels are based on a unique molecular biology that provides powerful solutions for detecting and screening clinically relevant mutations. Swift Biosciences' multiplex amplicon panels are comprised of 10's to 100's of primer pairs in a single-tube format which are optimized for sequencing on Illumina platforms. Primer pairs in the panels are designed for compatibility with the short DNA fragments from both formalin-fixed, paraffin-embedded (FFPE) and circulating, cell-free DNA (cfDNA) samples. A fast and easy single-tube workflow produces the best-in-class performance for on-target percentage and coverage uniformity, enabling variant discovery and confirmation.

針對基因中特定 amplicon 進行觀察

Features

- Single-tube, 2-hour workflow
- Inputs as low as 10 ng
- Amplicons sized 120-160 bp for compatibility with cfDNA and FFPE
- Limit of detection as low as 1%
- On-target specificity and coverage uniformity > 95%
- Leverages the high fidelity performance of the Illumina platform
- Includes sequencing adapters

Supported Panels

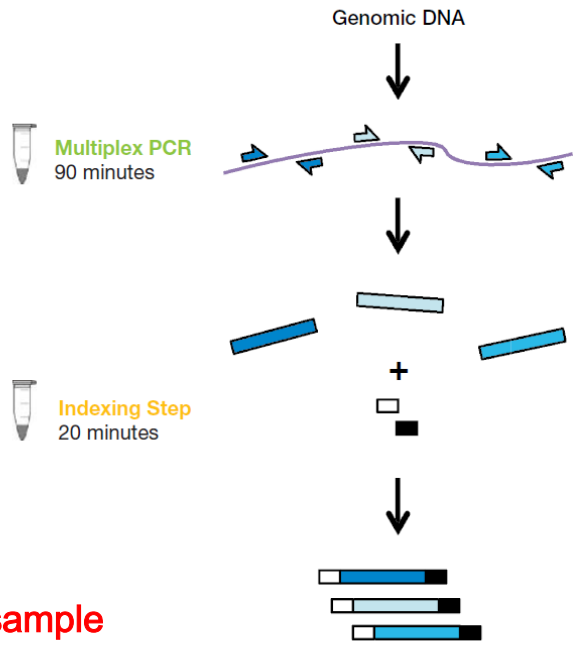
- 56G Oncology Panel
- Comprehensive TP53 Panel
- Custom panels

Sample Types

- FFPE
- cfDNA
- Fresh frozen
- Genomic DNA

適用許多不同來源 sample

Single-Tube, 2-Hour Workflow



Dual-Indexed Amplicon Library

The single-tube workflow includes two brief incubations to generate the multiplex amplicon targets and add a unique combination of Illumina-compatible indexed adapters, creating up to 96 uniquely-indexed libraries for multiplexing on a single sequencing run.

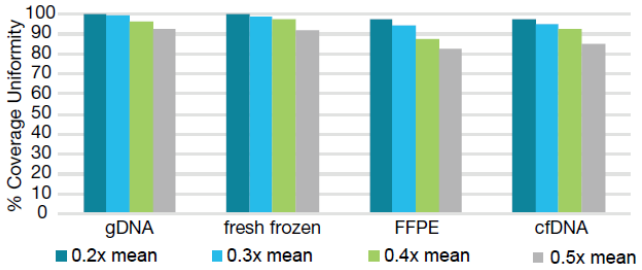
	Comprehensive TP53	56G Oncology
Packaging Options	12/48 reactions with dual indexing	12/48 reactions with dual indexing
Input DNA Required*	10-25 ng	10-25 ng
Time Required	2 hours	2 hours
Number of Amplicons	21	263
Amplicon Size	106-154 bp (average 140 bp)	92-184 bp (average 138 bp)
Number of Genes Covered	1	56
Target Size	1.8 kb	23.7 kb
FFPE/cfDNA Compatible	Yes	Yes
Percent On Target	> 95%	> 95%
Coverage Uniformity (>20% of Mean)	> 95%	> 95%

Accel-Amplicon 56G Oncology Panel

The Accel-Amplicon 56G Oncology Panel offers hotspot coverage of 56 clinically relevant oncology-related genes, using a 263-amplicon design to generate multiplex libraries compatible with Illumina sequencing platforms.

Product Specifications	
Input DNA Required	10-25 ng
Time Required	2 hours
Number of Amplicons	263
Amplicon Size	92-184 bp (average 138 bp)
Number of Genes Covered	56
Total Target Size	23.6 kb
FFPE/cfDNA Compatible	Yes
On Target Percentage	> 95%
Coverage Uniformity at > 20% of Mean	> 95%
Limit of Detection (at 10 ng for Base Substitutions)	1-5%
Multiplexing on MiSeq® v2 Nano @ 5000X Avg. Depth	1
Multiplexing on MiSeq v2 @ 5000X Avg. Depth	22

High Coverage Uniformity Across Sample Types



10 ng of input DNA from a variety of sample types was used to generate libraries with the Accel-Amplicon 56G Oncology Panel. The coverage uniformity, as the percentage of the bases covered at least 20%, 30%, 40%, or 50% of the average depth, was determined across four sample types. The percentage of reads on target was > 95% for all sample types.

針對 56 種 oncogen 特定 amplicon 進行觀察

Genes Represented in the 56G Oncology Panel

ABL1	5	CSF1R	2	FBXW7	6	GNAS	2	KIT	14	NPM1	1	SKT11	5
AKT1	2	CTNNB1	1	FGFR1	2	HNF1A	4	KRAS	3	NRAS	3	SMAD4	10
ALK	2	DDR2	1	FGFR2	4	HRAS	2	MAP2K1	5	PDGFRA	4	SMARCB1	14
APC	9	DNMT3A	1	FGFR3	6	IDH1	1	MET	6	PIK3CA	11	SMO	5
ATM	19	EGFR	9	FLT3	4	IDH2	2	MLH1	1	PTEN	14	SRC	1
BRAF	2	ERBB2	4	FOXL2	1	JAK2	2	MPL	1	PTPN11	2	TP53	21
CDH1	3	ERBB4	8	GNA11	2	JAK3	3	MSH6	4	RB1	1	TSC1	1
CDKN2A	2	EZH2	1	GNAQ	2	KDR	9	NOTCH1	3	RET	6	VHL	3

The Accel-Amplicon 56G Oncology Panel includes both clinically relevant hotspot loci and regions of contiguous coverage, depending on the allele distribution across each target gene. The table depicts the genes represented, followed by the number of amplicons for each gene. Contiguous, overlapping coverage is included for APC, ATM, EGFR, FBXW7, FGFR3, HNF1A, KIT, MSH6, PIK3CA, PTEN, and SMAD4. Comprehensive coding exon coverage is included for TP53.

Reproducible Variant Calling from Q-Seq HDx™ Quantitative Standards

Gene	AA	CHR	POS	REF	ALT	Expected Allele Frequency	Detected Allele Frequency (N=10)	Standard Deviation
EGFR	G719S	7	55241707	G	A	**24.5**	23.8	1.5
PIK3CA	H1047R	3	178952085	A	G	**17.5**	17.5	1.3
KRAS	G13D	12	25398281	C	T	**15**	15.0	1.8
NRAS	Q61K	1	115256530	G	T	**12.5**	13.4	1.2
BRAF	V600E	7	140453136	A	T	**10.5**	9.9	0.3
KIT	D816V	4	55599321	A	T	**10**	10.3	1.1
PIK3CA	E545K	3	178936091	G	A	**9**	8.5	1.1
KRAS	G12D	12	25398284	C	T	**6**	6.6	1.2
EGFR	L858R	7	55259515	T	G	**3**	2.7	0.5
EGFR	T790M	7	55249071	C	T	**1**	1.0	0.3

The Accel-Amplicon 56G Oncology Panel consistently detected validated variants at the expected frequency in replicates by five different users from 10 ng of the Horizon Diagnostics Quantitative Multiplex DNA Reference Standards HD701. The variants were called by LoFreq 2.1.1 (Genome Institute of Singapore) and GATK HaplotypeCaller (Broad Institute). When examining sporadic variants among the 10 replicates, the majority of background variants were present at less than 0.6%. No sporadic variants greater than 0.6% were detected.

Detection of Somatic Mutations in cfDNA and FFPE

Matched FFPE tumor, FFPE normal-adjacent, and cfDNA samples were obtained from Spectrum Health for analysis with the Accel-Amplicon 56G Oncology Panel. The data below shows concordance in variant allele frequencies across these matched samples.

Cancer Type	Gene	hg19 Coordinate	Amino Acid Change	% Mutant in FFPE Normal Adjacent	% Mutant in FFPE Tumor	% Mutant in cfDNA
Metastatic colorectal adenocarcinoma	PIK3CA	chr3:178936091	E545K	0%	23%	11%
	APC	chr5:112175576	Q1429*	0%	20%	5%
	TP53	chr17: 7579575	Q38* or intron	0%	21%	14%
	KRAS	chr12: 25398281	G13D	0%	22%	5%
Mammary carcinoma	PIK3CA	chr3:178952085	H1047R	0%	17%	0%
	TP53	chr17:7578488	D148H	0%	0%	9%
Ovarian cystadenofibroma	BRAF	chr7:140453136	V600E	0%	23%	1%
Fallopian tube adenocarcinoma	TP53	chr17:7577085	E285K	0%	48%	0%
	TP53	chr17:7578488	D148H	0%	0%	5%

In the above, cfDNA was extracted from 10 ml of blood and gDNA was obtained from FFPE normal or tumor tissues. The Accel-Amplicon 56G Oncology Panel was used to create libraries from 10 ng of cfDNA and 15 ng of FFPE gDNA. Sequencing was performed using V2 reagents on an Illumina MiSeq. Coverage uniformity and percentage of reads on target were greater than 95%. The average depth of coverage per base ranged from 2,500-5,000X. Somatic mutations were called using LoFreq 2.1.1 (Genome Institute of Singapore) and GATK HaplotypeCaller (Broad Institute).

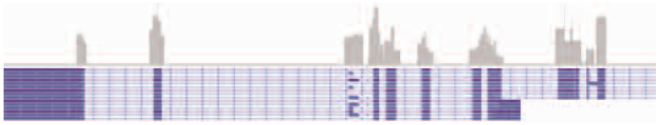
*Signifies a substitution leading to a nonsense mutation.

Accel-Amplicon Comprehensive TP53 Panel

The Accel-Amplicon TP53 Panel offers comprehensive, full-exon coverage of the TP53 gene.

Product Specifications	
Input DNA Required	10-25 ng
Time Required	2 hours
Number of Amplicons	21
Amplicon Size	106-154 bp (average 140 bp)
Total Target Size	1.8 kb
FFPE/cfDNA Compatible	Yes
On Target Percentage	> 95%
Coverage Uniformity at > 20% of Mean	> 95%
Limit of Detection (at 10 ng for Base Substitutions)	1-5%
Multiplexing on MiSeq v2 Nano @ 5000X Avg. Depth	19
Multiplexing on MiSeq v2 @ 5000X Avg. Depth	285

Comprehensive Coverage of TP53



Coverage of all coding regions of the TP53 gene by the Accel-Amplicon Comprehensive TP53 Panel are represented in a Sashimi plot (IGV; Broad institute).

Ordering Information

Accel-Amplicon Panel	Reactions	Catalog No.
Comprehensive TP53 Panel	12	AL-ILTP53-12
Comprehensive TP53 Panel	48	AL-ILTP53-48
56G Oncology Panel	12	AL-IL56G-12
56G Oncology Panel	48	AL-IL56G-48

Visit swiftbiosci.com for easy ordering.

This product is for Research Use Only. Not for use in diagnostic procedures.

Swift Biosciences, Inc.
58 Parkland Plaza, Suite 100 • Ann Arbor, MI 48103 • 734.330.2568 • www.swiftbiosci.com

Soar Above. Discover More.

Accel-NGS™ DNA Library Kit for Ion Torrent

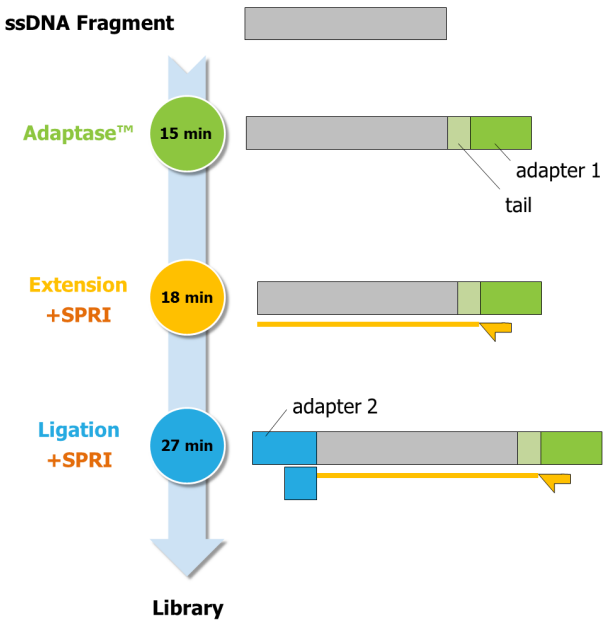
DNA Library Preparation for Next Generation Sequencing (NGS) on Ion Torrent Platforms

Innovative Swift technology improves sample prep for next gen sequencing by expediting the process and delivering higher quality data. The Accel-NGS DNA Library Kit for Ion Torrent platforms is the only commercially available kit capable of producing PCR-free libraries with as little as 5 ng of input DNA. PCR-free capability minimizes base composition bias and fidelity issues, while a highly efficient adapter ligation process reduces the input requirements.

- PCR-free to minimize base composition bias and fidelity issues
- Low input requirements: as little as 5 ng of DNA
- Fast - only 75 minutes start-to-finish
- Streamlined, 3-step protocol
- Reduces adapter dimer formation to maximize sequencing output
- Compatible with nicked, damaged, and denatured samples

Sample Types

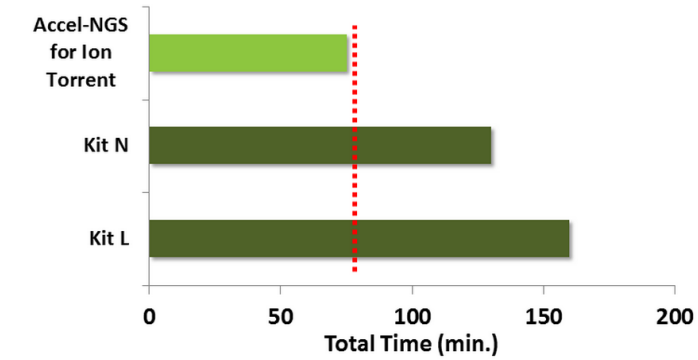
Single-stranded DNA
Double-stranded DNA
Heat-denatured samples
Amplicons
Nicked DNA
ChIP DNA
FFPE DNA
cDNA
Extremely AT/GC-rich Genomes

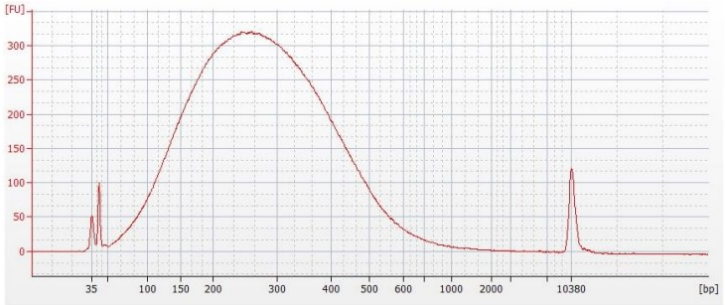


Instrument Compatibility

Ion Proton System
Ion PGM System

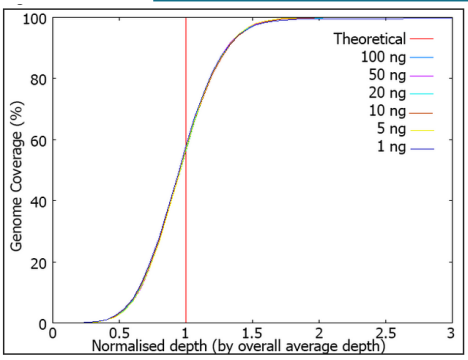
Total Turnaround Time Comparison Versus Other Kits

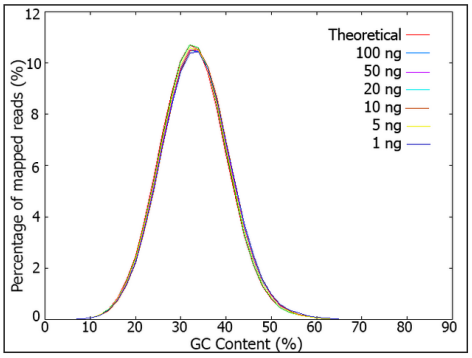




Example Library Size Distribution by Agilent Bioanalyzer for a 150 bp Insert Size Library Prepared from E. coli DNA .

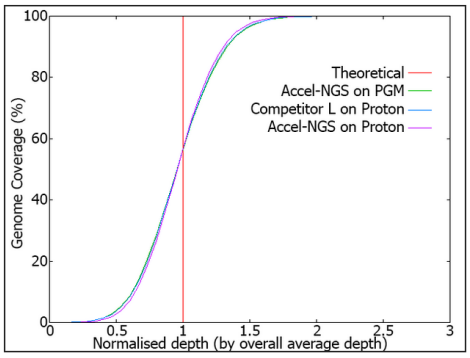
Even Coverage from 1 ng to 100 ng Input

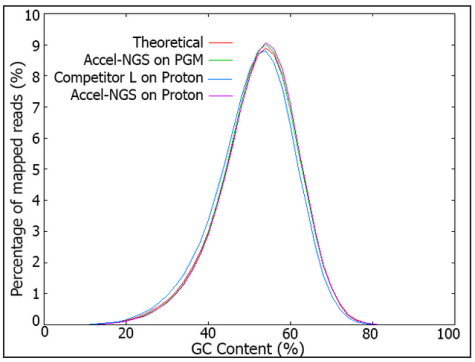




The Accel-NGS DNA Library Kit for Ion Torrent produces libraries from an AT-rich genome with near-theoretical coverage from 100 ng down to 1 ng of input DNA.

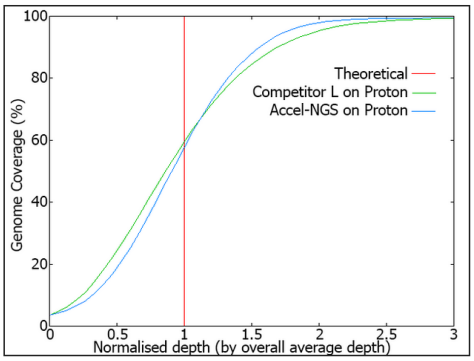
Balanced Composition Genome Per-

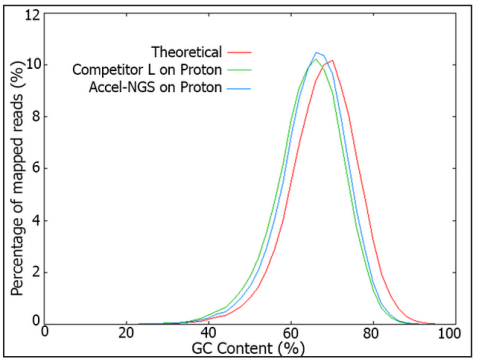




The Accel-NGS DNA Library Kit produces libraries from a balanced base composition genome with coverage comparable to Competitor L's kit on both the PGM and Proton platforms.

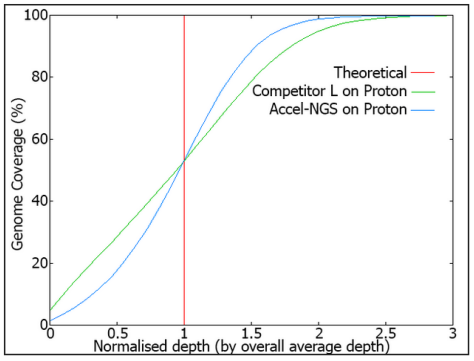
Outperforms Leading Ion Torrent Library Prep Kit for GC-rich Genomes

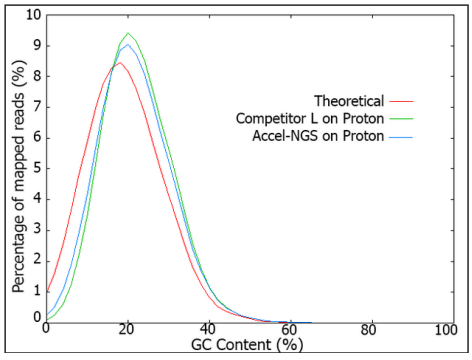




The Accel-NGS DNA Library Kit produces libraries from a GC-rich genome with coverage closer to the theoretical perfect result vs. Competitor L's kit on the Ion Proton.

Outperforms the Leading Ion Torrent Library Prep Kit for





The Accel-NGS DNA Library Kit produces libraries from AT-rich genomes with coverage closer to the theoretical perfect result vs. Competitor L's kit on the Ion Proton.

PCR-Free Libraries Prepared from 5 ng DNA

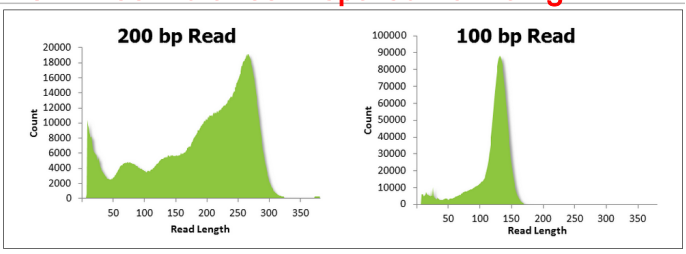


Figure 1. Read length histograms from 100bp and 200bp sequencing chemistries.

Sequencing Chemistry	Read Length	Fold Coverage	Adapter Dimer	Usable Sequence	Mean Accuracy
200 bp	189 bp	91.5	1.3%	56%	99.1%
100 bp	118 bp	78.0	5.3%	70%	99.6%

Table 1. Summary of sequencing run on 100bp and 200bp chemistries.

Accel-NGS DNA Library Kit for Ion Torrent produces sequenceable libraries PCR-free from 5 ng of input DNA. Similar performance has been obtained with input quantities ranging from 5 ng up to 8 µg.

PCR-Free versus Competitive Kit

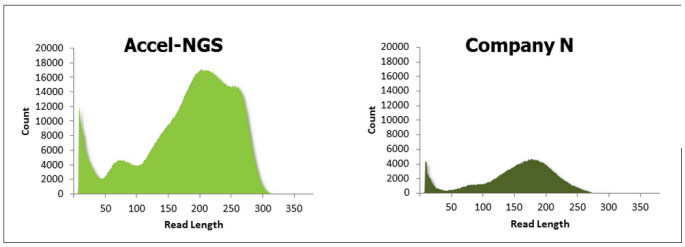


Figure 2. Read length histograms of Swift Biosciences versus Company N.

Product	PCR Cycles	Read Length	Fold Coverage	Adapter Dimer	Usable Sequence	Mean Accuracy
Accel-NGS DNA Library Kit	**0**	182 bp	**101.8**	**0%**	**61.0%**	99.1%
Company N	11	152 bp	17.7	64.4%	10.6%	99.1%

Table 2. Summary of sequencing run of Swift Biosciences versus Company N.

The majority of Company N kit results were adapter dimers. Accel-NGS DNA Library Kit produces sequenceable libraries from low input without PCR, while minimizing the formation of adapter dimers to maximize sequencing output.

Great Results in About Half the Time Versus Leading Kit

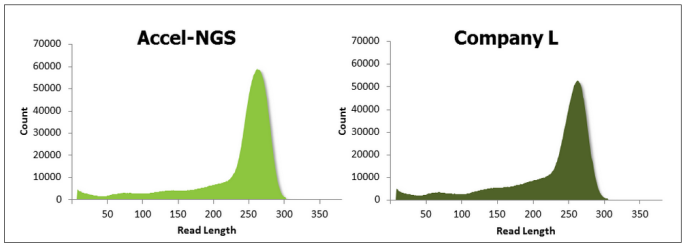


Figure 3. Read length histograms of Swift Biosciences versus Company L.

Product	Read Length	Fold Coverage	Adapter Dimer	Usable Sequence	Mean Accuracy
Accel-NGS DNA Library Kit	231 bp	156	0%	61%	99.4%
Company L	232 bp	118	0%	70%	99.4%

Table 3. Summary of sequencing run of Swift Biosciences versus Company L.

Accel-NGS DNA Library Kit achieves comparable performance to the leading competitive kit in about half the time.

Related Products

Product Name	Reactions	Catalog No.
Accel-NGS DNA Library Kit for Ion Torrent	10	DL-ION1-10
Accel-NGS DNA Library Kit for Ion Torrent	50	DL-ION1-50

Soar Above. Discover More.

NGS Advisors

To assist Swift in assuring that we are developing Next Generation Sequencing (NGS) sample preparation products that address the needs of this rapidly changing market, we have assembled an outstanding team of advisors, including the following individuals:

Chief Scientific Officer
Cofactor Genomics
St. Louis, MO

Jon Armstrong

Laboratory Head, Department of Developmental and Molecular Pathways
Novartis Institute of Biomedical Research
Cambridge, MA

Alex Gaither, PhD

Director, Genome Platform
The Broad Institute
Cambridge, MA

Stacey Gabriel, PhD

Head, Clinical Genomics, Genomics and Pathology Services
Medical Director, Cytogenomics and Molecular Pathology
Department of Pathology and Immunology
Washington University School of Medicine
St. Louis, MO

Shashikant Kulkarni, PhD

Director of Tech Dev, Clinical Applications and Automation Engineering, Genome Platform
The Broad Institute
Cambridge, MA

Niall Lennon, PhD

DNA library preparation kit

Select Institutions Using NEXTflex™ Sequencing Kits & Reagents

Brown Univ
CDC
Children's Hospital Boston
Cold Spring Harbor Lab
Columbia Medical Center
Dartmouth Medical School
Fred Hutchison Cancer Research Inst
Harvard Medical School
J. Craig Venter Institute
Johns Hopkins Univ
MD Anderson Medical Center
Medical Neurogenetics
Memorial Sloan Kettering Cancer Center
MIT
Mount Sinai School of Medicine
National Cancer Institute
National Institute of Health
New York Univ
Northern Arizona Univ
Oklahoma Medical Research Foundation
Rockefeller Univ
St Jude Children's Research Hospital
Stowers Institute
Translational Genomics Research Institute (TGEN)
Univ of British Columbia
Univ of California – Berkeley
Univ of California – Davis
Univ of California – Riverside
Univ of California - San Diego
Univ of California – Riverside
Univ of Illinois
Univ of Massachusetts
Univ of Massachusetts Med School
Univ of Montana
Univ of N. Carolina
University of Texas Health Science Center
Univ of Washington
University of Wisconsin, Madison
USDA
Vanderbilt University

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竹南: 037-680138 花蓮: 03-8463953

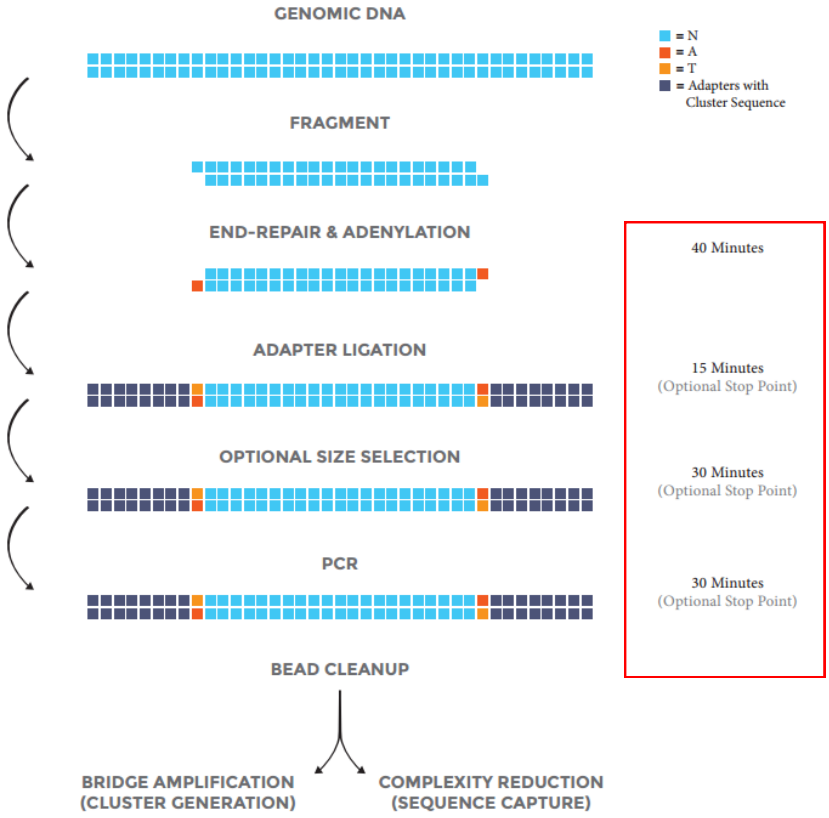
BIOO SCIENTIFIC NGS KITS

NEXTflex™ Rapid DNA Sequencing Kit
(1 ng – 1 µg)

(Illumina Compatible)
Catalog #5144-01 (8 reactions)

- Flexible amounts of input DNA from 1 ng to 1 µg
- Fast workflow requiring 2 hours or less, with minimal hands-on time.
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing reads
- Automation-friendly workflow
- Flexible barcode options – up to 192 barcodes available
- Compatible with Illumina® sequencing platforms including the MiSeq, HiSeq, NextSeq and HiSeq X Ten
- Prepare single, paired-end and multiplexed genomic DNA libraries

Sample flow chart with approximate times necessary for each step.



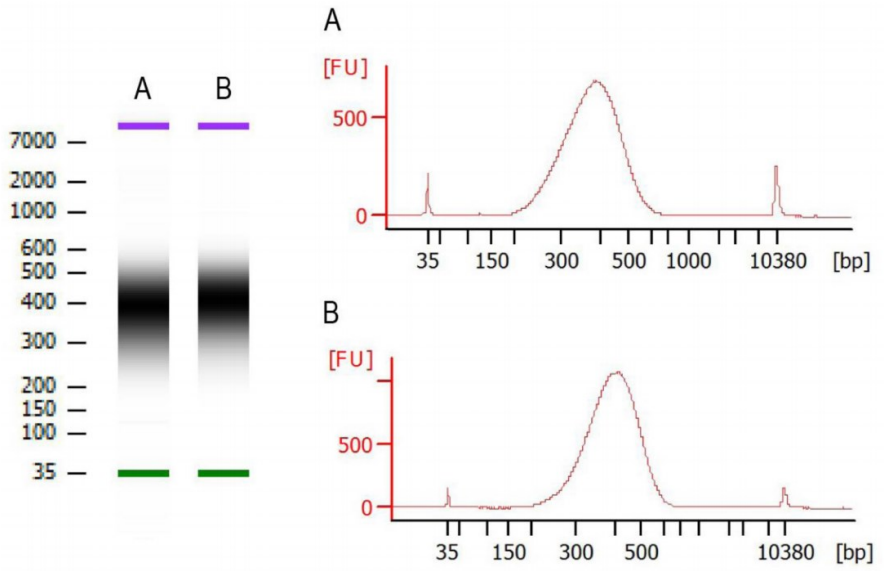
約 2 小時

End Polishing
↓
Adapter Ligation
↓
PCR

1 ng – 1 µg

2 hours or less

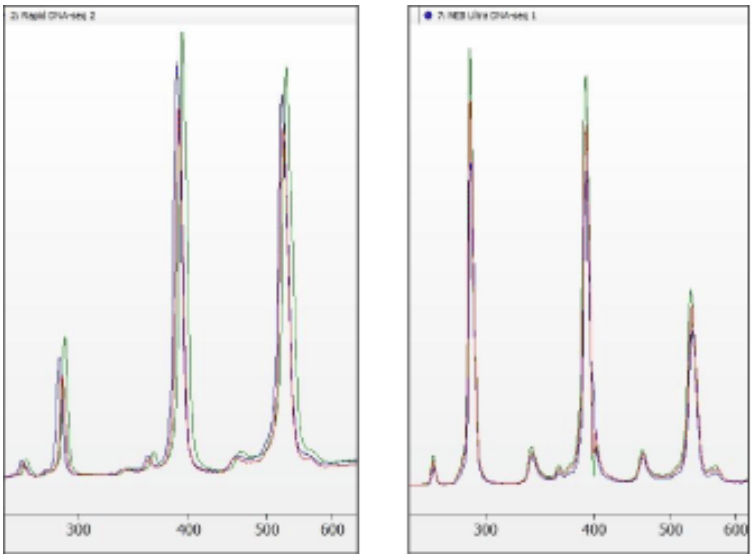
The NEXTflex Rapid DNA-Seq Kit features a streamlined library prep protocol to help you meet your research goals faster than you thought possible.



High Sensitivity DNA Chip Ladder / Electropherogram

A) 1 ng input NEXTflex™ 15 cycle PCR product.

B) 1 µg input NEXTflex™ 6 cycle PCR product.



Overlays of the triplicate samples of NEXTflex Rapid DNA-Seq (left) and Competitor N's kit (right)

Catalog#	Product Name	Quantity
5144-01	NEXTflex™ Rapid DNA Sequencing Kit	8 rxns
5144-02	NEXTflex™ Rapid DNA Sequencing Kit	48 rxns
5144-03	NEXTflex™ Rapid DNA-Seq Kit Bundle with DNA Barcodes 1 - 24	48 rxns
5144-04	NEXTflex™ Rapid DNA-Seq Kit Bundle with DNA Barcodes 25 - 48	48 rxns
	Barcodes for Use with 10 ng or more of DNA	
514101	NEXTflex™ DNA Barcodes - 6	48 rxns
514102	NEXTflex™ DNA Barcodes - 12	96 rxns
514103	NEXTflex™ DNA Barcodes - 24	192 rxns
514104	NEXTflex™ DNA Barcodes - 48	384 rxns
514105	NEXTflex™ DNA Barcodes - 96	768 rxns

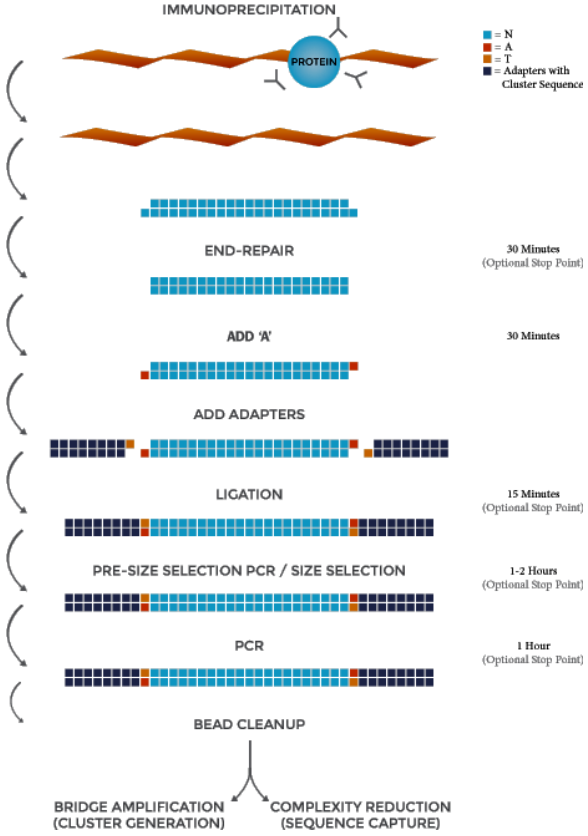
BIOO SCIENTIFIC NGS KITS

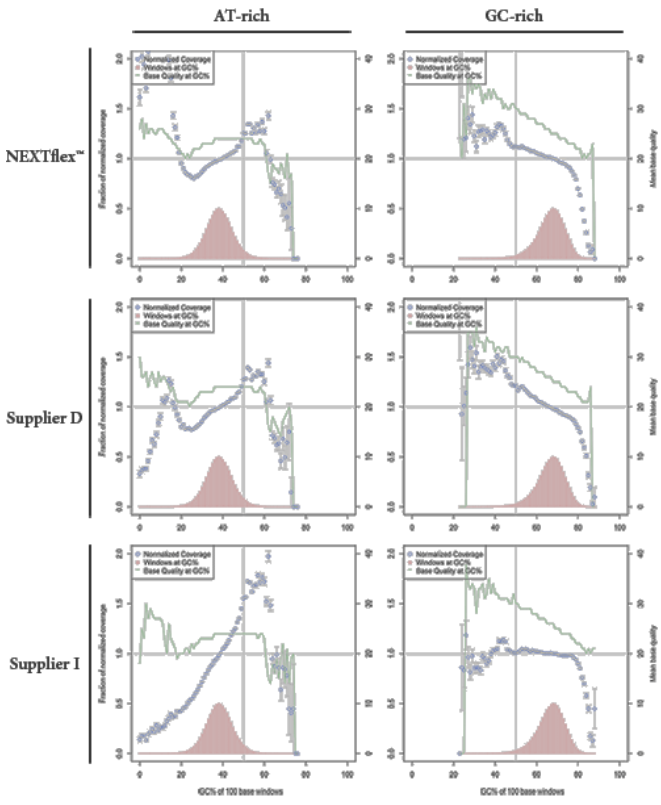
NEXTflex™ ChIP-Seq Kit

(Illumina Compatible)

Catalog #5143-01 (8 reactions)

- Optimized for down to 1 ng of DNA input
- For use with ChIP or genomic DNA samples
- Optimized for low DNA input with NanoQ™ enzymes and buffers
- Enhanced Adapter Ligation Technology offers optimal coverage and unique reads
- Flexible barcode options – up to 96 unique, single-index barcoded adapters available
- Compatible with Illumina's MiSeq, HiSeq, HiSeq, HiSeq X Ten and NextSeq500 sequencing platforms





Catalog#	Product Name	Quantity
5143-01	NEXTflex™ ChIP-Seq Kit	8 rxns
5143-02	NEXTflex™ ChIP-Seq Kit	48 rxns
514120	NEXTflex™ ChIP-Seq Barcodes - 6	48 rxns
514121	NEXTflex™ ChIP-Seq Barcodes - 12	96 rxns
514122	NEXTflex™ ChIP-Seq Barcodes - 24	192 rxns
514123	NEXTflex™ ChIP-Seq Barcodes - 48	384 rxns
514124	NEXTflex™ ChIP-Seq Barcodes - 96	768 rxns

BIOO SCIENTIFIC NGS KITS

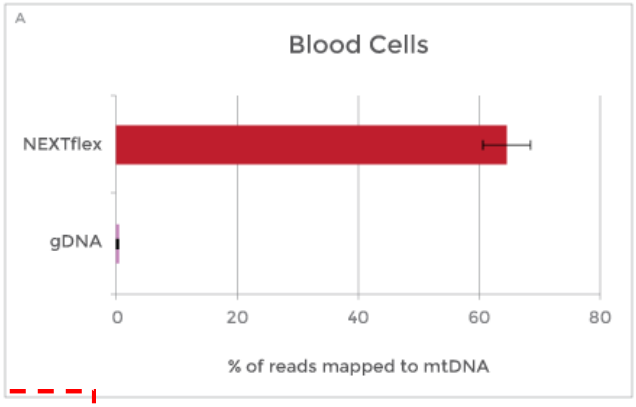
NEXTflex™ mtDNA-Seq Kit

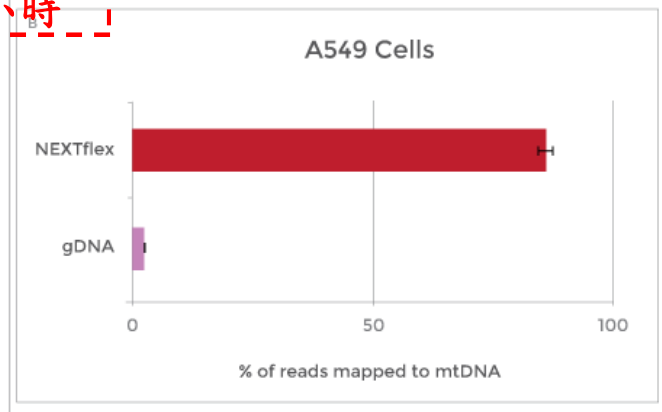
(Illumina Compatible)

針對粒腺體 DNA 進行觀察

- Enables targeted sequencing of the mitochondrial genome
- Offers efficient mtDNA isolation and library construction in a single workflow
- Produces a greater number of unique mtDNA reads compared to traditional PCR
- Enables heteroplasmy analysis
- Allows for increased multiplexing

約 2.5 小時





Enrichment of mtDNA reads in samples prepared using the NEXTflex mtDNA-Seq Kit. Triplicate libraries were made from mtDNA isolated using the NEXTflex mtDNA-Seq Kit (red) or untreated gDNA (purple). mtDNA was isolated from 4 µg blood or A549 gDNA. The bars represent mean and standard deviation.

Catalog#	Product Name	Quantity
5280-01	NEXTflex™ mtDNA-Seq Kit	8 rxns
5280-02	NEXTflex™ mtDNA-Seq Kit	48 rxns

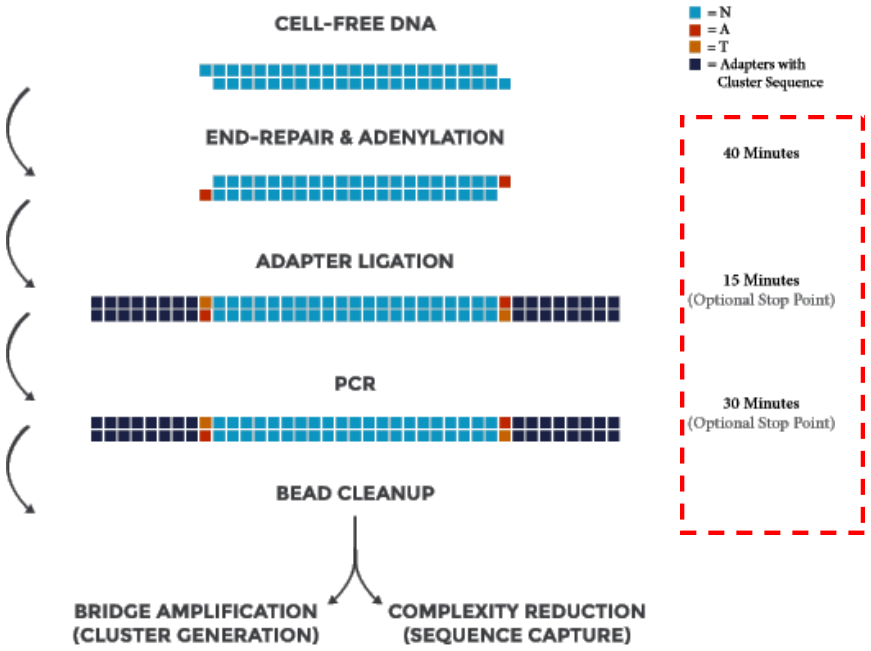
BIOO SCIENTIFIC NGS KITS

NEXTflex™ Cell Free DNA-Seq Kit

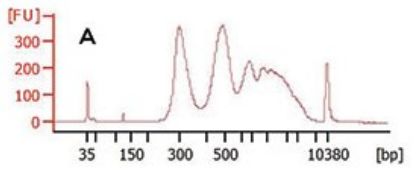
(Illumina Compatible)

針對游離 DNA 進行觀察

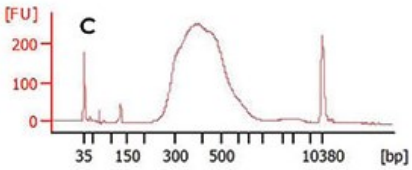
- Designed for low sample input - Only 1 ng of input DNA required
- Accelerated workflow requiring 2 hours or less, with minimal hands-on time
- Enhanced Adapter Ligation Technology offers a larger number of unique sequencing
- Flexible adapter barcode options – Kits containing up to 192 unique barcodes
- Compatible with Illumina® sequencing platforms Protocols available for automation



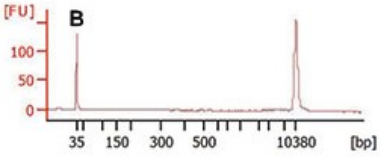
約 90 min



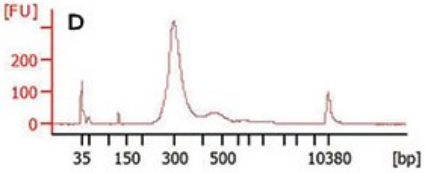
Panel A: Library from 32 µL (64% of prep) of cell-free DNA from a male donor amplified for 15 cycles. Note the broad size distribution, which reflects the discrete sizes of cell-free DNA fragments. The cell-free DNA was not fragmented prior to use.



Panel B: Analysis of the corresponding input cell-free DNA used to make the library shown in Panel A. Note, the concentration of cell-free DNA is too low to be detected, which is typical.



Panel C: Library made from 1 ng of sheared human genomic DNA, amplified for 15 cycles. Note the much different size distribution compared to the library made from cell-free DNA.



Panel D: Library made from cell-free DNA size-selected prior to library construction, using Ampure magnetic beads to enrich for small cell-free DNA. Library was amplified for 12 cycles. Sample was from a male donor. After adapter ligation, the desired library products are approximately 300 bp.

The metrics shown in the graphic below for Library 14 depict extremely high-quality sequencing data, as shown by the high Phred scores (Y-axis). The trend line shows average scores above 30, corresponding to >99.9% probability of accuracy, for the average of all reads in the position ranges corresponding to cfDNA sequence (as depicted along the X-axis), with the exception of the longest reads (positions 145-150), which have slightly lower scores (but which are still above 99% probability of being accurate). Results for Library 22 were similar.

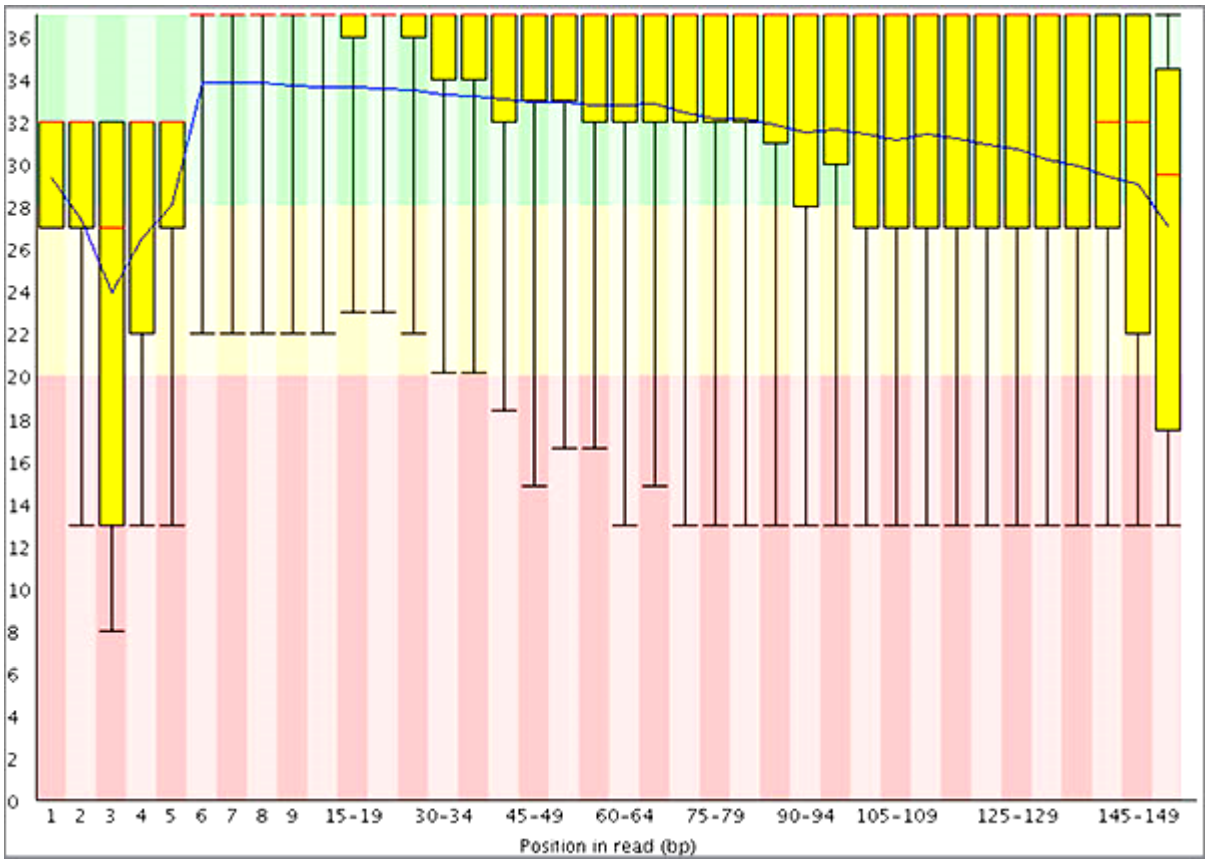


Table 1. Low % of adapter contamination and high % of reads mapping to human genome

Library	Total Reads	Reads after trimming adapter	% reads mapping to adapter	Reads after filtering duplicates	% duplication	Reads mapping to genome	% mapping to genome
Library 14	29,375,523	29,051,363	1.1%	24,338,715	16.22%	24020720	98.69%
Library 22	31,725,510	30,179,252	4.9%	12,651,028	58.08%	12321279	97.39%

Catalog#	Product Name	Quantity
5150-01	NEXTflex™ Cell Free DNA-Seq Kit	8 rxns
5150-02	NEXTflex™ Cell Free DNA-Seq Kit	48 rxns

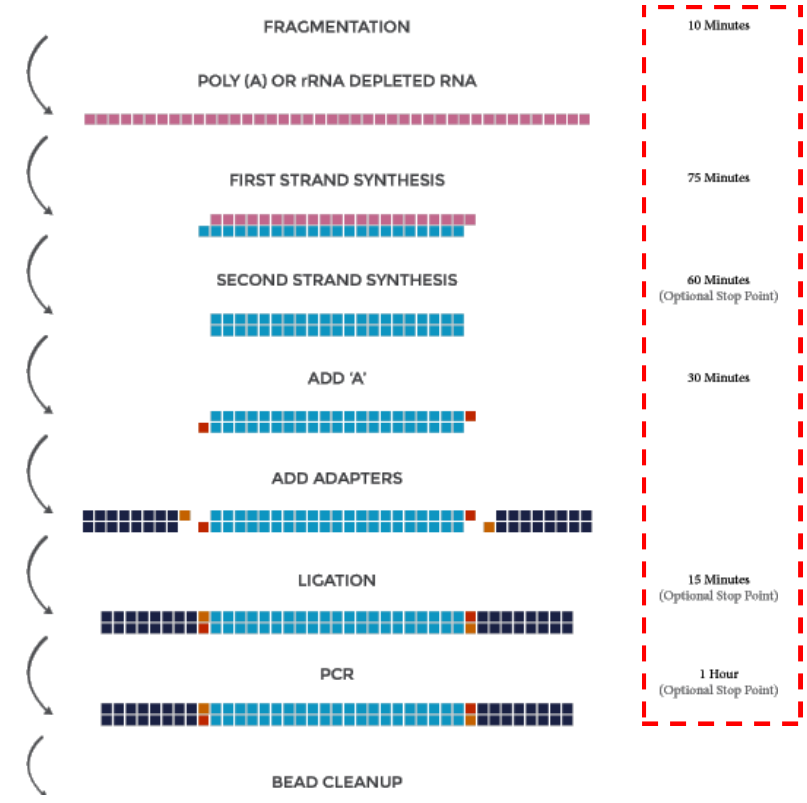
BIOO SCIENTIFIC NGS KITS

NEXTflex™ Rapid RNA-Seq Kit

(Illumina Compatible)

Catalog #5138-01 (8 reactions)

- Faster than traditional Illumina RNA library prep protocols
- Complete solution includes thermostable NEXTflex Rapid Reverse Transcriptase
- Input - 10 ng – 1 µg total RNA for enrichment by NEXTflex™ Poly(A) Beads or ~ 1 ng - 100 ng isolated mRNA or rRNA-depleted RNA
- Up to 96 barcodes available for multiplexing
- Automation-friendly workflow is compatible with liquid handlers Functionally validated with Illumina sequencing platforms



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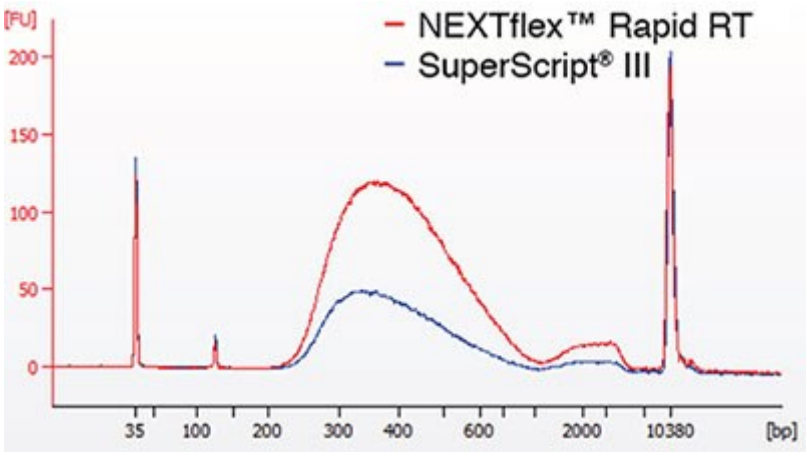
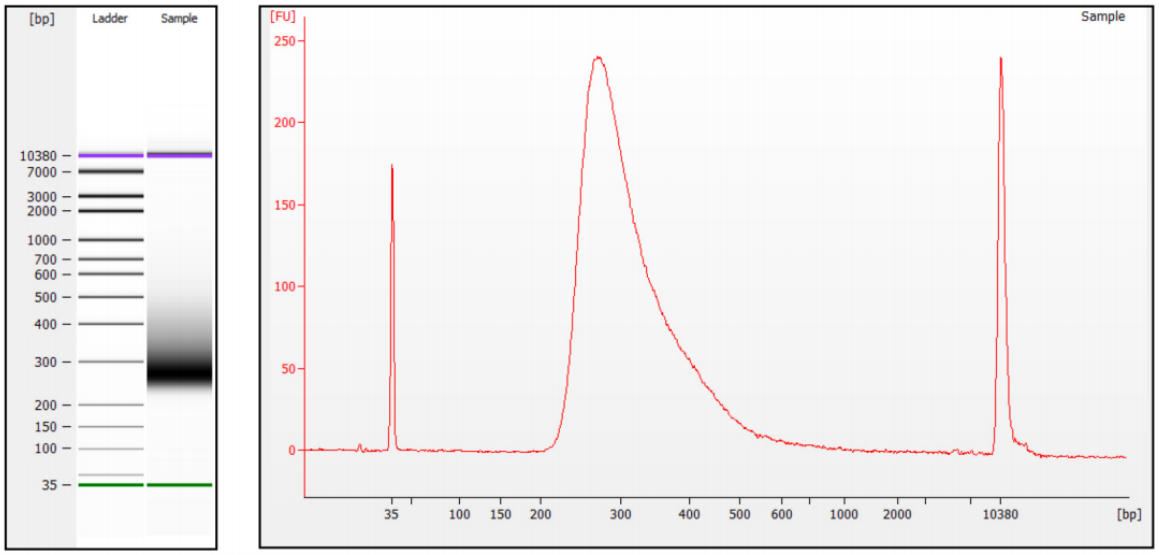
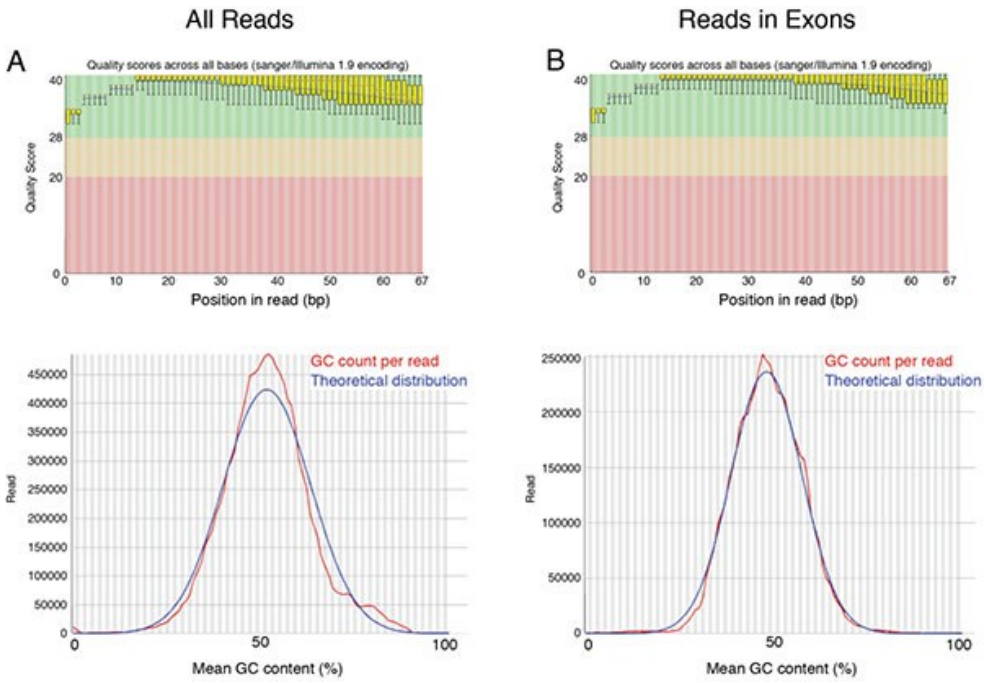


Improved library yield using NEXTflex Rapid RT. High Sensitivity DNA Bioanalyzer traces of RNA-Seq libraries constructed with NEXTflex Rapid RNA-Seq Kits. Libraries were constructed using 10 ng of fragmented, Poly (A)+ mRNA converted to cDNA using either NEXTflex Rapid RT (blue) or SuperScript® III (red).

Example of mRNA library size distribution. 1 µL of the library was run on an Agilent High Sensitivity DNA chip to verify size. Using a Qubit® 2.0 Fluorometer & Qubit® dsDNA HS Assay Kit, the concentration of the library was determined to be > 10 nM.





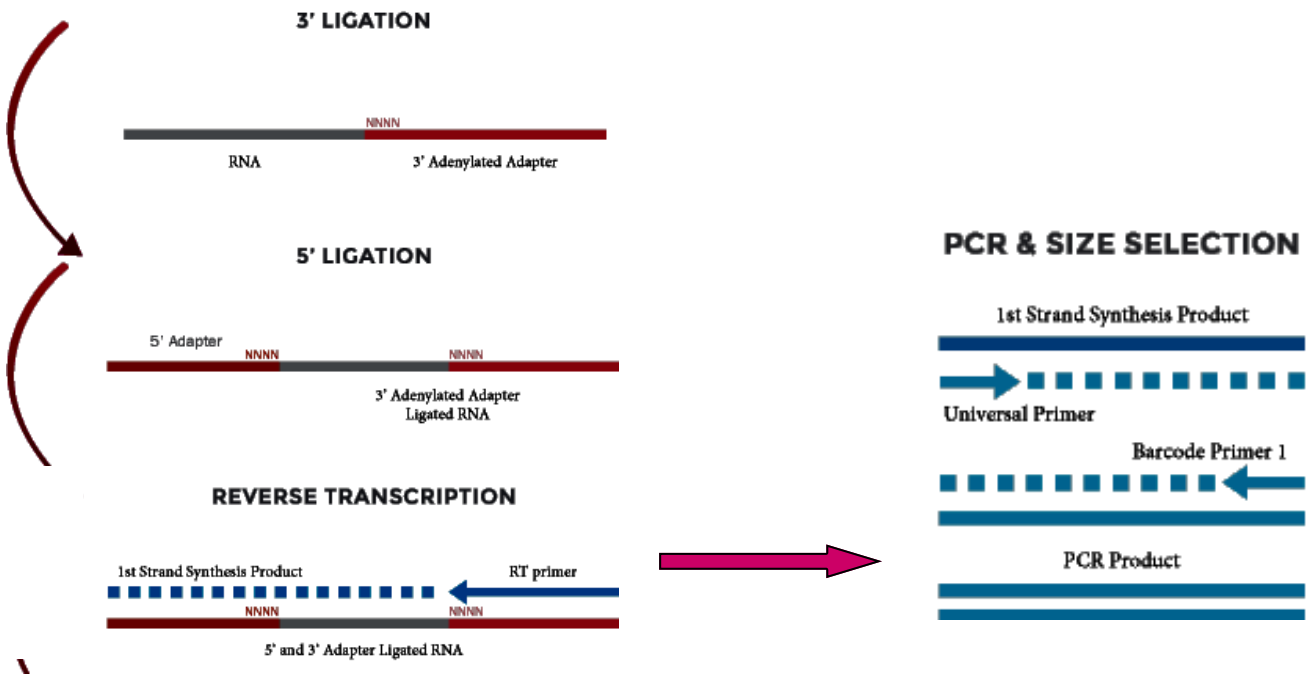
Catalog#	Product Name	Quantity
5138-01	NEXTflex™ Rapid RNA Sequencing Kit	8 rxns
5138-02	NEXTflex™ Rapid RNA Sequencing Kit	48 rxns
512911	NEXTflex™ RNA-Seq Barcodes - 6	48 rxns
512912	NEXTflex™ RNA-Seq Barcodes - 12	96 rxns
512913	NEXTflex™ RNA-Seq Barcodes - 24	192 rxns
512914	NEXTflex™ RNA-Seq Barcodes - 48	384 rxns
512915	NEXTflex-96™ RNA-Seq Barcodes - 96	768 rxns

BIOO SCIENTIFIC NGS KITS

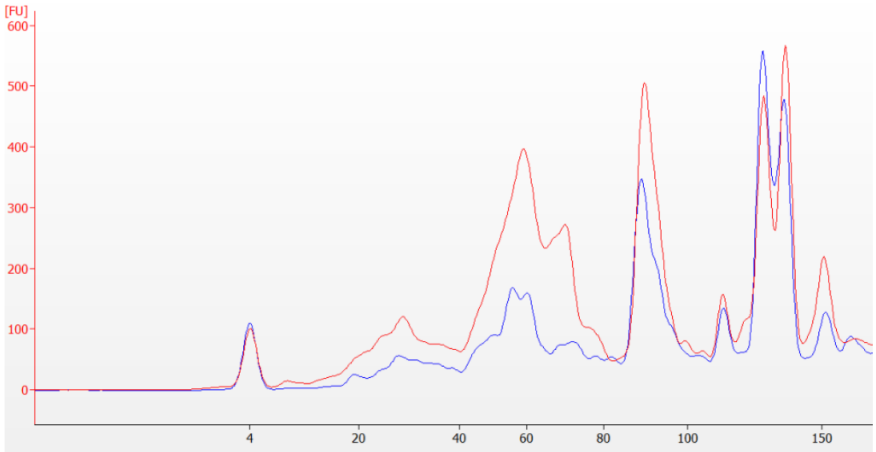
NEXTflex™ Small RNA Sequencing Kit v2

(Illumina Compatible)

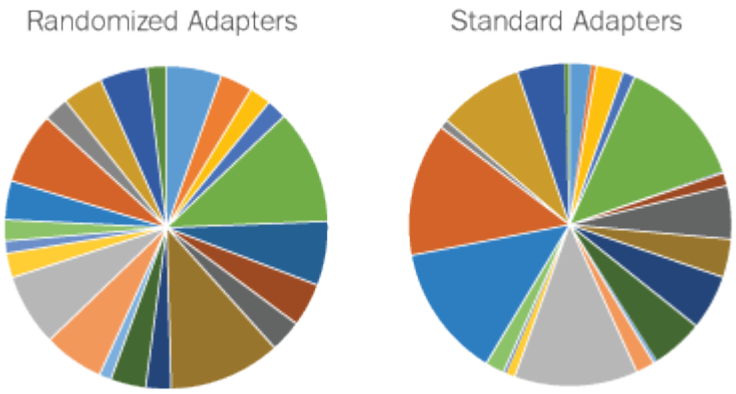
- Incorporates patent pending randomized adapters which reduce ligation bias, resulting in more accurate data than can be obtained using traditiona Illumina small RNA-seq library prep protocols
- Utilizes AIR™ Ligase, a highly efficient truncated T4 RNA Ligase for greater sequencing depth
- Simplified workflow reduces hands-on time 48 barcoded PCR Primers for multiplexing available
- 1-10 µg total RNA or purified small RNA from 1-10 µg total RNA input re quired
- Functionally validated with Illumina sequencing platforms



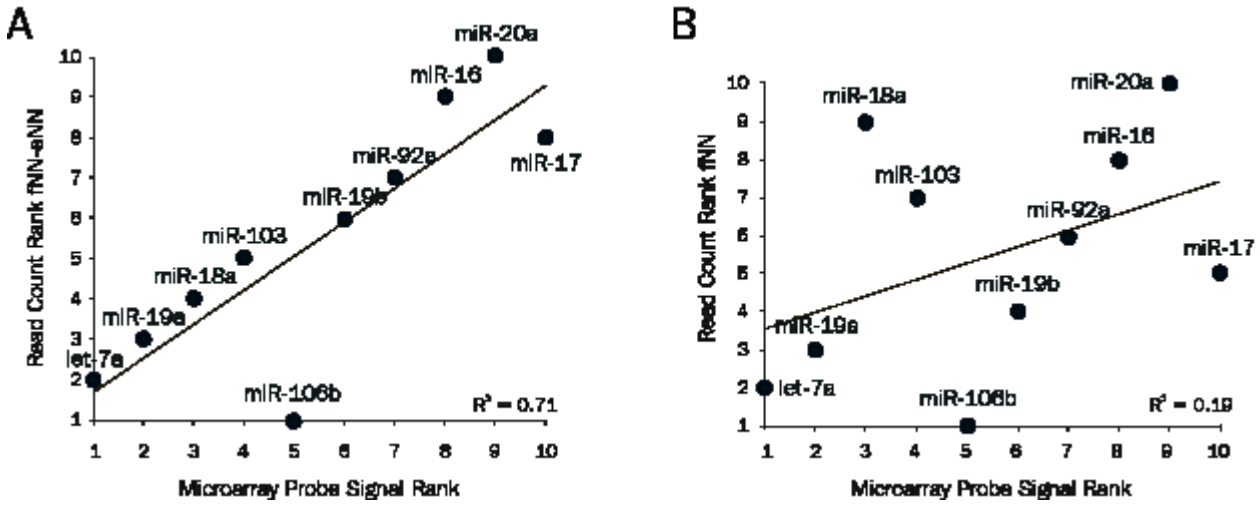
Small RNA Traces from Agilent Bioanalyzer



Bioanalyzer Small RNA assay results from 100 ng of human brain total RNA (red line) and MCF-7 total RNA (blue line). Mi-croRNAs are shown in the region from ~10 to 40 nts. Both of these RNA samples are suitable for library preparation with the NEXTflex™ Small RNA Sequencing Kit v2, but greater input amount or more PCR cy-cles will be required for library preparation from the MCF-7 RNA sample versus the human brain RNA sample.



Standard small RNA sequencing vs sequencing using the NEXTflex Small RNA Sequencing Kit v2 with randomized adapters. Libraries were prepared from an equimolar mixture of 23 synthetic miRNAs. Each slice in the pie graph represents one miRNA.



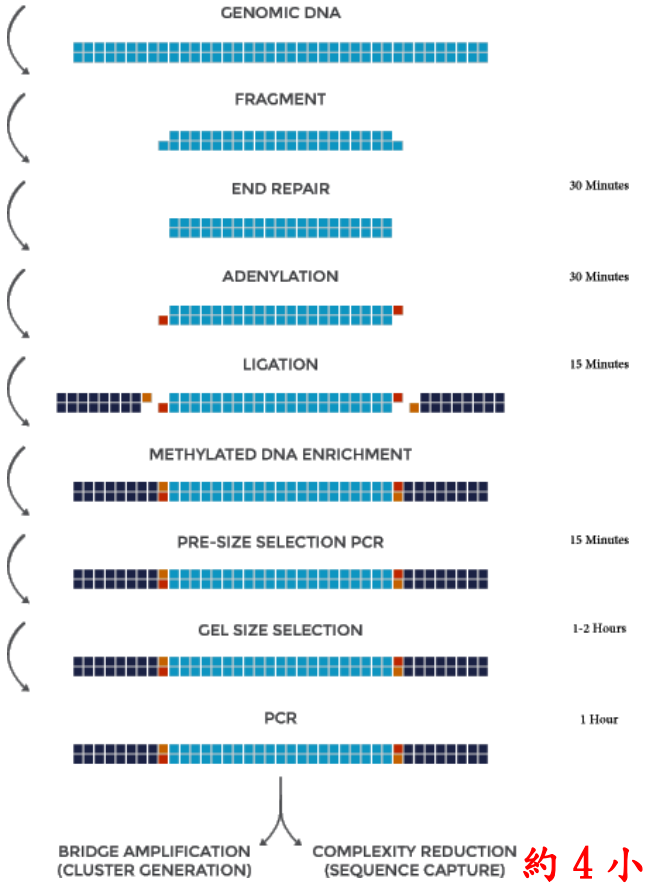
Small RNA sequencing libraries were constructed using 5' adapters with 2 random bases at the ligation junction (fNN) or 5' and 3' adapters with 2 random bases at the ligation junctions (fNN_eNN) and the resulting sequencing data compared to microarray results from the same RNA.

Catalog#	Product Name	Quantity
5132-03	NEXTflex™ Small RNA-Seq Kit v2	24 rxns
5132-04	NEXTflex™ Small RNA-Seq Kit v2	48 rxns
513305	NEXTflex™ Small RNA Barcodes Primers (Set A)	96 rxns
513306	NEXTflex™ Small RNA Barcodes Primers (Set B)	96 rxns
513307	NEXTflex™ Small RNA Barcodes Primers (Set C)	96 rxns
513308	NEXTflex™ Small RNA Barcodes Primers (Set D)	96 rxns

BIOO SCIENTIFIC NGS KITS

NEXTflex™ Methyl-Seq 1 Kit
MeDIP/MeCAP
(Illumina Compatible)

- Methylome-level assessment with broad genome coverage
- Make methyl rich libraries using methylated DNA immunoprecipitation (MeDIP) or MBD capture (MeCAP)
- Quantify absolute DNA methylation levels
- Identify differentially methylated regions (DMRs)
- Enhanced adapter ligation technology with NEXTflex™ Ligation
- Flexible barcode options– 6, 12, 24, 48, 96 unique adapters and 192 dual-indexed adapters
- Gel-Free and bead-based cleanup protocols
- Automation-friendly workflow is compatible with liquid handlers
- Barcoded adapters for multiplexing contain embedded index sequence
- Functionally validated with Illumina sequencing platforms



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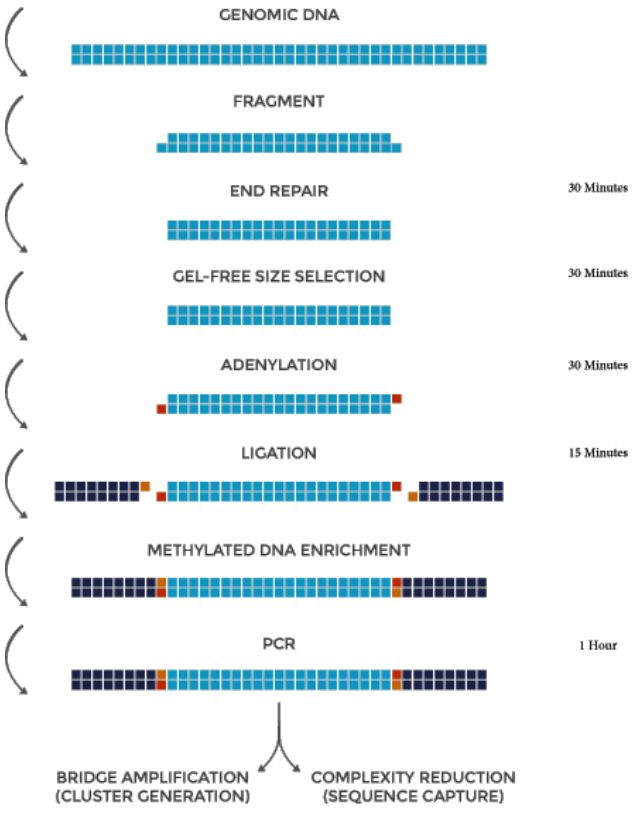
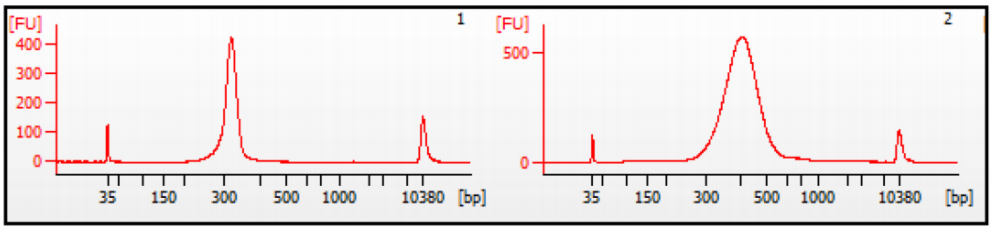
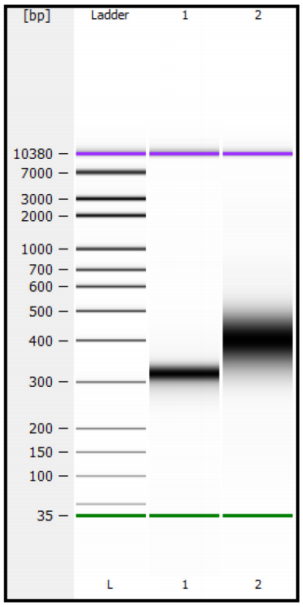


約 3 小時

(Left) is for users who are interested in size selecting a specific range of DNA fragments post ligation with an agarose gel. Proceed to Option 2 for the gel-free protocol.

(Right) Option 2 is a completely gel-free protocol that utilizes a magnetic bead based cleanup to size select DNA insert fragments between 300 – 400 bps.





Examples of Methyl-DNA Library Size Distribution.

1 µg of input DNA was used for both samples (1) and (2). A ~300 bp band was size selected (insert size = ~180 bp) with sample 1. The gel-free protocol was followed with sample 2, resulting in a ~400-500 bp product (insert size = ~ 300 – 400 bp). 1 µL of the resulting libraries were run on an Agilent High Sensitivity DNA chip to verify size. Using a Qubit® 2.0 Fluorometer & Qubit® dsDNA HS Assay Kit, the concentration of both samples was determined to be > 10 nM.

What applications can the NEXTflex Methyl-Seq Kit be used for?

The NEXTflex Methyl-Seq Kit can be used for either methylated DNA immunoprecipitation (MeDIP) sequencing or affinity purification using a methyl specific capture protein (MeCap) sequencing on any Illumina sequencing platform. These applications are designed to assess the methylation status of CpG rich regions in the genome (CpG islands). They offer global profiling of the methylome and are used to compare methylation patterns in different cell types. As only the rich methylated regions of the genome are sequenced, the amount of sequencing and coverage required is reduced in comparison to bisulfite sequencing applications.

Sequence Comparison between Differentiated and Non-differentiated Cells

Sample	Culture Passage	PCR Cycles	Capture Method	Format	Unique Mapped Reads	DMRs Covered	mCs Covered
H1	25-27	10	MeDIP	Single Read	120,154,231	87.89%	91.38%
HDF	-	9	MeDIP	Paired Read	79,286,174	88.93%	93.49%
HDF	-	9	MeCAP	Paired Read	76,884,138	89.63%	94.50%
HDF-iPSC	15	8	MeCAP	Paired Read	83,290,387	83.34%	87.64%

Catalog#	Product Name	Quantity
5118-01	NEXTflex™ Methyl Sequencing 1 Kit	8 rxns
5118-02	NEXTflex™ Methyl Sequencing 1 Kit	48 rxns
514101	NEXTflex™ DNA Barcodes - 6	48 rxns
514102	NEXTflex™ DNA Barcodes - 12	96 rxns
514103	NEXTflex™ DNA Barcodes - 24	192 rxns
514104	NEXTflex™ DNA Barcodes - 48	384 rxns
514105	NEXTflex-96™ DNA Barcodes - 96	768 rxns

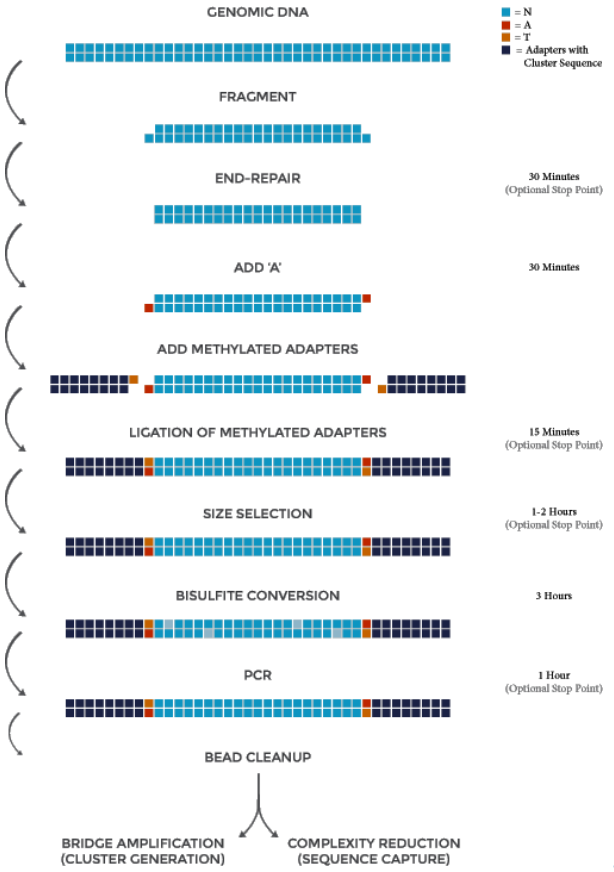
BIOO SCIENTIFIC NGS KITS

NEXTflex™ Bisulfite-Seq Kit

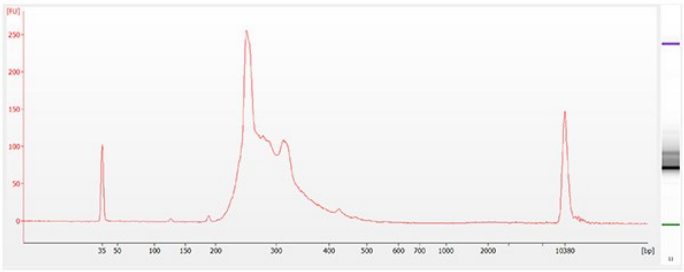
(Illumina Compatible)

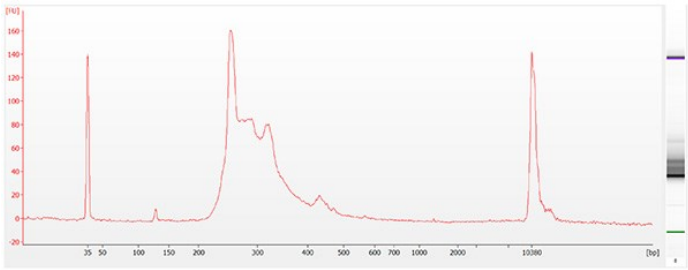
先進行 Bisulfite 以減少損失

- Compatible with total Bisulfite sequencing and reduced representation
- Single nucleotide resolution of methylation sites
- Uracil insensitive polymerase designed for bisulfite-converted DNA
- Methylome-level assessment with broad genome coverage
- Enhanced adapter ligation technology with NEXTflex™ Ligation
- Bead-based clean-up
- Automation-friendly workflow is compatible with liquid handlers
- Methylated barcoded adapters for multiplexing contain embedded index sequence
- Functionally validated with Illumina sequencing platforms



RRBS

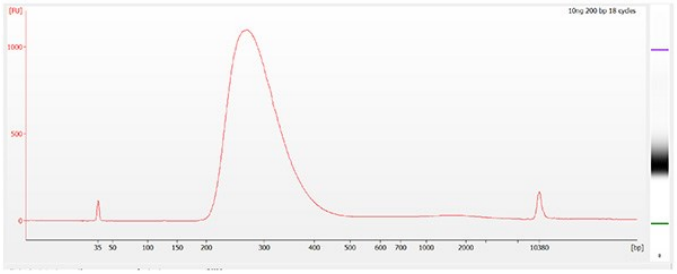


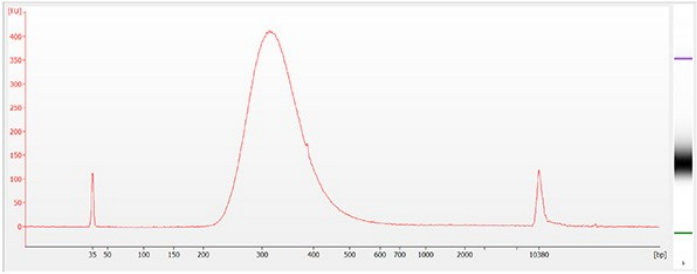


10 ng of Msp1 digested DNA input, 18 PCR cycles, 10 nm yield (upper)

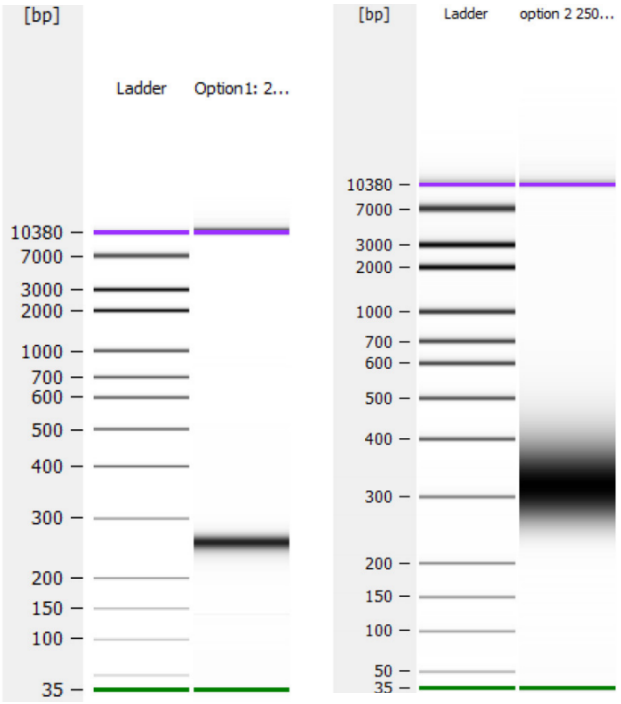
1 ug Msp1 digested DNA input, 18 PCR cycles, 32 nm yield (bottom)

WGBS





10 ng of 200 bp sheared DNA input, 18 PCR cycles, 58 nm yield ; 1 ug 200 bp sheared DNA input, 15 PCR cycles, 28 nm yield

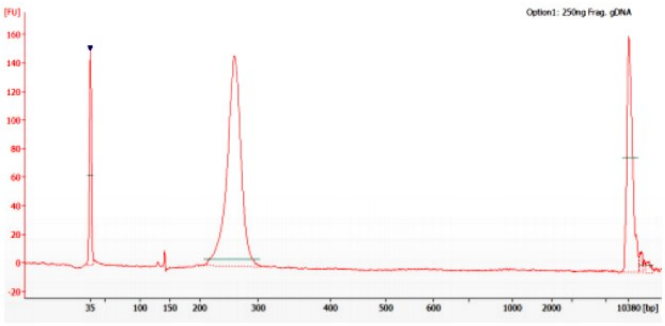


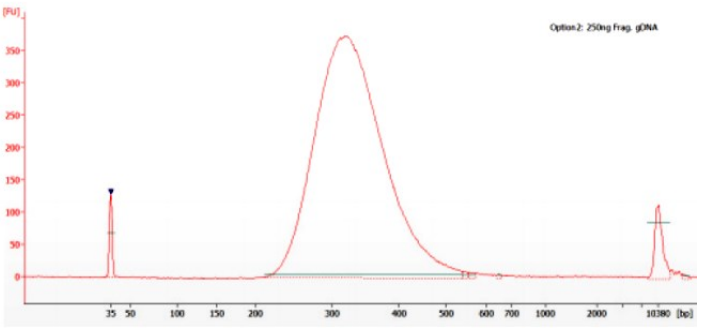
Following option 1, 250 ng of fragmented genomic DNA was used.

A ~250 bp band was size selected (insert size = ~130 bp) and a total of 15 cycles of PCR were performed after bisulfite conversion. 1 µL of the resulting library was diluted 3-fold and run on an Agilent High Sensitivity DNA chip to verify size.

Following option 2, 250 ng of fragmented genomic DNA was used.

A total of 15 cycles of PCR were performed after bisulfite conversion. 1 µL of the resulting library was run on an Agilent High Sensitivity DNA chip to verify size.



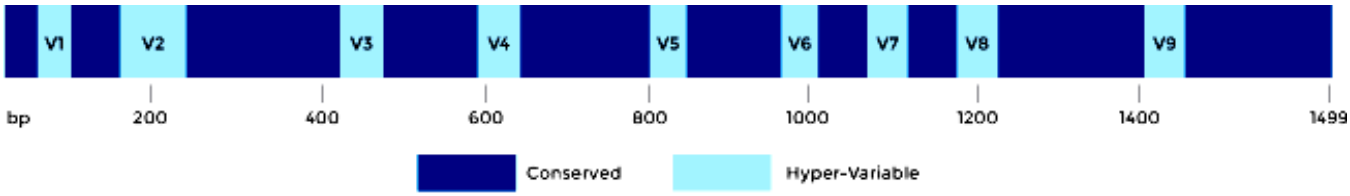


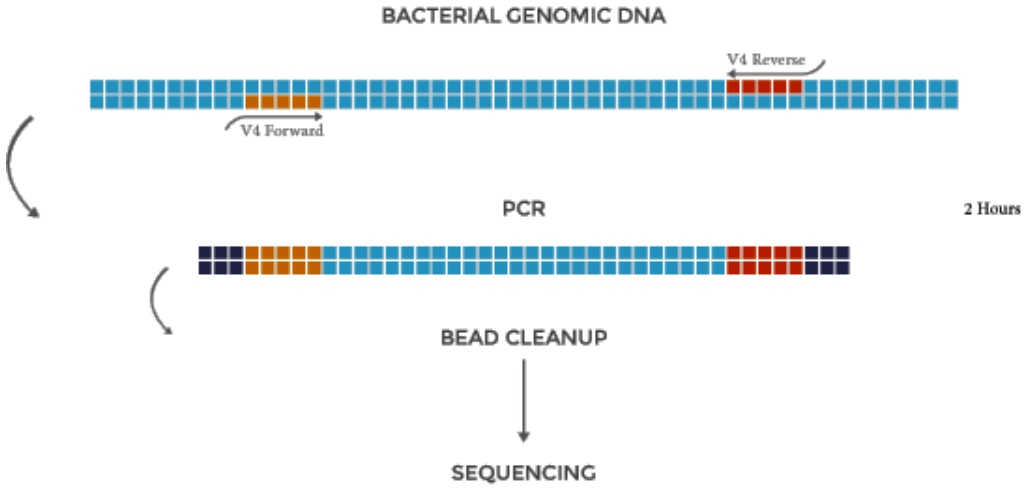
Catalog#	Product Name	Quantity
5119-01	NEXTflex™ Bisulfite Sequencing Kit	8 rxns
5119-02	NEXTflex™ Bisulfite Sequencing Kit	48 rxns
511911	NEXTflex™ Bisulfite-Seq Barcodes - 6	48 rxns
511912	NEXTflex™ Bisulfite-Seq Barcodes - 12	96 rxns
511913	NEXTflex™ Bisulfite-Seq Barcodes - 24	192 rxns
511921	NEXTflex™ Msp1 Restriction Enzyme	8 rxns
511922	NEXTflex™ Msp1 Restriction Enzyme	48 rxns

BIOO SCIENTIFIC NGS KITS

NEXTflex™ 16S V1-V3 Amplicon-Seq / NEXTflex™ V4 Amplicon-Seq Library Prep Kit

- Optimized protocol offers lower PCR bias and fewer off-target reads
- Fast library prep protocol
- Flexible barcode options
- Low input
- Automation-friendly workflow is compatible with liquid handlers
- Functionally validated with Illumina MiSeq





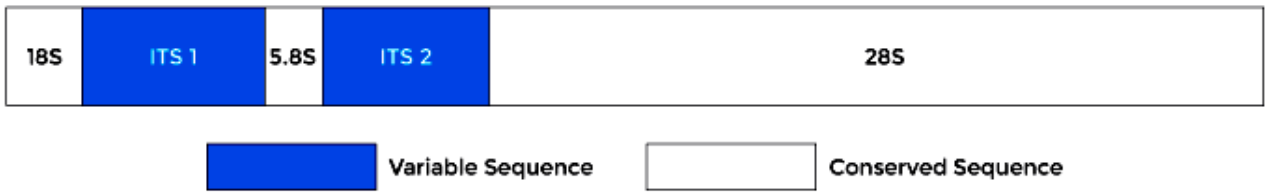
Catalog#	Product Name	Quantity
4201-01	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 4 barcodes)	16 rxns
4201-02	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 12 barcodes)	48 rxns
4201-03	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 24 barcodes)	96 rxns
4201-04	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 48 barcodes)	192 rxns
4201-05	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 96 barcodes)	384 rxns
4201-07	NEXTflex™ 16S V4 Amplicon-Seq Kit (with 288 barcodes)	1,152 rxns

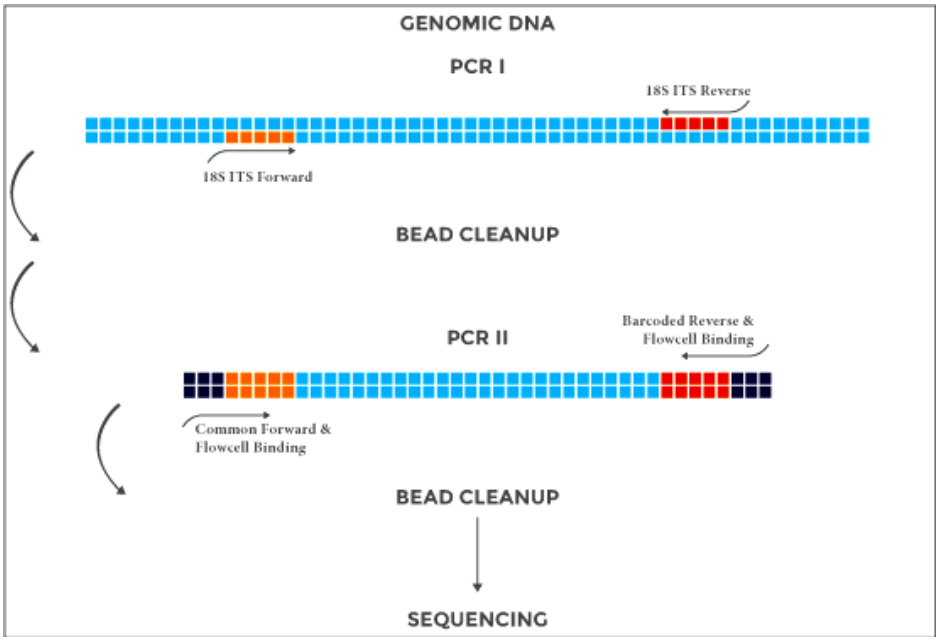
Catalog#	Product Name	Quantity
4202-01	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (4 Barcodes)	8 rxns
4202-02	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (12 Barcodes)	24 rxns
4202-03	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (48 Barcodes)	96 rxns
4202-04	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (Barcodes 1- 96)	192 rxns
4202-05	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (Barcodes 97 - 192)	192 rxns
4202-06	NEXTflex™ 16S V1-V3 Amplicon-Seq Kit (Barcodes 193 - 288)	192 rxns

BIOO SCIENTIFIC NGS KITS

NEXTflex™ 18S ITS Amplicon-Seq Kit

- Low PCR bias and high on-target reads
- Fast library prep protocol that covers both ITS1 and ITS2 of 18S rRNA gene
- Low input – As low as 1 ng of genomic DNA
- Flexible barcode options– Up to 384 unique barcodes available for multiplexing
- Compatible with regular Illumina sequencing primers
- Automation-friendly workflow
- Functionally validated with Illumina MiSeq





Catalog#	Product Name	Quantity
4210-01	NEXTflex™ 18S ITS Amplicon-Seq Kit (4 Barcodes)	8 rxns
4210-02	NEXTflex™ 18S ITS Amplicon-Seq Kit (12 Barcodes)	24 rxns
4210-03	NEXTflex™ 18S ITS Amplicon-Seq Kit (48 Barcodes)	96 rxns

DNA/RNA 純化

DNA/RNA 的萃取與純化一直是分子生物實驗中的重要課題。DNA 由於雙股結構以及去氧核醣的關係，使得保存上相對容易；而 RNA 的部分，單股結構相對於 DNA 來說較不穩定，即使保存在-80℃ 冰箱依舊容易造成裂解。加上分解 RNA 的 RNase 非常的穩定且存在於許多地方，使得經過萃取後的 RNA 更加不易保存。因此，如何縮短 RNA 萃取與純化的時間，避免操作過程遭受 RNase 降解，並且維持 RNA 完整性，是所有 RNA 純化套組(RNA purification kit)最主要的課題。

傳統質體 DNA 萃取方法修改自 Marmur 所提出之方法：

- **破菌：**以含有 lysozyme、EDTA 及 RNase 的溶液處理菌體。lysozyme 可水解細菌細胞壁中 peptidoglycan 的醣苷鍵，使得菌體無法承受滲透壓逆境而破裂； EDTA 可抑制 DNases 的活性，防止 DNA 受到 DNase 的作用； RNase 可水解細胞的 RNA。再加入 SDS 將細胞膜徹底瓦解，並使 DNase 及其它蛋白質發生變性。
- **去除蛋白質：**加入高濃度的鹽，使結合於 DNA 上的蛋白質完全脫離 DNA，再依序以酚/氯仿/異戊醇(phenol/chloroform/isoamyl alcohol) 及氯仿/異戊醇各萃取一次，離心後，溶液可被分離成有機溶液層及含有 DNA 之水溶液層，中間界面則聚集了被酚及氯仿變性的蛋白質。
- **分離沈澱 DNA ：**去除蛋白質後之 DNA 溶液，加入酒精使 DNA 沈澱，離心後之沈澱即為純化之 DNA。

傳統 RNA 萃取方法：

- 使用 **Guanidinium thiocyanate** 抑制 RNase 的活性，
- 有機溶劑 **phenol-chloroform (pH=4.5)**萃取 RNA 。

目前市面上一些試劑也是跟依據此原理所製作。不但能有效的移除蛋白質、脂類、細胞碎片等影響 RNA 純度的物質，也可以將溶解於試劑中的樣品保存於-80℃ (~1 month）待 RNA 實驗前再進行 RNA 萃取，但是對於不熟悉 RNA 萃取的人來說，由試劑中吸取上層 RNA 溶液時常會同時吸取到中間層，造成 RNA 被 genomic DNA 影響導致後續實驗誤判或失敗，且操作過程會接觸 phenol、chloroform 等有毒物質。

目前創世紀生技公司所提供的 DNA/RNA 萃取套組分成兩種形式：

一類是以 **silica column** 為主的的核酸萃取套組(MN)；

一類是由傳統法所改良的**溶液萃取方法** (Epicentre)，但是操作過程中不使用有機溶劑。

此兩類的 DNA/RNA 萃取套組，皆針對不同型式的樣本推出不同的解決方案~!!!!!

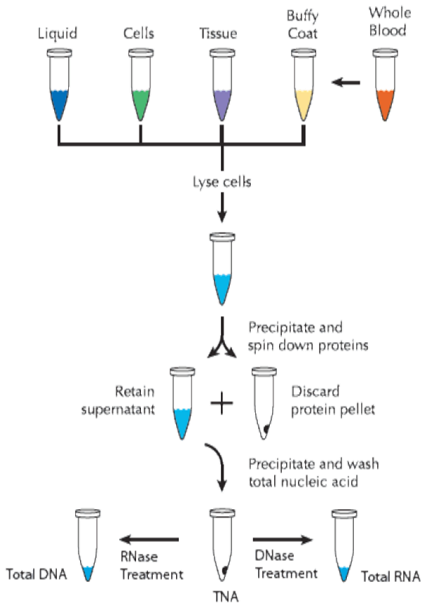
MACHEREY-NAGEL (MN) 的 Silica column

市面上最常見、也是最方便的的核酸萃取方式。其原理是利用 silica 帶正電、核酸帶負電使核酸留在 silica column 中，而 DNA/RNA 萃取套組在這個步驟使用酵素將不需要的核酸分解成小片段，再使用含有一定比例酒精的 wash buffer 清除短片段核酸及蛋白質，最後就能得到高品質的核酸。Silica column 能夠吸附的 RNA 片段大小及產量皆取決於 column 的材質，因此選擇 silica column 的核酸 萃取套組時，一定要注意 column 的 **nucleotide fragment size** 及 **binding capacity** 這兩個數據。

Epicentre 的 MasterPure RNA extraction kit 系列是 silica column 以外的另一種選擇。

MasterPure 是使用 lysis buffer 與 proteinase K 處理樣品，再以 isopropanol 將所有的 nucleotide 沈澱並以 RNase-Free DNase I 分解殘留的 DNA，最後的得到 total RNA。與 silica column 不同的是，MasterPure 一次萃取各種長度的 RNA。

epicentre®
an Illumina® company

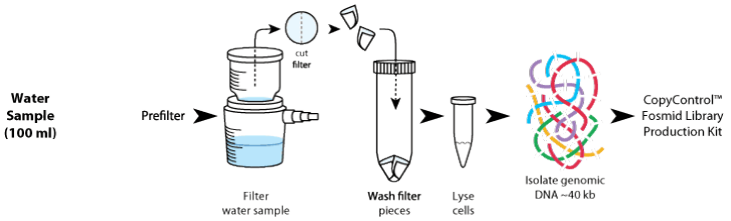


- 沒有分子量大小的限制：太大或太小的分子都無法和管柱結合 MasterPure 能收集的範圍更全面（可以收到<200base small RNA）
- 沒有 binding capacity 的問題：不會造成 sample 浪費
- 沒有無法 Elute 的問題：增加 DNA or RNA yield
- 可以隨 sample 量調整反應體積
- 適用範圍廣：細胞、組織、血液、植物、細菌
- 不使用有毒有機溶劑
- 純度高，$A_{260/280}$ ratio：1.8-2.0

Kit	Time	Samples Tested
MasterPure™ Complete DNA and RNA Purification Kit	< 1 hr	Whole blood, plasma, buccal cells, liver, mouse tail, kidney, saliva, urine, sputum, semen, cell lines, cervical cells, paraffin-embedded tissues, Guthrie cards
MasterPure™ DNA Purification Kit	< 1 hr	Whole blood, plasma, buccal cells, liver, mouse tail, kidney, saliva, urine, sputum, semen, cell lines, cervical cells, paraffin-embedded tissues, Guthrie cards
MasterPure™ Plant Leaf DNA Purification Kit	< 1 hr	Apple, fern, grape, maize, pine, quillwort, sugarcane, sunflower, tomato
MasterPure™ Yeast DNA Purification Kit	< 40 min	*Yeast (Candida, Saccharomyces, Pichia, Schizosaccharomyces), filamentous fungi (Aspergillus, Penicillium)*
MasterPure™ Gram-Positive DNA Purification Kit	30 min / overnight	*Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, Streptococcus mutans, Lactococcus lactis*
MasterPure™ DNA Purification Kit for Blood Version II	< 40 min	Whole blood, buffy coat

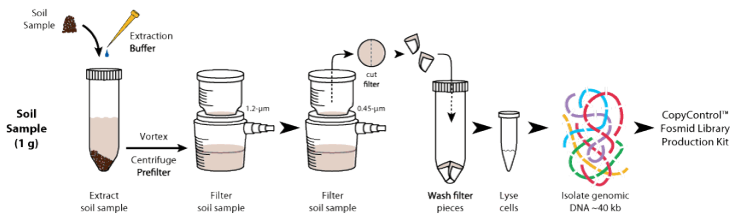
Kit	Time	Samples Tested
MasterPure™ Complete DNA and RNA Purification Kit	< 1 hr	Whole blood, plasma, buccal cells, liver, mouse tail, kidney, saliva, urine, sputum, semen, cell lines, cervical cells, paraffin-embedded tissues, Guthrie cards
MasterPure™ RNA Purification Kit	< 1 hr	HeLa/HL60 cells, liver, brain, heart, kidney, thymus, buffy coat, *E. coli*
MasterPure™ Plant RNA Purification Kit	< 1 hr	Banana, citrus, geranium, grape, maize, maple, raspberry, soybean, strawberry, and tomato leaves, alfalfa sprouts, soybean seedlings and seeds, maize seedlings and mature roots, pine needles, and field mustard seeds
MasterPure™ Yeast RNA Purification Kit	< 1 hr	*Yeast (Candida, Saccharomyces, Pichia, Schizosaccharomyces), filamentous fungi (Aspergillus, Penicillium)*
ArrayPure™ Nanoscale RNA Purification Kit	~2 hr	● sample as little as one cell. ● Captures low- and high-molecular-weight RNA.

Meta-G-Nome™ DNA Isolation Kit



- 利用過濾 技術及酵素分解步驟分離樣品中的 gDNA
- 適用土壤、水…
- 不使用 phenol/chloroform, CTAB 等溶劑
- 可到 randomly sheared gDNA（40 kb）

Metagenomic DNA Isolation Kit for Water



- 適用 water，可抽 bacteria and eukaryotes DNA
- 操作步驟簡單&溫和
- 不使用毒性有機溶劑
- 可得到大片段 gDNA
- 可得到小體積高濃縮 gDNA

Genomic DNA		
Genomic DNA from blood and biological fluids		血液、生物性液體檢體
43	NucleoSpin® Blood	qPCR, NGS**, blotting, enzymatic reactions
44	NucleoSpin® Blood QuickPure	qPCR, NGS**, blotting, enzymatic reactions
45	NucleoSpin® Dx Blood	qPCR, NGS**, blotting, enzymatic reactions
46	NucleoSpin® Blood L	qPCR, NGS**, blotting, enzymatic reactions
47	NucleoSpin® Blood XL	qPCR, NGS**, blotting, enzymatic reactions
48	NucleoSpin® 8 / 96 Blood, NucleoSpin® 8 / 96 Blood Core Kit*	qPCR, NGS**, blotting, enzymatic reactions
49	NucleoSpin® 8 / 96 Blood QuickPure	qPCR, NGS**, blotting, enzymatic reactions
50	NucleoMag® Blood 200 µL, NucleoMag® Blood 3 mL	qPCR, NGS**, blotting, enzymatic reactions
Genomic DNA from plasma		
51	NucleoSpin® Plasma XS	qPCR, NGS**, blotting, enzymatic reactions
Genomic DNA from tissue and cells		
52	NucleoSpin® Tissue	細胞或組織 qPCR, NGS*, blotting, enzymatic reactions
53	NucleoSpin® Tissue XS	qPCR, NGS*, blotting, enzymatic reactions
54	NucleoSpin® 8 / 96 Tissue, NucleoSpin® 8 / 96 Tissue Core Kit*	qPCR, NGS*, blotting, enzymatic reactions
55	NucleoMag® 96 Tissue	FFPE qPCR, NGS*, blotting, enzymatic reactions
Genomic DNA from FFPE samples		
56	NucleoSpin® DNA FFPE XS	qPCR
Genomic DNA from forensic samples		司法鑑定檢體
57	NucleoSpin® Forensic Filters NucleoSpin® Forensic Filters (Bulk)	DNA isolation
58	NucleoSpin® DNA Trace	qPCR, enzymatic reactions
59	NucleoSpin® 8 / 96 Trace	qPCR, enzymatic reactions
60	NucleoMag® 96 Trace	qPCR, enzymatic reactions
	NucleoMag® Forensic	PCR, STR analysis, enzymatic reactions
Genomic DNA from plant and fungi		
61	NucleoSpin® Plant II	qPCR, NGS**, blotting, enzymatic reactions
62	NucleoSpin® Plant II Midi	植物或 Fungi qPCR, NGS**, blotting, enzymatic reactions
63	NucleoSpin® Plant II Maxi	qPCR, NGS**, blotting, enzymatic reactions
64	NucleoSpin® 8 / 96 Plant II, NucleoSpin® 8 / 96 Plant II Core Kit*	qPCR, NGS**, blotting, enzymatic reactions
65	NucleoMag® 96 Plant	qPCR, NGS**, blotting, enzymatic reactions
Genomic DNA from soil		土壤或糞便
66	NucleoSpin® Soil	qPCR, NGS**, blotting, array technology
67	NucleoSpin® 96 Soil	qPCR, NGS**, blotting, array technology
Genomic DNA from food and feed		
68	NucleoSpin® Food	qPCR, blotting, enzymatic reactions
69	NucleoSpin® 8 / 96 Food	食物或飼料 qPCR, blotting, enzymatic reactions

MACHEREY-NAGEL

Since 1911

Filtration · Rapid Tests · Water Analysis · Chromatography · Bioanalysis
Filtration · Schnelltests · Wasseranalytik · Chromatographie · Bioanalytik

RNA		Fragment size	
RNA from cells and tissue			
23	NucleoSpin® RNA Plus	> 200 nt	qRT-PCR, NGS**, blotting, array technology
24	NucleoSpin® RNA	> 200 nt	qRT-PCR, NGS**, blotting, array technology
25	NucleoSpin® RNA XS	> 200 nt	qRT-PCR, NGS**, blotting, array technology
26	NucleoSpin® RNA Midi	> 200 nt	qRT-PCR, NGS**, blotting, array technology
27	NucleoSpin® 8/96 RNA, NucleoSpin® 8/96 RNA Core Kit*	> 200 nt	qRT-PCR, NGS**, blotting, array technology
28	NucleoMag® 96 RNA	> 200 nt	qRT-PCR, NGS**, blotting, array technology
MicroRNA			
29	NucleoSpin® miRNA	< 200 nt (small RNA), > 200 nt (large RNA)	qRT-PCR, NGS**, blotting, array technology
30	NucleoSpin® miRNA Plasma	< 1000 nt	qRT-PCR, NGS**, blotting, array technology
31	Exosome Precipitation Solution (Serum/Plasma)		miRNA isolation, exosome studies
32	Exosome Precipitation Solution (Urine)		miRNA isolation, exosome studies
RNA, DNA, and protein			
33	NucleoSpin® TriPrep	> 200 nt (RNA), < 30 kbp (DNA), 15–300 kDa (protein)	qRT-PCR, blotting, array technology, SDS-PAGE/Western blotting
34	NucleoSpin® RNA/Protein	> 200 nt (RNA), 15–300 kDa (protein)	qRT-PCR, blotting, array technology, SDS-PAGE/Western blotting
35	NucleoSpin® RNA/DNA Buffer Set	< 30 kbp (DNA)	qRT-PCR, NGS**, blotting, array technology, qPCR, enzyme reactions
RNA from blood			
36	NucleoSpin® RNA Blood	> 200 nt	qRT-PCR, NGS**, blotting, array technology
37	NucleoSpin® RNA Blood Midi	> 200 nt	qRT-PCR, NGS**, blotting, array technology
38	NucleoSpin® 8/96 RNA Blood	> 200 nt	qRT-PCR, NGS**, blotting, array technology
RNA and microRNA from FFPE samples			
39	NucleoSpin® totalRNA FFPE	Depending on sample material	qRT-PCR
40	NucleoSpin® totalRNA FFPE XS	Depending on sample material	qRT-PCR
RNA from plant			
41	NucleoSpin® RNA Plant	> 200 nt	qRT-PCR, NGS**, blotting, array technology
Poly(A) mRNA from total RNA			
42	NucleoTrap® mRNA Mini, NucleoTrap® mRNA Midi	50 nt–20 knt	qRT-PCR, NGS**, array technology

細胞或組織

抽 MicroRNA

血液

FFPE

植物

病毒

DNA/RNA

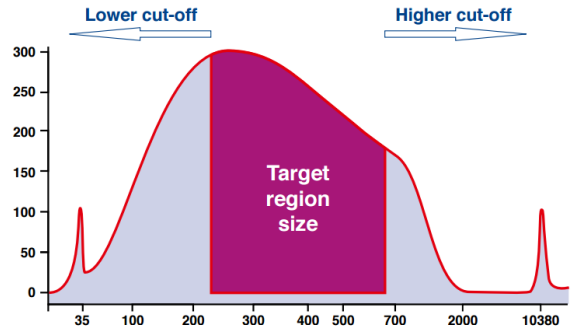
Viral RNA and DNA		Fragment size	
Viral RNA/DNA from cell-free body fluids			
70	NucleoSpin® Virus	100 bp–approx. 50 kbp	qRT-PCR, qPCR, enzymatic reactions
71	NucleoSpin® RNA Virus F	100 bp–approx. 50 kbp	qRT-PCR, enzymatic reactions
72	NucleoSpin® Dx Virus	100 bp–approx. 50 kbp	qRT-PCR, qPCR, enzymatic reactions
73	NucleoSpin® 8/96 Virus, NucleoSpin® 8/96 Virus Core Kit*	100 bp–approx. 50 kbp	qRT-PCR, qPCR, enzymatic reactions
74	NucleoMag® 96 Virus	100 bp–approx. 50 kbp	qRT-PCR, qPCR, enzymatic reactions
Viral RNA/DNA from blood, tissue, feces			
75	NucleoMag® VET	300 bp–approx. 50 kbp	qRT-PCR, qPCR, enzymatic reactions
Viral RNA/DNA from blood and biological fluids			
76	NucleoSpin® Blood	200 bp–approx. 50 kbp	qPCR, blotting, enzymatic reactions

NucleoMag® NGS Clean-up & Size Select

Clean-up and size selection for NGS library preps

Get highest recoveries for reliable sequencing

NucleoMag® NGS Clean-up and Size Select



Size selection of fragment mix

For single side size selection (left or right), the sample is mixed with the beads in pre-determined ratios for the desired exclusion of smaller or larger sized fragments. For the double sized size selection, two binding steps are performed to exclude larger fragments above the cut-off and smaller fragments below the lower cut-off.

Recovery of DNA fragments [%]

Size (bp)	0.5 : 1	0.55 : 1	0.6 : 1	0.65 : 1	0.7 : 1	0.75 : 1	0.8 : 1	0.85 : 1	0.9 : 1	0.95 : 1	1 : 1
100	0	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	0	3	5	6	8	13
200	0	0	0	2	3	6	9	20	32	42	59
250	0	1	2	4	8	21	31	54	77	86	91
300	0	0	3	10	21	53	66	82	95	97	95
400	0	5	14	49	75	95	93	94	99	99	95
500	3	20	48	90	109	103	98	99	103	102	98
600	7	45	81	96	96	99	93	96	98	98	94
700	18	70	92	95	95	97	91	93	96	96	92
800	40	81	93	94	94	95	89	91	95	94	91
900	64	84	93	94	95	96	89	91	95	95	90
1000	80	83	91	93	94	95	88	90	94	94	89

Recoveries of different fragment sizes

For DNA size selection, 100 µL gDNA (10 ng / µL) from E.coli was mixed with the NucleoMag® NGS Clean-up and Size Select beads to compose the shown ratios. Input DNA contained fragment sizes from 100 bp to 1000 bp. The different recoveries of the used ratios (beads: input DNA) are shown in percentage [%].

Sample input 可到 17.5 pg~!!

Efficient clean-up of NGS library preparation reactions

Sample input as low as 17.5 pg

Tunable size selection

150–800 bp

可篩選片段至 150 - 800 bps ~!!

Ordering information

Product	Volume	REF
NucleoMag® NGS Clean-up and Size Select Clean-up and size selection in NGS library preparations	5 mL 50 mL 500 mL	744970.5 744970.50 744970.500

創世紀生技 Bio-genesis Technology
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