Roche

KAPABIOSYSTEMS

NEXT GENERATION SEQUENCING

全新體驗
最優化的 NGS 試劑

KAPA HyperPlus kit

KAPA Hyper Prep Kit

KAPA Library Quantification Kits

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A/UV060418

KAPA HYPERPLUS GUIDE TO SUCCESS

1 INPUT DNA

Got EDTA?

- The enzymatic fragmentation reaction is sensitive to EDTA.
- The best strategy is to remove EDTA by means of a cleanup step before fragmentation.
- If your DNA contains EDTA, please see **Appendix 2** (p. 16) of the Technical Data Sheet (TDS).

How much DNA do I need?

Application	Sample type	Recommended input
WGS	Complex gDNA (high quality)	50 ng – 1 µg
Target capture (WES, custom panels)	Complex gDNA (high quality)	10 ng – 1 µg
WGS, target capture	FFPE DNA	≥50 ng (quality dependent)
WGS	Microbial DNA	1 ng – 1 µg
WGS (PCR-free)	High-quality DNA	≥50 ng (no SS)* ≥500 ng (w/SS)*
Targeted sequencing	Long amplicons	≥1 ng

*SS = size selection

setup is at 4˚C

2 ENZYMATIC FRAGMENTATION

variable time *(see below)*

incubate at 37˚C

Get to chopping.

- Mode and size distribution of DNA is controlled by fragmentation time and temperature.
- Try a range of fragmentation times to determine optimal insert size.
- For ease of sample processing, place samples with the longest fragmentation time in the thermal cycler first. Add samples with shorter fragmentation times at appropriate intervals.

Mode fragment length	Incubation time at 37°C*	Optimization range
600 bp	5 min	3 – 10 min
350 bp	10 min	5 – 20 min
200 bp	20 min	10 – 25 min
150 bp	30 min	20 – 40 min

*These parameters are a good starting point for high-quality genomic DNA. Please refer to **Appendix 2: Optimization of Fragmentation Parameters** of the TDS for guidelines on how to optimize fragmentation time and temperature, if needed.

It's not a typo!

- Ensure that you are adding the correct volume of KAPA Frag Buffer (5 µL) and KAPA Frag Enzyme (10 µL) to each reaction.

Component	Volume
Double-stranded DNA *(with KAPA Frag Conditioning Solution, if needed)*	35 µL
KAPA Frag Buffer (10X)	5 µL
KAPA Frag Enzyme	10 µL
Total volume	**50 µL**

KEEP GOING
assess size after amplification

place samples at 4˚C

3 END REP… AND A-TAI…

AD… LIG…

How much adapter…

- Adapter concentration a… as adapter and adapter-… ligation cleanup.

Input DNA	A… co…
1 µg	
500 ng	
250 ng	
100 ng	
50 ng	
25 ng	
10 ng	
5 ng	
2.5 ng	
1 ng	

最優化的試劑，提升 NGS 成功率

KAPA HyperPlus: single-tube fragmentation and library preparation workflow in less than 3 hours

5 0.8X POST-LIGATION CLEANUP

optional size selection

6 LIBRARY AMPLIFICATION

7 1X POST- AMP CLEANUP

AIR LING

A

incubate at 65°C

30 min

4 DAPTER GATION

incubate at 20°C

15 min

do I need?

ffects ligation efficiency, as well dimer carry-over during the post-

dapter stock oncentration	Adapter:insert molar ratio
15 µM	10:1
15 µM	20:1
15 µM	40:1
15 µM	100:1
15 µM	200:1
7.5 µM	200:1
3 µM	200:1
1.5 µM	200:1
750 nM	200:1
300 nM	200:1

Don't do more work than you have to.

- Determine how much final library material is required for your downstream application (e.g., capture, sequencing).

Input into library construction	Number of cycles required to generate	
	100 ng library	1 µg library
1 µg	0	0 – 1
500 ng	0	2 – 3
250 ng	0 – 1	3 – 5
100 ng	0 – 2	5 – 6
50 ng	3 – 5	7 – 8
25 ng	5 – 6	8 – 10
10 ng	7 – 9	11 – 13
5 ng	9 – 11	13 – 14
2.5 ng	11 – 13	14 – 16
1 ng	13 – 15	17 – 19

So did you make a good library?

This is where you decide if you made a good library by assessing both the size and concentration with an appropriate quantification method.

YES

8 TARGET CAPTURE OR SEQUENCING

Great! Now you want more HyperPlus, don't you?

NO

Do you have adapter-dimers?

NO

Uh-oh...it's okay, it happens. That's what we're here for.

YES

Repeat Step 7

on

0.8X post-ligation cleanup

optional size selection

library amplification

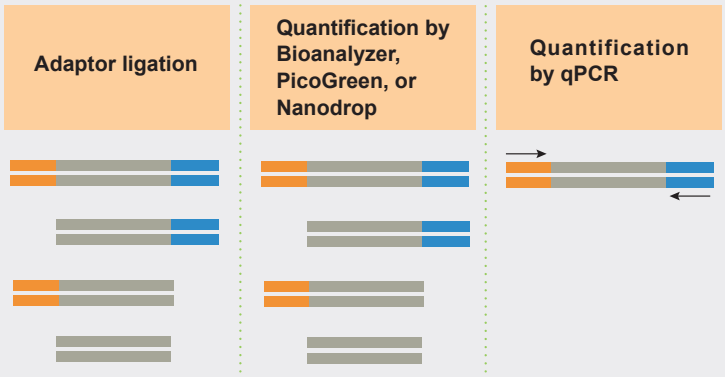
1X post-amp cleanup

target capture or sequencing

KAPA Library Quantification Kits

最佳的 cluster 密度或模板與磁珠的比率取決於 PCR 可擴增的 DNA 適當濃度。電泳和分光光度法都是測量總核酸濃度，而 real-time PCR 的定量方法，只會偵測有正確連接 adaptor 序列的 library 分子，提供精準的定量結果。

Real-time PCR 定量方式，完整連接 adaptor 的 libraries 分子



The ligation of adaptor sequences (orange and blue) to library DNA molecules (grey) results in a mixed population of molecules without the correct adaptor configuration. Electrophoresis and spectrophotometry measure total nucleic acid concentrations, whereas optimal cluster density or template-to-bead ratio depends on the appropriate concentration of PCR-amplifiable DNA molecules.

最精準的定量可使 cluster 達到最佳密度

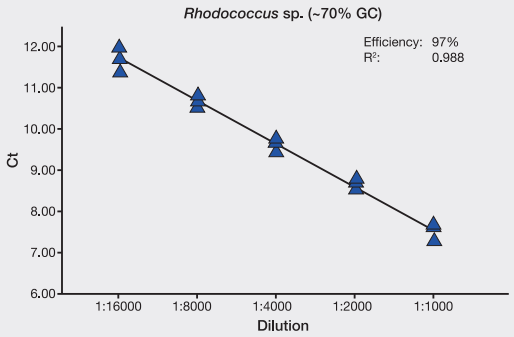
使 cluster 的大小與密度達到最佳並發揮儀器最佳效能

產生的 cluster 過少，無法完全發揮儀器使用效能

產生太多、太小的 cluster ，使區分不明顯，檢測困難

絕佳擴增效率，精準定量各種 GC 含量的 libraries

The KAPA Libary Quantificaiton Kit was used to determine the concentration of two Illumina GA libraries with unusual GC content (Rhodococcus sp.; ~70% GC - shown above, Staphylococcus sp.; ~35% GC - not shown). Both libraries amplified with efficiency >95%. Two-fold dilution series (1:1000 through 1:16000) were prepared in triplicate, and qPCR performed according to the recommendations in the product technical data sheet.



Cat No.	Product	Size
07960140001	Complete kit (Universal)	500 x 20 µl rxns
07960204001	Complete kit (ABI Prism®)	500 x 20 µl rxns
07960255001	Complete kit (Bio-Rad®)	500 x 20 µl rxns
07960298001	Complete kit (optimized for Roche® LightCycler 480)	500 x 20 µl rxns
07960336001	Complete Kit (ROX Low)	500 x 20 µl rxns

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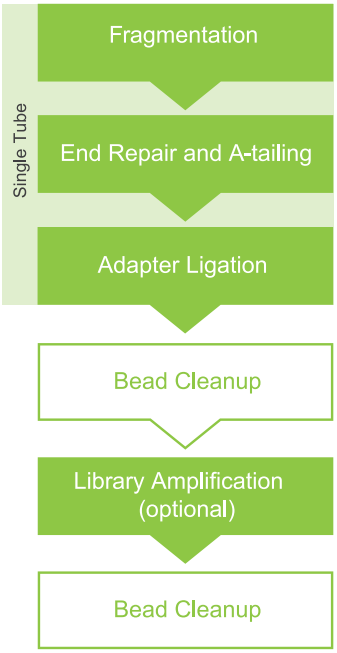
KAPA HyperPlus kit

卓越的精簡化單管流程，包含 fragmentation 與 library preparation 所需的試劑。可根據實驗需求調整 fragmentation 時間，以獲得最適當的片段大小，優化的試劑可降低 bias 並且有更均勻的覆蓋度。

快速、簡便的流程，輕鬆完成 library 構築

- **One-tube** 流程縮短操作時間
- **PCR-free** 流程 **<2** 小時，含 **PCR** 的流程 **<3** 小時即可完成
- 更少的處理步驟可改善一致性與再現性

Total time : ~2.5 hours



adapter 建議用量

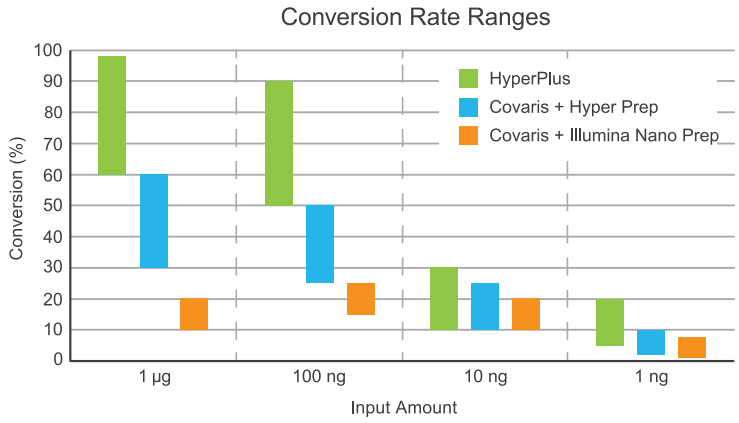
Fragmented DNA per 50 µL ER & AT reaction	Adapter stock concentration	Adapter:insert molar ratio	Fragmented DNA per 50 µL ER & AT reaction	Adapter stock concentration	Adapter:insert molar ratio
1 µg	15 µM	10:1	25 ng	7.5 µM	200:1
500 ng	15 µM	20:1	10 ng	3 µM	200:1
250 ng	15 µM	40:1	5 ng	1.5 µM	200:1
100 ng	15 µM	100:1	2.5 ng	750 nM	200:1
50 ng	15 µM	200:1	1 ng	300 nM	200:1

* Adapter:insert molar ratio calculations are based on a mode DNA fragment length of 200 bp, and will be higher for longer DNA fragments, or slightly lower for DNA fragmented o a mode size <200 bp. The lower adapter:insert molar ratios recommended for inputs >100 ng represent a fair compromise between library construction efficiency and cost; higher library yields will be achieved if a higher adapter concentration is used.

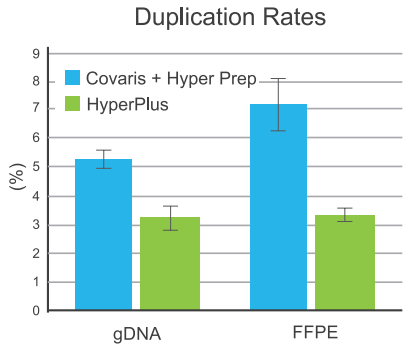
KAPA HyperPlus Kit 在 **high-input DNA** 以及 **low-input DNA** 均可獲得較高的 **conversion rate**

更高的 **conversion rate** 可以減少 **amplification** 時的 **cycles** 次數，並且降低 **duplication rate**

不同廠牌的 conversion rate 比較



conversion rate 是指有多少百分比的 input DNA 轉化成可定序的 library。adapter-ligated library 是 構築 library 的重要指標，其影響 library 的多樣性與品質



Libraries were prepared from 50 ng hgDNA or 50 ng FFPE DNA and captured with the Nimblegen™ SeqCap EZ HGSC VCRome panel.

Cat No	Product	Size
07962380001	KAPA HyperPlus Kit	8 rxns
07962401001		24 rxns
07962428001		96 rxns
08005702001	KAPA Single-Indexed Adapter Set A (30 µM)	12 x 40 µL
08005729001	KAPA Single-Indexed Adapter Set B (30 µM)	12 x 40 µL

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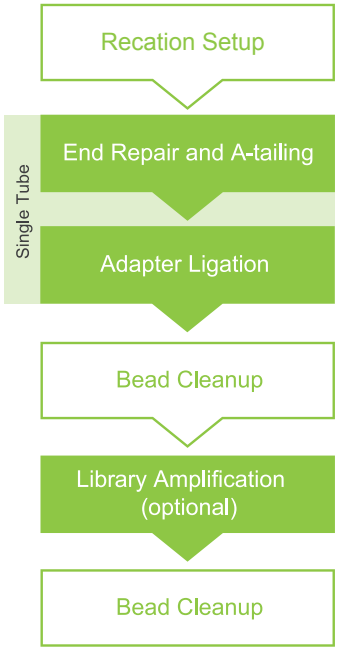
KAPA Hyper Prep Kit

創新單管設計，搭配優化的配方以及經由 “directed evolution” 技術篩選過的酵素，能夠使 adapter-ligated library 獲得高產率並且降低 amplification bias。特別是針對 FFPE 與 low-input 樣本可以獲得更高的 library 多樣性、降低 duplication rate 與更均勻的覆蓋率

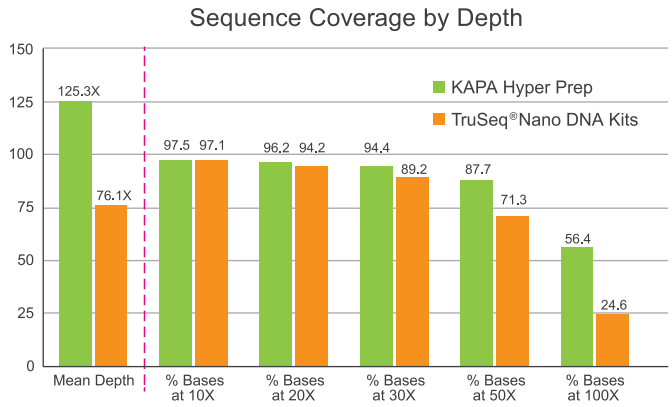
高表現，FFPE 樣本也能輕鬆定序

- 提升 **NGS library** 產率與序列品質
- 可由 **FFPE** 樣本構築高品質 **libraries**
- 操作簡便，不到 **3** 小時即可完成 **library** 構築

Total time : 2.75 hours



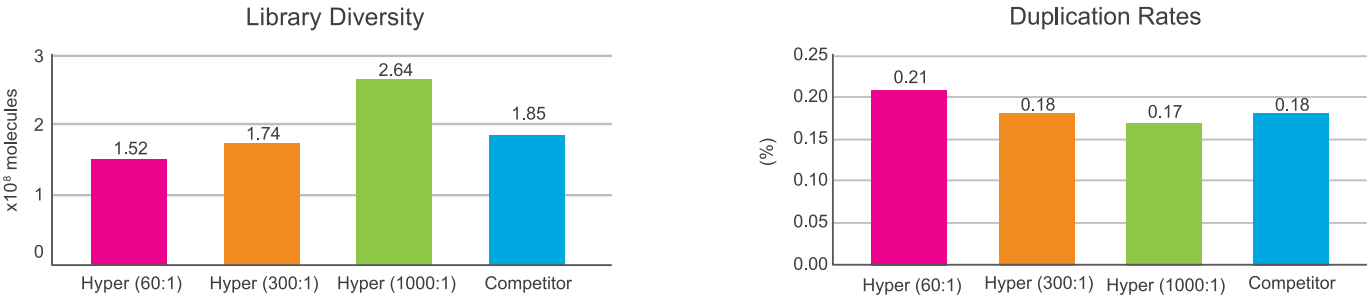
可從 FFPE 樣本建立高品質的 library



Libraries were prepared from 100 ng of FFPE DNA, and exome capture was performed with the SeqCap EZ HGSC VCRome Design, Roche NimbleGen.

使用 **KAPA Hyper Prep kit** 在高濃度的 **adapter** 條件下，可提升 **library** 構築的效率

改善 low-input 樣本的表現量



Library diversity and duplication rates for libraries prepared from 2 ng cell-free DNA, using the KAPA Hyper Prep Kit or a leading competitor kit optimized for low-input library construction.

Cat No	Product	Size
07962312001	KAPA Hyper Prep Kit for Illumina	8 rxns
07962347001		24 rxns
07962363001		96 rxns
08005702001	KAPA Single-Indexed Adapter Set A (30 µM)	12 x 40 µL
08005729001	KAPA Single-Indexed Adapter Set B (30 µM)	12 x 40 µL

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