KAPABIOSYSTEMS

Technical Data Sheet

# KAPA Dual-Indexed Adapter Kit

## Illumina® Platforms

KR1318 – v1.17

This Technical Data Sheet provides product information and guidelines for use of KAPA Dual-Indexed Adapter Kits for Illumina platforms.

This document applies to the KAPA Dual-Indexed Adapter Kit for Illumina platforms (08278555702), and the standalone KAPA Adapter Dilution Buffer (08278539001).

## Contents



| KAPA/*Roche* Kit Codes and Components | | |
|---|---|---|
| **KK8722**<br>*08278555702* | KAPA Dual-Indexed Adapter Plate (20 µL/well)<br>KAPA Adapter Dilution Buffer<br>Sealing foils | 96 x 15 µM<br>25 mL<br>3 foils |
| **KK8721**<br>*08278539001* | KAPA Adapter Dilution Buffer | 25 mL |

### Quick Notes

- KAPA Dual-Indexed Adapter Kits for Illumina platforms contain 20 µL of each indexed adapter, supplied at a concentration of 15 µM in plate format.
- The number of libraries that can be prepared with each KAPA Dual-Indexed Adapter Kit is dependent on the amount of input DNA, the average fragment size of the input DNA, and the kit used for library construction. Please refer to **Important Parameters** for guidelines on how to use KAPA Dual-Indexed Adapters in combination with different KAPA library preparation kits. With no dilution, 4 libraries can be prepared with each of the 96 dual-indexed adapters, for a total of 384 libraries per kit.
- KAPA Dual-Indexed Adapters are duplexed oligonucleotides and must not be exposed to temperatures above room temperature. Adapters must be diluted in the KAPA Adapter Dilution Buffer provided in the kit to avoid dissociation and ensure optimal performance.
- Employ best laboratory practices to avoid cross contamination of indexed adapters.
- To ensure equal read distributions in multiplexed sequencing applications, libraries must be carefully quantified and/or normalized prior to pooling for capture or cluster generation. qPCR-based quantification with the KAPA Library Quantification Kit constitutes the most accurate and reproducible method for the quantification of sequenceable molecules. This is particularly true for PCR-free workflows.

## Product Description

KAPA Dual-Indexed Adapter Kits for Illumina platforms are designed for use with KAPA DNA and RNA library preparation kits to construct libraries for sequencing on an Illumina sequencer. Each plate contains a set of 96 adapters, each with two, 8-nucleotide indexes (barcodes) for multiplexed sequencing applications.

The sequences of the sequencing indices (barcodes) included in KAPA Dual-Indexed Adapter Kits are given in **Important Parameters: Index Sequences and Pooling Guidelines** (p. 6). The highly dissimilar nature of indices minimizes the possibility of inaccurate index calling.

KAPA Adapter Dilution Buffer [10 mM Tris-HCl, (pH 8.0 – 8.5), 10 mM NaCl, 1 mM EDTA] is provided with the kit to ensure optimal performance when adapters require further dilution.

## Product Applications

KAPA Dual-Indexed Adapters are used to uniquely label sequencing libraries generated from individual biological samples. This allows for the pooling of libraries prior to target capture or cluster generation, to enable multiplexed sequencing—which simplifies sample preparation and reduces the cost of next-generation sequencing for a wide range of applications.

Primary applications for the use of KAPA Dual-Indexed Adapter Kits for Illumina platforms include:

- Human whole genome sequencing, performed with an Illumina HiSeq X instrument
- whole exome or targeted sequencing, using Roche® NimbleGen™ SeqCap™ EZ or IDT xGen® Lockdown® Probes or other hybridization capture systems, in combination with the appropriate blockers
- RNA-seq
- ChIP-seq
- other direct sequencing applications, e.g., microbial whole-genome sequencing on compatible platforms.

**NOTE:** KAPA Dual-Indexed Adapters are not methylated, and can therefore not be used for methyl-seq applications.

## Product Specifications

### Shipping and Storage

KAPA Dual-Indexed Adapter Kits are shipped on dry ice or ice packs, depending on the destination country. Upon receipt, immediately store the product at -15°C to -25°C in a constant-temperature freezer. Adapters must not be exposed to temperatures above room temperature. When stored under these conditions and handled correctly, the adapters will retain full functionality until the expiry date indicated on the kit label. The KAPA Adapter Dilution Buffer may be stored at 2°C to 8°C for short-term use, but -15°C to -25°C is recommended for long-term storage.

### Procedure for handling Dual-Indexed Adapter Plate

- Remove the KAPA Dual-Indexed Adapter plate from its packaging sleeve and thaw at room temperature for 10 min.
- Centrifuge the KAPA Dual-Indexed Adapter plate at 280 x g for 1 min at room temperature.
- Carefully remove the foil cover to avoid cross-contamination of the dual-indexed adapters. Discard the foil cover.
- Remove the desired volume of each dual-indexed adapter by following the guidelines in **Important Parameters** (pp. 3 – 7).
- If you are not processing the entire KAPA Dual-Indexed Adapter plate at the same time, apply a new adhesive foil seal (3 are supplied in the KAPA Dual-Indexed Adapter Kit).
  - If required, similar sealing film can be used for plates containing diluted adapters.
- Label the re-sealed Dual-Indexed Adapter plate and store at -15°C to -25°C in a constant-temperature freezer.

### Quality Control

KAPA Dual-Indexed Adapters are subject to stringent functional and barcode cross-contamination quality control. KAPA Adapter Dilution Buffer is free of detectable contaminating exo- and endonuclease activities, and meets strict requirements with respect to DNA contamination.

## Important Parameters

### Best Practices

- KAPA Dual-Indexed Adapters should be used on ice or in cooled reagent blocks, and must not be exposed to conditions above room temperature.
- Use the KAPA Adapter Dilution Buffer [10 mM Tris-HCl, (pH 8.0 – 8.5), 10 mM NaCl, 1 mM EDTA] provided in the kit to dilute KAPA Dual-Indexed Adapters. Adapters diluted in any other buffer or in PCR-grade water may not support optimal library construction efficiency.
- The KAPA Dual-Indexed Adapter plate contains an excess of each adapter, over and above the stated volume of 20 µL. For this reason, and because diluted adapters are less stable, adapter dilutions should not be performed in the plate in which the adapters are supplied. Dilute only the amount of each adapter needed for same-day usage, in a new plate. Long-term storage and multiple cycles of freezing and thawing of diluted adapter stocks are not recommended.
- Employ best laboratory practices to avoid cross-contamination of adapters and/or the dilution buffer.
- Always use plastics that are certified to be free of DNAses, RNAses, and nucleases. Low DNA- and RNA-binding plastics are highly recommended, especially for low-input DNA and all RNA-Seq library construction applications.

### Compatibility with KAPA Library Preparation Kits

KAPA Dual-Indexed Adapter Kits for Illumina platforms are recommended for use with the KAPA library construction kits listed below:

- KAPA HyperPrep Kits
- KAPA HyperPlus Kits
- KAPA HTP and LTP “with bead” Library Preparation Kits
- KAPA RNA and mRNA HyperPrep Kits
- KAPA RNA HyperPrep Kits with RiboErase (HMR)
- KAPA Stranded RNA-Seq Kit with RiboErase (HMR)
- KAPA Stranded RNA-Seq and mRNA-Seq Kits

- When selecting a KAPA Dual-Indexed Adapter Kit for a specific application, please consider the following:
  - Refer to the table corresponding to the KAPA Library Preparation Kit that will be used (p. 4). Recommended adapter stock concentrations for libraries constructed from different inputs and DNA fragment lengths with the listed kits listed are included in Tables 1 – 7.
  - Identify the recommended adapter stock concentration for the input and fragment length.
  - If the input and specific fragment length are not listed in the table, follow the instructions in **Adapter Concentration Calculations** (p. 5 ) to calculate the appropriate adapter stock concentration for the experiment.

- For each batch of libraries to be constructed, prepare an appropriate volume of diluted adapter using the KAPA Adapter Dilution Buffer provided in the kit. Standard protocols call for 5 µL of appropriately diluted adapter stock per library.
  - If an adapter stock concentration >15 µM is required, the volume of water in the ligation reaction may be reduced and the volume of adapter increased to the same extent, up to a total of 10 µL adapter per reaction.
  - Diluted adapters should be freshly prepared and must not be stored for long periods of time or subjected to repeated freezing and thawing.
  - An excess volume of each diluted adapter stock will be required to ensure accurate dispensing.The excess will be larger for automated vs. manual use.
  - All or some of the libraries in the batch may require a different index. Please refer to **Important Parameters: Index Sequences and Pooling Guidelines** (p. 6) for recommendations on multiplexing.
  - The Technical Data Sheet included with the library preparation kit contains specific guidelines for the optimization of adapter concentration when using that particular kit for different applications.

**Table 1. Recommended adapter concentrations for KAPA HyperPrep and HyperPlus Kits[1]**

| Fragmented DNA per 60 µL ER & AT reaction | Adapter stock concentration | Adapter:insert molar ratio | Fragmented DNA per 60 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 |

[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 to 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.

**Table 2. Recommended adapter concentrations for KAPA HTP and LTP Library Preparation Kits[1]**

| Insert DNA per 70 µL end repair reaction | Recommended adapter concentration for DNA sheared to an average size of | | | | | |
|---|---|---|---|---|---|---|
| | 175 bp | | 350 bp | | 500 bp | |
| | Stock | Final | Stock | Final | Stock | Final |
| 1 µg | 20 µM | 2 µM | 10 µM | 1 µM | 7 µM | 0.7 µM |
| 500 ng | 10 µM | 1 µM | 5 µM | 500 nM | 3.5 µM | 350 nM |
| 100 ng | 2 µM | 200 nM | 1 µM | 100 nM | 700 nM | 70 nM |
| 10 ng | 200 nM | 20 nM | 100 nM | 10 nM | 70 nM | 7 nM |

[1]Adapter concentrations are based on a ~10:1 adapter:insert ratio. For values not listed here, please refer to the calculation in **Important Parameters: Adapter Concentration Calculations** or visit **kapabiosystems.com/support** to obtain a calculator designed for this purpose.

**Table 3. Recommended adapter concentrations for KAPA RNA HyperPrep Kits**

| Quantity of starting material | Starting material quality | Adapter stock concentration |
|---|---|---|
| 1 – 49 ng | Partially degraded or FFPE-derived | 1.5 µM |
| | High-quality | 1.5 µM |
| 50 – 100 ng | Partially degraded or FFPE-derived | 1.5 µM |
| | High-quality | 15 µM |

**Table 4. Recommended adapter concentrations for KAPA mRNA HyperPrep Kits**

| Quantity of starting material | Adapter stock concentration |
|---|---|
| 50 – 499 ng | 1.5 µM |
| 500 – 1000 ng | 7 µM |

**Table 5. Recommended adapter concentrations for KAPA RNA HyperPrep Kits with RiboErase (HMR)**

| Quantity of starting material | Starting material quality | Adapter stock concentration |
|---|---|---|
| 25 – 499 ng | Partially degraded or FFPE-derived | 1.5 µM |
| | High-quality | 1.5 µM |
| 500 – 1000 ng | Partially degraded or FFPE-derived | 1.5 µM |
| | High-quality | 7 µM |

**Table 6. Recommended adapter concentrations for KAPA Stranded RNA-Seq Kits with RiboErase (HMR)**

| Input RNA | Adapter stock concentration | Final adapter concentration |
|---|---|---|
| 501 – 1000 ng | 280 nM | 20 nM |
| 251 – 500 ng | 210 nM | 15 nM |
| 100 – 250 ng | 140 nM | 10 nM |

**Table 7. Recommended adapter concentrations for KAPA Stranded RNA and mRNA-Seq Library Preparation Kits**

| Input RNA | | Adapter stock concentration | Final adapter concentration |
|---|---|---|---|
| RNA workflow | mRNA workflow | | |
| 201 – 400 ng | 2001 – 4000 ng | 1400 nM | 20 nM |
| 51 – 200 ng | 501 – 2000 ng | 700 nM | 15 nM |
| 10 – 50 ng | 251 – 500 ng | 350 nM | 10 nM |
| | 100 – 250 ng | 140 nM | 10 nM |

**Additional Notes on Adapter Concentration**

- Adapter concentration affects ligation efficiency as well as adapter and adapter-dimer carry-over in post-ligation cleanups. A molar excess of adapter is required to ensure optimal ligation efficiency. Low adapter:insert molar ratios (approaching 2:1) result in a significant proportion of insert molecules with an adapter ligated to only one end, leading to library construction failure.
- Adapter:insert molar ratios in the range of 10:1 – 40:1 are recommended for KAPA HTP and LTP Library Preparation Kits, whereas KAPA HyperPrep, KAPA HyperPlus Kits and KAPA RNA HyperPrep Kits are compatible with much higher ratios (≥100:1). Very high adapter:insert molar ratios (200:1 – 1000:1) may be beneficial for low-input library construction with KAPA HyperPrep and KAPA HyperPlus Kits.
- While it is not necessary to adjust adapter concentrations to accommodate moderate sample-to-sample variations, an adapter concentration that is appropriate for the range of input DNA concentrations is highly recommended.
- The best way to accommodate different adapter concentrations within a batch of samples processed together is to vary the concentration of adapter stock solutions and dispense a fixed volume (e.g., 5 µL) of each adapter. The alternative—using a single stock solution and dispensing variable volumes of adapter into ligation reactions—is not recommended and is not compatible with higher throughput or automated workflows.
- Post-ligation cleanup and size selection strategies should be informed by the choice of adapter concentration. Please refer to **Important Parameters: Post-ligation Processing** for more details.
- Ultimately, the optimal adapter concentration for a specific workflow represents a compromise between ligation efficiency, the potential negative impact of adapter/adapter-dimer carry-over, and cost.

**Adapter Concentration Calculations**

- The below calculation applies to DNA library construction. For RNA library construction, adapter stock concentrations are calculated based on input only.
- To calculate the optimal adapter stock concentration for DNA library construction, the amount of input DNA (in picomoles) must first be calculated. This is done with the following formula:

$$\text{Picomoles} = \frac{\text{mass of DNA (ng)}}{660} \times \frac{1000}{\text{median size (bp)}}$$

- Next, the picomole quantity of adapter required is calculated by multiplying the number of picomoles of input DNA by the desired adapter:insert ratio. Please refer to Tables 2 and 3 or the Technical Data Sheet included with the library preparation kit for optimal adapter:insert molar ratios for different applications.
  - The picomole quantity of adapter required is subsequently divided by the volume of adapter used per reaction, to obtain the desired adapter stock concentration (in µM or picomoles/µL).
- For example, 200 ng of input DNA with a mode fragment size of 250 bp represents 1.21 picomoles of insert DNA. For a 10:1 adapter:insert ratio, 12.1 picomoles of adapter is required. Therefore, when using 5 µL of adapter stock per ligation reaction, an adapter stock concentration of 2.42 µM is required.
- To obtain a calculator designed for the calculation of adapter:insert molar ratios and stock concentrations please contact Technical Support at **sequencing.roche.com/support**.

**Post-ligation Processing**

- It is important to remove excess unligated adapter and adapter-dimer molecules from Illumina libraries prior to library amplification or cluster generation. This is particularly important for libraries to be sequenced on Illumina platforms that employ patterned flow cells.
- Please follow the post-ligation cleanup instructions provided in the Technical Data Sheet for the library preparation kit. While a single post-ligation cleanup with KAPA Pure Beads or Agencourt® AMPure® XP removes most unligated adapter and adapter-dimer (as recommended for KAPA HyperPrep and HyperPlus protocols), a second cleanup or size selection step may be necessary to eliminate any remaining adapter species from the library. The amount of adapter and adapter-dimer carried through the first cleanup is dependent on the library construction chemistry and adapter concentration in the ligation reaction.
- If bead-based size selection is carried out after adapter ligation, a single post-ligation cleanup (with the appropriate bead-to-sample ratio; as per the library construction protocol) must first be performed. Ligation buffers contain high concentrations of PEG 6000, which will impact the length and distribution of library fragments recovered from post-ligation size selection.

### Index Sequences and Pooling Guidelines

- Sequencing indices (barcodes) included in KAPA Dual-Indexed Adapters are provided in Table 10.
- For low-plexity pooling applications (up to 16-plex) on Illumina sequencing platforms, specific index combinations must be used. For 17- to 96-plex pools, any combination of adapters can be used. Detailed multiplexing guidelines are provided in Table 9.
- To ensure equal read distributions in multiplexed sequencing applications, libraries must be carefully quantified and/or normalized prior to pooling for capture or cluster generation. qPCR-based quantification with the KAPA Library Quantification Kit constitutes the most accurate and reproducible method for the quantification of sequenceable molecules in an Illumina library, particularly for PCR-free workflows.

### Plate Format

Table 8 depicts the naming and placement of KAPA Dual-Indexed Adapters within the 96-well plate that is provided. Dual-indexed adapters are supplied in fully-skirted PCR plates, sealed with non-pierceable heat seals. Adapters are provided at a concentration of 15 µM. Each well contains 20 µL plus excess.

**Table 8. KAPA Dual-Indexed Adapter Plate layout**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H | H1 | H2 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

**Table 9. Detailed multiplexing guidelines**

| Plexity | Option | KAPA Dual-Indexed Adapters | | | |
|---|---|---|---|---|---|
| 6 | 1 | A1 – A3 and B1 – B3 | C1 – C3 and D1 – D3 | E1 – E3 and F1 - F3 | G1 – G3 and H1 – H3 |
| | 2 | A4 – A6 and B4 – B6 | C4 – C6 and D4 – D6 | E4 – E6 and F4 – F6 | G4 – G6 and H4 – H6 |
| | 3 | A7 – A9 and B7 – B9 | C7 – C9 and D7 – D9 | E7 – E9 and F7 - F9 | G7 – G9 and H7 – H9 |
| | 4 | A10 – A12 and B10 – B12 | C10 – C12 and D10 – D12 | E10 – E12 and F10 – F12 | G10 – G12 and H10 – H12 |
| 8 | 1 | A1 – A4 and B1 – B4 | C1 – C4 and D1 – D4 | E1 – E4 and F1 – F4 | G1 – G4 and H1 – H4 |
| | 2 | A5 – A8 and B5 – B8 | C5 – C8 and D5 – D8 | E5 – E8 and F5 – F8 | G5 – G8 and H5 – H8 |
| | 3 | A9 – A12 and B9 – B12 | C9 – C12 and D9 – D12 | E9 – E12 and F9 – F12 | G9 – G12 and H9 – H12 |
| 12 | 1 | A1 – A6 and B1 – B6 | C1 – C6 and D1 – D6 | E1 – E6 and F1 – F6 | G1 – G6 and H1 – H6 |
| | 2 | A7 – A12 and B7 – B12 | C7 – C12 and D7 – D12 | E7 – E12 and F7 – F12 | G7 – G12 and H7 – H12 |
| 16 | 1 | A1 – A8 and B1 – B8 | C1 – C8 and D1 – D8 | E1 – E8 and F1 – F8 | G1 – G8 and H1 – H8 |
| | 2 | A4 – A12 and B4 – B12 | C4 – C12 and D4 – D12 | E4 – E12 and F4 – F12 | G4 – G12 and H4 – H12 |
| >16 | | Any combination of the 96 available adapters | | | |

Table 10. KAPA Dual-Indexed Adapter Kit Index sequences

| Dual-Index | P5 Index | P7 Index |
|---|---|---|
| A1 | TATAGCCT | ATTACTCG |
| A2 | TATAGCCT | TCCGGAGA |
| A3 | TATAGCCT | CGCTCATT |
| A4 | TATAGCCT | GAGATTCC |
| A5 | TATAGCCT | ATTCAGAA |
| A6 | TATAGCCT | GAATTCGT |
| A7 | TATAGCCT | CTGAAGCT |
| A8 | TATAGCCT | TAATGCGC |
| A9 | TATAGCCT | CGGCTATG |
| A10 | TATAGCCT | TCCGCGAA |
| A11 | TATAGCCT | TCTCGCGC |
| A12 | TATAGCCT | AGCGATAG |
| B1 | ATAGAGGC | ATTACTCG |
| B2 | ATAGAGGC | TCCGGAGA |
| B3 | ATAGAGGC | CGCTCATT |
| B4 | ATAGAGGC | GAGATTCC |
| B5 | ATAGAGGC | ATTCAGAA |
| B6 | ATAGAGGC | GAATTCGT |
| B7 | ATAGAGGC | CTGAAGCT |
| B8 | ATAGAGGC | TAATGCGC |
| B9 | ATAGAGGC | CGGCTATG |
| B10 | ATAGAGGC | TCCGCGAA |
| B11 | ATAGAGGC | TCTCGCGC |
| B12 | ATAGAGGC | AGCGATAG |
| C1 | CCTATCCT | ATTACTCG |
| C2 | CCTATCCT | TCCGGAGA |
| C3 | CCTATCCT | CGCTCATT |
| C4 | CCTATCCT | GAGATTCC |
| C5 | CCTATCCT | ATTCAGAA |
| C6 | CCTATCCT | GAATTCGT |
| C7 | CCTATCCT | CTGAAGCT |
| C8 | CCTATCCT | TAATGCGC |
| C9 | CCTATCCT | CGGCTATG |
| C10 | CCTATCCT | TCCGCGAA |
| C11 | CCTATCCT | TCTCGCGC |
| C12 | CCTATCCT | AGCGATAG |
| D1 | GGCTCTGA | ATTACTCG |
| D2 | GGCTCTGA | TCCGGAGA |
| D3 | GGCTCTGA | CGCTCATT |
| D4 | GGCTCTGA | GAGATTCC |
| D5 | GGCTCTGA | ATTCAGAA |
| D6 | GGCTCTGA | GAATTCGT |
| D7 | GGCTCTGA | CTGAAGCT |
| D8 | GGCTCTGA | TAATGCGC |
| D9 | GGCTCTGA | CGGCTATG |
| D10 | GGCTCTGA | TCCGCGAA |
| D11 | GGCTCTGA | TCTCGCGC |
| D12 | GGCTCTGA | AGCGATAG |

| Dual-Index | P5 Index | P7 Index |
|---|---|---|
| E1 | AGGCGAAG | ATTACTCG |
| E2 | AGGCGAAG | TCCGGAGA |
| E3 | AGGCGAAG | CGCTCATT |
| E4 | AGGCGAAG | GAGATTCC |
| E5 | AGGCGAAG | ATTCAGAA |
| E6 | AGGCGAAG | GAATTCGT |
| E7 | AGGCGAAG | CTGAAGCT |
| E8 | AGGCGAAG | TAATGCGC |
| E9 | AGGCGAAG | CGGCTATG |
| E10 | AGGCGAAG | TCCGCGAA |
| E11 | AGGCGAAG | TCTCGCGC |
| E12 | AGGCGAAG | AGCGATAG |
| F1 | TAATCTTA | ATTACTCG |
| F2 | TAATCTTA | TCCGGAGA |
| F3 | TAATCTTA | CGCTCATT |
| F4 | TAATCTTA | GAGATTCC |
| F5 | TAATCTTA | ATTCAGAA |
| F6 | TAATCTTA | GAATTCGT |
| F7 | TAATCTTA | CTGAAGCT |
| F8 | TAATCTTA | TAATGCGC |
| F9 | TAATCTTA | CGGCTATG |
| F10 | TAATCTTA | TCCGCGAA |
| F11 | TAATCTTA | TCTCGCGC |
| F12 | TAATCTTA | AGCGATAG |
| G1 | CAGGACGT | ATTACTCG |
| G2 | CAGGACGT | TCCGGAGA |
| G3 | CAGGACGT | CGCTCATT |
| G4 | CAGGACGT | GAGATTCC |
| G5 | CAGGACGT | ATTCAGAA |
| G6 | CAGGACGT | GAATTCGT |
| G7 | CAGGACGT | CTGAAGCT |
| G8 | CAGGACGT | TAATGCGC |
| G9 | CAGGACGT | CGGCTATG |
| G10 | CAGGACGT | TCCGCGAA |
| G11 | CAGGACGT | TCTCGCGC |
| G12 | CAGGACGT | AGCGATAG |
| H1 | GTACTGAC | ATTACTCG |
| H2 | GTACTGAC | TCCGGAGA |
| H3 | GTACTGAC | CGCTCATT |
| H4 | GTACTGAC | GAGATTCC |
| H5 | GTACTGAC | ATTCAGAA |
| H6 | GTACTGAC | GAATTCGT |
| H7 | GTACTGAC | CTGAAGCT |
| H8 | GTACTGAC | TAATGCGC |
| H9 | GTACTGAC | CGGCTATG |
| H10 | GTACTGAC | TCCGCGAA |
| H11 | GTACTGAC | TCTCGCGC |
| H12 | GTACTGAC | AGCGATAG |

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KAPABIOSYSTEMS

**Manufacturing, R&D**
**Cape Town, South Africa**
Tel: +27.21.448.8200
Fax: +27.21.448.6503

**Technical Support**
sequencing.roche.com/support