4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit

For determining human single cell whole genome amplification success

HANDBOOK

4basebio

INDEX

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit	4 samples	CVC1004

KIT CONTENTS

DESCRIPTION	VOLUME
2x 4BB™ qPCR CovCheck™ Master Mix	1100 µl
PCR primers (96-well plate)	8 µl / well

SHIPPING AND STORAGE

The 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit is shipped in dry ice. Upon receipt, the reagents and the plate should be stored at -20°C immediately in a constant-temperature freezer. 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Master Mix should be protected from light. If stored correctly, the product can be kept for at least six months after shipping without displaying any reduction in performance. For longer periods, store the kit at -80°C.

HANDLING

This kit is sensitive to small amounts of DNA. Wear gloves at all times and prepare the reaction in a laminar flow hood or similar device to avoid contamination. Use molecular biology grade clean reagents, sterile reaction tubes and DNA free pipette tips. Due to the hot-start Taq DNA polymerase contained in the kit, it is not necessary to keep the samples on ice during reaction setup or while programming the real-time thermal cycler.

All chemicals should be considered as potentially hazardous. This material may contain substances or activities that are harmful to human health. It should not be ingested, inhaled, or brought into contact with skin, and handled with appropriate care in accordance with the principles of good laboratory practice as well as COSHH regulations. In case of skin contact, wash immediately with water. For more specific information, please consult the Material Safety Data Sheets (MSDS) available on-line at www.4basebio.com.

QUALITY CONTROL

Each batch of 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit is tested against predetermined specifications to ensure consistent product quality.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Sterile vials, pipettes and pipette tips. Use low-retention plasticware if possible.
- Microcentrifuge
- Real-time thermal cycler
- Vortex

INTRODUCTION

4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit provides a ready to use set of PCR primers in a convenient 96-well plate format, paired with optimized qPCR reagents.

The plate includes 4 identical sets of 24 different primer pairs that will amplify small portions from each human chromosome, allowing the coverage analysis in real-time of 4 independent single-cell whole genome amplifications simultaneously. Each primer pair number or letter reflects the chromosome that is amplified (see plate distribution in Figure 1).

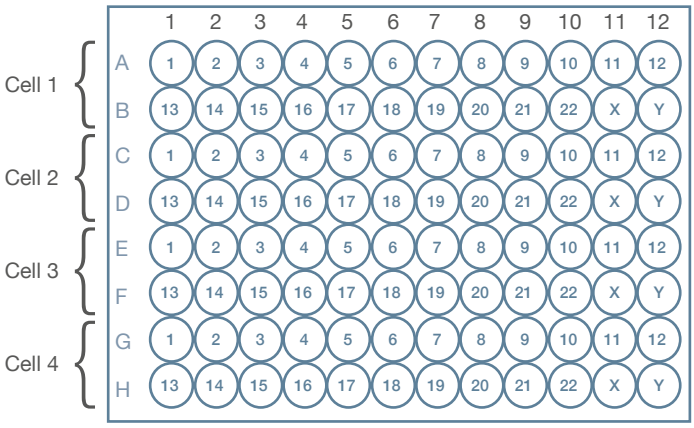

Figure 1. 96-well plate design containing the 4 sets of 24 primers pairs.

Simply prepare the master mix with the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Master Mix and a sample from your amplified single cell DNA. Aliquot 12 µl of the resultant mix to each well, run the qPCR reaction and visualize the result in real time.

A high fraction of successful amplifications per sample predicts a high coverage in sequencing.

Figure 2 below shows the correlation between the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit results and the coverage obtained through whole genome sequencing. In this example, single cells from the HEK293 cell line were amplified with 4BB™ TruePrime® Single Cell WGA kit and the coverage estimated using the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit. Several samples with either high or low success rates in the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex assay were selected for sequencing. Sequencing was carried out on an Illumina® HiSeq 2500 with 125 bp paired end reads. The fraction of the genome covered is shown on the y-axis and the fraction of positive qPCR reactions on the x-axis. As can be seen, the two methods of measure are tightly correlated.

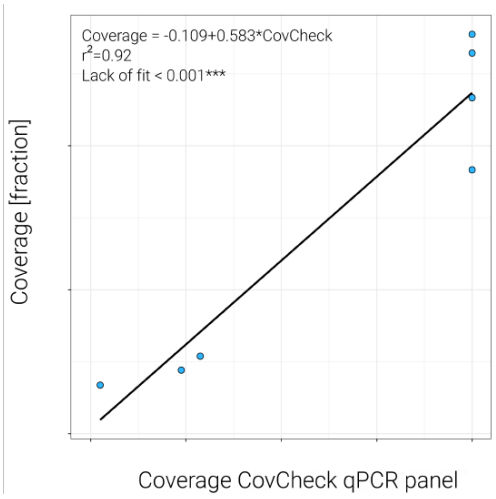

Figure 2. Excellent correlation between 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit results and results generated through sequencing.

The deviation of the sequencing coverage from a theoretically optimal coverage following a Poisson distribution model was also analyzed, further illustrating the good correlation to the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex assay (Figure 3 below).

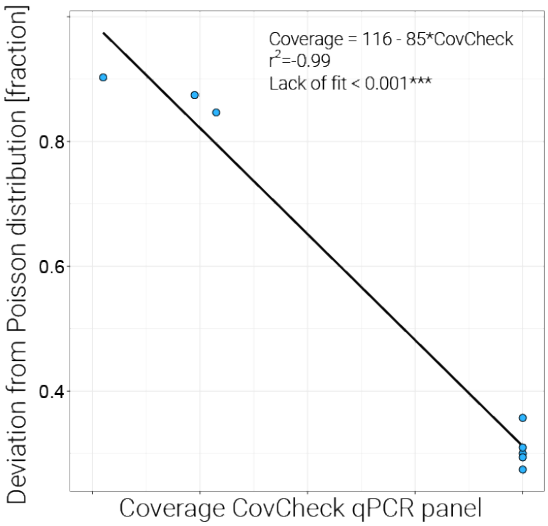

Figure 3. Graph depicting the correlation between Poisson distribution deviations and 4BB™ CovCheck™ qPCR panel coverage.

The Circos plot (Figure 4, left) depicts human genome coverage from a HEK293 cell from the correlation graphs above (Figures 2 and 3) that showed only a 22% success rate of the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex assay. Blue indicates the coverage obtained by sequencing, marked in red are the chromosomes for which a positive qPCR reaction was observed (i.e. an amplicon obtained). The overall genome coverage obtained by sequencing was not good, with complete drop-outs of several chromosomes and uneven representations of other chromosomes observed. By contrast a HEK293 cell showed a 100% success rate using the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex assay (Figure 4, right hand). In this case, the whole genome was amplified and an even coverage pattern observed. Since HEK293 cells are female, no coverage is observed for the Y chromosome.

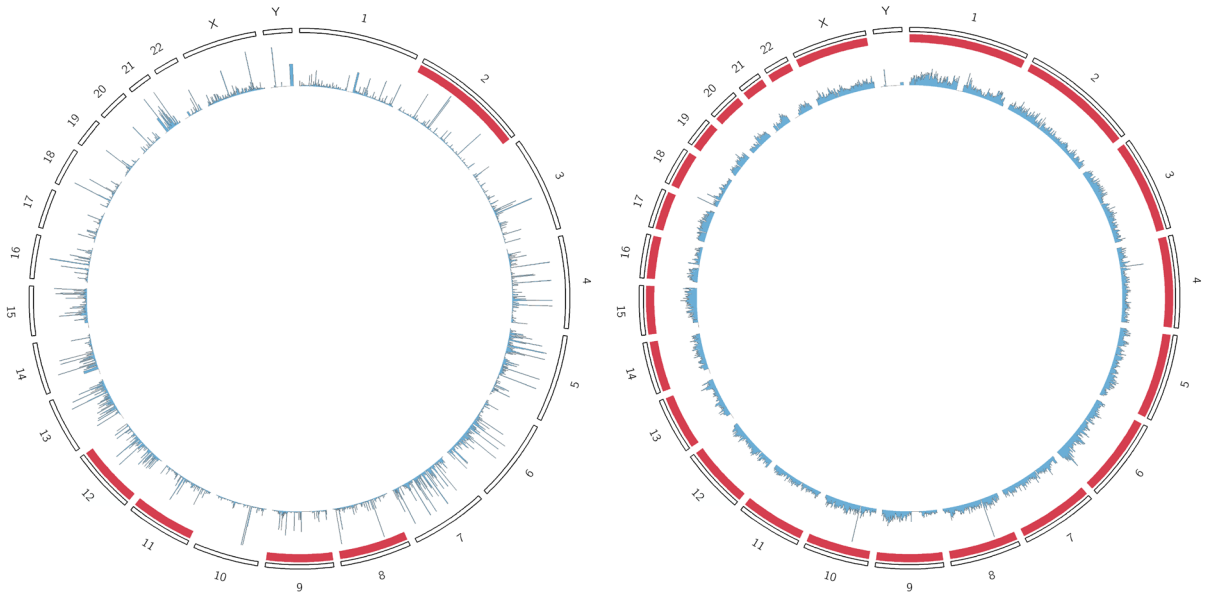

Figure 4. Human genome coverage obtained by sequencing versus 4BB™ qPCR CovCheck™ results.

PROTOCOL

The 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit provides a ready to use set of PCR primers in a convenient 96-well plate format, complete with optimized qPCR reagents (2x 4BB™ qCovCheck™ Genome Coverage WGA QC SinglePlex Master Mix containing hot-start Taq DNA polymerase, SYBR Green I, reaction buffer and dNTPs). The 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit is an easy and convenient approach to getting the best form your single cell experiments, enabling visualization of specific targets, thereby minimizing costs. 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit is optimized for use with 4BB™ TruePrime® Single Cell WGA, but is compatible with other amplification technologies.

NOTE: The 96-well plate containing the primer pairs is a single-use plate, so all 4 samples need to be run in one experiment.

1. DNA Purification and quantification:

   Remaining reagents from WGA reactions might affect the efficiency of the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex reaction, so it is recommended to purify the amplified DNA before running the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit. We recommend the Qiagen QIAquick PCR purification kit (Cat No./ID: 28104), the Qiagen QIAamp DNA Mini Kit (Cat No./ID: 51304) or ethanol precipitation. All have proven efficient, with slight advantages for the columns.

   Qubit™ fluorometer and PicoGreen® reagent are adequate methods for quantification of double stranded DNA. The Qubit™ procedure is recommended for use with the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Kit, as it has been found to produce reliable results during internal testing. Please follow the manufacturer's recommendations. Apparent yields will on average be approximately 25% lower using the Qubit™ method, since the PicoGreen® method has been found to overestimate DNA concentrations.

2. DNA dilution:

   Dilute purified DNA samples to 5 ng/µl with PCR-grade water to a final volume of 60 µl.

3. Preparation of the PCR master mixes:

   Prepare four qPCR master mixes (one per DNA sample), adding the components in the order listed in Table 1 below.

COMPONENT	VOLUME
2x 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex Master Mix	270 µl
DNA (5 ng/µl)	54 µl

Table 1. Preparation of amplification mix

   Mix the amplification mix by vortexing and centrifugate before use to remove bubbles. Due to the hot-start Taq polymerase contained in the kit, it is not necessary to keep samples on ice during reaction setup or while programming the real-time thermal cycler.

   Add 12 µl of each amplification mix to the corresponding wells in the rows selected for each DNA sample, according to the design shown in Figure 1. The final reaction volume is 20 µl.

   If bubbles are detected in the wells, centrifuge the plate (1000 rpm for 5 min) before running the qPCR program.

4. PCR reaction:

Seal the plate with the adhesive provided, place it in a real time thermal cycler and run the protocol outlined in Table 2 below. Select SYBR as the fluorophore to be read.

STEP	TIME	TEMPERATURE**	CYCLES
PCR initial activation step	2 min	95ºC	1
2-step cycling			40
Denaturation	5s	95ºC	
Annealing, extension and plate read	10s	60ºC	
Melting curve analysis		65 – 95ºC (Δ 0.5ºC / 5 s)	

Table 2. Thermal cycling protocol*

* Conditions optimized for Bio-Rad® CFX Connect™ real-time PCR system.
** Use maximal or fast mode ramp rates

5. Analysis:

5.1. Melting curve analysis of the PCR products:

Melting curve analysis of the PCR products, which is built into the software of real-time thermal cyclers, verifies the specificity and identity of PCR products.

Table 3 below shows the melt temperatures expected (± 1°C) for each amplicon.

	Melt Temperature* (°C)	Amplicon size (bp)
Chr1	84,5	303
Chr2	77,5	222
Chr3	80,5	275
Chr4	87,5	333
Chr5	79,0	252
Chr6	79,5	312
Chr7	84,5	340
Chr8	83,0	300
Chr9	75,5	399
Chr10	75,0	390
Chr11	76,5	238
Chr12	77,0	229
Chr13	78,5	214
Chr14	85,5	306
Chr15	86,0	316
Chr16	84,0	375
Chr17	85,5	356
Chr18	78,5	373
Chr19	83,0	232
Chr20	84,5	346
Chr21	80,5	241
Chr22	85,5	238
ChrX	81,0	320
ChrY	77,0	312

Table 3. Melt temperatures expected (± 1°C) and amplicon sizes (bp).
* Conditions optimized for Bio-Rad® CFX Connect™ real-time PCR system

5.2. Optional: check the specificity of the PCR products by agarose gel electrophoresis

Add 4 µl of DNA gel loading dye (6X) to each well of the plate. Prepare a 1% agarose gel and load 6 µl of each reaction. Alternatively, analyze the samples on an Agilent Bioanalyzer DNA chip following the manufacturer's recommendations.

A picture of a reference gel run to illustrate this application is shown in figure 5 below.

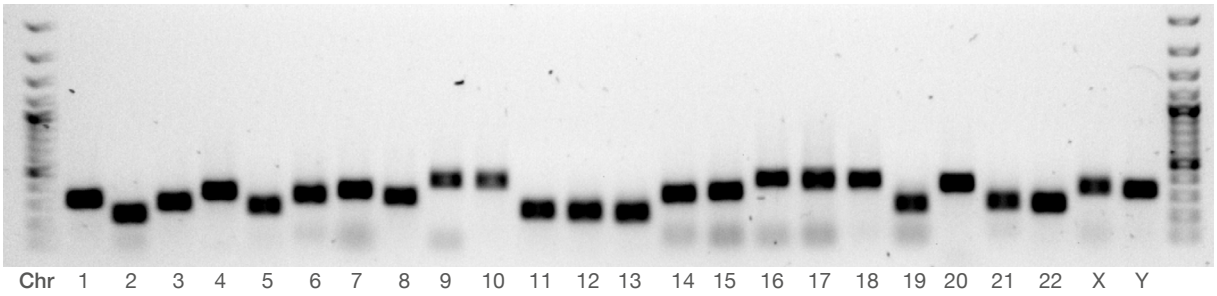

Figure 5. Agarose gel electrophoresis of the 4BB™ qPCR CovCheck™ Genome Coverage WGA QC SinglePlex amplicons.

Store the plate at 4ºC if required.

4basebio

orders@4basebio.com

www.4basebio.com