4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit

For determining human single cell whole genome amplification success

HANDBOOK

4basebio

INDEX

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit	16 samples	CVC000M

KIT CONTENTS

DESCRIPTION	CAP COLOR	VOLUME
5x Reaction Buffer	Yellow	500 µl
Magnesium Chloride solution	Blue	250 µl
dNTP mix	Green	40 µl
Polymerase	Orange	18 µl
96-well plate		12 µl / well

SHIPPING AND STORAGE

The 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit is shipped in dry ice. Upon receipt, the reagents and the plate should be stored immediately at -20°C in a non-frost-free (constant-temperature) freezer. The adhesive seal should be stored at room temperature. If stored correctly, the product can be kept for at least six months after shipping without displaying any reduction in performance.

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. Thaw Polymerase and dNTPs on ice. All other components can be thawed at room temperature.

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 should be handled with appropriate care in accordance with the principles of good laboratory practice. In case of contact with skin 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™ CovCheck™ Genome Coverage WGA QC Multiplex 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
- Cold block
- Thermocycler
- Vortexer

INTRODUCTION

4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit provides a ready to use set of end-point PCR primers in a convenient 96-well plate format, complete with optimized PCR reagents including a premium hot-start Taq polymerase. The plate includes 16 identical sets of 24 different primer pairs multiplexed in six reations (see Figure 1 and Table 1), that will amplify small portions from each human chromosome, allowing the coverage analysis of 16 independent single-cell whole genome amplifications simultaneously.

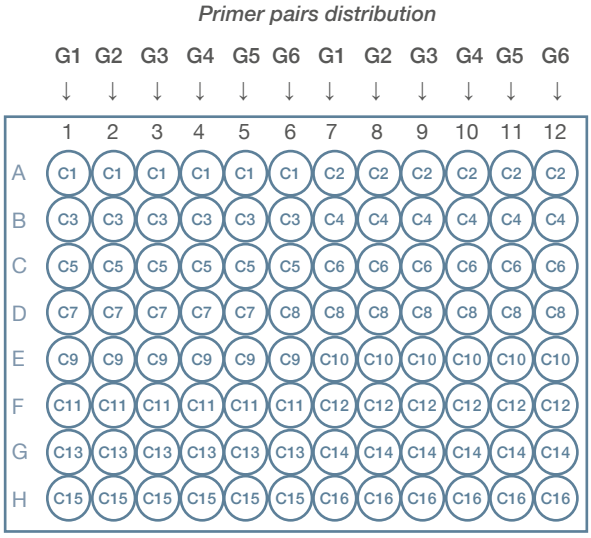


Figure 1. 96-well plate design containing the 6 groups of 4 primer pairs distributed in columns. Each row allows to check two samples, resulting in 16 samples per plate. Samples are coded with a "C" and a correlative number.

GROUP 1		GROUP 2		GROUP 3	
Chromosome	Amplicon size	Chromosome	Amplicon size	Chromosome	Amplicon size
16	901	17	861	6	900
13	637	15	656	14	758
9	483	20	501	8	569
Y	314	1	303	2	380
GROUP 4		**GROUP 5**		**GROUP 6**	
Chromosome	Amplicon size	Chromosome	Amplicon size	Chromosome	Amplicon size
10	869	7	916	X	964
12	718	18	742	11	775
19	486	21	436	22	490
3	275	5	252	4	333

Table 1. Chromosome and amplicon size correlation within each primer pair group.

You only need to prepare a master mix, add this and a sample from your amplified single cell DNA to each well, run a PCR reaction, and visualize the result on an agarose gel or an Agilent Bioanalyzer or TapeStation. A high fraction of successful amplifications per sample predicts a high coverage in sequencing.

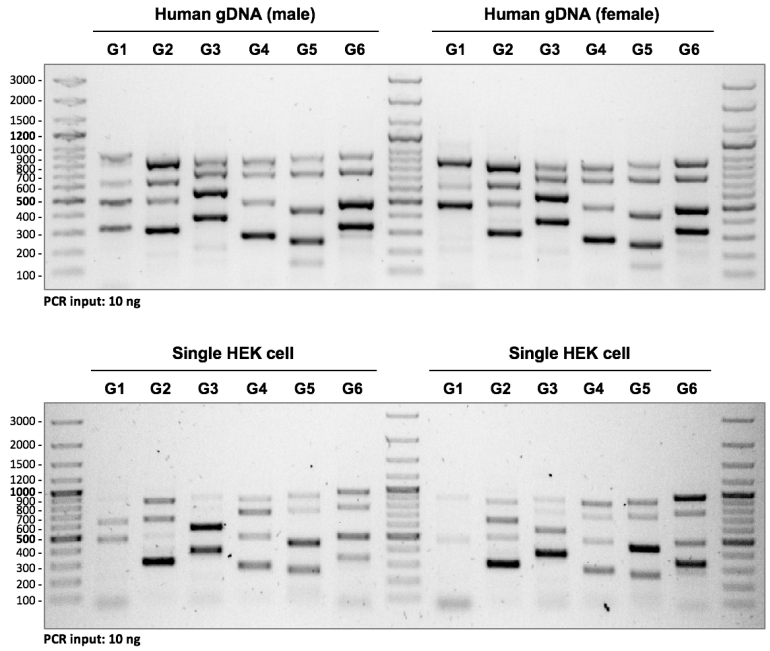
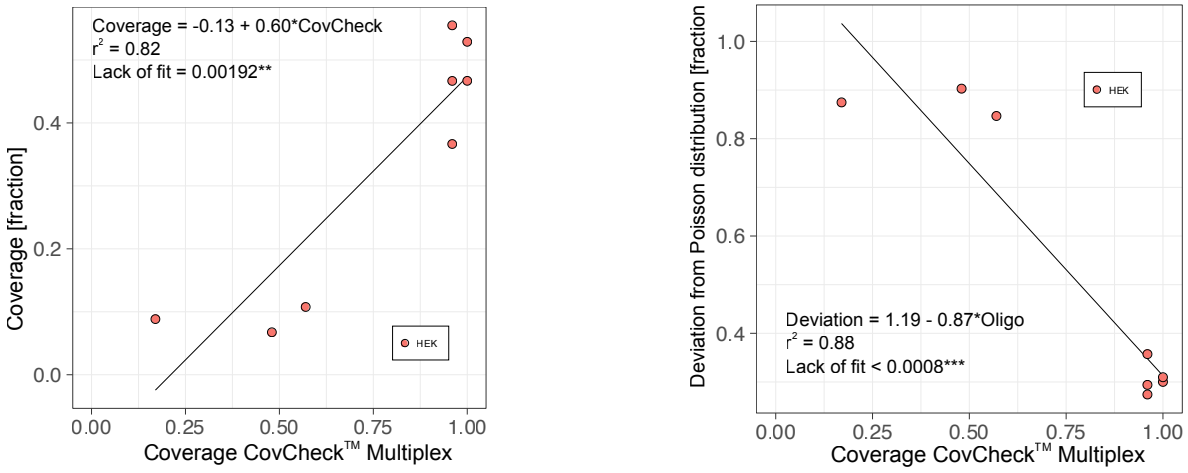


Figure 2. 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex results obtained with human genomic DNA (male and female), and DNA amplified with 4BB™ TruePrime® from single HEK293 cells. Each group (1-6) shows four different amplicons located in four different chromosomes (see Table 1).

Figure 3 shows the correlation between the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit results and the real coverage obtained through whole genome sequencing. In this example, single HEK293 cells were amplified with 4BB™ TruePrime® Single Cell WGA kit and the coverage estimated using the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit. Several samples with either high or low success rates in the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex assay were selected for sequencing. Sequencing was carried out on an Illumina® HiSeq 2500 with 150 bp paired end reads. The fraction of the genome covered is shown on the y-axis and the fraction of positive PCR reactions on the x-axis. The two measures are tightly correlated.



Figures 3 and 4. Excellent correlation between 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit results and real sequencing data

The deviation of the sequencing coverage from a theoretically optimal coverage following a Poisson distribution model was also analyzed, showing also a good correlation to the results from the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex assay (see figure 4).

Shown below is the correlation between the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex and Multiplex Kit results. In this example, 16 single HEK293 cells were independently amplified with 4BB™ TruePrime® Single Cell WGA kit and the coverage estimated using the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex and Multiplex Kits. The correlation between the results obtained with each kit is statistically significant ($p < 0.01$). A lower 4BB™ CovCheck™ percentage will not always show the same chromosomes for 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit and 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit, because some of the used PCR primers are located at different positions on the chromosome.

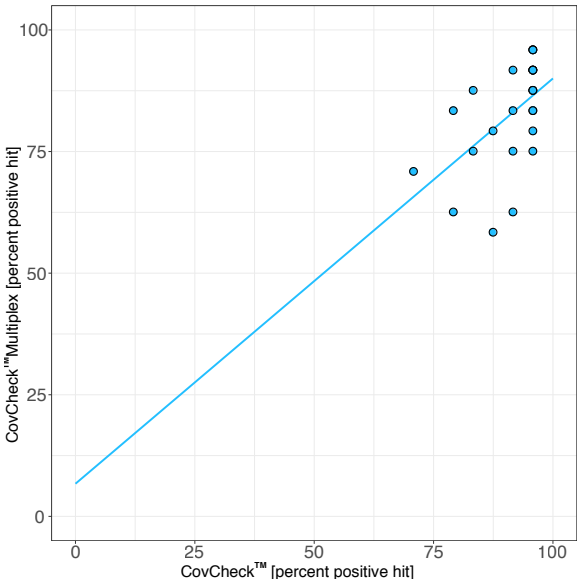


Figure 5. Correlation between 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex and Multiplex Kits. The correlation between the results obtained with each of the 16 single HEK293 cells used in the kits is statistically significant ($p < 0.01$).

The Circos plot in Figure 6 (left part) depicts human genome coverage from a HEK293 cell from the correlation graphs above (Figures 3 and 4) that showed only a 17% success rate of the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex assay. Blue indicates the coverage obtained by sequencing, marked in red are the chromosomes for which a positive PCR reaction was observed (i.e. an amplicon obtained). The overall genome coverage obtained by sequencing is not good, with complete drop-outs of several chromosomes and very spiky appearance of other chromosomes. In contrast, in the case of a HEK293 cell with near 100% success rate of the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex assay (Figure 6, right part), all parts of the genome have been amplified and the coverage pattern observed is very even. As HEK293 cells are female, no coverage is observed for the Y chromosome.

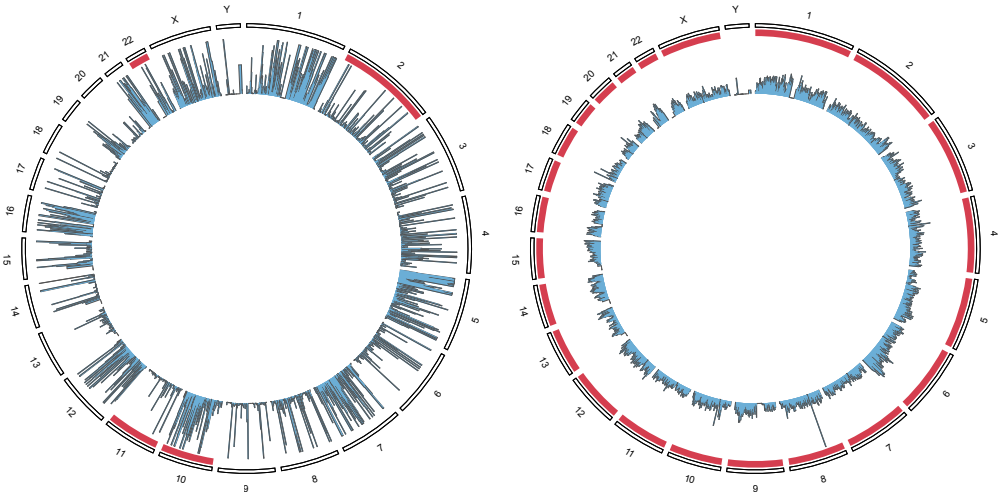


Figure 6. Human genome coverage obtained by sequencing versus 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex results.

PROTOCOL

4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit provides a ready to use set of end-point PCR primers in a convenient 96-well plate format, complete with optimized PCR reagents including a premium hot-start Taq polymerase. 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit is an easy and convenient approach to get out the best form your single cell experiments, and only sequence what you really want and save costs. Of course we recommend to use our 4BB™ TruePrime® Single Cell WGA v 2.0 technology for amplifying your cells of choice, but the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex Kit will also work for other amplification technologies with high probability.

NOTE: The 96-well plate containing the primer pairs is a single-use plate, so all 16 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™ CovCheck™ Genome Coverage WGA QC Multiplex Kit PCR reaction, so we highly recommend to purify the amplified DNA before running the 4BB™ CovCheck™ Genome Coverage WGA QC Multiplex 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 worked in our hands, with slight advantages for the columns.

   Qubit™ fluorometer and PicoGreen® reagent are adequate methods for quantification of double-stranded DNA. We recommend the Qubit™ procedure, as it produces more reliable results in our hands. Please follow the manufacturer's recommendations. Apparent yields will on average be about 25% lower with the Qubit™ method, since the PicoGreen® method likely overestimates DNA concentrations.

2. DNA dilution:

   Dilute purified DNA samples to 5 ng/µl with PCR-grade water to a final volume of 15 µl.

3. Preparation of the PCR master mixes:

   Prepare one PCR master mix per DNA sample adding the components in the order listed in Table 2.

COMPONENT	VOLUME
5x Reaction Buffer	26 µl
Magnesium Chloride solution	10.4 µl
dNTP mix	1.95 µl
Amplified DNA	13 µl
Polymerase	0.65 µl

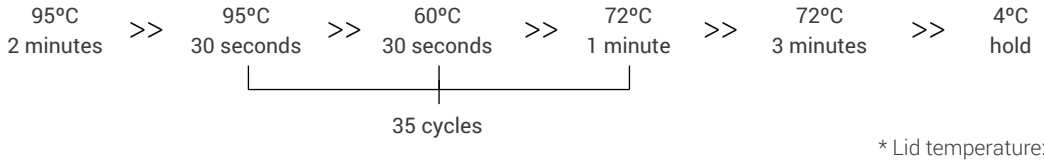
Table 2. Preparation of amplification mix

   Mix the PCR master mix by vortexing and store on ice until use.

   Add 8 µl of each PCR master mix to the corresponding wells of the row selected for each DNA sample, according to the design shown in Figure 1. The final reaction volume is 20 µl.

4. PCR reaction:

   Seal the plate with the adhesive provided, place it in a thermal cycler and run the following PCR program*:



   * Lid temperature: 105°C

5. Analysis:

   Centrifuge the plate after the PCR reaction if needed. Add 4 µl of DNA gel loading dye (6X) to each well of the plate. Prepare a 1,1% agarose gel and load 3 µl of each reaction. Alternatively, analyze the samples on an Agilent Bioanalyzer or TapeStation following the manufacturer's recommendations.

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