4BB™ 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™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit	4 samples	CVC0004

KIT CONTENTS

DESCRIPTION	CAP COLOR	VOLUME
5x Reaction Buffer	Yellow	450 µl
Magnesium Chloride solution	Blue	200 µl
dNTP mix	Green	35 µl
Polymerase	Orange	13 µl
PCR primers (96-well plate)		12 µl / well

SHIPPING AND STORAGE

The 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex 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.expedeon.com.

QUALITY CONTROL

Each batch of 4BB™ 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
- Cold block
- Thermocycler
- Vortexer

INTRODUCTION

4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex 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 4 identical sets of 24 different primer pairs that will amplify small portions from each human chromosome, allowing the coverage analysis of 4 independent single-cell whole genome amplifications simultaneously. Each primer pair number or letter reflects the chromosome that is amplified (see figure 1).

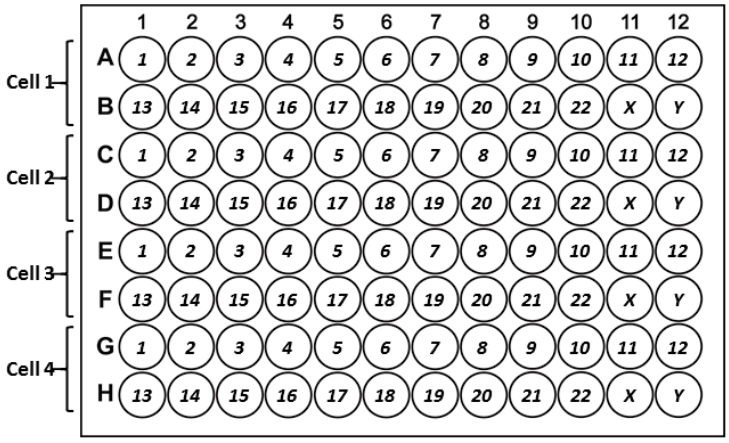


Figure 1. 96-well plate design containing the 4 sets of 24 primers pairs

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

Figure 2 shows the size ranges of the obtained amplicons (~300-400 bp), and the appearance in the case of a positive control (human genomic DNA) above, and a result from a random HEK293 cell amplified with the 4BB™ TruePrime® Single Cell WGA kit. Note that HEK293 cells are female, therefore no amplicon can be obtained for the Y chromosome.

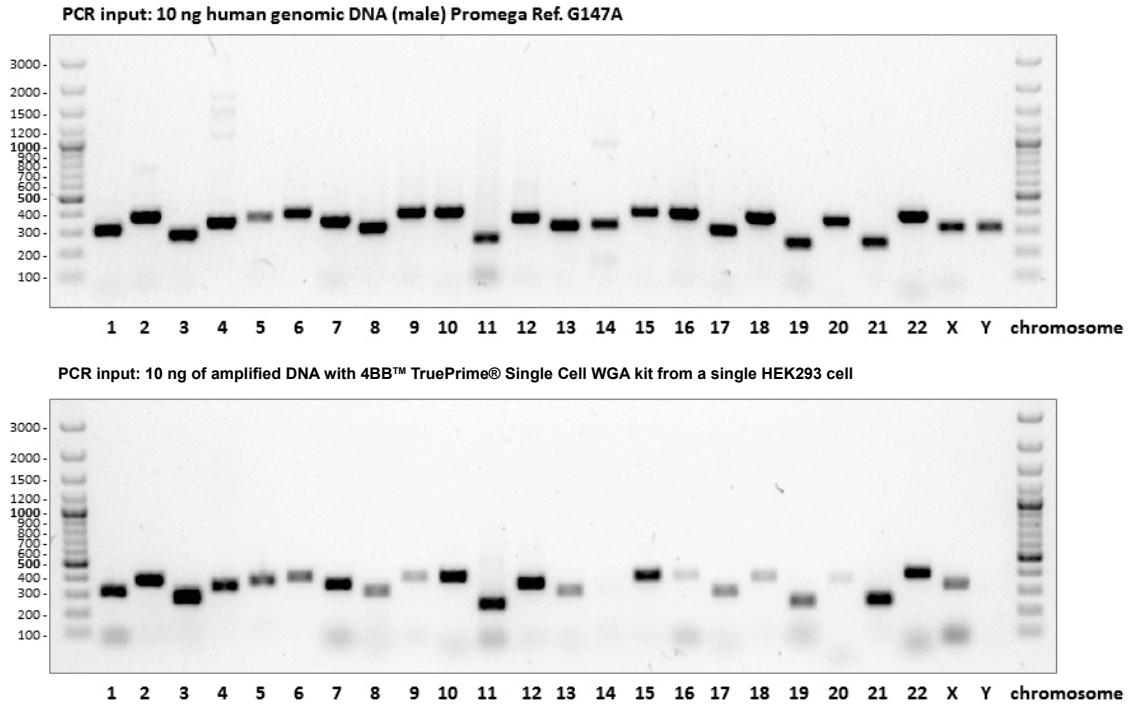


Figure 2. 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit result obtained with human genomic DNA and DNA amplified with 4BB™ TruePrime® from a single HEK293 cell

Figure 3 shows the correlation between the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit results and the real coverage obtained through whole genome sequencing. In this example, single cells from three human cell lines (HEK293, K562 and HeLa) were amplified with 4BB™ TruePrime® Single Cell WGA kit and the coverage estimated using the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit. Several samples with either high or low success rates in the 4BB™ 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 PCR reactions on the x-axis. The two measures are tightly correlated.

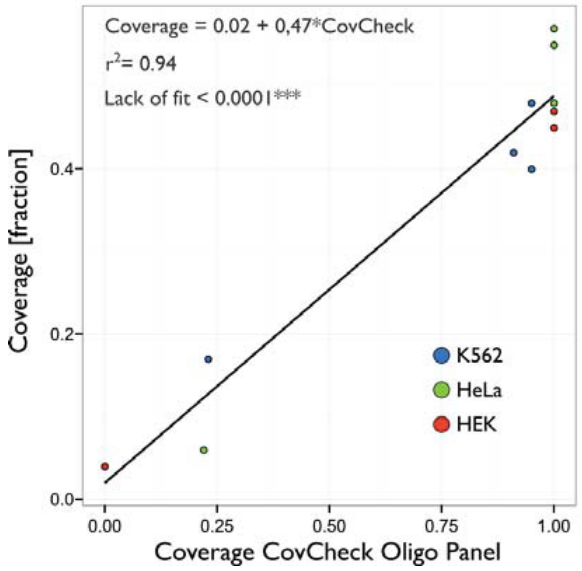


Figure 3. Excellent correlation between 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit results and real sequencing data

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 SinglePlex assay (see figure 4).

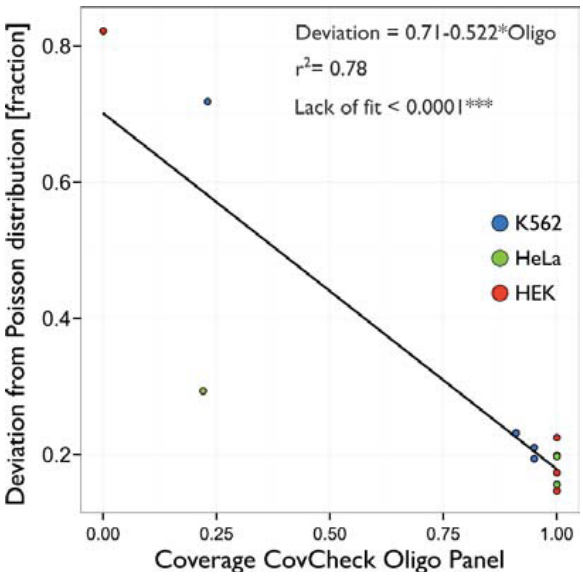


Figure 4. Excellent correlation between 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit results and real sequencing data

This coverage prediction has worked well for all three cell types, and we expect that the assay will work for any other human cell type as well.

The Circos plot in Figure 5 (left part) depicts human genome coverage from a K562 cell from the correlation graph above (Figures 3 and 4) that showed only a ~22% success rate of the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex 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 K562 cell with near 100% success rate of the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex assay (Figure 5, right part), all parts of the genome have been amplified and the coverage pattern observed is very even.

As K562 cells are female, no coverage is observed for the Y chromosome. In addition, chromosome 9 is heavily deleted and rearranged, and the target of the primers for this chromosome has been deleted. Therefore, no amplicon was obtained for chromosome 9.

The K562 cell line is derived from a bcr-abl translocation positive female CML (chronic myeloid leukemia) patient, and is heavily aneuploidic explaining the different coverage depths for individual chromosomes or chromosome parts (e.g. compare the proximal and distal part of the p arm on chromosome 3).

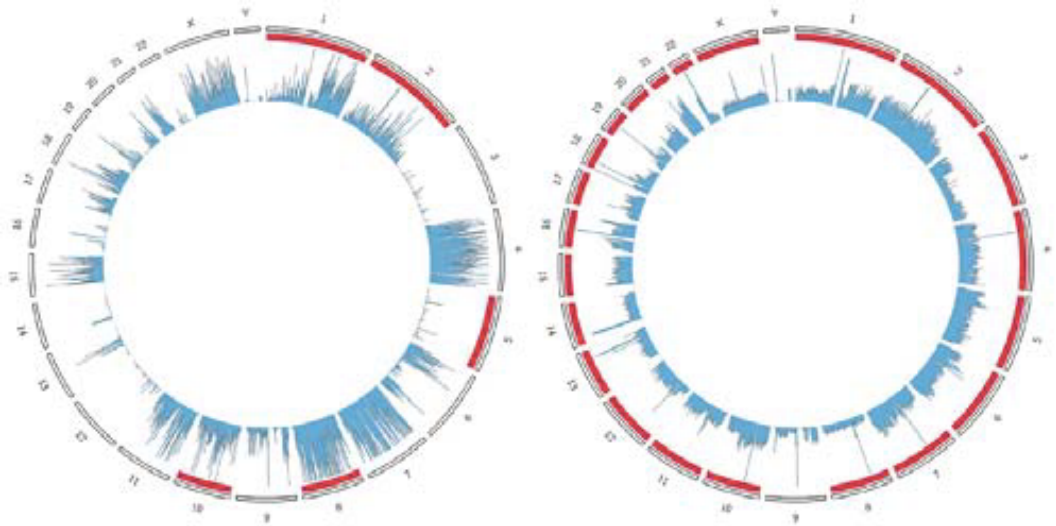


Figure 2. 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex results obtained with human genomic DNA and DNA amplified with 4BB™ TruePrime® from a single HEK293 cell

PROTOCOL

4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex 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 SinglePlex 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. We recommend using our 4BB™ TruePrime® Single Cell WGA v 2.0 technology for amplifying your cells of choice, but the 4BB™ CovCheck™ Genome Coverage WGA QC SinglePlex Kit has a very high probability of working for most 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™ CovCheck™ Genome Coverage WGA QC SinglePlex PCR reaction, so we highly recommend to purify the amplified DNA before running the 4BB™ 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 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 60 µl.

3. Preparation of the PCR master mixes:

3.A. Multichannel pipette protocol:

This protocol has been designed to minimize pipetting events.

Prepare two PCR master mixes per DNA sample adding the components in the order listed in Table 1. The eight total mixes should be prepared in a PCR 8-tube strip or in a column of a 96-well plate to be suitable for an 8-channel pipette, according to the designed shown in Figure 1.

COMPONENT	VOLUME
5x Reaction Buffer	52 µl
Magnesium Chloride solution	20.8 µl
dNTP mix	3.9 µl
Amplified DNA	26 µl
Polymerase	1.3 µl

Table 1. 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 well of the rows selected for each DNA sample, according to the diagram shown in Figure 1. The final reaction volume is 20 µl.

3.B. Single channel pipette protocol:

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

COMPONENT	VOLUME
5x Reaction Buffer	104 µl
Magnesium Chloride solution	41.6 µl
dNTP mix	7.8 µl
Amplified DNA	5.2 µl
Polymerase	2.6 µl

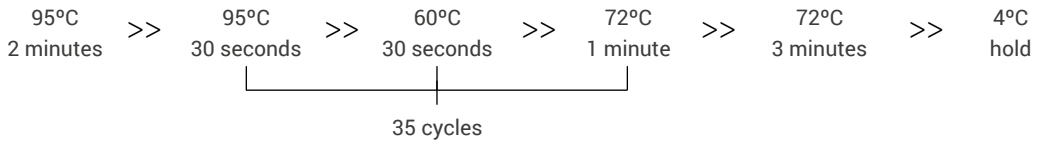
Table 2. Preparation of amplification mix

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

Add 8 µl of each PCR master mix to the corresponding well of the rows selected for each DNA sample, according to the diagram 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. PCR reaction:

   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% agarose gel and load 6 µl of each reaction. Alternatively, analyze the samples on an Agilent Bioanalyzer DNA chip following the manufacturer's recommendations.

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