



Quality control protocol

*For analyzing quality of whole genome
amplified material*

Version 1.0

Kit contents

The reagents in the kit are sufficient for 50 tests.

Table 1. Kit contents

| Component | Content |
|--------------------------|------------|
| VyCAP QC mix (WGA QC-50) | 1 x 100µl |
| Sterile H ₂ O | 1 x 1500µl |

Storage and handling

Store the VyCAP QC at -20°C upon arrival of the kit. All components can be thawed at room temperature, but must be stored on ice after thawing. Vortex briefly before using the components.

Procedure

The VyCAP quality control is a multiplex PCR that allows evaluation of presence and absence of ten key genes across ten different chromosomes of human origin simultaneously. It creates an overview of the quality of the whole genome amplified DNA with 1 nanogram of input material.

The QIAGEN Multiplex PCR PLUS kit mastermix (QIAGEN, 206152) is required to perform VyCAP's quality control and is not included. This mastermix must be ordered separately.

It is recommended to use the Qubit HS dsDNA assay (Invitrogen, Q32854) to determine the concentration of the input material.

Components 10 chromosome QC PCR master mix

Prepare the PCR master mix according to table 2. The total reaction volume for one reaction is 19 µl, multiply the volumes for the total number of reactions (including samples and controls). The amplified DNA does not have to be purified prior to the quality control, but should 1ng. Use sterile H₂O or RT-PCR grade water to dilute sample to a concentration of 1ng/µl if needed.

Table 2. 10 chromosome QC PCR master mix

| Component | Volumes per one reaction (µL) |
|---|-------------------------------|
| Multiplex PCR PLUS kit mastermix (QIAGEN, 206152) | 10 |
| RT-PCR Grade Water (Invitrogen, AM9335) | 7 |
| VyCAP QC mix (WGA QC-50) | 2 |
| Total reaction volume | 19 |

Once the PCR master mix has been prepared, briefly vortex and spin it down in order to collect all the volume at the bottom of the tube.

Quality control protocol

1. Prepare master mix for the quality control as stated in table 2 for the amount of DNA samples to be analyzed.
2. Dilute the DNA sample with sterile H₂O to obtain a concentration of 1ng/μl if needed.
3. In a 0.2ml PCR tube, add 19μl of master mix and 1μl of target DNA at 1ng/μl. Final volume of each reaction is 20μl.
4. Vortex and briefly spin down the samples.
5. Incubate all samples according to the thermal incubation profile of table 3.

Table 3. Thermal incubation profile of the 10 chromosome QC PCR

| Cycli | Temperature (°C) | Time (mm:ss) |
|-------|------------------|--------------|
| 1 | 95 | 05:00 |
| 35 | 95 | 00:30 |
| | 72 | 03:00 |
| 1 | 68 | 10:00 |
| | 4 | ∞ |

6. Analyze products on a 0.8% agarose gel.

Results evaluation

Results are evaluated by loading 5μl of each PCR product on a 0.8% agarose gel. Check the results by comparing the obtained amplicons basepair (bp) length and number of bands with figure 1.

Table 4. Expected amplicon lengths of 10 chromosome QC products, along with represented MseI fragment size obtained from Ampli1 Single Cell WGA kit

| PCR Product Identification | | | |
|----------------------------|------------|----------------------|-------------------------|
| Target | Chromosome | Amplicon length (bp) | MseI fragment size (bp) |
| 1 | 12 | 94 | 172 |
| 2 | 4 | 197 | 300 |
| 3 | 15 | 301 | 500 |
| 4 | 3 | 397 | 706 |
| 5 | 2 | 498 | 934 |
| 6 | 14 | 606 | 1 MseI cut present |
| 7 | 17 | 720 | 2 MseI cuts present |
| 8 | 19 | 800 | 1292 |
| 9 | 11 | 900 | 2050 |
| 10 | 7 | 1009 | 3434 |

Results interpretation

The VyCAP quality control is designed to detect key genes present on ten different chromosomes. Other than being able to detect the presence of these chromosomes, this quality control checks the performance of the Ampli1 WGA reaction and includes two amplicons that contain cutting sites of Ampli1's restriction enzyme, *MseI*. The amplicons that contain the restriction site will not appear on the agarose gel. In addition, Ampli1 cannot amplify large *MseI* fragments (>3kb), which results in dropout of target 10 of this QC. For other WGA kits, such as the QIAGEN REPLI-g WGA kit, maximum amount of 10 band are obtained due to the lack of *MseI*.

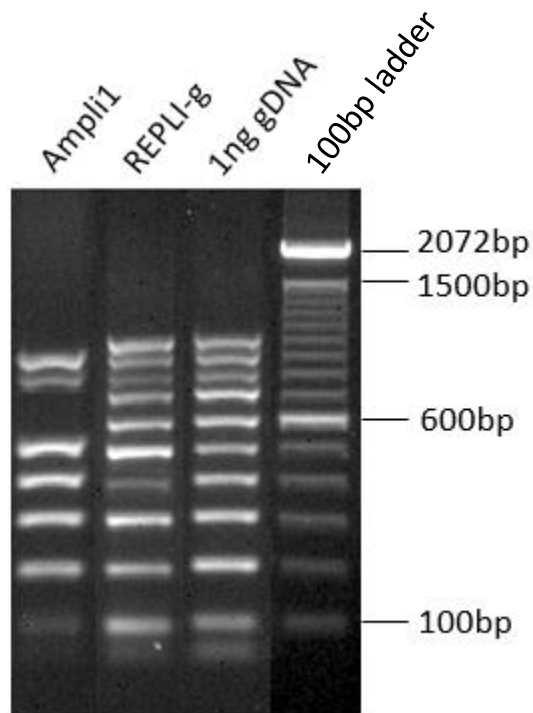


Figure 1. Optimal quality control products of 1ng of a VyCAP punched single cell after WGA using the Ampli1 or REPLI-g WGA kit, along with 1ng of isolated genomic DNA, put on a 0.8% agarose gel for analysis. A 100bp ladder of Invitrogen (15628-019) is added for reference of amplicon size.