BRCA MAQ USER GUIDE

Version 1.0

For Copy Number Analysis Assays

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Multiplicom

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Introduction to Multiplex Amplicon Quantification

Multiplex Amplicon Quantification (MAQ) is a straightforward method for the detection and analysis of copy number variations.

It consists basically of the simultaneous PCR amplification of several fluorescently labeled target and reference sequences. After fragment analysis, comparison of the relative intensities of the target amplicons in the test individual and a control individual result in the copy number of that target amplicon. More details (publications and application notes) about MAQ can be found at: www.agilent.com.

Chapter 1: Before you begin

This section describes the main prerequisites to perform a MAQ analysis, as detailed in section 3 of this user guide. Before starting a MAQ analysis, make sure that all the requirements listed in this section are met. This section is divided into the following:

Section 1.1: Physical installations
Section 1.2: What you should have before starting

1.1. Physical installations

Always take precautions to prevent contamination of DNA samples. To minimize the risk of contamination, make a clear-cut distinction between pre-PCR and post-PCR laboratories AND research workflows:

- Pre-PCR work:
  - Genomic DNA concentration measurements
  - PCR reaction setup
- Post-PCR work:
  - PCR cycling
  - Fragment analysis reaction setup
  - Fragment analysis on (automated) fragment analyzer
- Use physically separated pre-PCR and post-PCR laboratories
- Use distinct sets of pipettes and other consumables for pre-PCR and post-PCR research workflows

1.2. What you should have before starting

1.2.1. DNA samples

The quality and quantity of the DNA samples are critical to the success of this procedure. DNA degradation and PCR inhibitors will be reflected in the quality of the MAQ analysis. Both can be easily assessed using our QC-plex (art. no. QC-0520.100).

The protocol requires a total of 20-50 ng input DNA, meeting the following requirements:

- $OD_{260/280}$ ratio ≥ 1.7
- Concentration ≥ 10 ng/µl in H_2O
- No DNA degradation visible on agarose gel

To prevent degradation of DNA samples, always follow these guidelines:

- Prevent freeze-thaw cycles
- Long-term storage: -20 °C
- Short-term storage: 4°C
- Keep your DNA stocks at a high concentration (> 50 ng/µl) in TE or TE^{-4}
- Make your dilutions for working stocks in H_2O

1.2.2. MAQ kit

Each kit contains enough reagents for the analysis of **50** DNA samples:

Vial name	Storage	Components
2 x PCR mix	-20°C	PCR-buffer, dNTPs, primers
1 x Taq polymerase	-20°C	Taq DNA polymerase

! As the fluorescent labels used are light-sensitive, it is recommended to shield the PCR mix from light by wrapping the tube in aluminium foil.

1.2.3. Materials required but not provided

Materials	Requirements
Reference samples	See 1.2.1 DNA samples. These samples contain a normal copy number of the region under investigation.
Positive control	See 1.2.1 DNA samples. This sample contains a known deviation from the normal copy number of the region under investigation. This sample is not absolutely required.
HiDi™ formamide	ABI cat. no. 4311320
GeneScan®500Liz™ size standard	ABI cat. no. 4322682
GeneScan®500Rox™ size standard	ABI cat. no. 401734

Chapter 2: Procedure

A MAQ analysis comprises 2 steps:

Section 2.1: PCR reaction
Section 2.2: Fragment analysis

> **!** Attention: DNA contamination
>
> To reduce risk of contamination, perform all steps of section 2.1 until 2.1.4 (the PCR cycling) in a pre-PCR laboratory that is physically separated from the post-PCR laboratory and use pipettes that are exclusively assigned to pre-PCR research workflows.

2.1. PCR reaction

PCR setup should be performed on ice to obtain the best results. Start the PCR reaction as soon as possible after addition of the Taq DNA polymerase.

2.1.1. DNA sample dilution

1. Measure DNA concentration if unknown.
2. Dilute the DNA to 10-20 ng/µl, using sterile distilled water.
3. Include a negative control (only H_2O) and if possible a positive control. Always include reference individuals (≥2) with known normal copy number.

2.1.2. Preparation of master reaction mix

1. Remove the PCR mix from the -20°C freezer and allow complete thawing on ice.
2. Vortex thoroughly and centrifuge the vials at 12,000 x g for 10 s before use.
3. On ice, prepare a master reaction mix for the number of samples and controls to be run (minimum 8). In a microcentrifuge tube add, for each reaction, the following reagents in the order indicated:

5	µl	PCR mix
0.075	µl	Taq DNA polymerase
5.075	µl	Final volume per reaction

4. Vortex briefly and centrifuge the vials at 12,000 x g for 10 s.

> **!** Incomplete mixing of the viscous glycerol enzyme containing solution with the PCR mix is a major source of errors.

2.1.3. PCR reaction setup

1. Label 0.2-ml tubes or a 96-well PCR plate.
2. Prepare the PCR mix in the tubes/wells by adding the following reagents, in the order indicated:

2-10	µl	gDNA with a total DNA amount between 20-50 ng
0-8	µl	Sterile distilled water
5	µl	Master reaction mix*
15	µl	Final volume

! * Add the 5 µl master reaction mix to the DNA samples using separate pipette tips and mix by pipetting up and down.

3. Briefly vortex and centrifuge the vials or PCR plate until all liquid is at the bottom.

2.1.4. PCR cycling

1. In a post-PCR room, place all vials or PCR plate firmly in the thermal cycler block and start the PCR cycle program (use a ramp rate below 2.5°C/s).

98°C – 10 min

95°C – 45 s
60°C – 45 s
68°C – 2 min
} x23

72°C – 10 min

2. After completion of the PCR reaction, the MAQ PCR product may be stored at 4°C for up to 5 days before analysis by capillary electrophoresis.

! As the fluorescent labels used are light-sensitive, the PCR products should be stored in a dark box or wrapped in aluminium foil.

2.2. Fragment analysis

2.2.1. Preparation of size standard mix

1. Prepare a size standard mix for the number of samples to be analyzed. In a micro-centrifuge tube, add per sample the following reagents:

10	µl	HiDi-Formamide (not supplied; see section 2.2.3)
0.3	µl	GS500 size standard (not supplied; see section 2.2.3)

2. Briefly vortex and centrifuge the vial until all liquid is at the bottom.

2.2.2. Preparation of sample for fragment analysis

1. For each sample, dispense 10 µl of the size standard mix into a well of a 96-well plate compatible with the fragment analyzer used for MAQ analysis (not supplied).
2. Add 2 µl of the MAQ PCR product to a well containing the size standard mix.
3. Seal the plate with an aluminium sealing foil (not supplied).
4. Denature the samples at 95°C for 3 minutes and put on ice immediately.
5. Centrifuge the plate at 1,000 x g for 10 s to remove any bubbles in the wells and load onto the fragment analyzer

2.2.3. Fragment analysis

Module for the ABI3730XL sequence analyzer:

Run Module Settings

Name	Value	Range
Oven_Temperature	66	18...70 DegC
Buffer_Temperature	35	30...35 DegC
PreRun_Voltage	15.0	0...15 kV
PreRun_Time	180	1...1800 sec
Injection_Voltage	2.0	0...15 kV
Injection_Time	10	1...90 sec
First_ReadOut_Time	200	100...16000 ms
Second_ReadOut_Time	200	100...16000 ms
Run_Voltage	15.0	0...15 kV
Voltage_Number_Of_Steps	10	0...100 Steps
Voltage_Step_Interval	20	0...180 secs
Voltage_Tolerance	0.6	0...6.0 kV
Current_Stability	30.0	0...2000 uA
Ramp_Delay	1	1...1800 sec
Data_Delay	120	1...1800 sec
Run_Time	1200	300...14000 sec

! Please inquire to obtain the fragment analysis settings for other ABI sequencers. Some parameters can change when using other types of fragment analyzer.

2.2.4. Data analysis

For analyzing data, we recommend the MAQ-S software package (downloadable from www.agilent.com). A separate User Guide for MAQ-S is also available.

Preparation of master reaction mix

1. Remove the PCR mix from the -20°C freezer and allow complete thawing on ice.
2. Vortex thoroughly and centrifuge the vials at 12,000 x g for 10 s before use.
3. Mix **5 µl** PCR mix with **0.075 µl** Taq DNA polymerase.
4. Vortex briefly and centrifuge the vials at 12,000 x g for 10 s.

PCR reaction setup

5. For each sample, combine **20-50 ng** genomic DNA with **5 µl** of the master reaction mix.
6. Adjust with distilled water to final volume of **15 µl**.
7. Vortex briefly and centrifuge the vials at 12,000 x g for 10 s.

PCR cycling profile

98°C – 10 min
95°C – 45 s
60°C – 45 s } x23
68°C – 2 min
72°C – 10 min

Remark: Keep the ramp rate of the machine below 2.5°C/s.

Fragment analysis

8. Prepare the size standard mix by combining **10 µl** of HiDi-Formamide (not supplied) with **0.3 µl** GS500-liz size standard (not supplied).
9. For each reaction, dispense **10 µl** of size standard mix per well into a 96-well plate.
10. Add **2 µl** of the MAQ PCR product to a well containing the size standard mix.
11. Denature samples at 95°C for 3 minutes and put on ice immediately.
12. Centrifuge the plate at 1,000 x g for 10 s.
13. Load onto the fragment analyzer.
14. Analyse data with MAQ-S analysis software (free and downloadable from www.agilent.com).

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