Information

Chip Hybridization. For convenience and high signal reliability, hybridization is best performed using a CapitalBio BioMixer™ II Microarray Hybridization Station and HybSet™ Microarray Hybridization Cassette which both help to reduced edge-effects. The enhanced quality of hybridization is attested in recent publications such as Patterson *et al* (2006) *Nature Biotechnol*ogy 24:1140-1150 and Shi *et al* (2006) *Nature Biotechnology*, 24:1151-1161.

Ordering Information

Cat. No.	Product Name	Product Description
300065	CapitalBio Deafness Gene Mutation Detection Array Kit	24 tests

For research use only

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CapitalBio

CapitalBio Deafness Gene Mutation Detection Array Kit

Cat. No. 300065

User Manual

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7.2.9 **Washing**

Open the hybridization cassettes, discard the cover slips and transfer the chips to the Chip Washing Box 1 with **Wash Buffer 1**.

Put the Chip Washing Box into the BioMixer™ Ⅱ and wash at 30 rpm for 2 min.

Transfer chips to Chip Washing Box 2 with **Wash Buffer 2** and wash at 30 rpm for 2 min.

7.2.10 **Drying**

Transfer each of the chips into a CapitalBio Slide Centrifugation Tube. Cap the tubes and centrifuge at 800 rpm for 3-5 min to dry the chips.

7.3 Data Acquisition

After washing and drying, the chips should be stored at room temperature and analyzed as soon as possible to avoid fluorescence quenching. Scan the chip using LuxScan™ 10K-B Microarray Scanner. The results are generated automatically according to the instructions of the "CapitalBio Deafness Gene Mutation Detection Array Test System". If the chip cannot be analyzed immediately, it should be stored in dark and dry conditions, and analyzed within 24 hours.

2. Principle of the Test

CapitalBio Deafness Gene Mutation Detection Array Kit is designed to detect most important mutations about heredity hearing loss. Based on DNA microarray technology, mutations of the four genes (GJB2, GJB3, SLC26A4 and 12S rRNA) can be detected simultaneously. CapitalBio use its proprietary technology, named multiplex Allele-Specific PCR-based Universal Array (ASPUA), to accomplish the detection process. The first step is the multiplex amplification of the allele gene using allele-specific PCR primers. After amplification, the amplified gene is hybridized with specific tag probes immobilized on a chip. Wild type or mutations can be determined according to the hybridization results.

The DNA extracted from human whole blood sample is used as PCR template. Because the primer is tagged with fluorescence molecules, the amplified single chain PCR products obtained are fluorescently tagged. Then the PCR products are used for microarray hybridization in the reaction buffer provided. After washing and drying, the chips are scanned in a microarray scanner for image scanning. The interpretation software of the array kit is then used to analyze the data and to generate the test reports, based on the distribution of positive probe signals on the microarray.

3. Contents of the Kit

3.1 Six chips, packed in a plastic box. Each chip contains 4 identical blocks. Block layout is shown in **Figure 1** and detailed probe information in **Table 2**.
3.2 SmartCover™ cover slips (6 pieces, packed together with the chips in the same plastic box)
3.3 PCR Primer Mixture A1, 1 vial, 260 µl
3.4 PCR Primer Mixture B1, 1 vial, 260 µl
3.5 PCR Reaction Mixture A2, 1 vial, 80 µl
3.6 PCR Reaction Mixture B2, 1 vial, 80 µl
3.7 Wild type genomic DNA control, 1 vial, 20 µl
3.8 Hybridization Buffer,1 vial, 240 µl

7.2.2 **Preparation of PCR reaction solution**

Take an appropriate number of 200 μl microtubes or 8-strip tubes and mark with specimen codes. Take Tube A1, A2, B1, B2 from the kit and mix gently after thawing. Briefly centrifuge to collect contents to bottom.

Mix PCR Primer Mixture and PCR Reaction Buffer respectively at the ratio shown in **Table 3 and 4**. After vortex and short spin, dispense 13 μl aliquots of this mixture into the microtubes or 8-strip tubes.

Transfer the microtubes or 8-strip tubes to PCR template area. Two microlitres of extracted genomic DNA is added to each tube (each sample should be added both to PCR reaction solution A and B respectively). **Positive Control** (wild type genomic DNA template) and **Negative Control** (distilled or deionized water as template) is needed.

Table 3. The components of PCR reaction solution A

Tube code	Component	Volume (μl)
A1	PCR Primer Mixture A1	9.8
A2	PCR Reaction Buffer A2	3.2
---	Genomic DNA (50-100 ng/μl)	2.0
---	Total	15.0

Table 4. The components of PCR reaction solution B

Tube code	Component	Volume (μl)
B1	PCR Primer Mixture B1	9.8
B2	PCR Reaction Buffer B2	3.2
---	Genomic DNA (50-100 ng/μl)	2.0
---	Total	15.0

7.2.3 **PCR reaction**

Place the microtubes or 8-strip tubes into a PCR instrument. The amplification reaction is accomplished according to the thermocycling program shown in **Table 5**.

Table 5. PCR thermocycling program

Temperature (℃)	95	96	94	55	70	60	4
Time (s)	900	60	30	30	45	600	soak
Cycles	1	1	32			1	1
Cautions	Ramp from 94 to 55 ℃ (0.4 ℃/s); then ramp from 55 to 70 ℃(0.2 ℃/s)						

Table 2. Probe list

Category	Probe	Target
Control	QC	Chemical control (fabrication control)
	BC	Blank control (fabrication control)
	PC	External control (hybridization positive control)
	NC	Negative control (hybridization negative control)
Probe	35W	Wild type
	35M	Mutant type (35 del G)
	176W	Wild type
	176M	Mutant type (176 del 16)
	235W	Wild type
	235M	Mutant type (235 del C)
	299W	Wild type
	299M	Mutant type (299 del AT)
	538W	Wild type
	538M	Mutant type (538 C > T)
	547W	Wild type
	547M	Mutant type (547 G > A)
	1494W	Wild type
	1494M	Mutant type (1494 C > T)
	1555W	Wild type
	1555M	Mutant type (1555 A > G)
	2168W	Wild type
	2168M	Mutant type (2168 A > G)
	IVS7-2 W	Wild type
	IVS7-2 M	Mutant type (IVS 7-2 A > G)

1. Introduction

CapitalBio Deafness Gene Mutation Detection Array Kit is designed for rapid, high throughput screening of known hotspot mutations related to hereditary hearing loss. Ten mutations of four genes (GJB2, GJB3, SLC26A4 and 12S rRNA) are evaluated simultaneously.

Hereditary hearing loss may be inherited from one or both parents who may or may not have a loss of hearing themselves. The disease can be inherited in an autosomal dominant, an autosomal recessive, or an X-linked recessive manner, as well as by mitochondrial inheritance. Approximately 0.1% of children are born with profound hearing loss and more than 50% of prelingual deafness is genetic, most often autosomal recessive and nonsyndromic. Many different genes are known to cause hereditary hearing loss and deafness. Knowledge of the mutations can help identify hearing impairment at birth, so that educational programs that stimulate pronunciation can start immediately. Further more, it can also provide caution or help avoid taking certain types of antibiotics, which are known to cause deafness in children carrying certain gene mutations.

Using this kit, wild type and mutations or polymorphisms of the four genes can be detected simultaneously. Human genomic DNA extracted from whole blood samples is needed for test. The whole detection process is less than 5 hours, which is remarkably shorter than other methods. With the combination of special primer design, strictly controlled multiplex PCR procedure, standard array hybridization process and automatic software analysis, the kit offers a high throughput method to help diagnosis. The kit can be used for mutation carrier screen, prenatal diagnosis and neonatal hearing screening. Detection loci information of this kit is described in **Table 1**.

Table 1. Detection loci information of the kit

Gene	Loci	Description	Loci	Description
GJB2	35	35 del G	235	235 del C
	176	176 del 16	299	299 del AT
GJB3	538	538 C > T	547	547 G > A
SLC26A4	2168	2168 A > G	IVS 7-2	IVS 7-2 A > G
12S rRNA	1494	1494 C > T	1555	1555 A > G

8. Troubleshooting

Problem	Likely Cause	Solution
Absent signal on the array, including QC probe	• Insert chip in wrong direction while scanning • Damage to the chip • Scanner or software failure	• Adjust chip insert direction • Scan a control chip that worked well before
Only QC signal is present, but PC signal is absent	• Failed hybridization reaction • Incorrect manipulation in hybridization reaction • Preparation failure of chip wash buffer	• Check back chip code and the experiment record • Check hybridization temperature • Check chip wash buffer
Positive signal present in blank or negative control	• Dust present on the chip surface • Hybridization solution vaporized, generating bright background • Preparation failure of chip wash buffer • Sample prepared are likely contaminated and Reagents and/or pipettes are likely contaminated	• Ensure clean area • Work quickly with Hybridization solution • Check wash buffer • Decontaminate Pre-PCR clean area and PCR staging area including all equipment • Use new reagents and new pipettes
Positive signal present in QC and PC control probe, but all other probes show no signal in both samples and positive control	• PCR failure • Sample DNA contains enzymatic or chemical inhibitors	• Check PCR instrument and program • Check PCR products with Electrophoresis on the gel

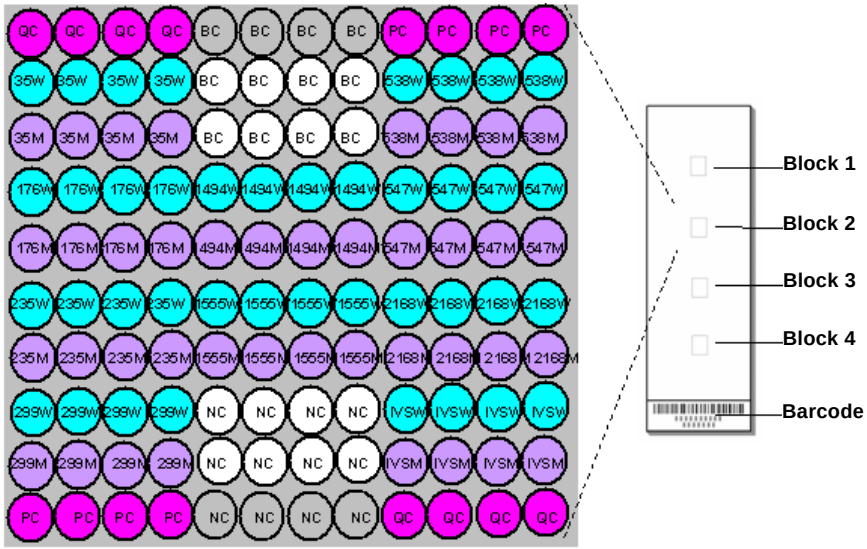

Figure 1. Array Layout. Each block contains 20 probes and 4 controls.

4. Storage and stability

20×SSC, 10% SDS and chips should be stored at room temperature (20-25℃). Other contents should be stored at -20℃.

The kit remains stable for at least 6 months from date of manufacture if handled properly. Do not use the kit after the stated expiry date. If the tubes are opened, the kit should be used within one month. The kit is stable during shipment under recommended shipping temperature conditions.

7.2.4 Turn on the Microarray Hybridization Station and set to 50 °C.

7.2.5 Determine the total number of chips needed and take the appropriate number of chips out of the package.

7.2.6 Fill in the hybridization cassette in the base with 200 µl of distilled water to keep it humid. Place the chip into the cassette with the barcode side facing up and the barcode towards the operator. Then cover the chip with the cover slip.

7.2.7 **Preparation of hybridization reaction**

Prepare the appropriate number of 200 µl microtubes or 8-strip tubes and mark with a specimen code.

Thaw out the **Hybridization Buffer** completely at 50 °C in the Microarray Hybridization Station. Aliquot 10 µl of the **Hybridization Buffer** into the microtubes, or the 8-strip tubes. Add 2.5 µl of PCR products A and B to corresponding tube to make up a final volume of 15 µl of hybridization reaction mixture. The components are shown in **Table 6**.The hybridization reaction mixtures are denatured at 95°C for 5 min (in a PCR instrument or incubator). Then immediately put the tubes onto the ice-water mix for 3 min.

Table 6. Components of hybridization reaction solution

Components	Volume (µl)
Hybridization Buffer	10
PCR products A+B	2.5+2.5
Total	15

7.2.8 **Hybridization reaction**

Pipette hybridization reaction mixes several times until the precipitate disappears. Transfer 13.5 µl of each mix to the chip surface through the sample port of the SmartCover™ cover slip. Close the hybridization cassette. Record the chip code and block location.

Place the closed hybridization cassette into the Microarray Hybridization Station at once and incubate at 50 °C for 60 min (count the time from the placing of the last cassette into the BioMixer™ Ⅱ).

5. Warnings and precautions

- This kit is for *in vitro* use only.
- Wear exposure suit, mask and disposable gloves while handling human whole blood, PCR and hybridization (use only disposable lab materials).
- The reagents should be mixed thoroughly prior to use and repetitive thawing and freezing should be avoided.
- **For all PCR steps**: sample preparation, reaction mix preparations and addition of templates should be performed in separate places to avoid false positive signals resulting from contamination.
- Use filtered pipette tips with this PCR assay kit. This precaution can prevent DNA aerosol contamination.
- The chips, the primer mix and the hybridization buffer are all light sensitive. Do not expose them to strong light sources.
- Cover slips are one-time use only.
- Avoid generating air bubbles when adding the samples to the chip surface.
- Incomplete or inadequate chip washing may cause high background or false positive results.
- Avoid touching or contacting the arrayed area.

6. Materials required but not provided

6.1 Equipments

- CapitalBio LuxScan™ 10K-B Microarray Scanner (Cat. No. 100020)
- CapitalBio BioMixer™ Ⅱ Microarray Hybridization Station (Cat. No. 120030)
- CapitalBio HybSet™ Microarray Hybridization Cassette (Cat. No. 430010)
- CapitalBio Slide Centrifugation Tubes: 6
- PCR instrument (ABI 9700, MJ PTC-200 or PTC225)
- Centrifuge (>1,000 rpm, for 1.5 ml microtubes)
- Centrifuge (for drying washed chips)
- Vortex mixer
- Laboratory timer
- Container for ice and water mix
- Chip Washing Boxes: 2

- Measuring cylinders: 25 ml × 1 and 500 ml × 1
- Reagent bottles: 5000 ml × 2
- Beakers: 1000 ml × 2

6.2 Others

- Micropipettes for 2.5 μl, 10 μl, 200 μl and 1000 μl
- Clean pipette tips for 2.5 μl, 10 μl, 200 μl and 1000 μl micropipettes
- Sterilized 8-strip 200 μl tubes and caps (optional)
- Sterilized 200 μl and 1.5 ml microtubes
- Tube rack for 200 μl microtubes and 1.5 ml microtubes
- Human whole blood genomic DNA extraction kit

6.3 Preparation of reagents

- Autoclaved distilled water

7. Assay procedure

7.1 Pre-test preparation

7.1.1 **Wash Buffer 1:**

Add 75 ml 20×SSC to 4875 ml distilled water into a 5000 ml reagent bottle and mix thoroughly. Then add 50 ml 10% SDS to make up a final volume of 5000 ml. Mix well by stirring or gentle shaking and set to 42°C before use.

7.1.2 **Wash Buffer 2:**

Add 15 ml 20×SSC to 4985 ml distilled water into a 5000 ml reagent bottle and mix thoroughly. Set to 42°C before use.

7.1.3 Use a 50:50 ice and water mix for the hybridization procedure.

7.2 Experimental procedure

7.2.1 Determine the total number of tests and prepare the appropriate volumes of reagents from the package.