



# Agena Bioscience's MassARRAY® System Excels in China Ministry of Health Comparison Study of *KRAS* Somatic Mutation Detection Methods

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## OVERVIEW

Many laboratories in China are running *KRAS* mutation detection assays to guide therapy selection for cancer treatment. To promote clinical *KRAS* mutation detection and improve the quality of laboratory testing, the National Center for Clinical Laboratories (NCCL) of the China Ministry of Health launched a *KRAS* External Quality Assessment project in 2014. In preparation for the project, the NCCL conducted a preliminary National *KRAS* Mutation Detection Inter-Lab Quality Evaluation Survey in 2013.

This preliminary study encompassed over 50 laboratories, including five labs which utilize the MassARRAY® System from Agena Bioscience. All five labs using the MassARRAY System produced highly concordant results, and were awarded a certificate by the Ministry of Health for passing the study criteria.

The MassARRAY System was the only technology in the study that covered all *KRAS* mutations and had all its sites pass the study criteria. In addition, Agena Bioscience's iPLEX® Pro Sample ID Panel was able to demonstrate that the single discrepant MassARRAY result was not a false negative, but in fact due to sample mix-up.

### Somatic Mutation on Agena Bioscience's MassARRAY System

The MassARRAY System uses iPLEX Pro biochemistry and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to detect somatic mutations. DNA extracted from tumor tissue is first amplified using region-specific primers spanning the mutated regions of interest. Next, extension primers which anneal directly upstream of suspected mutation sites, iPLEX Pro Enzyme, and mass-modified terminators are added to the amplified DNA, followed by single base extension. The MassARRAY Analyzer mass spectrometer then detects and quantifies the increased mass of each extension primer resulting from the addition of a wild-type or mutation-determined dideoxynucleotide. The exceptional sensitivity and resolution of the MassARRAY System makes it possible to detect mutation frequencies as low as 5% in the original tumor-derived template DNA.

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## METHODS

The National *KRAS* Mutation Detection Inter-Lab Quality Evaluation Survey encompassed over 50 laboratories, including five labs that utilize the MassARRAY System: Bioyong Technologies, Inc. in Beijing; DNA Lead in Beijing; Kindstar Global Co., Ltd. in Wuhan; Benegene Biotechnology Co., Ltd. in Shanghai; and DAAN Gene, Co., Ltd. in Guangzhou. Ten blinded FFPE samples, eight previously characterized as *KRAS*-positive and two with no *KRAS* mutations, were shipped to each site by the NCCL. DNA was extracted using Qiagen FFPE extraction kits at all sites. Assays by Agena Bioscience's Custom Services Laboratory scientists in Brisbane, Australia, led by Dr. Darryl Irwin, designed a *KRAS* assay panel and validated it internally. The panel was designed to be compatible with iPLEX Pro biochemistry, which detects at a mutation frequency of ~5-10%. The *KRAS* panel, along with Agena

Bioscience's iPLEX Pro Sample ID Panel, was shipped to the five MassARRAY System labs, which independently tested the 10 NCCL blinded study samples. The iPLEX Pro Sample ID Panel was used by each lab to check their sample quality and identity.

### iPLEX Pro Sample ID

The iPLEX Pro Sample ID Panel is designed to screen DNA extracted from FFPE samples, cell lines, and/or tissues in order to identify sample mismatch, sample duplication, and/or sample identification, and to quantify input DNA prior to further genotyping assays. The panel contains 44 SNPs and 3 gender markers for sample identification, and 5 DNA copy number assays for sample quantification.

## RESULTS

All five labs using the MassARRAY System showed highly concordant results (Table 1), except DAAN Gene's *KRAS* 6 sample, which showed no mutation. Using data from the iPLEX Pro Sample ID Panel, it was found that this sample was different from others with the same name. It was deduced that DAAN Gene received a different *KRAS* 6

sample than the other sites; it appears that an alternate wild-type sample was inadvertently shipped by the NCCL. DAAN Gene has appealed this reported false negative result with the NCCL, using Sample ID data as evidence of an incorrect sample being shipped.

**Table 1:** KRAS mutations detected by the MassARRAY System in the 2013 National KRAS Mutation Detection Inter-Lab Quality Evaluation Survey

Sample ID	Nucleotide Change	Protein Change	Cosmic ID	Mutation Frequency Detected				
				Bioyong	DNA Lead	Benegene	Kindstar	DAAN Gene
KRAS 1	c.35G>T	p.G12V	COSM520	93%	89%	98%	100%	83%
KRAS 2	c.34G>T	p.G12C	COSM516	67%	78%	74%	44%	86%
KRAS 3	c.34G>C	p.G12R	COSM518	62%	52%	82%	79%	77%
KRAS 4	no mut			no mut	no mut	no mut	no mut	no mut
KRAS 5	c.38G>A	p.G13D	COSM532	60%	42%	44%	52%	22%
KRAS 6	c.34G>A	p.G12S	COSM517	57%	40%	54%	100%	no mut
KRAS 7	c.35G>C	p.G12A	COSM522	64%	54%	67%	56%	44%
KRAS 8	c.35G>A	p.G12D	COSM521	69%	34%	71%	59%	56%
KRAS 9	c.38G>A	p.G13D	COSM532	75%	56%	67%	67%	63%
KRAS 10	no mut			no mut	no mut	no mut	no mut	no mut
gDNA	no mut			no mut	no mut	no mut	no mut	no mut
H2O	FAIL			FAIL	FAIL	FAIL	FAIL	FAIL

A number of somatic mutation detection technologies were used by the study participants in addition to MassARRAY MALDI-TOF, including real-time (ARMS), DNA sequencing, and Pyrosequencing (Qiagen) (Table 2).

Agena Bioscience's MassARRAY System was the only technology that covered all KRAS mutations and had all its sites pass the proficiency panel (detection accuracy of 80% or more).

**Table 2:** KRAS mutation detection results in the 2013 National KRAS Mutation Detection Inter-Lab Quality Evaluation Survey, by technology used (technologies with 3 or more sites)

Company Technology	KRAS Mutations able to be detected	Number of sites	Number of sites (% accurate samples)			
			100%	80-99%	60-79%	<60%
Agena Bioscience MassARRAY® System	G12D, G12A, G12V, G12S, G12R, G12C, G13D, G13C	5	4	1 (later determined to be a sample mix-up)	0	0
AmoyDx ARMS	G12D, G12A, G12V, G12S, G12R, G12C, G13D	14	0	8	6	0
Beijing ACCB Biotech Ltd. ARMS	G12D, G12A, G12V, G12S, G12R, G12C, G13D	5	4	1	0	0
Shanghai Yuanqi Bio-Pharmaceutical Company Ltd. ARMS	G12D, G12A, G12V, G12S, G12R, G12C, G13D, G13C	4	4	0	0	0
DNA Sequencing (companies not distinguished)	All mutations	18	13	4	0	1
Qiagen Pyrosequencing	All mutations	5	4	0	0	1

## RESULTS

Agena Bioscience's MassARRAY System performed exceptionally well in this independent China Ministry of Health KRAS somatic mutation study:

- All five MassARRAY System labs produced highly concordant results, and were able to detect all mutations in the samples, outperforming the other assays in the performance panel.
- The iPLEX Pro Sample ID Panel was able to detect and identify a sample mix up, explaining the only discordant result at a lab using the MassARRAY System.
- The MassARRAY System was the only technology in the study that covered all specified KRAS mutations and also had all sites using it pass the proficiency panel.

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