



# A comparison of MASS-PCR and ARMS-PCR for the detection of lung cancer gene mutation

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**Background:** Targeted therapy has been proven to be effective in lung cancer patients with specific driver gene mutations. At present, Sanger sequencing is still the gold standard in clinical practice to detect mutation, and amplification refractory mutation system PCR (ARMS-PCR) has become widely used due to its higher sensitivity and less limitation compared with Sanger sequencing. Mutation-selected amplification specific system PCR (MASS-PCR) is a novel gene detection technique with high specificity and sensitivity. This study aimed to compare the accuracy and sensitivity of ARMS-PCR and MASS-PCR and purposed to make an alternative choice in gene mutation detection in lung cancer.

**Method:** A total of 293 formalin-fixed paraffin-embedded (FFPE) tissues were collected from 293 patients with lung cancer from 2017 to 2018. The sample mutation statuses were evaluated by ARMS-PCR and MASS-PCR. Sanger sequencing was also conducted to confirm the results further. The consistency of ARMS-PCR and MASS-PCR were analyzed, and receiver operating characteristic (ROC) curve was drawn to assess the sensitivity and specificity of MASS-PCR.

**Results:** The consistency rate between the MASS-PCR and Sanger sequencing (kappa value =0.929) was higher than that between the MASS-PCR and ARMS-PCR (kappa value =0.821). There were 20 samples had inconsistent results among the three assays. For these samples, 11 positive samples were verified by the MASS-PCR and Sanger sequencing. Besides, 3 negative samples in Sanger sequencing were detected to be positive in MASS-PCR and ARMS-PCR. The ROC area under the curve (AUC) of assay panels was 0.930 referring to ARMS-PCR, and 0.967 as Sanger sequencing was referred to.

**Conclusions:** Our study demonstrated a higher accuracy and sensitivity of MASS-PCR than ARMS-PCR. Therefore, MASS-PCR could be used in clinical practice to detect gene mutations in lung cancer patients.

**Keywords:** Mutation-selected amplification specific system PCR (MASS-PCR); amplification refractory mutation system PCR (ARMS-PCR); lung cancer; mutation detection

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## Introduction

Lung cancer is the most common cause of cancer-related death around the world. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer patients (1). The prognosis of lung cancer is poor, with a 5-year survival rate of 4–17% depending on stage and regional differences (2). In recent decades, much progress has been made in comprehensive genomic profiling of lung cancer and divide the lung cancer into several molecular subtypes according to the oncogenic driver mutations identified in patients, thus contributing to the personalized therapy, especially targeted therapy. At present, the targeted therapies mainly targeted at the common mutations in lung cancer, including *EGFR*, *BRAF*, *HER2*, *KRAS*, *MET*, *ALK*, *ROS1*, *RET*, *NTRK* (3,4). Clinical practices have confirmed the success of targeted therapies in several molecular defined lung cancer subtypes (5). Whereas targeted therapy needs exact mutation detection.

In the past decades, Sanger sequencing was the most commonly used assay for mutation detection. At present, Sanger sequencing remains the gold standard in clinical practice and may detect unknown mutations (6,7). However, it is time-consuming and may delay the clinical-decision making (8). Besides, it has low sensitivity because the mutant tumor cells are required to more than 20% of all detected tumor cells, possibly leading to the false-negative result (9). Currently, many detection assays with higher sensitivity have been developed for mutation detection. Amplification refractory mutation system PCR (ARMS-PCR) has been approved by the China Food and Drug Administration (CFDA) and become a widely used method in clinic practice. ARMS-PCR is more sensitive than Sanger sequencing with its detection limit of about 1% (8). However, because the fluorescent signals are determined through calculating the value of  $\Delta C_t$ , sometimes it is highly subjective and difficult to make the determination of the conclusive results. Mutation-selected amplification specific system PCR (MASS-PCR) is a novel quantitative PCR (qPCR)-based assay to detect mutations. As only mutant gene emits a fluorescence peak in the cyclic amplification, it solved the defect of ARMS-PCR (10). In this study, we compared the accuracy and sensitivity of ARMS-PCR and MASS-PCR and evaluated the clinical feasibility of MASS-PCR, purposed to supply an alternative possibility to be more effective and exact in mutation detection.

## Methods

### *Samples collection and processing*

A total of 293 formalin-fixed paraffin-embedded (FFPE) tissues were collected from 293 lung cancer patients in Sun Yat-sen University Cancer Center and Foshan First People's Hospital from 2017 to 2018. The study was approved by the Institutional Review Board (IRB), Sun Yat-sen University Cancer Center and Foshan First People's Hospital. Written informed consent was obtained from all participants. Genomic DNA was extracted from FFPE tissue according to the manufacturer's instruction, and then, the genomic DNA was used to determine the genotype in the following methods.

### *MASS-PCR assay*

MASS-PCR is a qPCR-based assay with specific primers and probes that target at the common mutations of *EGFR*, *KRAS*, *BRAF*, *HER2*, *MET* exon 14 skipping mutation, *ALK* fusion, and *ROS1* fusion genes. MASS-PCR was performed in a StepOne™ PCR System (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. The one-step method was applied in all gene mutations detection, including *ALK* fusion and *ROS1* fusion genes. The kit was bought from the Shanghai Tongshu Biotechnology Co., Ltd.

### *ARMS-PCR assay*

The isolated DNA samples were re-tested with ARMS-PCR. ARMS-PCR was used to detect the same mutation as MASS-PCR assay. For the detection of *ALK* fusion and *ROS1* fusion genes, the two-step method was needed. Briefly, RNA was extracted from FFPE tissue and produced into complementary DNA (cDNA) according to the manufacturer's instruction. cDNA was then used to determine the genotype. The kit was brought from the Beijing ACCB Biotechnology Co., Ltd.

### *Sanger sequencing*

Sanger sequencing was performed to verify the genotype. DNA was used as a template to amplify the involved genes. PCR products were then sequenced using Sanger method.

**Table 1** Clinical information of patients in this study

Variables	Number (n=293)	Proportion, %
Gender		
Male	226	77.1
Female	67	22.9
Age (range, years)	29–86	
Histological types		
NSCLC	279	95.2
Squamous cell cancer	86	29.4
Adenocarcinoma	177	60.4
Large cell carcinoma	12	4.1
Adenosquamous carcinoma	1	0.3
SCLC	6	2.0
Undefined	8	2.7

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

### Statistical analysis

The statistical analysis and graphic generation were performed in SPSS 19.0 and MedCalc 19.0. The concordance rate of MASS-PCR, ARMS-PCR, and Sanger sequencing in identifying mutation status was analyzed by kappa test.  $P < 0.05$  was considered to indicate statistical significance.

## Results

### Clinical information on lung cancer patients

This study recruited 293 patients with lung cancer, in which there were 226 males (77.1%) and 67 females (22.9%) (Table 1). The age of patients ranged from 29 to 86 years old with the median age of 64-year-old. All the detected samples were originated from FFPE tissues. Most patients in our study were diagnosed as NSCLC accounting for 95.2%, in which, squamous cell cancer accounted for 29.4%, and adenocarcinoma accounted for 60.4%. Small cell lung cancer (SCLC) only accounted for 2.0%.

### Comparison between MASS-PCR and ARMS-PCR

Here, we conducted the gene mutation detection of all samples by the MASS-PCR and ARMS-PCR methods, and

**Table 2** Comparison of mutation detection results in a comparison between MASS-PCR and ARMS-PCR

Methods		MASS-PCR		Kappa value	P value
		Positive	Negative		
ARMS-PCR	Positive	51	5	0.821	<0.001
	Negative	12	225		
Sanger sequencing	Positive	59	3	0.929	<0.001
	Negative	4	227		

MASS-PCR, mutation-selected amplification specific system PCR; ARMS-PCR, amplification refractory mutation system PCR.

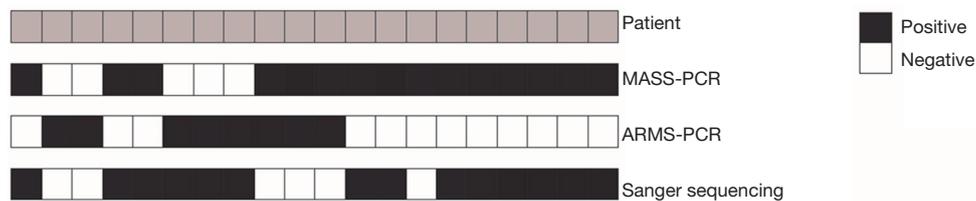
**Table 3** Mutation detection results between ARMS-PCR and Sanger sequencing

Methods		ARMS-PCR		Kappa value	P value
		Positive	Negative		
Sanger sequencing	Positive	51	11	0.830	<0.001
	Negative	5	226		

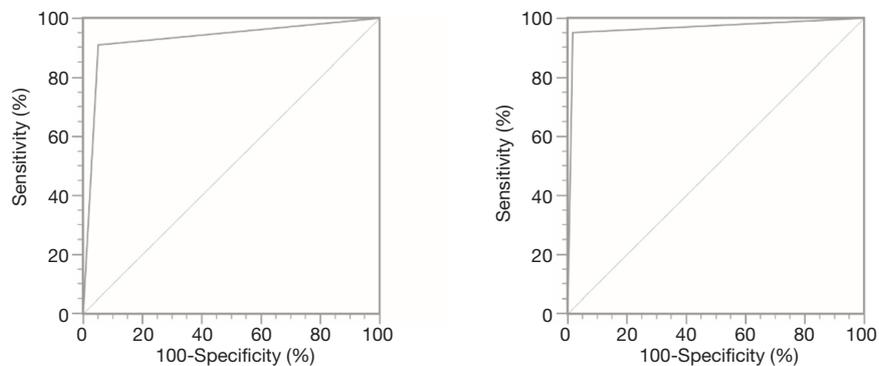
ARMS-PCR, amplification refractory mutation system PCR.

the results were summarized in Table 2. Results showed that there were 63 and 56 positive samples detected by MASS-PCR and ARMS-PCR, respectively. Besides, the kappa value was 0.821, with a significant difference ( $P < 0.001$ ). Although MASS-PCR showed high consistency with ARMS-PCR and Sanger sequencing, 17 samples showed different results, accounting for 5.8% of the total samples. Therefore, the mutation statuses of all samples were further confirmed by Sanger sequencing. Sanger sequencing detected a total of 62 samples that carried the common mutations. The MASS-PCR and Sanger sequencing showed a greater consistency rate (kappa value = 0.929,  $P < 0.001$ ), confirming the accuracy of MASS-PCR. Also, we also analyzed the consistency rate between ARMS-PCR and Sanger sequencing. Their consistency rate was 0.830 (Table 3), which was similar to that between MASS-PCR and ARMS-PCR.

By comparing the mutation statuses among the three detection methods, a total of 20 samples were revealed to be inconsistent. We summarized them in Figure 1. Among these samples, 11 samples were positive by the MASS-PCR and Sanger sequencing while negative by ARMS-PCR. Besides, 3 samples were detected to be positive in MASS-PCR and ARMS-PCR while negative in Sanger sequencing.



**Figure 1** Inconsistent detection results in patients. Patients detected to carry common mutations are represented with black blocks. Otherwise, they are represented by white blocks. MASS-PCR, mutation-selected amplification specific system PCR; ARMS-PCR, amplification refractory mutation system PCR.



**Figure 2** ROC curves of MASS-PCR referring to ARMS-PCR and Sanger sequencing. (A) ROC curve of MASS-PCR referring to ARMS-PCR, and the AUC was 0.930 (95% CI: 0.895–0.956); (B) ROC curve of MASS-PCR referring to Sanger sequencing, and the AUC was 0.967 (95% CI: 0.940–0.984). ROC, receiver operating characteristic; MASS-PCR, mutation-selected amplification specific system PCR; ARMS-PCR, amplification refractory mutation system PCR; AUC, area under the curve; CI, confidence interval.

### Receiver operating characteristic curve (ROC curve) of MASS-PCR

To further assessed the sensitivity and specificity of MASS-PCR, we drew the ROC curve with the reference of ARMS-PCR and Sanger sequencing, respectively. As shown in *Figure 2*, the areas under curve (AUC) were both larger than 0.9, irrespectively the reference, indicating the high sensitivity and specificity of MASS-PCR.

### Discussion

According to histologic features, lung cancer is mainly divided into NSCLC and SCLC, and NSCLC accounts for more than 80% of all lung cancer patients. Recently, with the fast development of genomic medicine, many oncogenic driver mutations have been identified. The common mutations are mostly found in NSCLC while infrequent in SCLC. Among these mutations, *EGFR* mutation has the highest occurrence rate with 10–15% frequency in

NSCLC (2,11), and the frequency was much higher in East Asian patients. In our study, we recruited 293 lung cancer cases with only 6 SCLC cases, and only 1 SCLC case was detected to harbor mutation. Based on the identified oncogenic driver mutations, lung cancer patients would be recommended to receive targeted therapy.

Clinically, before the targeted therapy, the patients need to detect if they harbor the gene mutations that had corresponding targeted drugs. Currently, Sanger sequencing and ARMS-PCR are the main technologies to determine the genotype in the clinic (12). Sanger sequencing (also named as direct sequencing), uses the target DNA single-strand as template to synthesize a new strand upon the catalysis of DNA polymerase. When the fluorescently labeled dideoxynucleotide is selectively synthesized into a new strand, the new strand synthesis is terminated, thus obtaining a series of DNA fragments with varying lengths. Subsequently, electrophoresis was performed to purify the PCR products, then conducting DNA sequencing (13). ARMS-PCR based on the principle that the 3'-terminal

nucleotides of the PCR primer must be complementary to its target sequence for efficient amplification. In the amplification system, allele-specific PCR primers and probes labeled with fluorescence are present, and specific fluorescent signals would be generated to indicate the mutant- or wild-type genes (10,14). Compared with Sanger sequencing, ARMS-PCR has a high sensitivity, high specificity and time-saving methods to detect mutations. Although the detection limit of ARMS-PCR is 1%, much lower than that of Sanger sequencing, Sanger sequencing is recognized as the gold standard in the mutation detection, because the positive result detected by Sanger sequencing is a real positive statue. Therefore, Sanger sequencing was used to verify the mutation results in our study. The main limitation of ARMS-PCR is that the determination of mutation status could be subjective when the peak shapes produced by mutant- and wild-type genes are different (15). To solve this defect, MASS-PCR appeared. MASS-PCR is developed based on ARMS-PCR. Only the mutant gene emits a fluorescence peak in the cyclic amplification, and the amplification of wild-type templates is suppressed. Thus, it is considered a positive result as long as the fluorescence peak is generated, reducing the subjectivity on the final determination of detection results (10).

In our study, we compared the performance of MASS-PCR and ARMS-PCR in 293 lung cancer cases. Results revealed a high consistency rate between the two methods. MASS-PCR had a higher consistency rate with Sanger sequencing than with ARMS-PCR. Further analyzing the samples with inconsistent results, we found that 11 of 20 samples were verified to harbor mutations by Sanger sequencing. These samples also detected to be positive by MASS-PCR but not ARMS-PCR. For the rest 9 samples that were detected to be negative by Sanger sequencing, 3 samples were showed positive results in both MASS-PCR and ARMS-PCR. The negative results revealed by Sanger sequencing in these 3 samples might be caused by the false negative. These results demonstrated the higher accuracy and sensitivity of MASS-PCR. The ROC curves also revealed the high sensitivity and specificity of MASS-PCR. Our findings were in line with the work of Zhu *et al.* (10).

Although we aimed to compare the accuracy and sensitivity of ARMS-PCR and MASS-PCR in both NSCLC and SCLC, only 6 patients were SCLC in our study. Furthermore, the common mutations usually occurred in NSCLC rather than in SCLC. Among the 6 SCLC patients, only 1 SCLC patient was detected to harbor mutation by Sanger sequencing. In the mutation detection

of this sample, MASS-PCR assay revealed the same positive result as Sanger sequencing, while, ARMS-PCR assay showed a negative result. This result suggested the higher accuracy and sensitivity of MASS-PCR than ARMS-PCR in the mutation detection of SCLC patients. However, the sample size of SCLC patients was too small, and more SCLC samples must verify our findings further.

In conclusion, we proved that MASS-PCR assay is reliable in the detection of gene mutations with high accuracy, sensitivity, and specificity. Therefore, MASS-PCR could be used in clinical practice to detect gene mutations in lung cancer patients.

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### Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.10.37>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This retrospective study was approved by the board-certified Research Ethics Committee of Sun Yat-sen University Cancer Center (No. B2017-50) and the Institute Research Medical Ethics Committee of Sun Yat-sen University Cancer Center (No. B2018-011 and GZR2019-302). Written informed consent was obtained from all participants.

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