

# C02 Novel Universal Primers to Identify the Expression of MAGE A1-A10 in the Core Biopsy of Lung Cancer

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## Novel Universal Primers to Identify the Expression of MAGE A1-A10 in the Core Biopsy of Lung Cancer

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### Abstract

**Background:** Recent studies have reported that melanoma antigen (MAGE) gene is expressed in a variety of cancers and testicular tissues. The expression of MAGE-A genes could be used for biomarkers with high tumor specificity; however, there is still a lack of data on most solid tumors. The objective of this study was to construct novel universal primers for detecting the mRNA of MAGE A1-10 genes in lung cancer patients.

**Method:** We conducted this cross-sectional study in 2017 at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. The specimens were a testicular tissue and 15 core biopsies of lung cancer tissues. We designed the universal primers to bind the mRNA of MAGE A1, A2, A2B, A3, A4, A5, A6, A8, A9, A9B, and A10 regions; the assay was performed by nested PCR and continued by direct sequencing.

**Results:** Using the universal primer MAGE A1-10, the PCR was able to detect the MAGE A mRNA of 10 subtypes of MAGE A from testicular and lung cancer tissues. The sequences analysis of individual MAGE A1-10 showed the same homology with MAGE A from GenBank data. Among the 15 lung cancer patients, 13/15 (86.67%) tested positive for GAPDH; subsequently, they were considered for MAGE-A gene detection; while, those testing negative for GAPDH were excluded. The PCR results showed that 12/13 (92.31%) had positive MAGE A1-10 tests and 3/13 (23.08%) tested positive for MAGE A1-6.

**Conclusion:** This finding showed that the novel universal primers could be applied as a new tool for detecting MAGE A1-10 expression in cancer cells.

**Keywords:** MAGE A1-A.10, Testicular tissue, Universal primer, Core biopsy, Lung cancer

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## Introduction

Melanoma antigen (MAGE) gene is well-known as a cancer testis antigen expressed in various tumors. Based on the tissue-restricted expression and gene structure, the MAGE gene is classified into two different types: the first type comprises MAGE A, MAGE B, and MAGE C, restricted to male germ-line tissues and located in clusters on the X chromosomes Xq28, Xp21, and Xp26-27, respectively.<sup>1</sup> The second type is MAGE D, expressed in body tissues and located in Xp11. MAGE D is expressed in normal tissues; whereas, MAGE A, B, C are expressed in tumor cells.<sup>2</sup>

Type I MAGE, including MAGE A, is the first known silent gene in all normal adult tissues except for the testis.<sup>3,4</sup> There are 12 subtypes of MAGE A: MAGE A1, A2, A3, A4, A5, A6, A7 (pseudo gene), A8, A9, A10, A11, and A12. The expression of each MAGE-A subtype, A1-A12, was positive in oral squamous cell carcinoma,<sup>5</sup> gastric cancer,<sup>6</sup> neuroblastoma tumors,<sup>7</sup> hepatocellular carcinoma,<sup>8</sup> benign and malignant neoplasms of the salivary glands,<sup>9</sup> epithelial ovarian cancer (EOC),<sup>10</sup> squamous cell vulvar neoplasms,<sup>11</sup> papillary thyroid carcinoma,<sup>12</sup> head and neck cancer tissues, breast cancer tissues, and lung cancer tissues.<sup>13</sup> MAGE A1 and A3 were expressed at primary non-small-cell lung cancer (NSCLC).<sup>14</sup> Several MAGE subgroup genes such as MAGE A, B, and D were primarily discovered to show differential expression in NSCLC.<sup>15</sup>

Subtypes MAGE-A1 to A12 had high expressions in some malignancies.<sup>8,11,16</sup> The patients with MAGE-A gene expression had a poorer prognosis compared to those with no MAGE-A expression.<sup>5,6,10,17-20</sup> This indicates that identifying individual subtypes of MAGE-A can increase the value of diagnosis and prognosis for cancer patients.

MAGE A1-6 expression in cancer tissues could be detected by reverse transcription polymerase chain reaction (RT PCR) and nested PCR.<sup>13,21</sup> In addition to MAGE A1-6, subtypes MAGE A8, A9, A10 are expressed in cancer tissues.<sup>15,22</sup>

Therefore, we decided to develop a novel universal primer to identify MAGE-A mRNA subtypes A1-10. This study designed the novel universal primer of MAGE A1-10 that could simultaneously bind to the mRNA of MAGE A1, A2, A2B, A3, A4, A5, A6, A8, A9, A9B, and A10. The PCR results showed that the universal primer for MAGE A1-10 for nested PCR could be used to identify MAGE A1-10 gene in the core biopsy samples of lung cancer tissues.

## Materials and Methods

### Specimens

This cross-sectional study made use of 15 specimens from the core biopsy of lung tissues taken from patients with lung cancer diagnosis at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia in 2017. As for the positive control, we utilized a testicular tissue derived from a patient, who received orchidectomy therapy. The Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia approved the study, (number 497/Panke.KKE/VII/2017); all subjects signed the informed consent form.

### Designing the primer

We obtained the MAGE-A gene sequences from GenBank data (National Centre for Biotechnology Information). In this study, we designed the novel universal primer MAGE A1-10 based on the mRNA sequences of the target gene through selecting the region between exons 1, 2, and 3 (Figure 1). The promoters and first exons of the MAGE-A gene (MAGE A1 to A12) show considerable variability<sup>23</sup> and the high homology domains were exons 2 and 3. Exon 3 was the coding region with similar sequences among all MAGE A gene families A. Accordingly, we designed the forward primer to bind to exon 2 joining with exon 3; the reverse primer was further designed to attach to exon 3.

Nested PCR identified the expression of all MAGE A1-10 subtypes using MF10/MR10 primers for the first round and MF10/MR12 primers for the second round (Table 1). The primer

MF10, as the outer forward primer, would bind to cDNA MAGE A1-10 at the exon 2 and exon 3. For the second round, we used MF10 and MR12 as inner primers to verify that the PCR product would only amplify the cDNA of MAGE A1-10. To compare this method with other studies, we identified MAGE A1-6 expression by using their primers:<sup>13</sup> MMRP1/MMRP1 primer for the first round and MMRP3/MMRP4 primer for the second round. We detected individual MAGE-A genes (from MAGE A1 until A10, separately) by use of MMRP3 as forward primer and M1, M2, M3, M4, M5, and M6 as reverse primers with product 377 bp for MAGE A1, 523 bp for MAGE A2, 569 bp for MAGE A3, 580 bp for MAGE A4, 478 bp for MAGE A5, and 628 bp for MAGE A6.<sup>13</sup> We identified MAGE A8, A9, and A10 subtypes using MF10 as forward and M8, M9, and M10 as reverse primers. The GAPDH PCR was performed using GAPDH-F and GAPDH-R (Table 1).

#### RNA extraction and reverse transcription-PCR

Using RNAeasy Plus Mini Kit (Qiagen, Germany), we extracted RNA from testicular and lung tissues based on core biopsy. We performed the procedures following the manufacturer's instruction protocols. Total RNA was stored at -20°C until further use. We conducted Reverse Transcription PCR (RT PCR) using ReverTraAce® qPCR RT Master mix with gDNA remover

(Toyobo, Japan).

For RNA denaturation, 25 µl of RNA template was kept in ice at 65°C for 5 minutes. 12 µl 4× DN master mix (with genomic DNA remover) and 3 µl random primer were added and then incubate 37°C for 5 minutes. It was directed for genomic DNA removal step. Finally, 10 µl 5×RT master mix II was added for cDNA synthesis. Total volume was 50 µl. The mix reaction was incubated at 37°C for 15 minutes and 50°C for 5 minutes. The reaction stopped at 98°C for 5 minutes. We stored cDNA at 4°C or -20°C until further use.

In this research, we performed PCR by using GoTaq(R) Green Master Mix (Promega, USA). In the first round, we carried out PCR in total volume 20 µl which content was 10 µl Gotaq green master mix, 1 µl primer forward, 1 µl primer reverse, 5 µl nuclease free water, and 3 µl cDNA template. Primer concentration was 10 pmoles/µl. PCR conditions was pre-denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds. The final extension was at 72°C for 7 minutes, with a subsequent visualization on 2% gel electrophoresis. In the second round, we used PCR, 3 µl PCR product from the first round, as template. Except for the primers and templates, the reactions and conditions of the second round PCR were the same as the first one.



Figure 1. This figure shows the position of MF10/MR10 and MF10/MR12 primers based on the sequences of MAGE A1-10 gene.

**Table 1.** The primers used for the identification of the MAGE A1-10 mRNA

Gene Target	Sequence of Primer (5'-->3')	F/R	Amplicon Length (bp)
GAPDH	GAPDH-F = TCG GAG TCA ACG GAT TTG GTC GTA	F	320
	GAPDH-R = CAA ATG AGC CCC AGC CTT CTC CA	R	
MAGE A1-10 (outer)	MF10 = GAA GAY CTG CCW GTG GGT C	F	823-919
	MR10 = CTC CAG GTA STT YTC CTG CAC	R	
MAGE A1-10 (inner)	MF10 = GAA GAY CTG CCW GTG GGT C	F	461-557
	MR12 = CCA GYA TTT CTG CCT TTG TGA	R	
MAGE A8	M8 = CAC TTT CTC ATC AAG TGC TTC CC	R	419
MAGE A9	M9 = CCT TCA ATT TCA GTG CTT CTT GG	R	407
MAGE A10	M10 = TGG GTA AAG ACT CAC TGT CTG G	R	464

MAGE = Melanoma antigen, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase, F= Forward, R = Reverse, bp = Base pair

We also performed PCR for housekeeping gene GAPDH for all samples. The master mix reaction and conditions of PCR is the same with PCR for MAGE A1-10 identification.

**Sequences analysis**

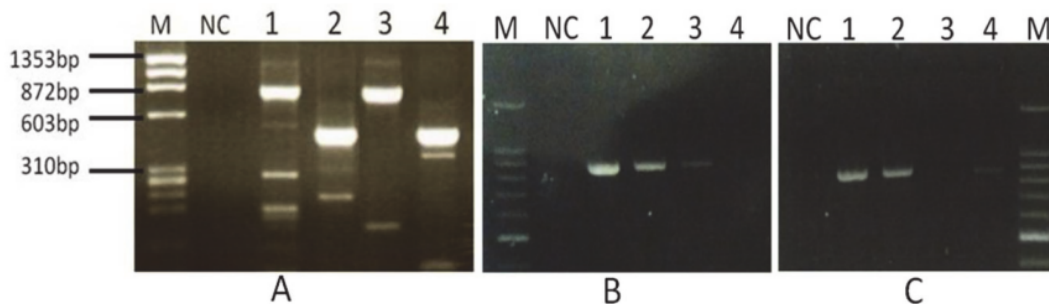
To verify the PCR results of MAGE A1-10 and MAGE A1-6, we separately performed PCR for each subtype of MAGE A1-10. We continued through direct sequencing to confirm the positive results of PCR using the Genetic Analyzer (Applied Biosystems, USA). The sequences were compared with those of GenBank data.

**Results**

*Analysis of the MAGE A1-10 primers*

The analysis indicated several results: PCR using the MF10/MR10 primers for MAGE A1-10 showed 823-919 base pair (bp), MF10/MR12 primers showed 461-557 bp, MMRP1/MMRP1 primers for MAGE A1-6 showed 852 bp, and MMRP3/MMRP4 primers showed 469-490 bp (Figure 2A). Figures 2B and 2C show the dilution test of the PCR using the MF10/MR10 and MMRP1/MMRP2 primers. The total measured RNA concentration from testicular tissue was 133,4 ng/μl. We used the total RNA in this concentration for dilution test with a ratio of 1:1, 1:10, 1:100, and 1:1000.

Figure 3 shows the results of PCR using the



**Figure 2.** The PCR product of MAGE A1-10 and MAGE A1-6 from testicular tissue specimen. Marker (M), Negative control (NC). The PCR product of MAGE A1-10 in first round PCR was ± 823 bp (lane 1, 2A) and second round PCR was ± 462 bp (lane 2, 2A), the PCR product of MAGE A1-6 in first round PCR was ± 852 bp (lane 3, 2A) and second round was ± 469 bp (lane 4, 2A). The dilution test for detecting MAGE A1-10 (2B) compared with MAGE A1-6 (2C), dilution 1:1 (lane 1, 2B, 2C), 1:10 (lane 2, 2B, 2C), 1:100 (lane 3, 2B, 2C) and 1:1000 (lane 4, 2B, 2C).

MAGE = Melanoma antigen, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

**Table 2.** Analysis of MAGE A1-10 subtypes with the sequences from GenBank

MAGE A Subtypes	Homolog with accession number
MAGE A1	NM_004988.4
MAGE A2	NM_001282501.1, NM_001282502.1, NM_001282504.1, NM_001282505.1, NM_005361.3, NM_175742.2, NM_175743.2, XM_011531159.1
MAGE A2B	NM_001321400.1, NM_001321401.1, NM_001321402.1, NM_001321403.1, NM_001321404.1, NM_153488.4, XM_006724814.2, XM_011531142.1, XM_011531141.1, XM_005277838.3, XM_005277839.2, XM_005277836.1, XM_011531143.1
MAGE A3	XM_005274676.2, XM_006724818.2, XM_011531160.1, XM_011531161.1
MAGE A4	NM_001011548.1, NM_001011549.1, NM_001011550.1, NM_002362.4, XM_005274677.2, XM_005274679.2, XM_005274678.3
MAGE A5	NM_021049.4
MAGE A6	NM_005363.3, NM_175868.2, XM_011531162.1
MAGE A8	NM_001166400.1, NM_001166401.1, NM_005364.4
MAGE A9	NM_005365.4, XM_005262335.2, XM_005262334.2
MAGE A9B	NM_001080790.1, XM_005278193.2, XM_005278192.2
MAGE A10	NM_001011543.2, NM_001251828.1, NM_021048.4

MAGE = Melanoma antigen

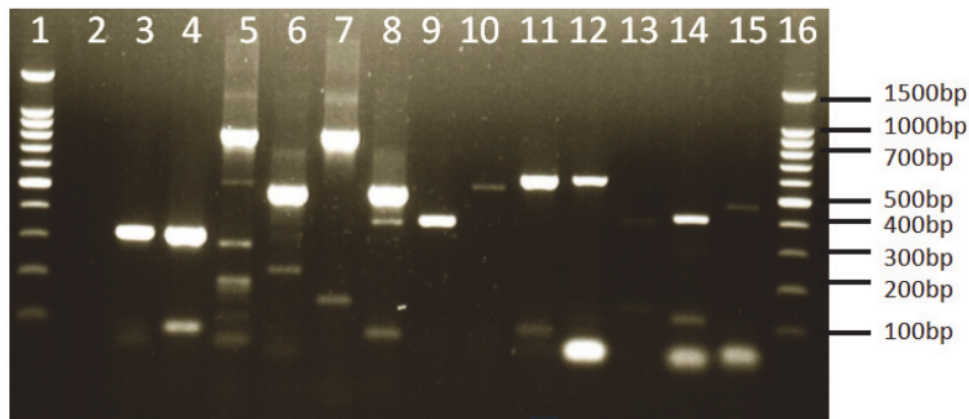
primer of each MAGE A1-10 subtype to identify the expression of MAGE A1, A2, A3, A4, A5, A8, A9, and A10 from testicular tissue specimens. The PCR products of each MAGE A1-10 subtype were used for direct sequencing.

The homology analysis showed that all MAGE-A genes from the lung cancer samples had the same homology as the MAGE-A from GenBank data with the accession number mentioned in

table 2. Figure 4 shows the result of homology analysis of MAGE-A3 gene, one of the MAGE-A subtypes.

#### Identifying MAGE A1-10 from the core biopsy of lung cancer tissues

The 15 specimens from the core biopsy of lung cancer tissues were as small as a string with the length of approximately half a centimeter.



**Figure 3.** The PCR product from testicular tissue specimen. Marker (lane 1), Negative control (lane 2), GAPDH (+) 320 bp (lane 3), MAGE A1-10 first Round 823 bp (-) (lane 4), MAGE A1-10 second round 461 bp (+) (lane 5), MAGE A1-6 first round 852 bp (+) (lane 6), MAGE A1-6 second round 469 bp (+) (lane 7), MAGE A1 377 bp (+) (lane 8), MAGE A2 523 bp (+) (lane 9), MAGE A3 569 bp (+) (lane 10), MAGE A4 580 bp (+) (lane 11), MAGE A5 478 bp (+) (lane 12), MAGE A8 419 bp (+) (lane 13), MAGE A9 407 bp (+) (lane 14), MAGE A10 464 bp (+) (lane 15), Marker (lane 16).

MAGE = Melanoma antigen, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

We employed these small specimens for PCR with GAPDH primers and the MAGE A1-10 primers. As illustrated in figure 5, 13/15 (86.7%) specimens had positive GAPDH, while 2/15 specimens (13.3%) had negative results. We excluded the specimens with negative GAPDH from this study. The results of PCR for MAGE A1-10 showed that 7/13 specimens (53.8%) were positive for the first round PCR, and 12/13 specimens (92.3%) were positive for the second round PCR. The results of PCR for MAGE A1-6

was 1/13 (7.7%) specimen for first round and 3/13 (23.1%) for second round. The GAPDH negative showed negative results for all MAGE A1-10 subtypes. The results of each MAGE A1-10 subtype showed that MAGE A1 was positive in 2/13 for specimens 8 and 9, MAGE A3 was positive in 1/13 for specimen number 8; MAGE A5 was positive in 12/13 for specimens 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, and 15; MAGE A8 was positive in 5/13 for specimens 3, 4, 5, 6, and 9; MAGE A10 was positive in 7/13 for specimens 2,

XM_005274676-X1	1	GTCATCATGCCTCTTGAGCAGAGGAGTCAGCAAGCCTGAAGAAGGCCTTGAGGCC
XM_011531160-X2	1	GTCATCATGCCTCTTGAGCAGAGGAGTCAGCAAGCCTGAAGAAGGCCTTGAGGCC
XM_006724818-X3	1	GTCATCATGCCTCTTGAGCAGAGGAGTCAGCAAGCCTGAAGAAGGCCTTGAGGCC
MAGEA3-seq	1	GTCATCATGCCTCTTGAGCAGAGGAGTCAGCAAGCCTGAAGAAGGCCTTGAGGCC
XM_005274676-X1	61	CGAGGAGAGGCCCTGGGCCTGGTGGGTGCGCAGGCTCCTGCTACTGAGGAGCAGGAGGCT
XM_011531160-X2	61	CGAGGAGAGGCCCTGGGCCTGGTGGGTGCGCAGGCTCCTGCTACTGAGGAGCAGGAGGCT
XM_006724818-X3	61	CGAGGAGAGGCCCTGGGCCTGGTGGGTGCGCAGGCTCCTGCTACTGAGGAGCAGGAGGCT
MAGEA3-seq	61	CGAGGAGAGGCCCTGGGCCTGGTGGGTGCGCAGGCTCCTGCTACTGAGGAGCAGGAGGCT
XM_005274676-X1	121	GCCTCCTCCTCTTCTACTCTAGTTGAAGTCACCCTGGGGAGGTCCTGCTGCCGAGTCA
XM_011531160-X2	121	GCCTCCTCCTCTTCTACTCTAGTTGAAGTCACCCTGGGGAGGTCCTGCTGCCGAGTCA
XM_006724818-X3	121	GCCTCCTCCTCTTCTACTCTAGTTGAAGTCACCCTGGGGAGGTCCTGCTGCCGAGTCA
MAGEA3-seq	121	GCCTCCTCCTCTTCTACTCTAGTTGAAGTCACCCTGGGGAGGTCCTGCTGCCGAGTCA
XM_005274676-X1	181	CCAGATCCTCCCCAGAGTCTCAGGGAGCCTCCAGCCTCCCCACTACCATGAACTACCCT
XM_011531160-X2	181	CCAGATCCTCCCCAGAGTCTCAGGGAGCCTCCAGCCTCCCCACTACCATGAACTACCCT
XM_006724818-X3	181	CCAGATCCTCCCCAGAGTCTCAGGGAGCCTCCAGCCTCCCCACTACCATGAACTACCCT
MAGEA3-seq	181	CCAGATCCTCCCCAGAGTCTCAGGGAGCCTCCAGCCTCCCCACTACCATGAACTACCCT
XM_005274676-X1	241	CTCTGGAGCCAATCCTATGAGGACTCCAGCAACCAAGAAGAGGAGGGGCCAAGCACCTTC
XM_011531160-X2	241	CTCTGGAGCCAATCCTATGAGGACTCCAGCAACCAAGAAGAGGAGGGGCCAAGCACCTTC
XM_006724818-X3	241	CTCTGGAGCCAATCCTATGAGGACTCCAGCAACCAAGAAGAGGAGGGGCCAAGCACCTTC
MAGEA3-seq	241	CTCTGGAGCCAATCCTATGAGGACTCCAGCAACCAAGAAGAGGAGGGGCCAAGCACCTTC
XM_005274676-X1	301	CCTGACCTGGAGTCCGAGTTCCAAGCAGCACTCAGTAGGAAGGTGGCCGAGTTGGTTTCAT
XM_011531160-X2	301	CCTGACCTGGAGTCCGAGTTCCAAGCAGCACTCAGTAGGAAGGTGGCCGAGTTGGTTTCAT
XM_006724818-X3	301	CCTGACCTGGAGTCCGAGTTCCAAGCAGCACTCAGTAGGAAGGTGGCCGAGTTGGTTTCAT
MAGEA3-seq	301	CCTGACCTGGAGTCCGAGTTCCAAGCAGCACTCAGTAGGAAGGTGGCCGAGTTGGTTTCAT
XM_005274676-X1	361	TTTCTGCTCCTCAAGTATCGAGCCAGGGAGCCGGTCACAAAGGCAGAAATGCTGGGGAGT
XM_011531160-X2	361	TTTCTGCTCCTCAAGTATCGAGCCAGGGAGCCGGTCACAAAGGCAGAAATGCTGGGGAGT
XM_006724818-X3	361	TTTCTGCTCCTCAAGTATCGAGCCAGGGAGCCGGTCACAAAGGCAGAAATGCTGGGGAGT
MAGEA3-seq	361	TTTCTGCTCCTCAAGTATCGAGCCAGGGAGCCGGTCACAAAGGCAGAAATGCTGGGGAGT
XM_005274676-X1	421	GTCGTCGGAAATGGCAGTATTTCTTTTCCTGTGATCTTCAGCAAAGCTTCCAGTTCCTTG
XM_011531160-X2	421	GTCGTCGGAAATGGCAGTATTTCTTTTCCTGTGATCTTCAGCAAAGCTTCCAGTTCCTTG
XM_006724818-X3	421	GTCGTCGGAAATGGCAGTATTTCTTTTCCTGTGATCTTCAGCAAAGCTTCCAGTTCCTTG
MAGEA3-seq	421	GTCGTCGGAAATGGCAGTATTTCTTTTCCTGTGATCTTCAGCAAAGCTTCCAGTTCCTTG
XM_005274676-X1	481	CAGCTGGTCTT
XM_011531160-X2	481	CAGCTGGTCTT
XM_006724818-X3	481	CAGCTGGTCTT
MAGEA3-seq	481	CAGCTGGTCTT

**Figure 4.** This figure shows the alignment analysis of MAGE-A3 gene from testicular tissue with the MAGE A3 sequences from the GenBank data. <sup>12</sup>  
MAGE = Melanoma antigen, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

**Table 3.** Identification of MAGE A1-10 from the core biopsy of lung cancer tissues

No Sample	GAPDH	MAGE A1-10	MAGE A1-6	MAGE A1	MAGE A2	MAGE A3	MAGE A4	MAGE A5	MAGE A6	MAGE A8	MAGE A9	MAGE A10	MAGE A Positive
1	+	-	-	-	-	-	-	-	-	-	-	-	- 55
2	+	+	-	-	-	-	-	+	-	-	-	+	A5, A10
3	+	+	-	-	-	-	-	+	-	+	-	+	A5, A8, A10
4	+	+	-	-	-	-	-	+	-	+	-	-	A5, A8
5	+	+	-	-	-	-	-	-	-	+	-	+	A5, A8, A10
6	+	+	-	-	-	-	-	+	-	+	-	+	A5, A8, A10
7	+	+	-	-	-	-	-	+	-	-	-	+	A5, A10
8	+	+	+	+	-	+	-	-	-	-	-	-	A1, A3, A5
9	+	+	-	-	-	-	-	+	-	+	-	+	A5, A8, A10
10	+	+	+	+	-	-	-	+	-	-	-	-	A1, A5
11	-	x	x	x	x	x	x	x	x	x	x	x	x
12	+	+	-	-	-	-	-	+	-	-	-	-	A5
13	-	x	x	x	x	x	x	x	x	x	x	x	x
14	+	+	-	-	-	-	-	+	-	-	-	-	A5
15	+	+	+	+	-	-	-	+	-	-	+	+	A5, A10
Positive result	13/15 (86.7%)	12/13 (92.3%)	3/13 (23.1%)	2/13 (15.4%)	-	1/13 (33.3%)	-	12/13 (92.3%)	-	5/13 (38.5%)	-	7/13 (53.8%)	

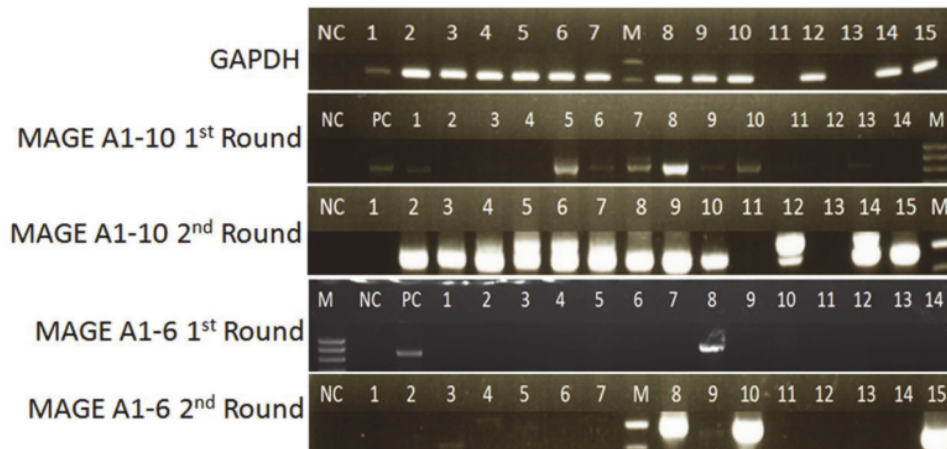
Note: +: symbol for positive result, -: symbol for negative result, x: symbol for excluded sample, MAGE = Melanoma antigen, GAPDH = Glyceraldehyde-3-phosphate dehydrogenase.

3, 5, 6, 7, 9, and 15 while MAGE A2, A4, A6, and A9 were negative for all specimens (Table 3).

## Discussion

The objective of this study was to construct a novel universal primer to identify the expression of MAGE A1-10 which could simultaneously bind to the cDNA of MAGE A1, A2, A2B, A3, A4, A5, A6, A8, A9, A9B, and A10 by nested PCR. The pair primers of MF10/MR10 and MF10/MR12 had already been assessed by RT-PCR using the testicular tissue, resulting in a

single target band. The PCR for the individual MAGE-A subtypes showed positive results for MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A8, MAGE-A9, and MAGE-A10, but negative for MAGE-A6. The sequences of MAGE-A6 primers utilized in the present study were the same as previously published.<sup>13</sup> The negative results for MAGE-A6 might be attributed to the atrophy testicular tissue which did not express MAGE-A6. As stated before, we collected the testicular specimen from the atrophy testis which had received orchidectomy therapy.



**Figure 5.** Expression analysis of GAPDH, MAGE A1-10, and MAGE A1-6 from the core biopsy of lung cancer patients. PC = positive control for PCR with template total RNA from a testicular tissue.

M = marker, NC = negative control, lane 1-15 are the specimens from 15 patients. MAGE = Melanoma Antigen, GAPDH = Glyceraldehyde-3-Phosphate Dehydrogenase.



The MAGE A1-10 expression in this study was positive in the lung tissues of patients diagnosed with lung cancer. As reported in the previous studies, MAGE-A gene is often expressed in some cancers, including oral squamous carcinoma,<sup>5,24</sup> gastric cancer,<sup>6,25</sup> renal cancer,<sup>26</sup> papillary thyroid carcinoma,<sup>12</sup> lung cancer,<sup>14,27</sup> and NSCLC.<sup>15</sup> The previous results showed that MAGE-A1 was expressed in hepatocellular carcinoma<sup>28</sup> and testicular tissue.<sup>29</sup> The expression of MAGE-A1 was evaluated by Reverse Transcription PCR.<sup>30</sup>

The expression of MAGE A family genes, including subtype MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A8, MAGE-A9, and MAGE-A10 in this study, showed the single expression, which one specimen was positive for one subtype MAGE A and multiple expression, which one specimen was positive for more than one subtype MAGE A. Other research showed a similar trend that the multiple expression of MAGE-A1-10 subtype often occurred in some cancers. The multiple expression of cancer testis antigens was also reported to be highly frequent in hepatocellular carcinoma,<sup>8</sup> primary and recurrent vulval tumours,<sup>11</sup> and medulloblastoma.<sup>16</sup>

Patients with MAGE-A expression had a worse prognosis compared to those with no MAGE-A expression NSCLC.<sup>18</sup> Expression of MAGE-A1 or -A10 antigens in epithelial ovarian cancer (EOC) resulted in a poor progression of free survival.<sup>10</sup> The expression of MAGE A3, -A4, -A5, -A9, and -A11 was significantly associated with lymph node metastasis; MAGE A1, -A3, -A4, -A5, -A9, and -A11 was significantly related to clinically advanced stages of oral squamous cell carcinoma.<sup>5</sup> MAGE-A family was involved in gastric cancer progression, indicating the poor prognosis of gastric cancer patients.<sup>6</sup> MAGE A1 expression might be a predictive marker of poor prognosis in gastric cancer.<sup>19</sup> MAGE-A gene expression in peripheral blood served as a poor prognostic marker for lung cancer patients.<sup>17</sup> MAGE A1-6 expression of bone marrow in lung cancer patient correlated with poor survival rates. MAGE A1-6 positive patients showed a poorer

overall survival and overall disease-free survival rates compared with MAGE A1-6 negative patient. MAGE A1-6 might be considered as a novel prognostic factor for lung cancer, resulting in effective follow-up and treatment.<sup>20</sup> Therefore, the expression of MAGE A could improve the prognosis of some cancers.

## Conclusion

The novel universal primer could detect the expression of MAGE A1-10 in the core biopsy of lung cancer tissues, which expressed at least one of the ten subtypes of MAGE-A mRNA. This universal primer might be applied as a new tool for detecting the expression of MAGE A1-10 in solid cancer cells.

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## Conflict of Interest

None declared.

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