



# Molecular Detection of *Toxoplasma Gondii* in *Haemaphysalis* Ticks in Korea

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**Abstract:** *Toxoplasma gondii* are intracellular protozoa that can cause neurological disease or death in fetuses and even in immunocompromised human adults. Ticks are recognized as vectors of many microorganisms including viruses, bacteria, and protozoa. Recent studies detected *T. gondii* in various tick species in many countries. In this study, we performed PCR detection of the *T. gondii* B1 gene from *Haemaphysalis* ticks collected from vegetation in 4 localities, Wonju, Gunsan, Miryang, and Yangsan, in Korea. We analyzed DNA from 314 ticks (268 *Haemaphysalis longicornis* and 46 *Haemaphysalis flava*) and the B1 gene of *T. gondii* was detected in 13 of these. The detection of *T. gondii* in ticks differed significantly by region ( $P=0.021$ ). *T. gondii* was detected in the following percentages of collected ticks: 3.7% (7 of 189) in Gunsan, 10% (5 of 50) in Wonju, 16.7% (1 of 6) in Yangsan, and 0% (0 of 69) in Miryang. The detection of *T. gondii* in ticks was not associated with tick species or development stage. This is the first report of *T. gondii* detection in ticks in Korea. Our results provide important information necessary to understand toxoplasmosis transmission.

**Key words:** *Toxoplasma gondii*, *Haemaphysalis*, tick-borne disease, tick-borne pathogen, Korea

*Toxoplasma gondii* can cause severe neurological disease or death in developing human fetuses and in immunosuppressed patients; however, infected immunocompetent individuals are usually asymptomatic [1]. The seroprevalence of *T. gondii* in Korean people was reported as 5.6% in 1960, 7.2% in 1983, 7.7% in 1999, 6.6% in 2000, 6.7% in 2009, and 8.6% in 2016 [2-7].

*T. gondii* has been detected in Korean wild animals such as feral cats, Chinese water deer, roe deer, and raccoon dogs [8-10], and in domesticated animals such as cattle, horses, rabbits, and dogs in Korea [11-14]. These animals include herbivores, which raises the possibility that bloodsucking arthropods such as ticks could transfer *T. gondii* [15]. Ticks are vectors of many microorganisms including viruses, bacteria, and protozoa.

Recently, *T. gondii* has been detected in various tick species [15], such as *Dermacentor reticulatus* and *Ixodes ricinus* in Poland [16-23], *Amblyomma* in the Republic of Chad [24], and

*Haemaphysalis longicornis* in China [25]. *H. longicornis* is the dominant tick species in Korea. In addition, many experiments have demonstrated the possibility of toxoplasmosis transmission via ticks [25-30].

In this study, we detected *T. gondii* DNA in *Haemaphysalis* ticks collected from vegetation in 4 localities in Korea. Ixodid tick *H. longicornis* is endemic to China, Korea, Japan, Russia, Australia, and New Zealand and is an important vector of protozoal parasites including *Babesia ovata*, *Babesia gibsoni*, *Theileria sergenti*, and *Theileria mutans* [31,32]. However, *T. gondii* has not been found in Korean ticks to date.

Ticks were collected from the vegetation by flagging. The tick collection areas were selected based on topographical, temperature, seasonal and weather aspects, among others. Ticks were collected from August 2014 to October 2016 in various Korean provinces to investigate possible regional characteristics: Wonju (Gangwon-do Province; 37.389545, 127.801770), Gunsan (Jeollabuk-do Province; 36.006237, 126.807751), Miryang (Gyeongsangnam-do Province; 35.475082, 128.780564), and Yangsan (Gyeongsangnam-do Province; 35.286111, 129.027625) (Fig. 1; Table 1). Species identification of collected ticks was performed by examination under a dissecting microscope according to Yamaguti et al. [33], and confirmed by PCR amplification of the tick DNA and sequencing of the 5.8S rRNA in-

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**Table 1.** Summary of *Haemaphysalis* ticks collected from vegetation in four localities in Korea

Locality	No. of screened ticks	Stage	Species	<i>T. gondii</i> positive No. (%)	Collection date
Gunsan	189	Adult: 6 Nymph: 52 Larva: 131	<i>H. longicornis</i> : 153 <i>Haemaphysalis flava</i> : 36	3.7	2014-August
Wonju	50	Adult: 5 Nymph: 45 Larva: 0	<i>H. longicornis</i> : 50	10	2015-July
Miryang	69	Adult: 1 Nymph: 2 Larva: 66	<i>H. longicornis</i> : 65 <i>H. flava</i> : 4	0	2016-October
Yangsan	6	Adult: 2 Nymph: 4 Larva: 0	<i>H. flava</i> : 6	16.7	2015- October
Total	314	Adult: 14 Nymph: 103 Larva: 197	<i>H. longicornis</i> : 268 <i>H. flava</i> : 46	4.1	

**Fig. 1.** Four localities of tick collection. Ticks were collected from the vegetation by flagging in Wonju, Gunsan, Miryang, and Yangsan in Korea.

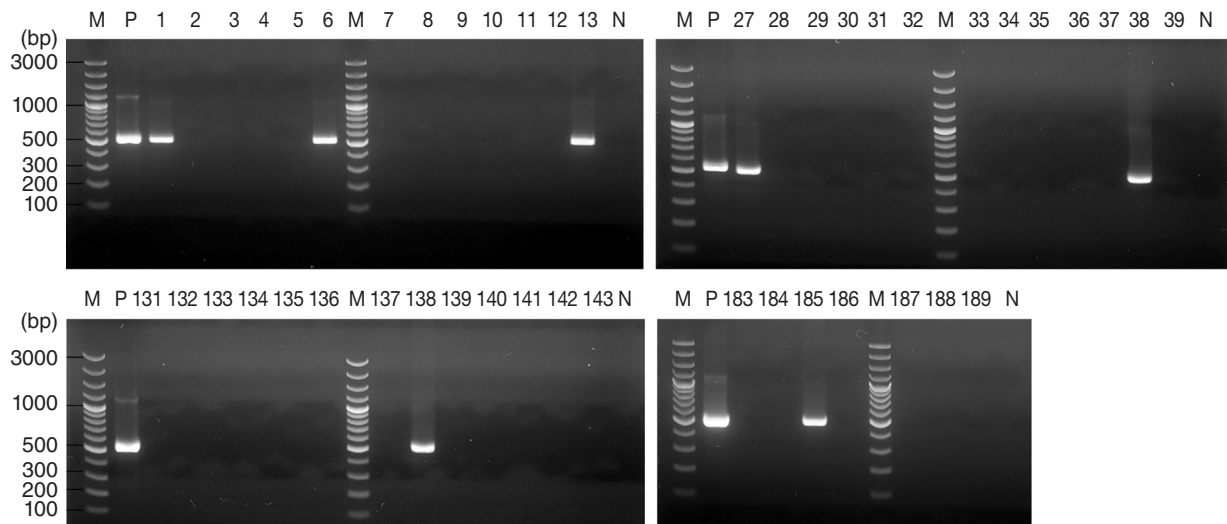
ternal transcribed spacer 2 region using the primers HITS2-F (5'-GGTGCTCGAGACTCGTTTTG-3') and HITS2-R (5'-ATTC-

GCGGTTTACGAGAGAA-3') [34]. DNA was extracted from each collected tick using a NucleoSpin DNA Insect kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions, and stored at -20°C until use. To detect the *T. gondii* B1 gene in ticks, nested PCR was performed with 2 primer pairs: S1 (5'-TGTTCTGTCCATCGCAAC G-3') and AS1 (5'-ACGGATGCAGITCCTTTCTG-3'), which amplify a 580-bp fragment; and S2 (5'-TCITCCCAGACGTGGATTTC-3') and AS2 (5'-CTCGACAATACGCTGCTTGA-3'), which amplify a 530-bp fragment [35]. The PCR products were sequenced (Bionics Co., Seoul, Korea). A BLAST search was used to compare the obtained sequences to those available in GenBank (USA).

A total of 314 ticks from 4 regions were analyzed in this study, comprising 268 *H. longicornis* and 46 *H. flava* (Table 1). For 13 of these 314 ticks, agarose gel electrophoresis of PCR products revealed a band pattern at 530 bp (Fig. 2), which indicated the presence of the *T. gondii* B1 gene. The DNA sequence of these PCR products showed greater than 99.7% identity with the published B1 gene sequence of *T. gondii* (MH744807.1) (Table 2).

The detection of *T. gondii* in ticks differed significantly by collection locality ( $P=0.021$ ) (Table 3). The *T. gondii* B1 gene was detected in 7 (3.7%) of 189 ticks collected from Gunsan, in 5 (10%) of 50 ticks collected from Wonju, and in one (16.7%) of 6 ticks collected from Yangsan. *T. gondii* was not detected in the 69 ticks collected from Miryang.

The detection rate of *T. gondii* was 4.1% (11 of 268) in *H. longicornis* and 4.3% (2 of 46) in *H. flava*. This indicated there was no association between the species of tick and the detec-



**Fig. 2.** Agarose gel electrophoresis results of *Toxoplasma gondii* B1 gene PCR products from *Haemaphysalis* ticks collected from Gunsan, Korea. PCR products from 7 of 189 tick DNA samples indicated the presence of *T. gondii*. M, marker; P, positive control (*T. gondii* DNA); N, negative control.

**Table 2.** Details of *Toxoplasma gondii*-positive *Haemaphysalis* ticks

No.	Locality	Collection date	Tick species	Development stage	Sequence identity (%) to B1 gene (MH744807.1)
1	Gunsan	2014-August	<i>H. longicornis</i>	Larva	99.7
2	Gunsan	2014-August	<i>H. longicornis</i>	Larva	100
3	Gunsan	2014-August	<i>H. longicornis</i>	Larva	99.7
4	Gunsan	2014-August	<i>H. longicornis</i>	Larva	100
5	Gunsan	2014-August	<i>H. longicornis</i>	Larva	99.7
6	Gunsan	2014-August	<i>H. longicornis</i>	Larva	100
7	Gunsan	2014-August	<i>Haemaphysalis flava</i>	Adult	99.7
8	Wonju	2015-July	<i>H. longicornis</i>	Nymph	99.7
9	Wonju	2015-July	<i>H. longicornis</i>	Nymph	100
10	Wonju	2015-July	<i>H. longicornis</i>	Nymph	99.7
11	Wonju	2015-July	<i>H. longicornis</i>	Nymph	99.7
12	Wonju	2015-July	<i>H. longicornis</i>	Adult	99.7
13	Yangsan	2015-October	<i>H. flava</i>	Nymph	99.7

tion of *T. gondii* (Table 3). The detection rates of *T. gondii* in tick adults, nymphs, and larvae were 14% (2 of 14), 4.9% (5 of 103), and 3.0% (6 of 197), respectively. However, the detection rate of *T. gondii* among these 3 development stages was not statistically different ( $P=0.113$ ).

In this study, the detection rates of *T. gondii* in *H. longicornis* and *H. flava* were lower than the previously reported detection rate of *T. gondii* in *H. longicornis* (9.2%; 39 of 422) from Qingdao, Shandong Province, China [25]. This study did not find *T. gondii* in *Haemaphysalis* larvae, but our results showed the presence of *T. gondii* in 3% (6 of 197) of larvae. Previous studies have reported the presence of *T. gondii* in many tick species. In

north-western Poland, 12.7% of 259 *Ixodes ricinus* were infected with *T. gondii* [16]. The same study demonstrated that inoculation with homogenate of ticks caused toxoplasmosis in 44 of 60 mice [16]. *T. gondii* DNA was detected in 2.8% of adult *I. ricinus* in eastern Poland [17], and in 64.9% of *I. ricinus* adults and nymphs in Upper Silesia, Poland [18]. *T. gondii* DNA was also found in 3% (52 of 1,737) of *I. ricinus* feeding on ponies and in 10.2% (38 of 371) of *I. ricinus* from vegetation including 7 larvae [19]. *T. gondii* DNA was detected in 3.2% (21 of 664) of adult *Dermacentor reticulatus*, a human-biting tick, collected from vegetation [20].

Several laboratory experiments have demonstrated the trans-

**Table 3.** Association of variables related to *Toxoplasma gondii*-positive of ticks

	No. of screened	<i>T. gondii</i> positive (%)	P-value <sup>a</sup>
Locality			0.021
Gunsan	189	3.7	
Wonju	50	10	
Miryang	69	0	
Yangsan	6	16.7	
Total	314	4.1	
Tick Species			1.000
<i>Haemaphysalis longicornis</i>	268	4.1	
<i>Haemaphysalis flava</i>	46	4.3	
Total	314	4.1	
Stage			0.113
Adult	14	14.3	
Nymph	103	4.9	
Larva	197	3.0	
Total	314	4.1	

<sup>a</sup>P-values were calculated using chi-square or Fisher exact tests.

mission of toxoplasmosis by ticks. *Amblyomma americanum*, *Dermacentor variabilis*, and *Dermacentor andersoni* transmitted *T. gondii* by blood feeding in the laboratory animals [26]. *D. andersoni* showed transovarial transmission of *T. gondii* to offspring [26]. *I. ricinus* can transmit *T. gondii* to mice through blood feeding [27]. *T. gondii* was reported to survive in the body of *H. longicornis* for more than 10 days [25,28] and *T. gondii* was transmitted to the host by ingestion, but not by blood-feeding, of infected ticks [25].

*T. gondii* has previously been detected in wild animals and livestock in Korea. *T. gondii* oocysts were found in 0.89% (5 of 563) of feral cat feces [8]. *T. gondii* IgG test results were positive in 12% (6 of 50) feral cats [9]. A PCR assay indicated the presence of *T. gondii* in 47.2% (50 of 106) of feral cats and 46.3% (64 of 138) of dogs [14]. The incidence of *T. gondii* seropositivity was 10.8% in Chinese water deer, 4.3% in raccoon dogs, 16.7% in roe deer [10]. Furthermore, the *T. gondii* B1 gene was detected in the blood of 16.2% (23 of 142) of rabbits from breeding farms in Korea [11]. The incidence of *T. gondii* seropositivity was 0.5% (3 of 568) in cattle [12], and 2.9% (24 of 816) and 2.6% (5 of 191) in 2 studies on horses [13,36].

It is well known that *T. gondii* infection can be caused by contact with *T. gondii*-oocyst-shedding cats and ingestion of *T. gondii*-contaminated food [37]. However, we suggest the possibility that the tick is a potential vector for transmission of *T. gondii*, based on the worldwide presence of *T. gondii* in ticks, experimental evidence of toxoplasmosis transmission by ticks,

and cases of *T. gondii* infection in several herbivorous wild animals and livestock [15].

This is the first report of *T. gondii* detection in ticks in Korea. *T. gondii* was found in all lifecycle stages of both *H. longicornis* and *H. flava* ticks collected from vegetation in Gunsan, Wonju, and Yangsan. This study provides important information necessary to understand toxoplasmosis transmission. We suggested a new transmission cycle of *T. gondii* as vector-borne in wild and domestic animals.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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**Supplementary Table S1.** DNA sequences of B1 gene of *Toxoplasma gondii* detected in ticks

No.	Locality	DNA Sequence
1	Gunsan	GTTCTGTTCCGTGCTGTCTAGGGCCACCCTTACTGCAAGAGAAAGTATTTGAGGTCATATCGTCCCATGAAGTGGACCACCCTGTTTCCTCTCTTCACTGTCCAGTACGACATC- GCATTTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAAGAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGAT
2	Gunsan	CGTGTCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCA CGTACGACATCG- CATTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGA
3	Gunsan	CTGTTCTGTTCCGTGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCCAGTACGACATCG- CATTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGA
4	Gunsan	TCTGTTCCGTGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCCAGTACGACATCG- CATTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGA
5	Gunsan	TTCTGTTCCGTGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCCAGTACGACATC- GCATTTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTTG- CATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGA
6	Gunsan	ATATCAGGCTTCGTGTTCTGTTCCGTGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCCAG- GTACGACATCGCATTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGGATTCA
7	Gunsan	CTGTTCTGTTCCGTGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATGTCCTCCATGAAGTGGACCACCTGTTTCCTCTCTTCACTGTCCAGTACGACATCG- CATTCAAGGGGAAGATCCAG CAGATCTCGTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTC TTGTGCTGCCTCTCATGGCAAAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATTT CCCGCTGGCAAAATACAGGTGAAATGTACCTCCAGAAAAGCCACTAGTAGTCGTGGGCAATGTGCCACCTGCCTCTTTGGGAGAAAAAGAGAAAGAGACGCTGCCGCTGTTTTG- CAATGAAAAGTATTC

(Continued to the next page)

**Supplementary Table S1.** Continued

No.	Locality	DNA Sequence
8	Wonju	TTCTGTTTCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTTCATATCGTCCCATGAAGTGGACCACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATC- GCATTTCAAGGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAATGAAAAGATTAC
9	Wonju	GGCCCTCTGTTCTGTTGGCTGTCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCATGAATGGACCACTGTTTCCCTCTCTTCACTGTCAACGTACGACGA- CATCGCATTTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAAA
10	Wonju	CACCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGACCACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCGTATTTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGACGCTGCCGCTG CGCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGCAACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCG- CATTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGACGCTGCCGCTG CTGTTGGCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGCAACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCG- CATTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCACCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTTG- CATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAAA
11	Wonju	CGCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGCAACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCG- CATTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGACGCTGCCGCTG CTGTTGGCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGCAACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCG- CATTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCACCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTTG- CATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAAA
12	Wonju	GGGACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGACCACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCGGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGACGCTGCCGCTG CGCTGCTGCTAGGGCACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGCAACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCG- CATTCAAGGGAAGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCACCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTTG- CATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAAA
13	Yangsan	GGGACCCCTTACTGCAAGAGAAGTATTTGAGGTCATATCGTCCCATGAAGTGGACCACCTGTTTCCCTCTCTTCACTGTCAACGTACGACATCGGATCCAG CAGATCTCGTTTCGTGTTTCGAGACAAGAGAGGTCGGCCCCCACAAAGACGGCTGAAGAATGCAACATTCCTTGCTGCCTCTCATGGCAATGCCAGAAAGGGTACGTGTT- GCATCATAACAAGAGCTGTATT CCCGCTGGCAATACAGGTGAAATGTACTCCAGAAAAGCCACTAGTAGTGGGGCAATGTGCCACCTGCCCCTTTGGGAGAAAAGAGAAAGAGAGCGCTGCCGCTGTTTTG- CAAA

