Advanced primer design

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1. Precautions in primer design

1.1 When too many primer sets are generated

a) Adjust the primer GC content.

When the primer GC content is 50 - 60%, favorable amplification performance will be obtained experimentally.   Thus, the conditions are adjusted so that the GC content is in this range. Narrowing the permitted range for the GC content will be able to reduce the number of candidates.

b) The differences in the Tm are set to about 5°C for the primers (regions F2 and F1c, regions B2 and B1c).

Under the principles of the LAMP reaction, the F2 of FIP (or B2 of BIP) anneals to the target gene and initiates the gene synthesis.   The conditions are set so that F2 (B2) anneals to the target gene more frequently than F1c (B1c) does.   For this reason, it is preferred that the Tm at region F2 (B2) be about 5°C lower than that at region F1c (B1c).   When less stringent conditions (wider range of Tm's at each primer location) are used to design the primers, primer sets are generated, which consists of the primers with various Tm value.   For this reason, the difference in the Tm in each primer region may be 3°C or less.   Also, best results are obtained if the Tm's match between regions F2 and B2, regions F1c and B1c, and regions F3 and B3.

1.2 When too few primer sets are generated

If only small number of primer sets is generated for GC rich or AT rich sequences, it is plausible that the primer design conditions for the given target sequence are too stringent.   In PrimerExplorer V3, the primer design conditions are automatically selected for GC rich or AT rich sequences, but for some sequences, in spite of these conditions only a few primer sets are generated.   In such cases, the range of primer length or the range of Tm should be adjusted.

a) For AT rich sequences

For AT rich sequences, the Tm is calculated to be lower than non-AT rich sequences of the same length.   For this reason the Tm based on the default primer length may be lower than the lower limit of default Tm value, and prevent primers from being designed.   Thus, the primer length should be increased and/ or the Tm should be decreased.

b) For GC rich sequences

In contrast, for GC rich sequences, the Tm is calculated to be higher than non-GC rich sequences of the same length.   For this reason the Tm calculated from the default primer length may be higher than the default Tm upper limit, and prevent primers from being designed.   Thus, the primer length should be decreased and/or the Tm should be increased.   Because how the Tm or the length is adjusted would be determined on a case-by-case basis, the length of each primer should be changed by one base at a time and the Tm should be changed 1°C at a time.   Once a large number of primers have been generated, then stop the adjustment and select the primers.

2. Designing wild type and mutant type primers

In PrimerExplorer V3, it is possible to introduce mutations into the target sequence and then design primers. However, if there are too many mutations, the primer design conditions become too stringent and either the primers are not generated or the variety is insufficient. In such a case, one can design the primer with less stringent condition, for example reduce the number of the mutation point entered or completely eliminate the mutation sites from the target sequence. Appropriate primer sets could be selected, while identifying where the mutation points in the target sequence are located relative to the primer.

2.1 Detecting wild type and mutant type by amplification using common primers

In general, the primers are design to exclude mutation within the primer region, but if there are numerous mutations, it may not be possible to design primers that satisfy these conditions. For this reason, primers are designed that allow (contain) mutations and if possible, try to design primers that are not likely influenced by the mutation.

Under the principles of the LAMP reaction, F2 of FIP (or B2 of BIP) anneals to the target gene and initiates the gene synthesis. If the mutation is at the 3' end of F2 (B2), the DNA polymerase has difficulty in recognizing the double strand formed between the primer and the target gene, thus inhibit the gene amplification. Similar principles apply to the 5' end of F1c (B1c) and the 3' end of F3 (B3). Therefore, primers are selected so that mutations are not located in these regions.

On the other hand, if primers are selected so that the mutations are outside of the 3' end in F2 (B2), 5' end of F1c (B1c), or 3' end of F3 (B3), the primers are less susceptible to the effect of the mutation and the both wild type and mutant type are detectable by a common set of primers. Thus, primers are selected by permitting the mutation to be located within the following locations (Table 2- 1).

        a) 3' end of F1c or B1c and in the internal region

        b) 5' end of F2 or B2 and in the internal region

        c) 5' end of F3 or B3 and in the internal region

Here, we will design common primers that detect M13 and its mutant. Figure 2-1 shows an alignment of the wild type and the mutant type. In the entire length of 510 bp there are seven mutations. The region containing these mutations is the target region for amplification.

Figure 2- 2 shows an example of primer selection. Under default, primers have been designed for the wild type strain. Here, we focus on the 25 primer set candidates that contain the mutations and select the common primers. A star shows the locations of the mutations, and the locations of the mutations applicable to the primers designed are enclosed by a dotted line. This can be used to confirm the location of the primers for a given mutation. Table 2- 2 shows these results. The black dot indicates the location of the primer region (5' end, internal region, 3' end) for each primer set (F3, F2, F1, B1, B2, B3) for a given mutation. It can be determined that No 1 - 5, No 9 - 13, and No 25 are the primers less susceptible to the effects of the mutations at amplification. These are selected from the primer list, and their detailed information is then assessed for a final selection of the primer sets.

2.2 Highly specific primers (specific primers that distinguish between wild-type and mutant type)

If the mutant type and wild type need to be distinguished, a method opposite to the aforementioned method is used.   Thus, by selecting primers with the mutation in the locations mentioned below, one can select highly specific primers.   If the primers include a mutation in this location, the mutant type is generally amplified while the wild type amplification is hindered, thus improving the specificity toward the mutant type (table 2- 1).

> a) 5' end of F1c or B1c
>
> b) 3' end of F2 or B2
>
> c) 3' end of F3 or B3

As in (1), from the primer list designed under default, primer sets are selected that fulfill the above a), b), or c).   In Table 2- 2, No 6 – 8 and No 14 - 24 are mutant type-specific primer sets. Then, their detailed information is examined for final selection of the primer set.

Table 2-1 Select Common primers and specific primers

Primer region	F3			F2			F1c			B1c			B2			B3		
Position	5'[a]	In[b]	3'[c]	5'	In	3'	5'	In	3'	5'	In	3'	5'	In	3'	5'	In	3'
Common primer [d]	+ [f]	+		+	+			+	+		+	+	+	+		+	+	
Specific primer [e]			+			+	+			+					+			+

a) 5' terminal region, b) internal region, c) 3' terminal region, d) Common primer: Mutation sites that can be permitted for the amplification of wild type and mutant type using common primers. e) Specific primer: Sites for mutation for distinguishing between wild type and mutant type.   f) +; The mutation points are included.

```
M13_3.nuc     1: GCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTG 60
M13_3M1.nuc   1: GCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTGCAACGTCGTGACTGGGATAACCCTG 60

M13_3.nuc    61: GCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCG 120
M13_3M1.nuc  61: GCGTTACCCAACTTAATCGACTTGCAGCACATCCCGCTTTCGCCAGCTGGCGTAATAGCG 120

M13_3.nuc   121: AAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCT 180
M13_3M1.nuc 121: AAGAGTCCCGCACCGATCGCCCTTCCCAACACTTGCGCAGCCTGAATGGCGAATGGCGCT 180

M13_3.nuc   181: TTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTG 240
M13_3M1.nuc 181: TTGCCTGGTTTCCGGCACCAGAAGCGGTGCAGGAAAGCTGGCTGGAGTGCGATCTTCCTG 240

M13_3.nuc   241: AGGCCGATACGGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCT 300
M13_3M1.nuc 241: AGGCCGATACGGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCT 300

M13_3.nuc   301: ACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGA 360
M13_3M1.nuc 301: ACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGA 360

M13_3.nuc   361: CGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGC 420
M13_3M1.nuc 361: CGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGC 420

M13_3.nuc   421: GAATTATTTTTGATGGCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAA 480
M13_3M1.nuc 421: GAATTATTTTTGATGGCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAA 480

M13_3.nuc   481: CGCGAATTTTAACAAAATATTAACGTTTAC                               510
M13_3M1.nuc 481: CGCGAATTTTAACAAAATATTAACGTTTAC                               510
```
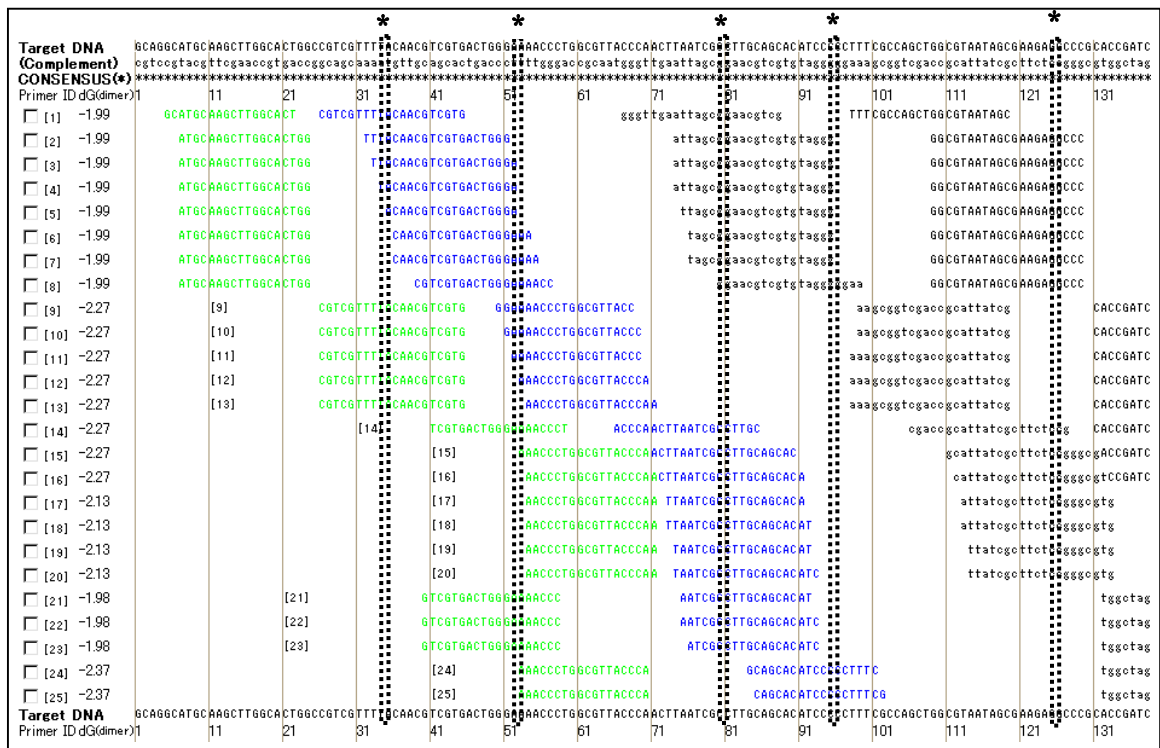
Figure 2-1 Alignment of wild type with mutant type

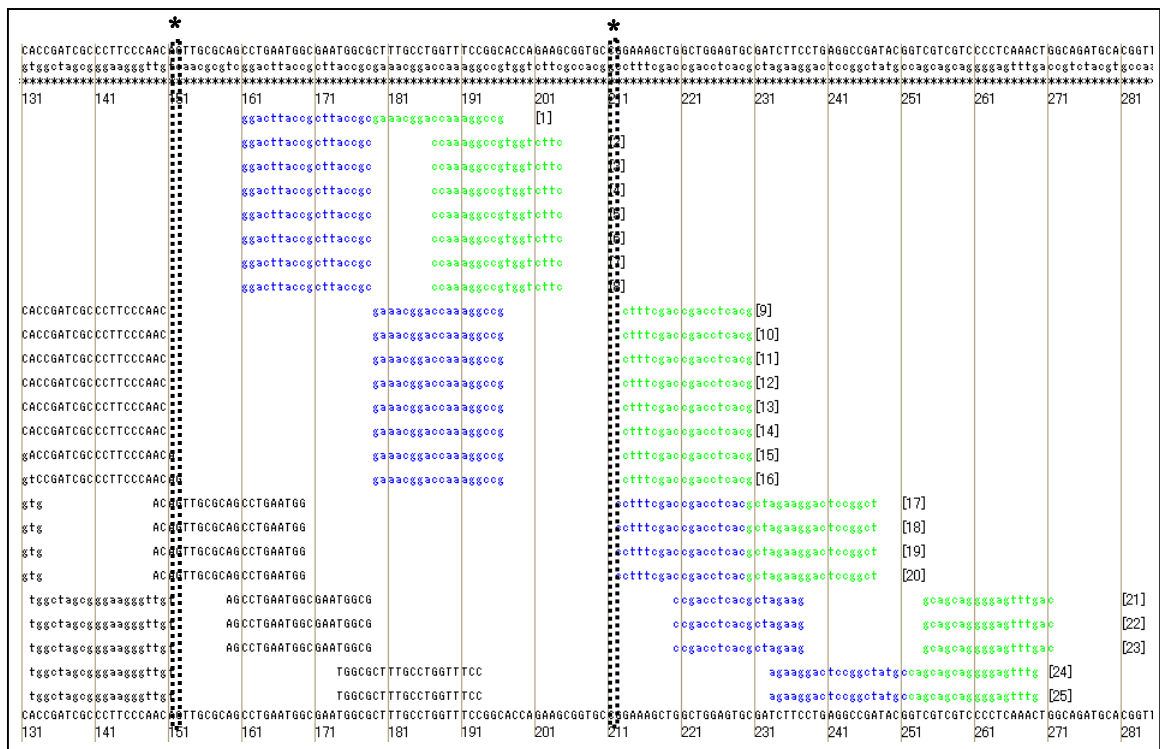**Figure 2-2 Primer sets and location of mutations**

Figure 2-2 continued
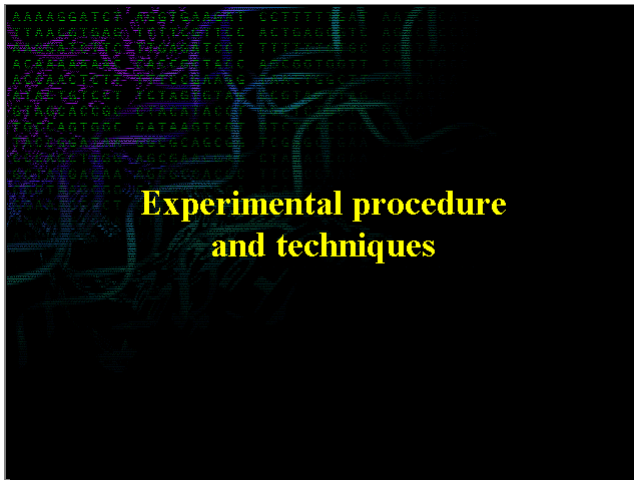
Table 2-2 Primer sets depending on location of mutation

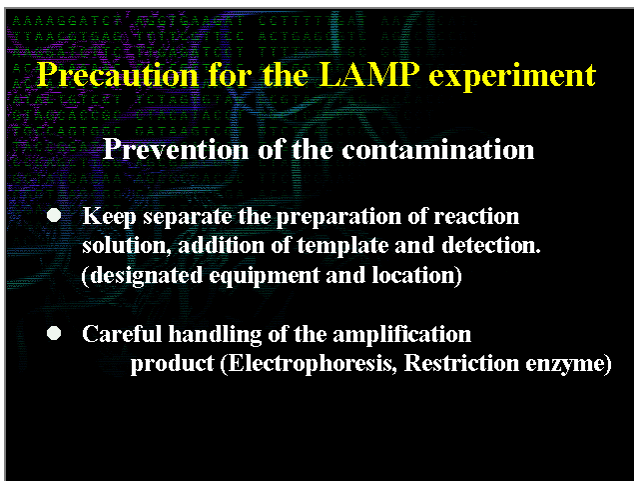| No.	F3 5'	F3 In.	F3 3'	F2 5'	F2 In.	F2 3'	F1c 5'	F1c In.	F1c 3'	B1c 5'	B1c In.	B1c 3'	B2 5'	B2 In.	B2 3'	B3 5'	B3 In.	B3 3'	Primer
1					+			+											Common*
2				+				+				+							Common
3				+				+				+							Common
4				+				+				+							Common
5				+				+				+							Common
6						+	+					+							Specific**
7						+	+					+							Specific
8						+	+					+							Specific
9		+		+															Common
10		+		+															Common
11		+		+															Common
12		+		+															Common
13		+																	Common
14						+	+												Specific
15	+				+		+												Specific
16					+			+				+							Specific
17					+			+		+					+				Specific
18					+			+		+					+				Specific
19					+			+		+					+				Specific
20					+			+		+					+				Specific
21			+		+														Specific
22			+		+														Specific
23			+	+															Specific
24						+													Specific
25	+				+														Common

∗Common: primer set candidates that can amplify both wild type and mutant type with a common primer set

∗∗Specific: primer set that distinguishes mutant type from wild type

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Experimental procedure and techniques

of LAMP method
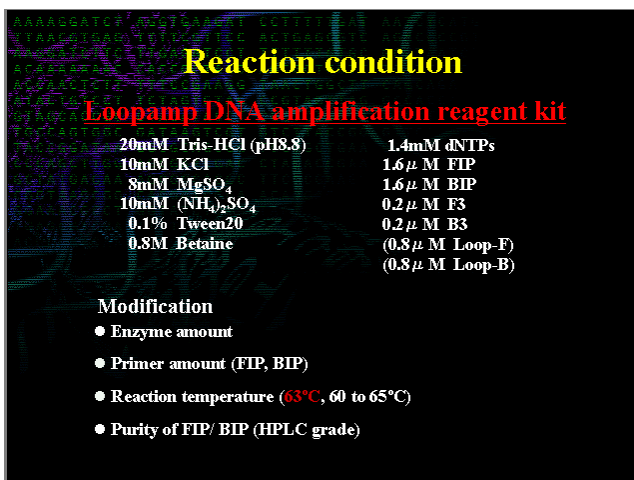
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Experimental procedure and techniques

Precaution for the LAMP experiment

Prevention of the contamination

- Keep separate the preparation of reaction solution, addition of template and detection. (designated equipment and location)

- Careful handling of the amplification product (Electrophoresis, Restriction enzyme)

In conducting the LAMP method, the greatest care is needed to prevent contamination.

One preventive measure is to keep separate the preparation of reaction solutions, addition of template, and detection. The experimental equipment and experimental locations should be kept physically separate. If separately experimental locations are not available, at least conduct the preparation of reaction solutions and the addition of templates in separate clean benches. However, the detection should be done in a separate room.
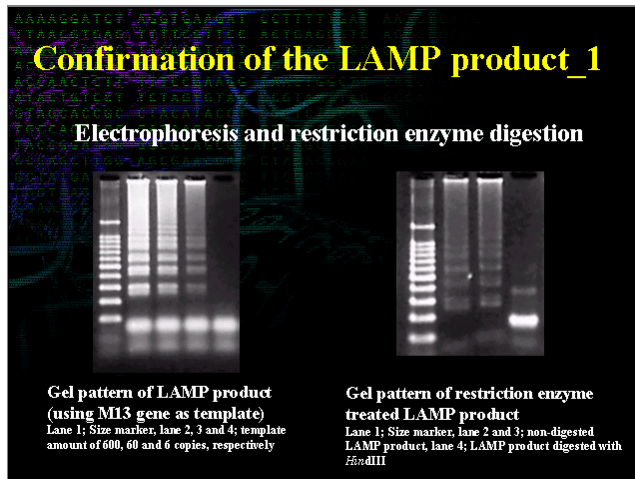
Handling of the amplification product might cause contamination, thus sufficient care is needed when performing electrophoresis or restriction enzyme digests for the purpose of identifying the amplification products.

Reaction condition

Loopamp DNA amplification reagent kit

20mM Tris-HCl (pH8.8)	1.4mM dNTPs
10mM KCl	1.6 μ M FIP
8mM MgSO$_4$	1.6 μ M BIP
10mM (NH$_4$)$_2$SO$_4$	0.2 μ M F3
0.1% Tween20	0.2 μ M B3
0.8M Betaine	(0.8 μ M Loop-F)
	(0.8 μ M Loop-B)

Modification
- Enzyme amount
- Primer amount (FIP, BIP)
- Reaction temperature (63°C, 60 to 65°C)
- Purity of FIP/ BIP (HPLC grade)

In the LAMP method, the amplification conditions of Loopamp DNA Amplification Kit are regarded as the fundamental experiment conditions.

To increase the amplification speed or sensitivity, consider the following factors.

・ Amount of enzyme, amount of primer (particularly the Inner primer)

・ Reaction temperature (In addition to the recommended temperature of 63°C, temperature in the 60 - 65°C range can also be considered)

・ Purity of the Inner primer (for screening the primer sets, the de-salting grade purity is sufficient, but for further assessments, HPLC-grade purity should be used.)

Confirmation of the LAMP product_1

Electrophoresis and restriction enzyme digestion

Gel pattern of LAMP product (using M13 gene as template)
Lane 1; Size marker, lane 2, 3 and 4; template amount of 600, 60 and 6 copies, respectively

Gel pattern of restriction enzyme treated LAMP product
Lane 1; Size marker, lane 2 and 3; non-digested LAMP product, lane 4; LAMP product digested with *Hin*dIII

A basic procedure for confirmation of LAMP product is restriction enzyme digest and electrophoresis.

The Figure on the left is the electrophoresis result of the LAMP products obtained using M13 as template. From the left are the size marker, 600 molecules, 60 molecules, 6 molecules of the template and negative control.

There exists a single *Hin*dIII site in the target M13 sequence. In the figure on the right, from the left are: size markers, two lanes of untreated sample, and a sample digested with *Hin*dIII. The apparent digestion result by *Hin*dIII treatment has confirmed the amplification of the target template.

Confirmation of the LAMP product_2

Real-time fluorescent detection

Real-time turbidity detection
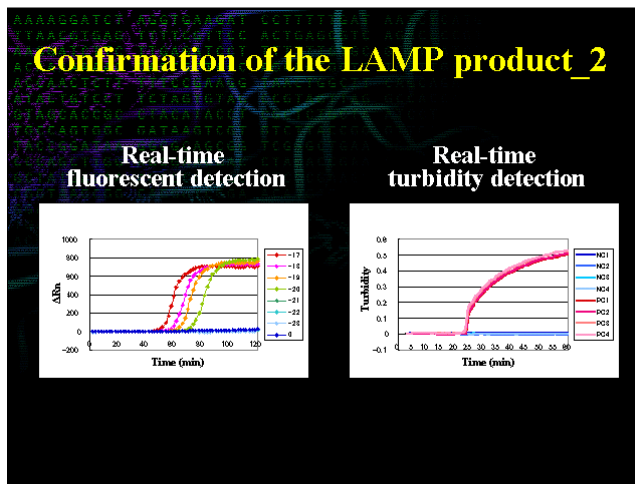
A basic procedure for confirmation of LAMP is by electrophoresis, but this requires that the tube lid be opened after completion of the reaction, resulting in a high risk of contamination. Thus, we recommend that electrophoresis is only for the initial confirmation of amplification and afterwards detection can be conducted within the tube. Examples include a fluorescent real-time detection or real time turbidity detection.

The figure on the left shows the results obtained with real time fluorescent detection with M13 as the template. The amount of template is stated as 10^{-17} mol/tube to 10^{-23} mol/tube, and the speed of amplification relates to the quantity of the template.

The figure on the right is real time turbidity detection with λ DNA as the template. Samples NC1 to NC4 are negative controls, and PC1 to PC4 are positive controls, indicating a high degree of reproducibility.

Precaution for the reagent

● **Experiment precaution for LAMP is similar to those for PCR.**

● **Keep the reagent at –20ºC.**

● **Store undiluted Primer at –80ºC.**

● **dNTP gradually deteriorates.**

● **Template DNA and primer should be resolved in TE buffer (pH8 to 9) and stored.**

● **Diluted template DNA could easily be degraded.**

There may be circumstances where the LAMP amplification proceeds quite effectively in the beginning, but as time passes it begins to perform poorly. In such circumstances, the deterioration of the reagents might be a possible cause, therefore, reagents should be handled with sufficient care. Generally the precautions required are similar to those for PCR, storing the reagents at -20°C, and storing the undiluted primer stock solution at -80°C. The substrate dNTP also deteriorates gradually and thus needs to be handled with care. If DNA such as the template or primer is reconstituted in water, the deterioration may be accelerated, thus they should be stored in a buffer such as TE. Specifically, the target template DNA at low concentration can easily be degraded and thus sufficient care is needed.

List of terms

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AT rich, GC rich:

The GC content of nucleic acids can vary for different creatures, or it can differ depending on whether the nucleic acid is derived from prokaryotic cell or non-prokaryotic cell.   Those with a low GC content is said to be AT rich, and those with a high GC content is said to be GC rich.

bp:

Abbreviation for base pairs.   Every nucleic base can specifically combine with its counterpart nucleic base through hydrogen bonds.   This plays an important role in nucleic acid replication, transcription and interaction between mRNA and tRNA.   In DNA the pairing is between adenine (A) and thymine (T) or between guanine (G) and cytosine (C), while in RNA the pairing is between A and uracil (U), or between G and C.   The length of double-stranded DNA is often expressed as the number of bases (bp).

dNTP:

A solution containing equal amounts of dATP, dTTP (or dUTP), dGTP and dCTP, and is used as substrate in nucleic acid synthesis.   During nucleic acid synthesis, if the dNTP concentration is too high in the reaction solution, it is said that the mismatch of nucleotide will increase.

FASTA format:

FASTA is a computer program that, through database searches, identifies similarities in gene or protein sequences.   It is appropriate for searching through long sequences for homologies. The FASTA format is a format most often used in sequence analysis programs and is in the following format.

```
>AA987701(genbank-upd) ←  The header is a comment beginning with > (sequence name or origin)
taaagaagtaagcctttatttccttgttttgca ←  Second line and beyond is the data
tggcttcaaccttagctggggctgcagcagcac
>AA987701(genbank-upd) ←  Multiple sequences are entered in this format
taaagaagtaagcctttatttccttgttttgca
tggcttcaaccttagctggggctgcagcagcac
```

Forward side, backward side:

At the start of DNA synthesis, primers are needed; PCR requires the minimum of two, while in the LAMP method the minimum of four primers are needed.   With respect to the double-stranded DNA, with the coding region of the relevant gene as shown in the 5' end on the left and the 3' end on the right, the 5' → 3' direction is the forward side and the reverse is the backward side.

For PCR

Forward Primer

5' ▬▬▶ 3'
3' ▬▬▬▬▬▬▬▬▬▬▬▬ 5'

5' ▬▬▬▬▬▬▬▬▬▬▬▬ 3'
3' ◀▬▬ 5'

Backward Primer
(Reverse Primer)
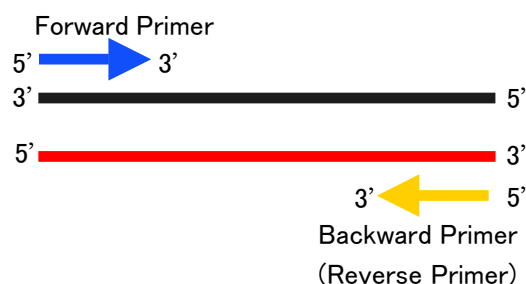
For the LAMP method
⇨ p.47 the LAMP method to legend (1)

45

GC content：

In expressing the base composition of nucleic acids, the proportion (percent) of G and C in the entire sequence.　The GC content of primers is selected so that that it is not AT rich to ensure stability of binding to the target gene.　For duplex nucleic acids, the base pairing is fixed, so this indicates the proportion of (G+C) in the entire sequence.　The GC content is one index of a property of nucleic acids.　The DNA GC content differs depending on the organism and in higher animals is in a narrow range centered at 42%, while in bacteria, it ranges from 75-25%.

GenBank format：

GenBank is an internationally known public DNA database maintained by the US NCBI (National Center for Biotechnology Information).　GenBank uses the database entry format indicated below.

LOCUS	Locus name, sequence length, molecule type, GenBank division, and modification date
DEFINITION	Brief description of sequence
ACCESSION	Original accession number
KEYWORDS	Key words to describe the entry for searches
SOURCE	Organism from which DNA is derived
ORGANISM	Formal scientific name for the source organism
REFERENCE	Literature reference
COMMENT	Biological function or database information
FEATURES	Information about genes and gene products, as well as regions in the sequence
source	A region of sequence, source organism
misc_signal	A region of sequence, function or signal type
mRNA	A region of sequence, mRNA
CDS	A region of sequence, protein coding region
intron	A region of sequence, intron location
Mutation	Sequence change due to mutation within the sequence
BASE COUNT	The number of A, C, G, T, or other letter in a sequence
ORIGIN	Letter corresponding to sequence start

```
  1  gaattcgata  aatctctggt  ttattgtgca  gtttatggtt  ccaaaatcgc
 51  atatactcac  agcataactg  tatatacacc  caggggggcgg  aatgaaagcg
```

`//`　　　Symbol indicating sequence end

*Hin*d III:

Type of restriction enzyme often used in experimental procedures.　This enzyme is produced from *Haemophilus influenzae* Rd and thus the enzyme is so named.　The recognition sequence and location of the cuts are indicated below.

```
5'……A │ AGCTT……3'
3'……TTCCA │ A……5'
```
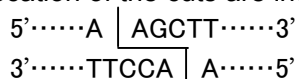
HPLC purification:

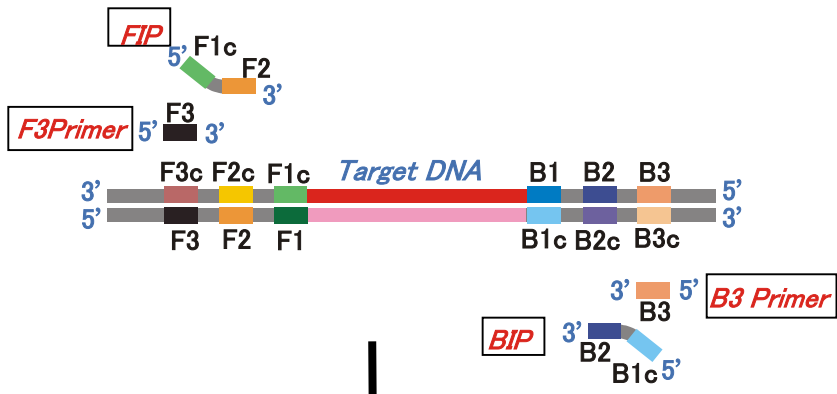A purity grade for synthetic oligonucleotides.

The LAMP method:

The LAMP method (Loop-mediated isothermal Amplification) is a simple, rapid, specific and cost-effective nucleic acid amplification method solely developed by Eiken Chemical Co., Ltd. As a gene amplification technology, compared to PCR, it has higher specificity and amplification efficiency, and has the advantage that it can perform the amplification at a constant temperature

around 65°C.

　The two important features of isothermal amplification are: ① DNA synthesis occurs by DNA polymerase with strand displacement activity without the need for thermal cycles for double-strand denaturation ⇒annealing -⇒DNA synthesis ② Four primers (recognizing 6 distinct regions in the target gene) are used to amplify the gene, whereby the loops formed at the end of the synthesized strand proceed to self-primed DNA synthesis.　The following Figure describes the steps in amplification.
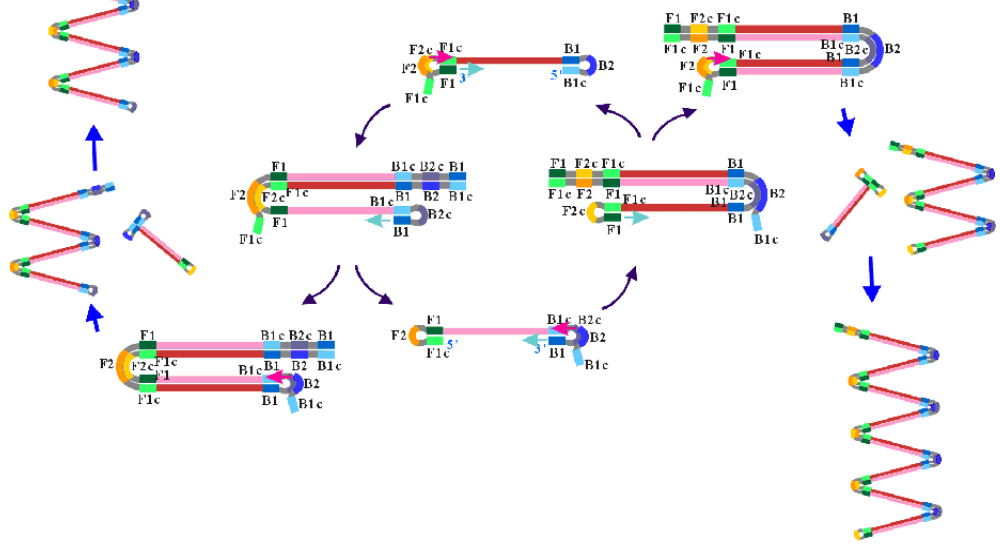
(1)

(2)

(3)

By adding four primers (1), after a few steps of reaction, single stranded structure with a loop structure at each end is formed. (2) This serves as the starting point where primers will anneal to proceed to the amplification. As a result of this process, various sized structures consisting of alternately inverted repeats of the target sequence on the same strand are formed.

Loopamp DNA Amplification kit:

A reagent kit designed for LAMP amplification for research use.   The contents are: the buffer, substrate and DNA polymerase with strand displacement activity.   The user prepares the LAMP primers designed for the target gene of interest.   The system can then be used in a variety of applications.

Loop primers:

The Loop Primers (either Loop Primer B or Loop Primer F), containing sequences complementary to the single stranded loop region (either between the B1 and B2 regions, or between the F1 and F2 regions) on the 5' end of the dumbbell-like structure, provide an increased number of starting points for DNA synthesis by the LAMP method.   Loop primers provide an increased number of starting points for DNA synthesis resulting in shorter amplification time and higher specificity.

M13 phage:

Filamentous single-stranded DNA phage.   Infects the host E coli through the F pili, and is incorporated into the bacteria.   In the host, the single-stranded DNA changes into a double-stranded replicative form, and this template is used to form single-stranded DNA.   Once taken up by the newly formed progeny phage particles, the host bacteria do not lyse but rather releases phage particles.   This phage is also useful as a cloning vector, and is widely used to prepare single stranded DNA for use in DNA sequencing by the dideoxy method.

Nearest-Neighbor method:

A method for estimating the Tm of DNA and has now come to be the standard.   The Tm is determined by taking into account the thermodynamic factors of the neighboring bases under the following formula.

$$Tm = \Delta H \times 1000/(\Delta S + R \ln(C/4)) - 273.15 + 16.6 \log[Na^+]$$

R: gas constant = 1.987cal/°C/mol

ΔH: enthalpy (kcal/mol)

ΔS: entropy (eu)

C: oligonucleotide concentration (M)

$[Na^+]$: sodium ion concentration

PCR:

PCR (polymerase chain reaction) involves repeated DNA synthesis reactions of a specified DNA sequence bracketed by 2 primers and can amplify the specified DNA region by several 10,000 folds.   The amplification primers used are usually the synthetic oligonucleotides containing the bases at both ends of the region to be amplified, and the reaction involves repeated steps of 1) denaturation of double-stranded DNA, 2) annealing of oligonucleotide, and 3) DNA polymerase-mediated complementary strand synthesis (repeated usually 20-30 times). Developed by Cetus Co., in 1985.

TE buffer：

Nucleic acid buffer (10mM Tris- HCl (pH 8.0), 1 mM EDTA).   Contains divalent metal ion (Mg^{2+} etc) chelator EDTA (ethylenediamine- N,N,N',N'- Tetraacetic acid, a chelator which can remove divalent metal ions in samples), which inhibits the divalent metal-ion dependent nucleases (nucleic acid degrading enzyme) and preserves the nucleic acids.

Tm:

Refers to the melting temperature of biological polymers. For nucleic acid in solution, elevation in temperature leads to destruction of the hydrogen bonds responsible for the base pairing. The temperature at which the DNA loses the double stranded structure, so that 50% is double stranded structure and 50% is single stranded, is the Tm. There are three hydrogen bonds in the GC pair and two in the AT pair, so that DNA with higher content of GC base pairs is resistant to thermal denaturation and thus has higher Tm. The efficiency of binding to primers is generally expressed as Tm.

Annealing:

After the double-stranded DNA has been denatured to the single-stranded form, annealing refers to the re-binding of the denatured single-stranded DNA back into a double-stranded DNA. The DNA-specific double stranded helix is formed, so that the annealing can be called renaturation. With double-stranded DNA, heat or alkaline treatment causes denaturation into single-stranded DNA. Denatured double stranded DNA under certain conditions forms the hydrogen bonds and forms a complete double helical structure.

Oligo concentration:

In the text, the oligo concentration in the slide on p43 refers to the oligonucleotide concentration, namely the primer concentration.

5' end, 3' end:

In nucleic acids all nucleotides are bound to the next nucleotide via phosphate diesterification between the 5-carbon on the 5-carbon sugar to the 3-carbon on the next sugar. At both ends these exist as -OH groups, called the 5' end and 3' end. For a given nucleic acid, the left hand side is generally the 5' end or upstream, while the right hand side is the 3' end or downstream.

Cloning:

Gene cloning refers to the isolation of DNA in which unfractionated DNA fragments are inserted into vectors to form recombinants, which are introduced and propagated in the hosts as colonies or plaques and then the desired DNA is identified, isolated and purified.

Free energy:

A type of thermodynamic coefficient. This refers to the thermodynamic equilibrium standard under standard experimental conditions. In systems where change of state is possible, the change tends to go in the direction of lowest free energy. As with chemical reactions, in the chemical equilibrium state, the free energy of the system is minimized. Currently used free energy terms include the Gibbs free energy and the Helmholtz free energy.

Restriction enzyme:

Name of enzyme that recognizes and cuts DNA at specific sequences. Classified as types I, II and III depending on the needed cofactors for enzyme activity and the type of cut made. Widely distributed among microorganisms, the enzyme type or recognition sequence differs depending on the microbial strain, so that numerous types are available.

Real-time turbidity detection:

Gene amplification is conducted by LAMP and its detection can be done by simultaneously monitoring the white turbidity caused by the existence of magnesium pyrophosphate, the amplification by-products. Thus, real time detection can be achieved. The detection of the magnesium pyrophosphate turbidity is facilitated by the efficiency and specificity of the LAMP amplification reaction.

Electrophoresis:

Method for separation and analysis of DNA. It takes advantage of the observation that when electric current applied to a substances, it can result in the movement of substances to the positive or negative electrode. In applying the current, the buffer, filters, gels, carrier ampholytes, are used.

In nucleic acid electrophoresis, relatively large molecular weight DNA (60 - 100kbp) are separated by agarose gels, while smaller molecular weight DNA (1 kbp or less) are separated using acrylamide gels.

Secondary structure:

The primer secondary structure refers to the hairpin structure that can form if the primer is complementary to itself. Depending on the primer sequences, the likelihood for hairpin formation can differ greatly. If the primer itself forms hairpin structures, the primer becomes unable to bind to the target gene or can bind to unexpected genes, thus resulting in false positives.

Primer:

In general, the term primer refers to the oligonucleotide that forms a double strand with the target gene and supplies the 3'-OH needed to initiate the DNA polymerase-mediated elongation reaction. Through the activity of the DNA polymerase, the complementary nucleotides are added to the 3' –OH group of the primer on the template DNA sequence, so that the elongation proceeds from the 5' side to the 3' side.

Primer dimer:

This term refers to the structure that forms when a primer hybridizes to another primer. In the gene amplification method involving DNA synthesis in a test tube, it is necessary to have the primer concentration in the reaction mix at a concentration that is far greater than the Target gene concentration, so that if the structure of the primer permits the hybridization of a primer to itself, primer dimers can form and thus inhibit the hybridization to the Target gene.

Plain text format:

Containing only the sequences information in the following format.

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ctcgaggact ggggaccctg caccgaacat ggagaacaca acatcaggat tcctaggacc
cctgctcgtg ttacaggcgg ggtttttctt gttgacaaga atcctcacaa taccacagag
tctagactcg tggtggactt ctctcaattt tctagggga gcacccacgt gtcctggccc
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Mutation:

Refers to spontaneously occurring gene mutation. Changes occurring in the base sequence of a gene can result in a change in the genetic makeup. Gene mutation can be at the level of the genome, chromosome, a part of a chromosome, gene or nucleotide. Depending on how a gene has changed, the mutation can be classified also as a point mutation, deletion, duplication, inversion, insertion, translocation, etc. Mutations can occur in various forms, so its effect at the level of expression can vary greatly from noticeable significant changes to those only detectable after statistical analysis.

Terminal end stability:

The stability of the formation (ease of formation) of double stranded region formed between the template gene and each primer at the 3' end and the 5' end. The LAMP Primer designing software determines the stability as the ΔG (change in free energy) calculated by the Nearest-Neighbor method.

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