Cat. # 9104

For Research Use

TaKaRa

TaKaRa DEXPAT™ Easy

(DNA Extraction from Paraffin-embedded Tissue Easy)

Product Manual

v201903Da

Table of Contents

I. Description

TaKaRa DEXPAT Easy is designed for simple and rapid extraction of PCR-ready DNA from Formalin-fixed paraffin-embedded (FFPE) tissues. This kit provides a ready-to-use optimized mixture of a special surfactant and PCR inhibitor-absorbent resin predispensed in 1.5-ml microtubes. Simply add paraffin-embedded tissue sections into one of the microtubes, incubate at 100℃, centrifuge at 4℃ and cool on ice for 5 min to obtain PCR-ready DNA in approximately 30 min. The refrigerated centrifugation following heat treatment allows the formation of a gel-like layer above the absorbent resin. When collecting the DNA solution, the gel-like layer prevents contamination of the absorbent resin into the DNA solution. The absorbent resin may inhibit PCR amplification.

Extraction of DNA from paraffin-embedded tissue sections usually involves a very time-consuming and labor intensive process. TaKaRa DEXPAT Easy, however, offers a rapid and simplified procedure with the following advantages :

1. No deparaffinizing step
2. Takes Only 30 min for the entire process
3. Yields PCR template-grade DNA (for amplification of DNA <400 bp)
4. No toxic substances
5. Increased analytical precision by site-specific extraction
6. Good separation of the DNA aqueous solution and the absorbent resin

This product retains the performance of TaKaRa DEXPAT (Cat. #9091), and has the added benefits of one reaction mixture predispensed into each tube and an improved composition designed to well separate the DNA aqueous solution and the absorbent resin during DNA recovery.

Note: Since DNA extracted from paraffin-embedded tissue sections likely had been degraded during fixation or paraffin embedding, PCR amplifications of ≥400 bp are difficult in most cases. The quality of extracted DNA depends on the conditions used to fix and embed tissue.
This product is for DNA extraction from mammalian paraffin section to detect mammalian genes. It cannot be available for the detection of bacteria or fungi genes.

II. Components

TaKaRa DEXPAT Easy	500 μl x 50

III. Storage

4℃

IV. Materials Required but not Provided

- Micropipettes
- Refrigerated microcentrifuge: A capability of about 17,000*g**1 is preferable.
  A centrifuge that runs at ≥15,000*g**2 will work, but the yield of DNA aqueous solution will be lower than that from a 17,000*g* centrifuge.
  - *1 17,000*g*: about 13,500 rpm with a 9 cm radius rotor
  - *2 15,000*g*: about 12,500 rpm with a 9 cm radius rotor

  Please refer to the manual for your centrifuge to check the rpm.
- Block heater (100℃)
- Microtubes (for recovered DNA solution)
- Laboratory gloves

V. Intended Use

DNA extraction from Formalin-fixed paraffin-embedded (FFPE) tissues for use as PCR templates.

VI. Protocol

1. Extraction from paraffin-embedded tissues

a. Prepare paraffin-embedded tissue sections that are about 5 μm thick and use sterilized tweezers to place 1 to 3 pieces into a 1.5 ml microtube containing TaKaRa DEXPAT Easy.
   - The size of tissue sections should at least be 6 mm x 6 mm and the thickness may be 4 - 10 μm.
   - Wear laboratory gloves during all procedures to avoid nuclease contamination, which causes DNA degradation.
   - Disinfect microtomes used to cut sections with a hydrogen peroxide disinfectant and then wipe with ethanol.
   - Treat apparatuses such as blades or tweezers in the same manner as described above and irradiate with UV for at least 10 min to avoid cross contamination from residual DNA.

b. Cap the microtube and incubate at 100℃ for 10 min. Gently invert the microtube two to three times 5 min later from incubation starting to ensure even mixing.
   - Use a block heater for convenience.
   - The microtube is hot. Please handle with care to avoid burn injuries.

c. Place the microtube in a microcentrifuge precooled to 4℃ immediately after heat treatment and centrifuge at 17,000*g* for 10 min at 4℃.

d. Remove the microtube immediately upon completion of microcentrifugation and place on ice. Let stand on ice for 5 min.

e. Use a micropipette to collect the aqueous layer while avoiding the paraffin thin layer formed at the top. (Figure 1 and 2) You will see a gel-like layer above the absorbent resin. Collect the aqueous layer on top of this gel-like layer.
   - The collected aqueous layer (DNA solution) may be used directly as a template for PCR reactions.
   - When using a conventional PCR enzyme such as *TaKaRa Ex Taq*®, the DNA solution volume should be no more than 1/10 of the PCR reaction mixture (5 μl for a 50 μl PCR reaction).
   - PrimeSTAR® GXL DNA Polymerase, which is fairly resistant to inhibitors, may improve the PCR reactivity. Also in this case, the DNA solution volume should be no more than 1/10 of the PCR reaction mixture.
   - With MightyAmp™ DNA Polymerase, the DNA solution volume may be up to about 4/10 of the reaction mixture (20 μl for a 50 μl PCR reaction system). This is because MightyAmp DNA Polymerase is designed for crude samples and shows great resistance to inhibitors.

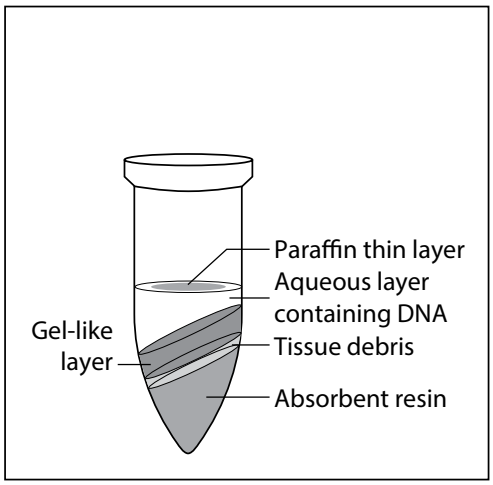


Figure 1

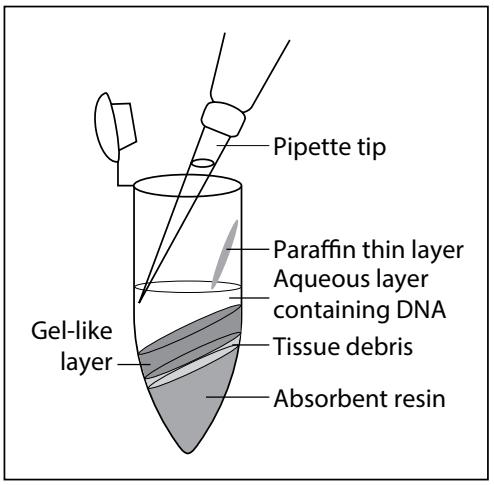


Figure 2

Flow Chart Comparing Extraction Methods

<TaKaRa DEXPAT Easy>

Place 4 - 10 μm thick paraffin-embedded tissue sections (1 - 3 pieces) into a 1.5-ml microtube containing TaKaRa DEXPAT Easy

↓

Incubate at 100℃ for 10 min

↓

Centrifuge at 17,000*g* at 4℃ for 10min

↓

Place on ice for 5min

↓

Collect DNA solution (→ PCR reacion)

TIme required: 30 min

<Conventional Extraction Method>

(Paraffin-embedded tissue sections)
Deparaffinizing

↓

Drying

↓

Protein removal for 1 - 2 days

↓

Phenol-chloroform extraction

↓

Ethanol precipitation

↓

Drying, dissolution

↓

DNA solution (→ PCR reaction)

Time required: 2 - 3 days

2. Site-defined extraction

Site-defined extraction is used to recover DNA from part of a paraffin-embedded tissue section fixed on a glass slide.

a. Use a felt-tip marker to draw on the underside of the glass slide an outline of the part of tissue from which DNA is to be recovered.

b. Incubate a 1.5 ml microtube containing TaKaRa DEXPAT Easy at 100℃ for 1 min.
   - Use a block heater for convenience.
   - The microtube is hot. Please handle with care to avoid burn injuries.

c. Pipette TaKaRa DEXPAT Easy with a 1 ml pipette tip and place a small amount on the outlined paraffin section. Scrape this section off the slide using the pipette tip and recover all of the TaKaRa DEXPAT Easy and debris.

   Note: As long as the unmarked part of the tissue is left unscraped, there is no risk of contamination and no need to mask the unmarked part. If you need to recover both the lesion and the normal tissue separately from the same slide, recover the lesion first and then the normal tissue in the same manner.

d. Cap the microtube and incubate at 100℃ for 10 min. Gently invert the microtube two to three times 5 min later from incubation starting to ensure even mixing.
   - The microtube is hot. Please handle with care to avoid burn injuries.

e. Place the microtube in a microcentrifuge precooled to 4℃ immediately after heat treatment and centrifuge at 17,000*g* for 10 min at 4℃.

f. Remove the microtube immediately upon completion of microcentrifugation and place on ice. Let stand on ice for 5 min.

g. Use a micropipette to collect the aqueous layer while avoiding the paraffin thin layer formed at the top (Section VI.1, Figure 1 and 2). You will see a gel-like layer above the absorbent resin. Collect the aqueous layer on top of this gel-like layer.
   - The collected aqueous layer (DNA solution) may be used directly as a template for PCR reactions.
   - When using a conventional PCR enzyme such as *TaKaRa Ex Taq*, the DNA solution volume should be no more than 1/10 of the PCR reaction mixture (5 μl for a 50 μl PCR reaction system).
   - PrimeSTAR GXL DNA Polymerase, which is fairly resistant to inhibitors, may improve the PCR reactivity. Also in this case, the DNA solution volume should be no more than 1/10 of the PCR reaction mixture.
   - With MightyAmp DNA Polymerase, the DNA solution volume may be up to about 4/10 of the reaction mixture (20 μl for a 50 μl PCR reaction system). This is because MightyAmp DNA Polymerase is designed for crude samples and shows great resistance to inhibitors.

Flow chart (Extraction from a defined region)

Use a felt-tip marker to draw an outline on the underside of the slide of the part of tissue to be recovered.

↓

Use heated TaKaRa DEXPAT Easy to recover the target part of the section on the slide.

↓

Incubate at 100℃ for 10 min

↓

Centrifuge at 17,000*g* at 4℃ for10 min

↓

Place on ice for 5 min

↓

Collect DNA solution (→ PCR reaction)

VII. Precautions for Use

The feasibility of PCR is directly dependent on the conditions used for tissue fixation and embedding. Please refer to the general precautions below for tissue fixation and embedding.

1. The recommended fixative solution is 10% formalin. The penetration rate is about 1 mm per hour. Small pieces of biopsy specimen are directly soaked in the fixative. Large pieces of specimens may be nicked to help fixative penetration. The recommended fixation time is no more than 3 days. Please also refer to the AMeX fixation method recommended by Sato, *et al.*[2)]
2. Follow the conventional embedding method consisting of ethanol dehydration, chloroform replacement, followed by embedding in paraffin with a melting point of 56 - 58℃. Use fresh reagents when the target is an exogenous gene, such as a viral gene.

VIII. Frequently Asked Questions

Q1 : Can RNA be extracted with this reagent?

A1 : No. This reagent is designed for DNA extraction and therefore cannot be used to extract RNA.

Q2 : Can this reagent be used for DNA recovery from samples other than paraffin-embedded tissues?

A2 : Yes. It has been shown to work on frozen tissue sections (Section IX.3). Takara Bio has not used this reagent on deparaffinized tissue sections.

Q3 : Can DNA be extracted with this reagent from tissues fixed on slides?

A3 : It does not work on specimens scraped off slides or removed by xylene. To extract DNA from tissues on slides, follow the protocol for site-defined extraction (Section VI.2). Note that the dye from stained tissues may inhibit PCR reactions.

Q4 : Can extracted DNA be measured by absorption spectrometry?

A4 : The recovered DNA cannot be measured by UV absorbance because this reagent is for recovery of DNA, but not for purification of DNA.

Q5 : Can extracted DNA be analyzed by electrophoresis?

A5 : The amount of extracted DNA is too small to run on gel electrophoresis.

Q6 : How long can extracted DNA be preserved?

A6 : In some cases extracted DNA are stored for up to 3 months at 4℃ and up to 1 year at -20℃.

Q7 : How much aqueous layer can be collected?

A7 : Generally, 200 μl or more can be collected.

Q8 : Incubation and centrifugation following the protocol yielded no aqueous layer. What could be done to improve the yield?

A8 : The amount of tissue section used may be too much. Try the following to see if they help.

- Decrease the size of the tissue section.
- Increase centrifugation speed (run the centrifuge at the maximum rpm).
- Make sure TaKaRa DEXPAT Easy is mixed well during incubation.

When processing a large number of samples (microtubes), the thin paraffin layer on the top may sometimes not solidify after 10 min of refrigerated centrifugation. If that happened, either prolong the centrifugation time or reduce the number of samples (microtubes) in each batch of centrifugation to allow adequate cooling.

Q9 : No target DNA band was observed after PCR amplification with *TaKaRa Ex Taq* using recovered DNA as template. What was the cause and what can be done?

A9 : 1) The extracted DNA may be contaminated with PCR inhibitors.

→ Use a PCR enzyme such as MightyAmp DNA Polymerase Ver.2 (Cat. #R071A/B)* and MightyAmp DNA Polymerase Ver.3 (Cat. #R076A/B)* that can better withstand inhibitors. The amount of DNA solution to use is up to 4/10 of the reaction mixture MightyAmp.

* Not available in all geographic locations. Check for availability in your area.

2) The expression level of the target gene may be low. Recovered DNA may have suffered extensive breakage during fixation and embedding.

→ Use more DNA solution by switching to MightyAmp DNA Polymerase Ver.2 as PCR enzyme.

Note: When using a conventional PCR enzyme, the amount of DNA solution that can be used is no more than 1/10 of the reaction mixture. Purification of the extracted DNA solution by ethanol precipitation can remove inhibitors and allow more DNA to be added to a reaction mixture. Alternatively, use of MightyAmp DNA Polymerase, which is designed for crude samples, may raise the DNA solution volume to 4/10 of the reaction mixture volume without compromising the reaction.

< Method for DNA purification by ethanol precipitation >

1. Estimate the volume of the recovered DNA extract.
2. Add 3 M sodium acetate in an amount equivalent to 1/10 of the estimated DNA volume.
3. Add ethanol or isopropanol in an amount equivalent to 2.5 times the estimated DNA volume or in equal volume, respectively.
4. Invert to mix evenly.
5. Let stand at -20℃ for 30 - 60 min.
6. Centrifuge at 4℃ , 12,000*g* for 10 - 15 min.
7. Remove the supernatant and add 1 ml of 70% ethanol.
8. Centrifuge at 4℃ and 12,000*g* for 10 - 15 min.
9. Carefully discard the supernatant and allow to air dry.
10. Dissolve in an appropriate amount of buffer (e.g. TE buffer).

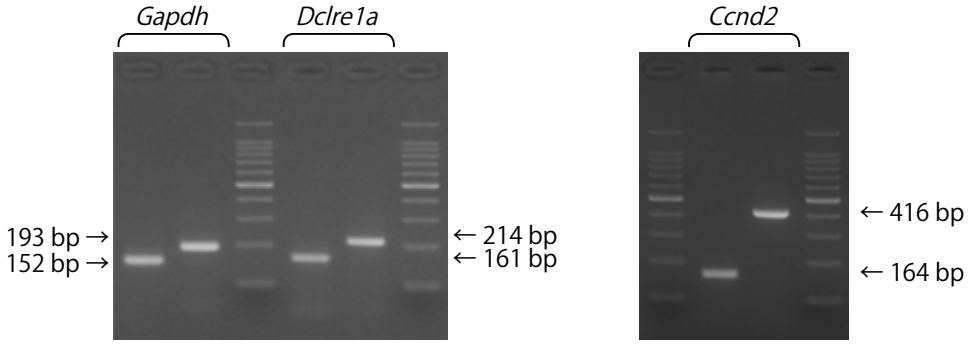
IX. Experimental Examples

1. PCR amplifications of *Gapdh, Dclre1a* and *Ccnd2* from rat testis tissue.

DNA was extracted using TaKaRa DEXPAT Easy from a paraffin-embedded rat testis tissue section .The DNA solution was used as a template in PCR amplifications with *TaKaRa Ex Taq.*

Template : 2.5 μl of extracted DNA
PCR Mixture : 25 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :
94℃ 30 sec
54℃ 60 sec
72℃ 60 sec
] 35 cycles
↓
72℃ 5 min



M：100 bp DNA Ladder (400 ng)

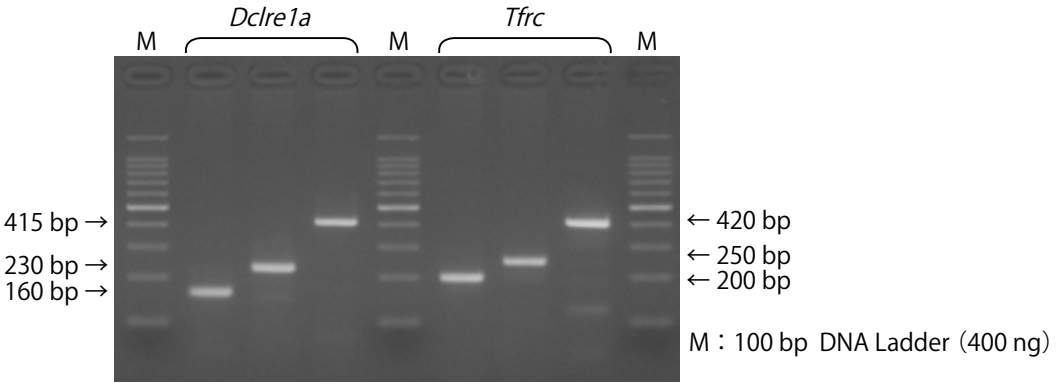
Result: PCR amplification of extracted DNA. yielded products of expected size.

2. PCR amplification of *Dclre1a* and *Tfrc* genes from mouse foot tissue

A DNA solution extracted by TaKaRa DEXPAT Easy from a paraffin-embedded mouse foot tissue section was used as template for PCR amplification of *Dclre1*a and *Tfrc* genes.

Template : 2.5 μl of extracted DNA
PCR Mixture : 25 μl
Polymerase : *TaKaRa Ex Taq*

PCR condition :
94℃ 30 sec
54℃ 60 sec
72℃ 60 sec
] 35 cycles
↓
72℃ 5 min



M：100 bp DNA Ladder（400 ng）

Result: PCR amplification of extracted DNA yielded products of expected size.

3. PCR amplification of *Dclre1a* and *Tfrc* genes from frozen mouse tissue sections

Sample : Frozen mouse tissue sections (1-day old, whole body)

DNA extraction :

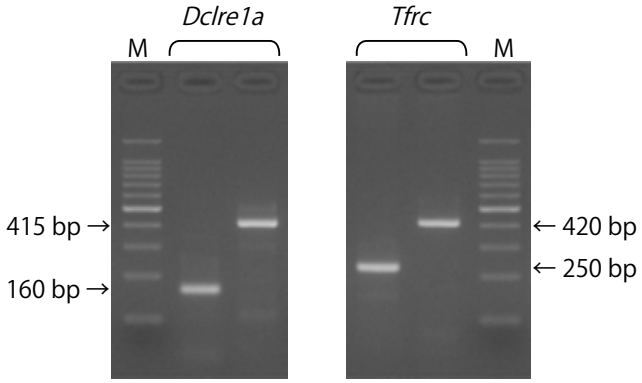
Preincubate 500 μl of TaKaRa DEXPAT Easy to 100℃
↓
Place the aqueous layer of DEXPAT on part of the tissue section on a glass slide, scrape off the section and transfer to a microtube
↓
Incubate at 100℃ for 10 min (mix once after 5 min)
↓
Centrifuge (17,000*g*, 4℃, 10 min)
↓
Place on ice, 5 min
↓
Transfer the aqueous layer to a new microtube

PCR amplification :

Template : 2.5 μl of extracted DNA
PCR Mixture : 25 μl
Polymerase : *TaKaRa Ex Taq*

PCR conditions :

94℃ 30 sec
54℃ 60 sec
74℃ 60 sec
] 35 cycles
↓
72℃ 5 min



M：100 bp DNA Ladder（400 ng）

Result: Amplification of each target DNA was confirmed.

M : 100 bp DNA Ladder (400 ng)

4. Real-time PCR using DNA extracted from paraffin-embedded rat and mouse tissue sections on glass slides

DNA template : Add 2.5 μl of each of the 5 serial 10 - fold dilutions, up to a 10^4 - fold dilution, of a DNA solution extracted from rat or mouse tissue sections

Target genes : Rat *Gapdh* and mouse *Tfrc*

PCR Mixture : 25 μl

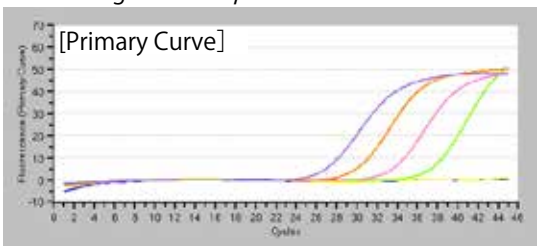
Reagent : TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. # RR420A)

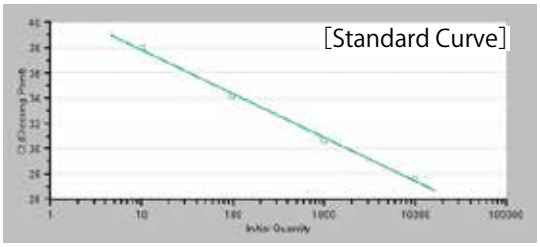
Apparatus : Thermal Cycler Dice™ Real Time System *II* (Cat. # TP900)

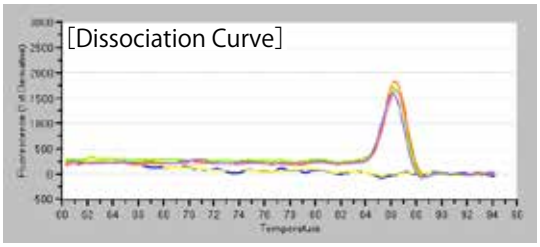
PCR condition :

95℃ 30 sec
↓
95℃ 5 sec
60℃ 30 sec] 35 cycles
↓
Dissociation

Target : Rat *Gapdh*

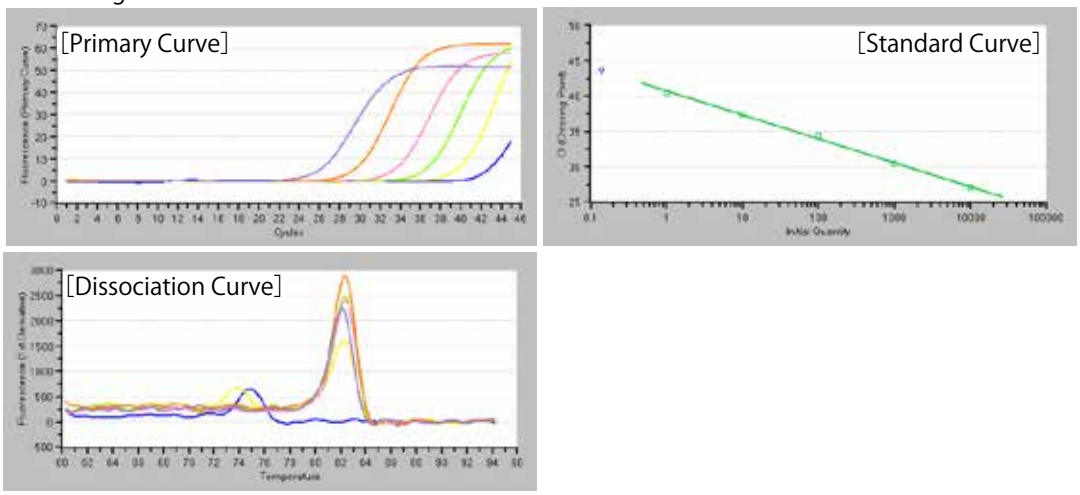






Result: Real-time PCR using DNA extracted from a paraffin-embedded rat tissue section resulted in detection of the *Gapdh* gene in diluted stock solution up to a 10^3 - fold dilution.

Target : Mouse *Tfrc*



Result: Real-time PCR using DNA extracted from a paraffin-embedded mouse tissue section resulted in detection of the *Tfrc* gene in diluted stock solution up to a 10^4 - fold dilution.

X. References

1) Goelz, S.E. *et al. BBRC*. (1985) **130**: No. 1, 118-126.
2) Sato, Y. *et al* Byori to Rinsho [Japanese] (supplementary volume),(1990) **8**: 432-453.

XI. Related Products

TaKaRa DEXPAT™ (Cat. #9091)
MightyAmp™ DNA Polymerase Ver.2 (Cat. #R071A/B)*1
MightyAmp™ DNA Polymerase Ver.3 (Cat. #R076A/B)*1
TaKaRa Ex Taq® (Cat. #RR001A/B/C)
PrimeSTAR® GXL DNA Polymerase (Cat. #RR050A/B)
TB Green® *Premix Ex Taq* ™ (Tli RNaseH Plus) (Cat. #RR420A)*2

*1 Not available in all geographic locations. Check for availability in your area.

*2 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series". These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.