AmpEasy™ Tissue PCR Kit

Cat. No.:	YG101	YG102
Product Name:	AmpEasy™ Tissue PCR Kit	
Reactions:	10	100
Sample:	All biological samples, such as animal tissues, yeast, bacteria, blood, buccal swab, hair, plant tissues, seeds...etc.	
Format:	Reagent format	
Operation:	Incubation	
Operation Time:	10 Minutes	

Introduction

AmpEasy™ Tissue PCR Kit contains all the reagents necessary to rapidly extract genomic DNA from a wide range of biological samples and amplify targets of interest by PCR. Genomic DNA could be extracted within 10 minutes by single-step. The novel one-step extraction eliminates the needs for freezing of cells or tissues with liquid nitrogen, mechanical disruption, organic extraction, column DNA purification, or alcohol precipitation. RBC CoolTaq™ DNA Polymerase and PCR buffers are all supplied for amplification directly after the extraction.

Features

One-tube & single-step extraction of genomic DNA from biological samples.
Cells, tissues or plant tissues to PCR in 10 minutes.
Polymerase and PCR buffers are all supplied for amplification directly after the extraction.

Contents

ITEM	YG101	YG102
AmpEasy™ Extraction Buffer	500ul	5ml
AmpEasy™ Extraction Buffer Enhancer	25ul	250ul
Primer U18S-F(10uM)	10ul	20ul
Primer U18S-R(10uM)	10ul	20ul
Primer P18S-F(10uM)	10ul	20ul
Primer P18S-R(10uM)	10ul	20ul
10X PCR Reaction Buffer(with 20 mM Mg^{2+})	100ul	500ul
10mM dNTPs Mix	10ul	50ul
RBC CoolTaq™ DNA Polymerase	10ul	50ul

Applications

Purified DNA is ready for direct use in detection of low copy genes, PCR-based genotyping, such as Standard PCR, Multiplex PCR, RAPD PCR, SSR PCR.

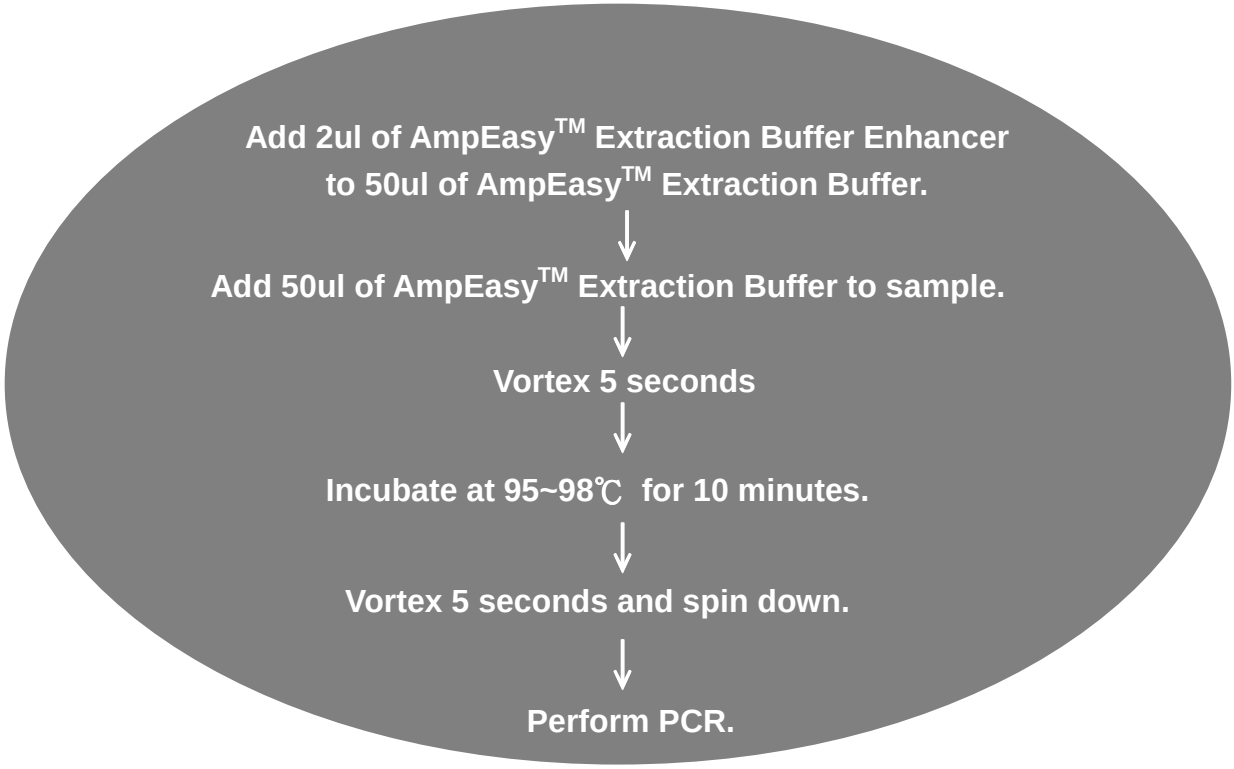
Quality Control

The quality of AmpEasy™ Tissue PCR Kit is tested on a lot-to-lot basis.

Storage Conditions

AmpEasy™ Tissue PCR Kit should be stored upon receipt at -20℃ in a constant temperature freezer. AmpEasy™ Tissue PCR Kit can be stored for up to 12 months without showing any deduction in performance and quality with proper storage.

Protocol



1. DNA Extraction

(1) Add 2ul of AmpEasy™ Extraction Buffer Enhancer to 50ul of AmpEasy™ Extraction Buffer. Mix the extraction buffer well before use. (If precipitates have formed in the AmpEasy™ Extraction Buffer Enhancer, warm the enhancer solution in a 37℃ water bath to dissolve, and then mix well.)

(2) Add 50 µl of AmpEasy™ Extraction Buffer to sample up to 1 mm^3, 1 mg or 2 µl, in a microcentrifuge tube and mix well by tapping the bottom of the tube or pipetting several times in the tube if necessary. Make sure the sample block is submerged in the buffer. (For samples like 0.1X serum, saliva and sputum, up to 50 µl of the samples can be extracted using 50 µl of the buffer.)

(3) Vortex 5 seconds. (optional for liquid sample)

(4) Heat at 95-98℃ for 10 minutes.

(5) Vortex and centrifuge briefly.

(6) 1-2 ul of the mixture can then be used immediately for PCR amplification or qPCR analysis.

Note:

(1) If necessary, extracted DNA can be stored at -20°C for at least one week without losing DNA quality for PCR amplification.

(2) For those difficult samples that contain paraffin, phenolic compounds, heavy metals or some unknown inhibitory metabolites, a 10~100X serial dilution of the lysate is recommended before PCR amplification. The dilution can be done simply using PCR-grade water.

2. PCR Amplification

A. Components for standard PCR.

Components	Volume	Final Concentration
10X PCR Reaction Buffer	2.5 ul	1X
10mM dNTPs Mix	0.5 ul	0.2 mM
Primer F (10 µM)	0.5 ul	0.2 µM
Primer R (10 µM)	0.5 ul	0.2 µM
Mixture of sample and extraction buffer	1-2 ul	n/a
RBC CoolTaq™ DNA Polymerase (5u/µl)	0.5 ul	2.5 units
ddH_2O	Add to 25 µl	

B. Suggested Reaction Parameters.

Segment	No. of Cycles	Temperature	Duration
1	1	94°C	5 minutes*
2	35-38	94 °C (Denaturation)	45 seconds
		Primer Tm-5 °C	45 seconds
		72 °C (Extension)	1 minute/kb
3	1	72 °C	10 minutes
4	n/a	4 °C	∞

* For HotStart DNA Polymerase, such as RTH01, RTH02, RT101, RT101, use 15 minutes.

3. Post-amplification analysis

After amplification, PCR products can be analyzed using 1% agarose gel.

Primer Information

Primer	Forward sequence	Reverse sequence
16S	5'-CTCCTACGGGAGGCAGCAG-3'	5'-GWATTACCGCGGCKGCTG-3'
U18S	5'-GCTTGTCTCAAAGATTAAGCC-3'	5'-TGATCCTTCTGCAGGTTCACCTAC-3'
P18S	5'-AACGGCTACCACATCCAAGG-3'	5'-CCGAAGGCCAACACAATAGG-3'
β-GBN	5'-CAACTTCATCCACGTTCACC-3'	5'-GAAGAGCCAAGGACAGGTAC-3'

Primer	Specificity	*Length of PCR products*
16S	Bacteria	198 bp
U18S	Yeasts, Fungi, Animals	~1750 bp
P18S	Higher Plants	446 bp
β-GBN	Animals	268 bp

Sample		Primers
Bacteria	*E. coli DH5α*(single colony or cell pellet)	16S
Yeast	*Yarrowia lipolytica* (single colony)	U18S
	Saccharomyces cerevisiae (single colony)	
Fungi	Auricularia polytricha (1 mm3)	
	Pleurotus eryngii (1 mm3)	
Insect	One Entire Drosofila (Fruit Fly)	
ICR mouse	Mouse blood (2 ul)	
	Mouse Tail Tip (1 mm3)	
	Paraffin Embedded Mouse Liver (1 mm3)	β-GBN
Human	Hair (root end of 3 pieces of hair)	
	Oral mucosal cells	
	1ul of human blood treated with EDTA or Heparin (fresh ~ 4 weeks stock)	Human IP-10 Gene
Higher Plant	A variety of samples ex: leaves or seeds (1 mm3)	P18S
	Green tea or oolong tea bag (10X~100X serial dilution from 1 mg sample)	
E. Coli Plasmid	4~36 transformed E. Coli cells (containing pUC18 derived plasmids)	pUC18 FR
Fish	Meat of Grouper (less than 1 mg)	β-Actin