EZ-Genotyping™ Kit

Cat# GE2XXA-1

User Manual

Store kit at -20°C on receipt

Contents

I. Introduction 1
A. Overview of EZ-Genotyping™ Kit 1
B. Highlights of EZ-Genotyping™ Kit 2
II. Product Information 2
Table 1. Components of EZ-Genotyping Kit (GE200A-1) 2
III. Genotyping Protocol 3
A. Overview of Workflow 3
Figure 1. Workflow of the EZ-Genotyping Kit 3
B. Protocol 3
Table 2. Recommended Amounts of CellExtract Solution for Different Well Sizes 3
IV. Troubleshooting 5
Table 3. Recommended Solutions for Troubleshooting 5
V. Applications 5
VI. Performance Data and Applications 5
Figure 2. Comparison of PCR amplification in genomic DNA extracts using EZ-Genotyping Kit vs purified genomic DNA 6
Figure 3. Successful amplification and allelic discrimination of multiple genomic markers (DNA fingerprinting) 6
Figure 4. Genotyping results for validation of allele status in gene targeting assay using homologous recombination (HR) targeting vector 7
Figure 5. PCR results for positive control primers in EZ-Genotyping Kit demonstrates robust and consistent amplicon formation in different mammalian samples 7
VII. Technical Support Information 7
VIII. Licensing and Warranty 8

I. Introduction

A. Overview of EZ-Genotyping™ Kit

The EZ-Genotyping Kit provides reagents to effectively determine the genotypes of target alleles in most mammalian samples using a robust endpoint PCR reaction. The complete kit contains sufficient reagents for genomic DNA extraction from 50 samples and subsequent PCR reactions. This genotyping kits offers a fast and streamlined solution for genotyping analysis of cultured mammalian cells. A major advantage of the kit is that genomic DNA

extraction is carried out in a single tube - mitigating chances for sample cross-contamination for subsequent PCR analysis. The kit is amenable to high throughput-based screening and suitable to several downstream applications such as identification of genotypes in mixed/clonal cell populations, DNA fingerprinting, and cell identity/contamination analysis

B. Highlights of EZ-Genotyping™ Kit

1) **Easy to use**- no need for overnight digestion or column purification of genomic DNA.

2) **Complete Package** - contains both genomic DNA template preparation and 2X PCR Master Mix for PCR reaction.

3) **Robust Results** - the complete solution was tested to successfully amplify multiple targets on different chromosomes.

4) **Reliable** - positive control primers are included to ensure successful preparation and subsequent amplification of genomic targets to assure the absence of false negative results.

5) **Economical**- The kit provides enough reagents for carrying out ~50 genotyping reactions.

II. Product Information

Product Name: **EZ-Genotyping Kit™**
Catalog Number: **GE200A-1**
Shipping Condition: **Blue Ice or Dry Ice**
Storage Condition: **-20 ^{0}C**

Table 1. Components of EZ-Genotyping Kit (GE200A-1)

COMPONENTS	DESCRIPTION	VOL
CellExtract Solution (GE210A-1)	Simple, one-step solution for sample DNA extraction without DNA purification	5 mL
2x PCR Master Mix	2xPCR Mix contains all the components (e.g. dNTPs, MgCl2, Taq) necessary to perform PCR reaction	500 µl
Positive Control Primer Set	10uM of each primer designed to give 212 bp PCR product in most species	25 µl/each

Note: Please avoid multiple freeze-thaw cycles for the 2X PCR Master Mix. The 2X PCR Master Mix can be kept at + 4^0 C after thawing for a period of ~3 month without significant change in performance.

III. Genotyping Protocol

A. Overview of Workflow

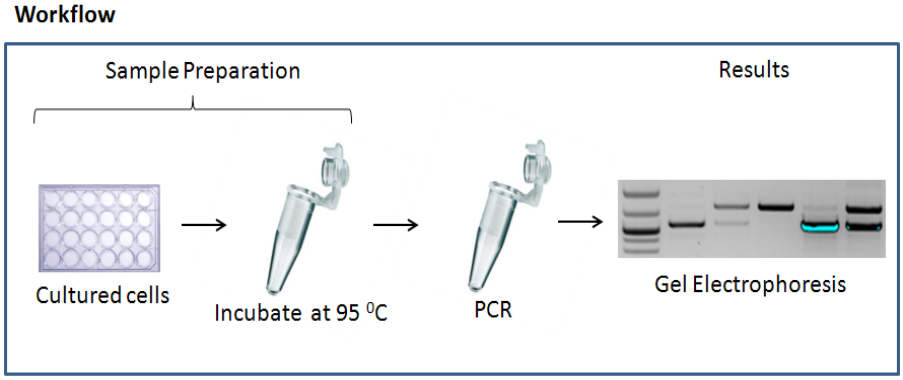


Figure 1. Workflow of the EZ-Genotyping Kit

B. Protocol

1. Genomic DNA Extraction

(Example of cells in culture using a 24-well plate, volume can be scaled up/down depending on plate size)

1. Remove culture medium and wash cells 2x in each well with 0.5 ml of PBS
2. Remove all of the PBS from the wells
3. Add 100 µl of CellExtract Solution to each well
4. Dislodge the cells in each well by pipetting the solution up and down several times to form a cell extract
5. Transfer the cell extract to an Eppendorf tube and pipette up and down the extracts an additional 3-5 times
6. Heat the extract at 95 ^{0}C for 2 min.
7. Centrifuge the heated extract at 14, 000 RPM for 2 min.
8. Save the supernatant as it contains the genomic DNA, which is ready for PCR amplification

Table 2. Recommended Amounts of CellExtract Solution for Different Well Sizes

CULTURE DISH	SURFACE AREA (cm^2)	CELL NUMBER	CELLEXTRACT SOLUTION (µL)
96-Well	0.3	1-4x10^4	25~50
48-Well	1	2-8x10^4	50
24-Well	2	0.5-2x10^5	100
12-Well	4	1-4x10^5	250
6-Well/35mm	9.5	2-9x10^5	400
60mm/T25	28	5-30x10^5	500

2. Genotyping PCR Setup

Add the following reagents to a 0.2 or 0.5 ml PCR tube in the following order:

REAGENT	VOLUME	POSITIVE CONTROL
2xPCR Mix	10 µL	10 µL
Forward Primer (10 µM)	1.0 µL	1.0 µL (10 µM of each)
Reverse Primer (10 µM)	1.0 µL	
Template	2 µL	2 µL
ddH2O	6 µL	7 µL
Total Volume	20 µL	20 µL

PCR Conditions:

1. Denature the template at 94C for 3 min.
2. Denature at 94C for 30 s, Anneal primers at 60C for 30 s, Extension at 72C for 10 s per every 100bp
   32-34 cycles of amplification are recommended
3. Store at 4C indefinitely.

3.Agarose Gel Electrophoresis

The percentage of agarose gel (1~2.5%) to use for a given PCR product size.

Resolution of Linear DNA on Agarose Gels.

Recommended % Agarose	Optimum Resolution for Linear DNA
0.5	1,000–30,000bp
0.7	800–12,000bp
1.0	500–10,000bp
1.2	400–7,000bp
1.5	200–3,000bp
2.0	50–2,000bp

IV. Troubleshooting

Table 3. Recommended Solutions for Troubleshooting

PROBLEM	REASON	POSSIBLE SOLUTIONS
Non-specific bands	Mispriming	A) Prepare PCR reaction on ice B) "Hotstart " by pre-heating the thermocycler to 94 degree before adding samples or use a hotstart-specific Taq polymerase
No bands	Extracts contain no genomic DNA Primers errors	A) Run positive control B) Design and order new primers in case primer degradation or incorrrectness
Poor Amplification	Too much or too little DNA	Run a dilution series of DNA samples including a non-diluted sample
Weak or absent bands	Low number of cycles	Increase the number of cycles or decrease the annealing temperature

V. Applications

The follow applications have been successfully tested by using the EZ-Genotyping Kit.

1. Genotype analysis in mixed/clonal cell populations
2. DNA fingerprinting analysis
3. Culture cell identity and contamination checking

VI. Performance Data and Applications

1. Reliable and unambiguous genotyping results:

Reliability and consistency are important factors in choosing a genotyping method. Due to less-than-optimal genotyping conditions for many commercial and "homemade" protocols, the PCR analysis sometimes lead to weak or ambiguous bands. SBI's EZ-Genotyping kit provides a reliable solution for amplifying various amounts of genomic DNA in extracts compared to those of purified DNA templates (Figure 2 below).

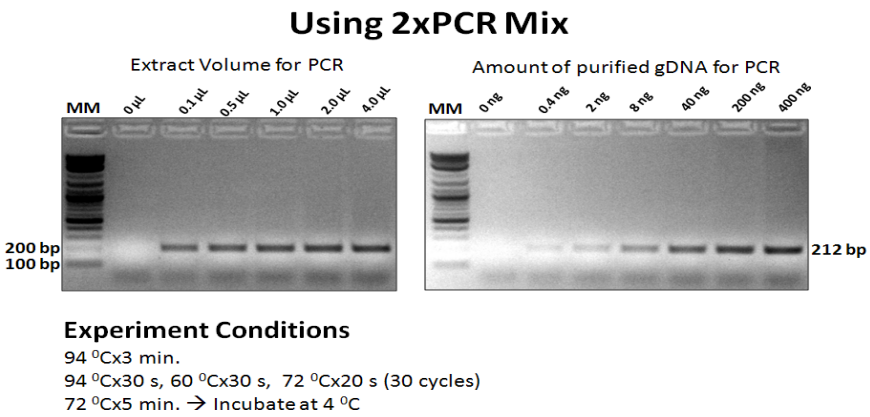


Figure 2. Comparison of PCR amplification in genomic DNA extracts using EZ-Genotyping Kit vs purified genomic DNA

2. Genes with different allele sizes and chromosome locations can be successful amplified:

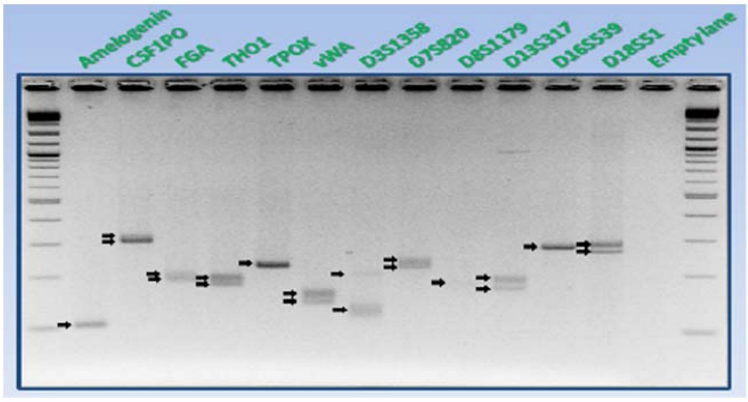
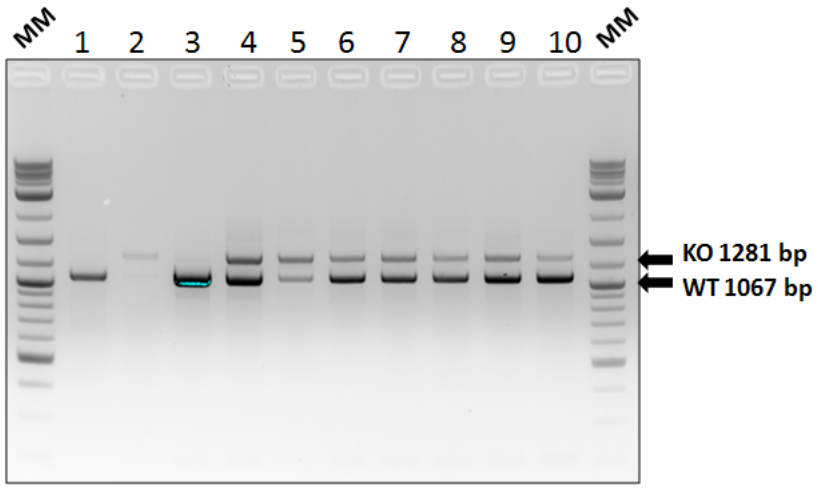


Figure 3. Successful amplification and allelic discrimination of multiple genomic markers (DNA fingerprinting)

3. Confirmation of mono- or bi-allelic modifications by genotyping



WT: Samples 1 and 3;
KO Mono- allele: Samples #4, #5, #6, #7, #8, #9, #10
KO Bi-allele: Sample #2

Figure 4. Genotyping results for validation of allele status in gene targeting assay using homologous recombination (HR) targeting vector

4. Positive controls:

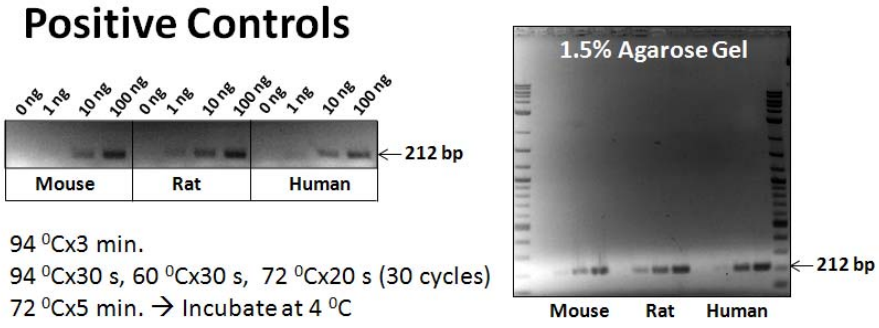


Figure 5. PCR results for positive control primers in EZ-Genotyping Kit demonstrates robust and consistent amplicon formation in different mammalian samples

.

VII. Technical Support Information

For more information about SBI products and to download manuals in PDF format, please visit our web site:
http://www.systembio.com
For additional information or technical assistance, please call or email us at:
System Biosciences (SBI)
265 North Whisman Rd.
Mountain View, CA 94043

Phone: (650) 968-2200
(888) 266-5066 (Toll Free)
Fax: (650) 968-2277
E-mail:
General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VIII. Licensing and Warranty

Limited Use License

Use of the EZ-Genotyping Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a credit. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a credit limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.