Ten-minute DNA Release Kits

- A novel approach to obtain DNA easily in modern biological science

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Abstract: Genomic DNA study has been used widely in biology, genetics, reproduction, clinical medicine and clinical medicolegal. This article describes characterization and protocol of the new product 10-minute DNA Release Kit that provides a reliable, simple, and quick approach, by taking 10 minutes at 86˚C for DNA release and desired DNA extract obtaining. The product will save scientists' time and give better result on the DNA extract. [Nature and Science. 2006;4(2):58-70].

Keywords: DNA; extract; knowledge; PCR

1. Introduction

Genomic DNA study has been used widely in biology, genetics, reproduction, clinical medicine and clinical medicolegal. It is very important for the genomics study to obtain the desired DNA extract easily and quickly. The 10-minute DNA Release Kit provides a reliable, simple, and quick approach, by taking 10 minutes at 86˚C for DNA release and desired DNA extract obtaining.

The 10-minute DNA Release Kit not only offers a wide range on the desired DNA extract (Figure 1), but also gives a good expression of multi-primers in polymerase chain reaction (PCR) (Figure 2). And, it is not only used for on genome expression by the analysis of PCR, but also used on mitochondrial DNA mutation for the diagnosis and treatment observation of mitochondrial disease, which can be done by the analysis of PCR/RFLP (Restriction Fragment Length Polymorphism) and point mutation (Figure 3).

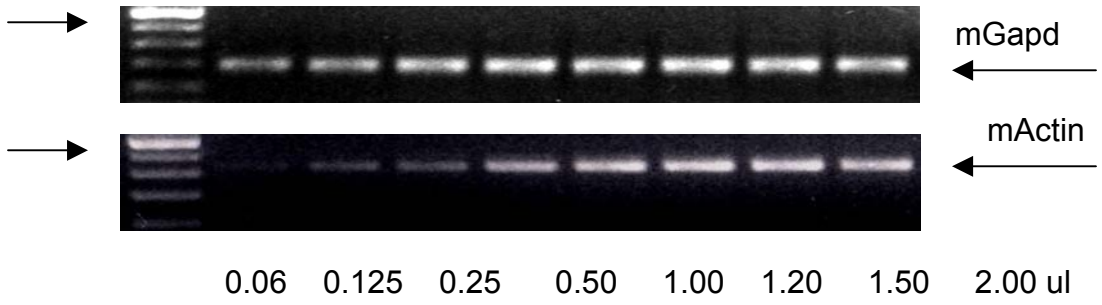
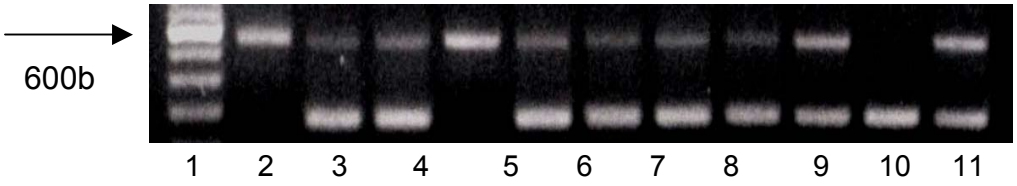


Figure 1. mGapdh and mActin expression on the DNA volume-dependent effective with the DNA Extract from mice tail



The expression of P53 +/+ at No. 1, 4;
P53 +/- at No. 2, 3, 5, 6, 7, 8, 9, 11;
P53 -/- at No. 10

Figure 2. Genotyping the P53 transgenic mouse with 10 Minute DNA Kit

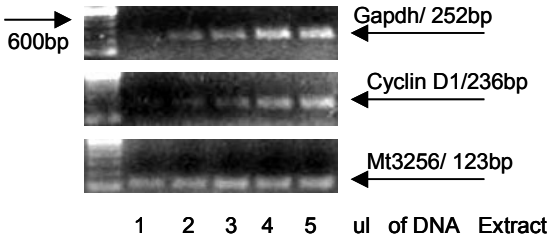


Figure 3A. Primer expression on DNA volume-dependent effective with the DNA extract from human blood

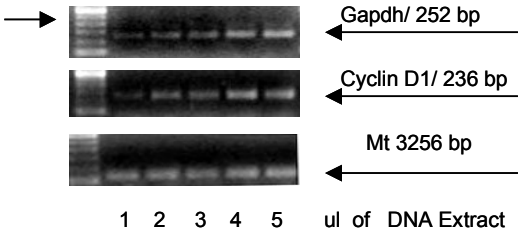


Figure 3B. Primer expression on DNA colume-dependent effective with the DNA extract from human urine

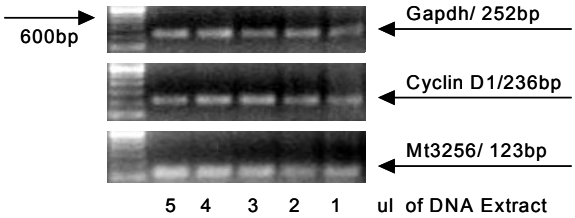


Figure 3C. Primer expression on DNA volume-dependent effective with the DNA extract from human Saliva

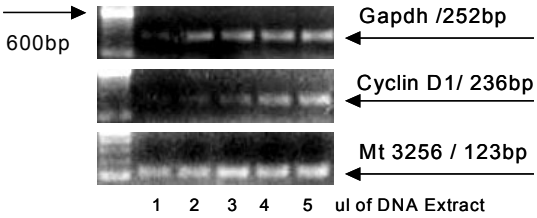


Figure 3D. Primer expression on DNA volume-dependent effective with the DNA extract from human Hair Follicle

Figure 3. Primer expression on DNA volume-dependent effective with the DNA extract

(1) Primer, Gapdh and Cyclin D1 are designed from human Genomic DNA Sequence.

Gapdh	Start	Sequence	Size
Left prime	108	GAA GGT GAA GGT CGG AGT CA	252bp
Right primer	359	TTG ATT TTG GAG GGA TCT CG	
Cyclin D1			
Left primer	2096	CCA TTC CAT TTC CAA GCA CT	236bp
Right primer	2331	TCA TCCTGG CAA TGT GAG AA	

(2) Primer mt3256 is designed from human mitochondrial DNA sequence.

This is to analyze myDNA mt3256 (C->T) mutation. Normally, mt3256 point is the C-Cytosine. The C is replaced with T-thymine when the 3256 point mutation occurred. The mt3256 mutation is associated with MELAS (Mitochondrial Encephalomyopathy, lactic Acidosis, and Strokelike Episodes).

Mt3256	Start	Sequence	Size
Left primer	3230	GTT AAG ATG GCA GCG CCC GGT AAG CG	123bp
Right primer	3353	GCG ATT AGA ATG GGT ACA ATG	

PCR program: 33 cycles; 50 seconds at 95˚C; 50 seconds at 56˚C; 60 second at 72˚C.

2. The Kits 1-7 function description and protocols

2.1 Ten-minute DNA Release Kit-1, for mouse tail, ear-DNA-PCR. Cat# JZ-001.

From the mouse tail, ear and any organs tissue to obtain the desired DNA to run PCR for the transgenic mouse genetyping, PCR/RFLP-point mutation and any gene expression.

2.2 Ten-minute DNA Release Kit-2, for mouse tail and tissue–purified DNA-PCR and Southern etc. The catalogue number is Cat#JZ-002.

From the mouse tail or any tissue to obtain the purified DNA within 20 minutes after the DNA release. The ratio of DNA OD260/280 nm is higher than 1.70. With the purified DNA to run PCR and Southern for the transgenic mouse genetyping, PCR/RFLP-point mutation and any other gene expression.

2.3 Ten-minute DNA Release Kit-3, for blood-DNA-PCR. Cat# JZ-003.

2.4 Ten-minute DNA Release Kit-4, for urine-DNA-PCR. Cat# JZ-004.

2.5 Ten-minute DNA Release Kit-5, for saliva-DNA-PCR. Cat# JZ-005.

2.6 Ten-minute DNA Release Kit-6, for hair follicle-DNA-PCR. Cat# JZ-006.

From the blood, urine, saliva and hair follicle to obtain the desired DNA to run PCR for PCR/RLFP-point mutation and any other gene expression. The desired DNA extract shows the volume dependent effective on the different genomic DNA expression (Figure 3A,B,C,D).

2.7 Ten-minute DNA Release Kit-7, for culture cells-DNA-PCR-Southern etc. Cat# JZ-007.

From the cultured cells to obtain the purified DNA easily and quickly. The purified DNA could be used for any purpose on the genomic DNA expression.

3. Detail Descriptions of Ten-minute DNA Release Kits 1-7

3.1 Ten-minute DNA Release Kit-1

Ten-minute DNA Release Kit-1 is for mouse tail or ear-DNA-PCR, and for the smallest tissue, body liquid pellet and the material stained by biologic trace also. Catalogue number of Ten-minute DNA Release Kit-1 is JZ-001.

The preliminary protocol of Ten-minute DNA Release Kit-1 is as the following

3.1.1 Aim

3.1.1.1 It is easy and quick from mouse tail or ear to obtain the desired DNA to run PCR for transgenetic mouse genetyping.

3.1.1.2 It is also easy and quick to obtain the desired DNA extract form any organ tissue and body liquid pellet.

3.1.1.3 It is special to obtain the desired DNA extract from material which was stained by blood on any biologic body liquid.

3.1.1.4 For the detail of items b and c to see the protocols 5 and 6 on the purpose of Kit-1 gene expression, please read the Kit-1 protocol 5 first.

3.1.2 Have been ready for the equipments?

3.1.2.1 It is fine for this kit to have any type of thermo machine or water bath which could set up the constant temperature between 86-90˚C.

3.1.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g should be available.

3.1.2.3 1.5 ml Eppendorf tubes, tips and micro-pipettor with the scalar 10-200 ul.

3.1.2.4 Vortex or mixture machine is available.

3.1.3 Contents Table (Table 1)

Table 1. Ten-minute DNA Release Kit-1 contents table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified for 6 moths at 5-29? C.
JZ-001-1	Kit1-B1	4 ml	100	2.Warm to RT before using if it was kept at 4? C.
JZ-001-2	Kit1-B2	6 ml		

3.1.4 Work Table (Table 2)

Table 2. Ten-minute DNA Release Kit-1 work table

Steps	Action	Show
Step 1	Place a mouse tail 1.5-3 mm, or ear tissue 2x3 mm, (or any tissue and/or material stained the body liquid) to a eppendorf tube. Add 40 ul of Kit-1 B1 to the tube. See show.	
Step 2	Put the tube at 86?C in a Thermo-Mathine or Water Bath, not shaking, for 6-8 minutes (86-90?C is allowed).	
Step 3	Add 60 ul of Kit-1 B2 to the tube, flick it 3-5 times, then keep the tube at room temperature (RT) for 2-3 minutes.	
Step 4	Centrifuge the tube at 15,000 g X 3 minutes. in RT. (The 0-4?C is not allowed).	
Step 5	Pipette the clear aqueous phase 60 ul to a fresh tube, which is the desired DNA extract for PCR. Take 1-3 ul DNA as the templet to run PCR in 20-25 reaction volume.	
Note	If you use the kit first, it's better to do expression on your primer by taking the DNA extract 1-3 to run PCR separately to test the working range.	

3.1.5 Treating for Another Organs Tissue:

3.1.5.1 Cut organ tissue to 1-3 mm^3 (1-12 mg).

3.1.5.2 If the organ tissue is with blood or another dirty trace, please:

3.1.5.2.1 Rinse 2-3 times with PBS without Ca++ and Mg++.

3.1.5.2.2 Touch dry the tissue on the paper towel, then follow the work table.

3.1.5.3 If the organ tissue is clean, like skin etc, go to follow the work table.

3.1.5.4 For the other small tissue:

3.1.5.4.1 The tissue is punching from human skin, animal ear or any other organs

3.1.5.4.2 The tissue is obtained by laser punching or scrape from human or animal.

3.1.5.4.3 If the tissue is embedded by paraffin, you can scrap a few pieces to an eppendorf tube.

3.1.5.5 For the material that is stained by biologic trace: Cut the material to the smallest pieces and place the smallest pieces to an eppendorf

tube, then follow the work table to process it like to do mouse tail.

3.1.5.6 For any deposit pellet which is from any body liquid: Place the body liquid to an eppendorf, centrifuge at 5000 g X 3, get rid of the supernatant, keep the pellet in the tube, and go to follow the work table.

3.1.6 Buffer Volume for the Another Organs Tissue

The ratio of Kit-1 B1 and Kit-1 B2 is 1:1.5, which means according to the tissue or liquid pellet size, if you choose to use 1 ul of Kit1-B, you need to use 1.5 ul of Kit1-B2.

3.2 Ten-minute DNA Release Kit-2

Ten-minute DNA Release Kit-2 is for mouse tail and tissue-purified DNA—PCR-Southern. It is for 100 samples. Its catalogue number is JZ-002.

The preliminary protocol of Ten-minute DNA Release Kit-2 is as the following:

3.2.1 Aim

3.2.1.1 It is easy and quick to obtain purified DNA from mice tail or any tissue after the DNA release within 20 minutes. The ratio of DNA OD260/280 nm is between 1.70-1.90. The purified DNA can be used to run PCR or Southern for the transgenic mice genetyping etc.

3.2.1.2 If you want to use the Kit to obtain purified DNA from any tissue for any purpose of gene expression, please read protocol Kit 2-6 first.

3.2.2 Have been ready for the equipments?

3.2.2.1 It is fine for this kit to have any type thermo-machine, or water bath if they can set up the constant temperature between 86-90˚C.

3.2.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g is needed for the running.

3.2.2.3 1.5 ml eppendorf tubes, tips and micro-pipettor with the scalar of 10-200 ul are needed.

3.2.2.4 Vortex or mixture machine is needed.

3.2.3 The reagents are prepared by you

3.2.3.1 Isopropanol (2-propanol, molecular biology reagent, minimum 99%) is needed. This could be ordered from any chemical company.

3.2.3.2 0.1 x TE buffer that is made by 1/10 of 1 x TE plus 9/10 of distilled water.

To prepared 100 ml of 1 X TE Buffer (pH 7.3) following the below:

Reagents	Final Concentration	Volume	Stock solution
Tris-HCl	10 mM	1.0 ml	1 M Tris-HCl, pH 7.3
EDTA	1 mM	0.2 ml	0.5M EDTA, pH 8.0
Distilled water		98.8 ml	
Total volume		100 ml	

3.2.4 Content Table (Table 3)

Table 3. Ten-minute DNA Release Kit-2 contents table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified fro 6 moths at 5-29? C.
JZ-002-1	Kit2-B1	15 ml	100	2.Warm to RT before using if it was
JZ-002-2	Kit2-B2	6 ml		kept at 4? C.

3.2.5 Work Table (Table 4)

Table 4. Ten-minute DNA Release Kit-2 work table

Step	Action	Show
Step 1	Place a mouse tail 4-6 mm (or tissue 6-20 mg) in eppendorf tube, add 150 ul of Kit-2 B1 to the tube, as the tube 1. See show 1.	Show 1 
Step 2	Put tube 1 at 86?C in a thermo-machine or a water bath, not shaking, for 10 minutes. The 86-90?C is allowed.	
Step 3	Place tube 1 at room temperature (RT) for 2 minutes. Add 60 ul of the Kit-2 B2 to tube 1. Flick the tube 4-6 times (or Vortex 3-5 seconds), then place the tube at RT for 2-3 minutes.	
Step 4	Centrifuge tube 1 at 15,000 g X 3 minutes at RT. Pipette the clear aqueous phase 170 ul from tube 1 to a fresh tube, as tube 2.	
Step 5	Add Isopropanol (2-propanol) 150 ul to tube 2, invert the tube 2 6-10 times. Then, centrifuge it at 15,000g X 3 minutes at RT.	
Step 6	The DNA pellet* should be seen in bottom of the tube 2. Romove the supernatant with micro-pipettor. Then add 1 ml of 70% alcohol to tube 2, wash the DNA pellet by inverting the tube 4-6 times and centrifuge it again, or not.**	Show 2 
Step 7	Pour the alcohol out from the Tube 2, then place it on the paper towel to dry the DNA pellet 4-6 minutes at RT. Show 2	
Step 8	Add 32 ul of 0.1X TE buffer to Tube 2. keep it at RT for 8-10 minutes. Flick the tube 6-8 time, the DNA should be dissolved well.	
Step 9	1) To dilute the purified DNA 5-15 times with 0.1 X TE buffer. Then take 1-2 ul as the templet to run PCR in 20-25 reaction volume. 2) To measure the DNA ratio and concentration by Southern blot etc. 3) To keep at 4?C for long time using.	
*	It is easy to see the DNA pellet in the 70% alcohol.	
**	It is unnecessary to centrifuge if the DNA pellet is sticking on bottom of the tube with 70% alcohol.	

3.2.6 Treating for Another Organ Tissue:

3.2.6.1 Cut another organ tissue to 2-3 mm^3 (6-20 mg).

3.2.6.2 If the organs tissue is with blood or another dirty trace, please do the following:

3.2.6.2.1 Rinse 2-3 times with PBS without Ca++ and Mg++.

3.2.6.2.2 Touch dry the tissue on the paper towel. Then, to follow the work table.

3.2.6.3 If the tissue isn't adhered the blood etc., like the clean skin or muscle, it is fine to follow the work table to obtain the desired purified DNA extract.

3.3 Ten-minute DNA Release Kit-3

The Ten-minute DNA Release Kit-3 is for blood-DNA-PCR. The catalogue number is JZ-003.

The preliminary protocol of Ten-minute DNA Release Kit-3 is as the following:

3.3.1 Aim

3.3.1.1 With Ten-minute DNA Release Kit-3 it is easy and quick (5-6 minutes) to obtain desired DNA from blood to run PCR.

3.3.1.2 The DNA could be used for any gene expression to follow your purpose.

3.3.2 Have been ready for the equipments?

3.3.2.1 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g setting up.

3.3.2.2 1.5 ml eppendorf tubes, tips and micro-pipettor with the scalar of 10-200 ul.

3.3.2.3 Vortex or mixture machine.

3.3.3 Content Table (Table 5)

Table 5. Ten-minute DNA Release Kit-3 content table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified for 6 moths at 5-29? C.
JZ-003-1	Kit3-B1	1.2 ml	100	2.Warm to RT before using if it was kept at 4? C.
JZ-003-2	Kit3-B2	1.8 ml		
JZ-003-3	Kit3-B3	5.0 ml, 10 X*	* diluted with D.W 45 ml to be 1X before using	

3.3.4 Work Table (Table 6)

Table 6. Ten-minute DNA Release Kit-3 work table

Step	Action	Show
Processing 1, The red blood cells lysis		Show1 
Step1	Ready a Eppendorf tube with 500ul of Kit3-B3. Place one drop(15-30ul) of whole blood (heaprinized or not) or bone marrow to the tube. Invert it 4-6 times.Centrifuge the tube at 10,000 g x 1 min. in room temperature.	
Step2	The white blood cell pellet with red cells piece(Blood Cells Pellet)should be seen on the tube bottom. With a pipet to remove the red supernatant. Show 1. **(If your blood sample is 15 or 20 ul/each. Go ahead from step2 to step4 directly.)**	
Step3	Add D.W 500 to the tube. Vortex or flick it 6-8 sec.(times), centrifuge again.	
Step4	Remove the the supernatant as clean as possible. Keep the blood cells pellet in the tube bottom. Show 2.	Show1 
Processing 2, 2 minute DNA release		
Step5	Add 12 ul of Kit3-B1 to the tube with the ready blood cells pellet. Flick it until the pellet that was dissolved. And add 18 ul of Kit3-B2 to the tube again.	
Step6	Flick it 3-5 time to be mixtured well. The total 30 ul volume has be ready DNA extract. Take 1-3 ul to run PCR in a 20-25 ul reaction volume.	
Note	If using the kit first. It is better for a good experiment result by taking 1-3 ul ready DNA extract to run PCR separately to test the working range on the primer target.	

3.4 Ten-minute DNA Release Kit-4

The Ten-minute DNA Release Kit-4 is for urine-DNA-PCR. Its catalogue number is JZ-004.

The preliminary protocol of Ten-minute DNA Release Kit-1 is as the following:

3.4.1 Aim

3.4.1.1 It is easy and quick from urine to obtain desired DNA to run PCR.

3.4.1.2 The DNA can be used for any gene expression to follow your purpose.

3.4.2 Have been ready for the equipments?

3.4.2.1 It is fine for this kit to have any type of thermo-machine or water bath which can set up the constant temperature between 86-90˚C.

3.4.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g.

3.4.2.3 1.5 ml eppendorf tubes, tips and micro-pipettor with the scalar of 10-200 ul.

3.4.2.4 Vortex or mixture machine which is fine to have or have not.

3.4.3 Content Table (Table 7)

Table 7. Ten-minute DNA Release Kit-4 content table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified for 6 moths at 5-29?C.
JZ-004-1	Kit4-B1	1.4 ml	100	2.Warm to RT before using if it was kept at 4?C.
JZ-004-2	Kit4-B2	2.1 ml		
JZ-004-3	Kit4-B3	5.0 ml, 5 X*	* diluted with D.W 20 ml to be 1X before using	

3.4.4 Work Table (Table 8)

Table 8. Ten-minute DNA Release Kit-4 work table

Processing 1. Collect the urine deposit pellet		
Step	**Action**	**Show**
Step 1	Place urine 1 ml to a eppendorf tube. Add 150 ul of Kit-4 B3 to the tube, mixtured. Centrifuge at 12,000 g X 1 minute.	
Step 2	The urine deposit pellet is in the tube bottom. Remove the supernatant. Add 100 ul of Kit-4 B3 again, resuspend the pellet by flicking or pipette, centrifuge as above.	
Step 3	Remove supernatant and keep pellet in the tube bottom.	
	See show	
Processing 2. 10 Minute DNA release		
Step 4	Add 14 ul of Kit-4 B1 to the tube with urine deposit pellet. Flick it 4-6 time (or vortex). Put it in a thermo-machine or a water bath, not shaking, at 86?C (to 90?C is allowed) for 10 minutes. Then, put it at room temperature for 2 minutes.	
Step 5	Add 21 ul of Kit-4 B2 to the tube, flick it 4-6 times. Centrifuge it at 12,000 g x 3 minutes.	
Step 6	Transfer the supernatant 30-36 ul (DNA is inside) to a fresh eppendorf tube. The desired DNA extract has bee ready. Take 1-3 ul is as the template to run PCR in 20-25 reaction volume.	
Note	If you are first time to use the kit, it is better for the gene expression by trying 1-3 ul separate to run PCR to test the working range for the primers target.	

3.5 Ten-minute DNA Release Kit-5

The Ten-minute DNA Release Kit-5 is for Saliva-DNA-PCR. Its catalogue number is JZ-005.

The preliminary protocol of Ten-minute DNA Release Kit-5 is as the following:

3.5.1 Aim

3.5.1.1 It is easy and quick to obtain desired DNA from saliva to run PCR.

3.5.1.2 The DNA can be used for any gene expression for your purpose.

3.5.2 Have been ready for the equipments?

3.5.2.1 It is fine for this kit to have any type thermo-machine or water bath which can set up the constant temperature between 86-90˚C.

3.5.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g.

3.5.2.3 1.5 ml eppendorf tubes, tips and micro-pipettor with the scalar of 10-200 ul.

3.5.2.4 Vortex or mixture machine is fine to have or have not.

3.5.3 The PBS is made by you

Making the 1 X, PBS without Ca++ and Mg++:

Reagents	1 x, working solution, pH 7.3±	Weight
NaCl	137 mM	8.0 g
KCl	2.7 mM	0.2 g
Na_2HPO_4-$7H_2O$	4.3 mM	1.15 g
KH_2PO_4	1.4 mM	0.2 g .

Dissolved in 1000 ml of distilled water.

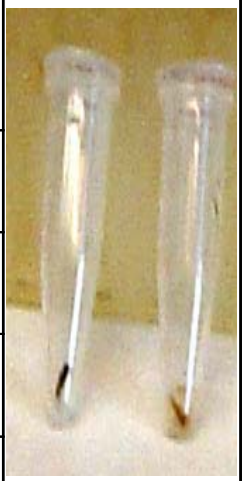
3.5.4 Content Table (Table 9)

Table 9. Ten-minute DNA Release Kit-5 content table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1. It is qualified for 6 moths at 5-29?C.
JZ-005-1	Kit1-B1	1.4 ml	100	2.Warm to RT before using if it was kept at 4?C.
JZ-005-2	Kit1-B2	2.1 ml		

3.5.5 Work Table (Table 10)

Table 10. Ten-minute DNA Release Kit-5 work table

Steps	Action	Show
Step 1	Place a mouse tail 1.5-3 mm, or ear tissue 2x3 mm, (or any tissue and/or material stained the body liquid) to a eppendorf tube. Add 40 ul of Kit-1 B1 to the tube. See show.	
Step 2	Put the tube at 86?C in a Thermo-Mathine or Water Bath, not shaking, for 6-8 minutes (86-90?C is allowed).	
Step 3	Add 60 ul of Kit-1 B2 to the tube, flick it 3-5 times, then keep the tube at room temperature (RT) for 2-3 minutes.	
Step 4	Centrifuge the tube at 15,000 g X 3 minutes. in RT. (The 0-4?C is not allowed).	
Step 5	Pipette the clear aqueous phase 60 ul to a fresh tube, which is the desired DNA extract for PCR. Take 1-3 ul DNA as the templet to run PCR in 20-25 reaction volume.	
Note	If you use the kit first, it's better to do expression on your primer by taking the DNA extract 1-3 to run PCR separately to test the working range.	

3.6 Ten-minute DNA Release Kit-6

Ten-minute DNA Release Kit-6 is for hair follicle-DNA-PCR. Its catalogue number is JZ-006.

The preliminary protocol of Ten-minute DNA Release Kit-1 is as the following:

3.6.1 Aim

3.6.1.1 It is easy and quick to obtain desired DNA from hair follicle to run PCR.

3.6.1.2 The DNA can be used for any gene expression for your purpose.

3.6.2 Have been ready for the equipments?

3.6.2.1 It is fine for this kit to have any type thermo-machine or water bath which could set up the constant temperature between 86-90˚C.

3.6.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g.

3.6.2.3 1.5 ml eppendorf tubes, tips and micro-pipette with the scalar 10-200 ul.

3.6.2.4 Vortex or mixture machine which is fine to have or have not.

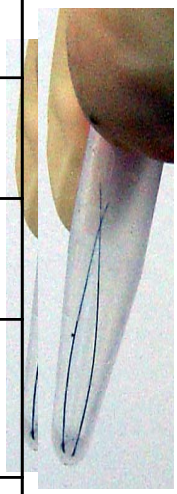
3.6.3 Content Table (Table 11)

Table 11. Ten-minute DNA Release Kit-6 content table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified for 6 moths at 5-29? C.
JZ-006-1	Kit6-B1	1.2 ml	100	2.Warm to RT before using if it was kept at 4? C.
JZ-006-2	Kit6-B2	1.8 ml		

3.6.4 Work Table (Table 12)

Table 12. Ten-minute DNA Release Kit-6 work table

Step	Action	Show
Step 1	Ready an eppendorf with 12 ul of Kit-6 B1. Place hair follicle 2-5 in the buffer. See show.	
Step 2	Put the tube at 86?C in a thermo-machine or water bath, not shaking, for 10 min.	
Step 3	Flick the tube 3-5 times, then keep it at room temperature for 2 minutes.	
Step 4	Add 18 ul of Kit-6 B2 to the tube, flick it again. Throw the follicles out. The 30 ul DNA extract in the tube has been ready.	
Step 5	Take 1-3 ul as the template to run PCR in 20-25 ul reaction volume.	
Note	If you use the kit first time, it is better expression on your primer by taking the DNA extract 1-3 to run PCR separately to test the working range.	

3.7 Ten-minute DNA Release Kit-7

Ten-minute DNA Release Kit-7 is for cells-DNA-PCR and Southern. The catalogue number is JZ-007.

The preliminary protocol of Ten-minute DNA Release Kit-7 is as the following:

3.7.1 Aim

3.7.1.1 It is easy and quick from the adhered culture cells to obtain purified DNA for PCR or Southern.

3.7.1.2 The DNA can be used for any gene expression for your purpose.

3.7.2 Have been ready for the equipments?

3.7.2.1 It is fine for this kit to have any type thermo-machine or water bath which can set up the constant temperature between 86-90˚C.

3.7.2.2 A room temperature eppendorf centrifuge machine with the speed 10,000-15,000 g.

3.7.1.3 1.5 ml eppendorf tubes, tips and micro-pipettor with the scalar of 10-200 ul.

3.7.1.4 Vortex or mixture machine which is fine to have or have not.

3.7.3 The PBS is made by yourself

Making the 1X, PBS without Ca++ and Mg++:

Reagents	1 x, working solution, pH 7.3±	Weight .
NaCl	137 mM	8. 0 g
KCl	2.7 mM	0.2 g
Na_2HPO_4-$7H_2O$	4.3 mM	1.15 g
KH_2PO_4	1.4 mM	0.2 g .

Dissolved in 1000 ml of distilled water.

3.7.4 Content Table (Table 13)

Table 13. Ten-minute DNA Release Kit-7 content table

Catalogue Number	DNA Release Buffer		Work for Samples	Store and Use
	Name	Volume		1.It is qualified for 6 moths at 5-29? C.
JZ-007-1	Kit7-B1	1.4 ml	100 times	2.Warm to RT before using if it was
JZ-007-2	Kit7-B2	2.1 ml	or 40 dishes	kept at 4? C.

3.7.5 Work Table (Table 14)

Table 14. Ten-minute DNA Release Kit-7 work table

Processing 1 for 5 x 10^4-1 X 10^6 of the adhered or suspention cells	
Step	**Action**
Step1	The adhered cells are trypsinized and harvested with new growth media. Count
	and transfer the cells to a eppendorf tube, Centrifuge it at 5000 g X 3 min. remove
	the supernatant. Add 1 ml of PBS. Centrifuge it again. Remove PBS from the tube,
	keep cells pellet in the tube bottom.
Step2	Add 14 ul of Kit7-B1 to the tube. Flick it until the cells pellet that was dissolved.
	Place the tube at 86? C in a Thermo-machine or Water Bath,not shaking,
	for 8 min. then, put it at room temperature(RT) for 2 min.
Step3	Add 21 ul of Kit7-B2 to the tube, mixtured well by flicking 3-5 times.
	Centrifuge it at 12,000 g X 3 min.in RT.
Step4	Transfer the supernatant 25-30 ul(DNA is inside) to a fresh eppendorf tube.
	The DNA extract has been ready for PCR, Southern etc.
Processing 2 for bigger cells pellet from a 100 mm cell culture dish	
Step	**Action**
Step1	The cells Trypsinized. Harvest the cells with Growth Media 5-10 ml to a 10 ml
	centrifuge tube. Centrifuge the tube at 1000 rpm X 5 min.
Step2	Get rid of supernatant, add 1 ml of PBS to the the tube. Mixtured with pipettor,
	then, transfer the PBS with cells to a eppendorf tube.
Step3	Centrifuge the tube at 5000 g X 5 min. in RT. Remove the PBS and keep
	the cells pellet in the tube bottom.
Step4	Add 30 ul of Kit7-B1 to the tube, mixtured well with pipettor.Put it at 86? C in
	a Thermo- machine of Water Bath, not shaking, for 8 min. then,at RT for 2 min.
Step5	Add 45 ul of Kit7-B2 to the tube, mixtured well by flicking 3-5 times.
Step6	Centrifuge it at 12,000 g x 3 min.Transfer the supernatant 70 ul(DNA is inside)
	to a fresh eppendorf tube. The DNA extract hes been ready for PCR,Southern etc.
Note	1.you must use the trypsin to digest the adhered cells to obtain the cell pellt .
	2.It is not guarantee to have a good DNA extract with scraper to obtain cells.
	3.The kit also is using for suspension cells

4. Reference to the DNA Quantity to Match Your Action (Table 15, Figure 4)

Table 15. Kit-7 DNA quantity on the cell amount dose-dependent effective with adhered cells

Cells	Concentration (ug/ul) Range	Average	Volume/each	Ratio on 260/280 nm
5 X 10^4	0.12-0.16	0.14	32-36 ul	1.7-2.0
1 X 10^5	0.22-0.26	0.24	32-36 ul	
5 X 10^5	0.7-0.9	0.8	32-36 ul	
1 X 10^6	0.9-1.5	1.2	32-36 ul	
100 mm cell	1-1.6	1.3	70 ul	
culture dish	2.93-2.99	2.96	30 ul	

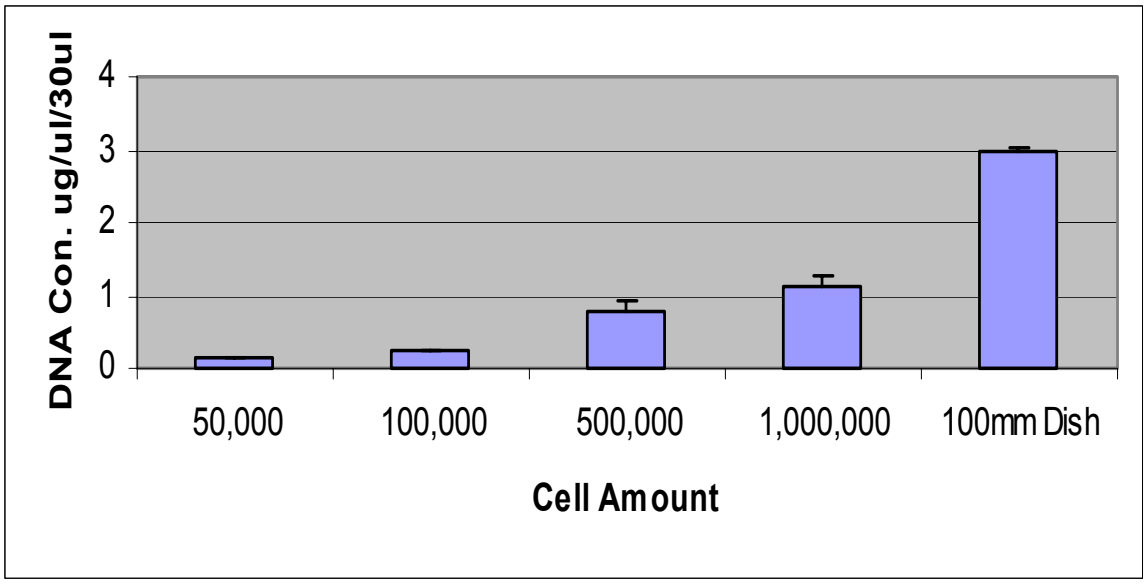


Figure 4. Kit-7 DNA quantity on the cell amount dose-dependent effective with the adhered cells

5. Troubleshooting

If you do not get a good result, please check the process you have done by following:

5.1 If the buffers are kept at 5-28° C?

5.2 If the sample is frozen sample? The samples must be thawed to the room temperature, then start the process following the protocol.

5.3 If you use the centrifuge that is below 4° C? You have to use the centrifuge at room temperature for your good result.

5.4 If the buffers are used wrongly?

5.5 If you use the incorrect temperature for DNA releasing? You must put the tube at 86-90° C, not shaking in any thermo-machine or water bath.

5.6 If the experimental steps are done following the protocol exactly?

References

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