APPENDICIES
Appendix 1A

Preparation of an Agarose Gel for Gel Electrophoresis

Solutions Needed:

- DNA samples
- Agarose
- 1x TAE buffer
- 10,000x SYBR Safe DNA gel stain (Invitrogen)
- 50 ng/μL DNA ladder with loading dye (Invitrogen)
- 6x Loading dye containing xylene cyanol and/or bromophenol blue dyes

Materials Needed:

- Pipettes (P-20)
- Pipet tips (regular, non-filter tips)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge tube rack
- 250 mL Erlenmeyer flask
- 25 mL Erlenmeyer flask
- Saran wrap
- Scale
- Microwave
- 55°C water bath
- Hot hand protector
- Gel cast
- Gel comb
- Round bubble level
- Gel box
- Cables
- Electrophoresis power supply
- Plastic container for carrying the gel
- Gel document system (Bio-Rad)

PROCEDURE

*Note: SYBR Safe gel stain is unstable in UV or bright room light. If possible, keep the gel in the **dark** by either turning off the lights, covering the gel with a cardboard box or aluminum foil, or run the gel inside of a drawer. Realistically, hours of constant UV or bright room light exposure are required to cause any significant loss of signal.*

1. For a **1% agarose gel**, weigh out **1 gram** of agarose (powder) into a weigh boat on the scale in room 4128A2. *Note: The **percentage** of agarose in the gel reflects the amount of agarose (in **grams**) in **100 mL** of 1x TAE buffer.*
   Example: If you want to make a 0.7% agarose gel (0.7 g/100 mL, w/v), weigh out 0.7 g of agarose for 100 mL of 1x TAE buffer.
2. Carefully, pour the agarose into a 250 mL Erlenmeyer flask.
3. Measure **100 mL** of **1x TAE** buffer using a graduated cylinder, and add it to the flask in step 2.
4. Cover the flask with an inverted 25-mL Erlenmeyer flask or saran wrap. Swirl the solution to break up any lumps of agarose. *Note: The inverted flask will collect condensation from the steam produced during microwaving.*
5. Microwave the solution for **1-2 minutes** or until the agarose granules have completely melted and the solution looks **clear**. Gently **swirl** the solution every 15 sec during microwaving to help melt the agarose evenly. *Caution: The solution gets **very hot**. Use a hot hand protector. Note: Constantly watch over the solution because if it starts boiling, it might overflow.*
6. Cool down the agarose solution for **10 min** in a **55°C water bath** until it is cool enough to handle.
7. While the agarose solution is cooling, prepare the gel cast with the appropriate comb. Use the round bubble level to make the gel cast level.
   *Note: **The choice of comb depends on the number of samples to be loaded on the gel**. For example, if there are ≤ 18 samples, then use a 20-tooth comb; but, if there are ≥ 19 samples, then use a 30-tooth comb. Note: Remember that at least **two wells** will be for loading **DNA ladder** (on left and right sides of loaded samples).*
8. After the agarose solution has cooled down, add **10 µL** of **10,000x SYBR Safe DNA gel stain** to 100 mL of agarose solution and **swirl** the flask **GENTLY** to mix. *Note: Do NOT swirl vigorously to avoid generating bubbles. Note: Add 5 µL of 10,000x SYBR Safe DNA gel stain for a small (50 mL) gel.*
9. Pour the **agarose/gel stain** solution into the gel cast with the appropriate gel comb. Wait **30 min** for the agarose solution to solidify. *Note: IMMEDIATELY after pouring the agarose solution, inspect the agarose solution's surface for the present of*

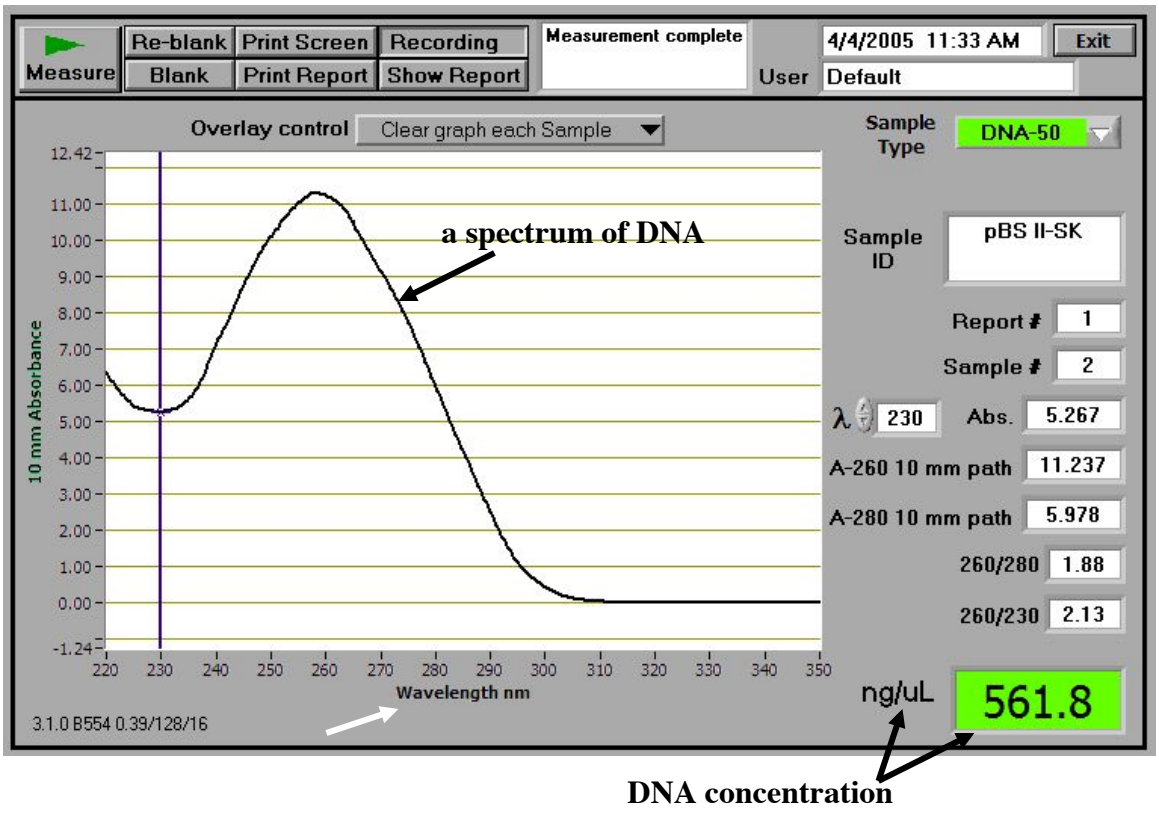
bubbles. If there are bubbles floating on the surface of the gel solution, use a pipette tip to pop them or move them to the sides of the gel before the gel has completely solidified.

10. Pour ~600 mL of **1x TAE** buffer into the gel box.
11. After the agarose has solidified into a gel, take out the comb by **gently** pulling it side to side and out of the gel. Put the gel in its cast into the gel box containing the 1x TAE running buffer.
12. Add **6x loading dye** to your samples. *Note: Do not use a loading dye that will travel to the same place as your DNA on the gel because the dye will obscure the DNA band. Xylene cyanol runs at ~3-4 kb, and bromophenol blue runs at ~400 bp on a 1% agarose gel.*
13. Load **10 µL** of **50 ng/µL DNA ladder with loading dye**.
14. Load samples and record the identity of the sample in each lane.
15. Add **10 µL** of **10,000x SYBR Safe DNA gel stain** to the running buffer at the anode (red) side of the gel box (near the bottom of the gel). Pipet up and down to mix. *Note: Add 5 µL of 10,000x SYBR Safe DNA gel stain for a small (50 mL) gel.*
16. Put the lid on the gel box and firmly connect the electrodes to the power supply (**RED** to **RED** and **BLACK** to **BLACK**).
17. Run the gel at **105 volts** for **1-2 hours** or until the front dye (bromophenol blue) has migrated one-half or two-thirds of the gel length.
18. After 1-2 hours of running the gel, turn **off** the power supply.
19. Remove the lid of the gel box. Put the gel in its gel cast into a small plastic container and bring the container to room 4128A2. ***Caution:*** *It is a* ***MUST*** *to put the gel into a plastic container so that the gel cannot slide off the gel cast, fall on the floor and be broken into pieces while walking.*
20. Take a picture of the gel using the Bio-Rad Gel Document System. Label the picture using the text program of the Gel Document System. (*Your TA will show you how.*) *Alternatively: Print out the picture. Tape it to a piece of paper by putting a piece of white tape at a position immediately above the wells. Label the wells with the sample names.*
21. Print out the picture. Store the labeled picture in your lab notebook.

Appendix 1B

What is a **spectrophotometer**?

It is an **instrument** that **measures** the **amount** of **molecules absorbing a given wavelength of energy**. In this exercise, we measure the amount of DNA molecules in a given volume in the **ultraviolet wavelengths** of **200 – 280 nm** (**nm** stands for **nanometer**, which is **1 billionth** of a **meter**). The bases of DNA absorb at a wavelength of 254 nm. The absorbance of DNA molecules over the wavelength range of 220 - 350 nm is represented as a **spectrum** with a **peak** at **near 260 nm** as illustrated by the **NanoDrop spectrophotometer readout** below:



OPERATION OF NANODROP SPECTROPHOTOMETER ND-1000

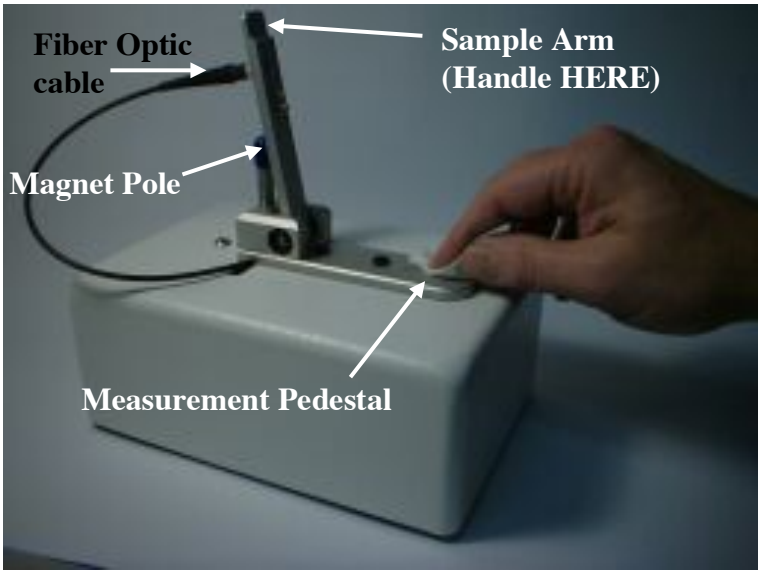
Note: The NanoDrop is powered by the laptop via the USB port.

1. **Open** the NanoDrop program by clicking the "**ND-1000 v3.7.1**" icon on the computer desktop. Wait for a few seconds for the program to open. You see the NanoDrop 1000 3.7.1 menu panel with **user field** set to **default.**
2. To read the concentration of DNA or RNA solutions, **click** on the "**Nucleic Acid**" button on the top left column.

   Note: You see the following message: "Ensure sample pedestals are clean and then load a water sample. After loading water sample, click OK to initialize instrument."
3. **Clean** the **measurement pedestals** as following:
   a. **Raise** the **sample arm** by holding its end.

      Caution: ***NEVER*** *hold the fiber optic cable when lifting and lowering the sample arm because the cord is fragile, and it is very expensive to replace it.*

   b. **Blot BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes slightly wetted with distilled water.



c. **Pipet 1.5 - 2 µL** of water onto the **measurement pedestal**.

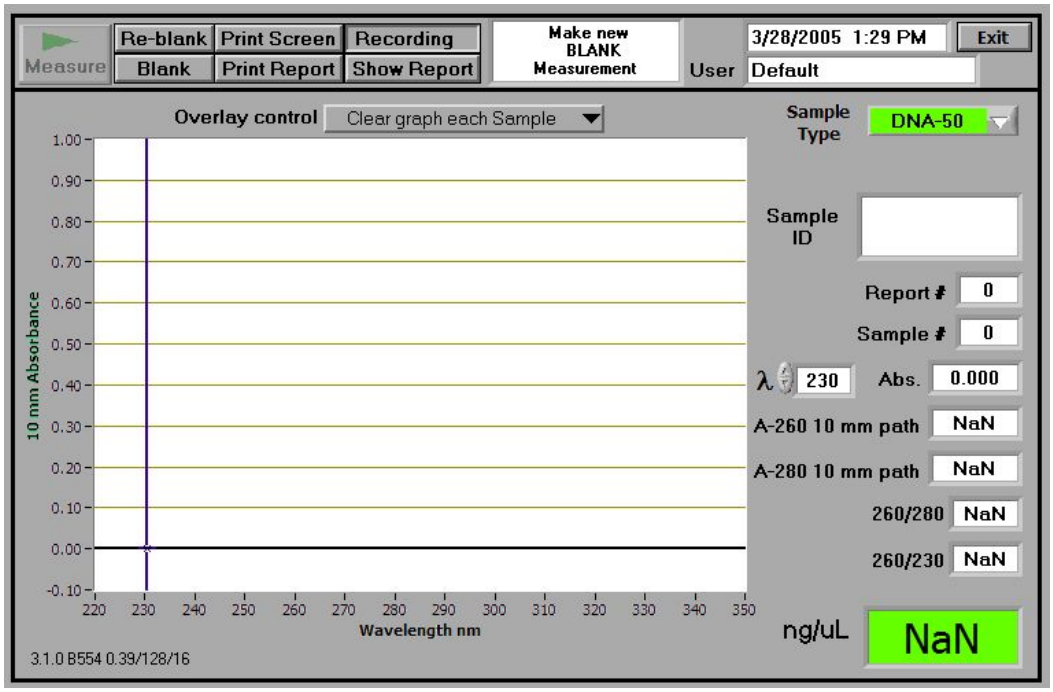
*Note: Even though NanoDrop Inc. claims that the NanoDrop can read as low as 1 µL, the concentration reading is NOT consistent at this volume. Therefore, the **minimal** volume for the concentration reading is **1.5 µL.***

d. **Slowly lower** the sample arm to its horizontal position.

Caution: *NEVER let the arm fall freely.*

e. **Click** the **OK** button.

*Note: Now the NanoDrop is **INITIALIZED.** You will see the **dialog panel** as shown below.*



f. Raise the sample arm to the vertical position.

g. **Wipe off** the **liquid** from **BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes.

4. **Change SAMPLE TYPE** (if necessary) from **DNA-50** (default, for DNA) to **RNA-40** (for RNA) or **ssDNA-33** (for oligonucleotides), depending on your sample.
5. (Optional) **Change** the **OVERLAY CONTROL** field from the default setting of "CLEAR GRAPH EACH SAMPLE" to "CLEAR GRAPH ON NEW REPORT" or "ACCUMULATE UNTIL CLEAR" or "CLEAR GRAPH NOW."
6. Make a **NEW BLANK measurement.**
    a. Pipet 1.5 - 2.0 µL of either double-distilled water, TE or EB (depending on what solution your sample is dissolved in) on the measurement pedestal.
    b. Lower the sample arm to the horizontal position.
    c. **Click** the **BLANK** button. *The blank was made.*
    d. After the reading is done, raise the sample arm to the vertical position.
    e. **Blot off** the **liquid** from **BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes.
7. **Confirm** that the blank was made.
    a. Pipet 1.5 - 2.0 µL of either double-distilled water, TE or EB (depending on what solution your sample is dissolved in) on the measurement pedestal.
    b. Lower the sample arm to the horizontal position.
    c. In the **SAMPLE ID** field, **type** the **identity** of the solution that you are measuring (either ddH_2O, TE or EB).
    d. **Click** the **MEASURE** button.

    *Note: After the reading is done, a **concentration** (in **ng/µL**) and a **spectrum** of the absorbance, along with other information, are shown. The reading should be **less than 1 ng/µL**. If it is not, make a new blank measurement by repeating step 6.*
    e. Raise the sample arm to the vertical position.
    f. **Blot off** the **liquid** from **BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes.
8. Measure the **samples**.

a. In the **SAMPLE ID** field, **type** the **identity** of the sample solution.
b. Pipet 1.5 - 2.0 μL of SAMPLE on the measurement pedestal.
c. Lower the sample arm to the horizontal position.
d. **Click** the **MEASURE** button to determine concentration of your sample.
e. After the reading is done, a **sample concentration** (in **ng/μL**) and a **spectrum** of the sample, along with other information, are shown. You can either:
    i. **Save** the **window** of measured sample.
        1. Click on FILE.
        2. Choose SAVE WINDOW.
        3. Select an existing folder or create a new folder (give a name for the new folder).
        4. Type in a file name in the FILE NAME field.
        5. Click the SAVE button to save the file.
    ii. **Or**, **print** the **window** by **clicking** the **PRINT SCREEN** button.
    Note: To print the spectrum of the current sample, you MUST print it before reading the next sample. Otherwise, you need to repeat the sample reading.
f. Raise the sample arm to the vertical position.
g. **Blot off** the **liquid** from **BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes.

9. **Repeat** step 8 for other samples.
10. After reading the **last sample**, **click** the **PRINT REPORT** button to print the concentrations of all samples.
11. If you are done with the NanoDrop, **click** the **EXIT** buttons.
12. **Blot BOTH** the **measurement pedestal** and the **sample arm pedestal** with a piece of Kimwipes slightly wetted with distilled water.

Appendix 1C
100 bp DNA Ladder

Invitrogen
life technologies

100 bp DNA Ladder

Cat. No. 15628-019 **Size: 50 µg**
Concentration: 1 µg/µl **Store at -20°C.**

Description:

The 100 bp DNA Ladder consists of 15 blunt-ended fragments between 100 and 1500 bp in multiples of 100 bp and an additional fragment at 2072 bp. The 600 bp band is approximately 2 to 3 times brighter than the other ladder bands to provide internal orientation. This ladder is not designed for quantitation.

Storage Buffer:

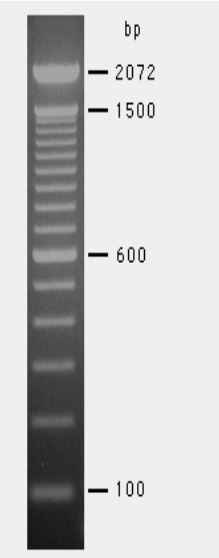
10 mM Tris-HC1 (pH 7.5)
1 mM EDTA

Recommended Procedure:

A final concentration of 20 mM NaCl is recommended for gel electrophoresis. Apply approximately 0.1 µg of ladder per mm lane width. **Do not heat** before loading.

Quality Control:

Agarose gel analysis shows that the bands between 100 to 1500 bp are distinguishable. The 600 bp band must be more intense than any other band except the band at 2072 bp.



Note:

During 2% agarose gel electrophoresis with tris-acetate (pH 7.6) as the running buffer, bromophenol blue migrates near the 100-bp fragment. The 100-bp band migrates behind the bromophenol blue marker on 6% polyacrylamide gels with tris-borate (pH 8.0) as the running buffer.

Part of the 600-bp band may migrate anomalously slowly in polyacrylamide gels (1,2,3). This band may appear as an extra band near or on top of the 700-bp band.

References:

1. Hsieh, C., et al. (1991) *Mol. Gen. Genet. 225*, 25.
2. Stellwagen, N.C. (1983) *Biochemistry 22*, 6186.
3. Jordan, H. and Hartley, J. (1997) *Focus® 19*, 9.

100 bp DNA Ladder
0.5 µg/lane
2% agarose gel stained with ethidium bromide.

Cat. No. 15628-019

Appendix 1D
1 Kb Plus DNA Ladder

invitrogen
by life technologies

1 Kb Plus DNA Ladder

Cat. no. 10787-018 **Size** 250 µg at 1 µg/µL **Store at –30°C to –10°C**

Doc. Part no. 10787018.pps **Pub. no.** MAN0000898 **Rev.** 2.0

Description

Use the 1 Kb Plus DNA Ladder is to size linear double-stranded DNA fragments from 100 bp to 12 kb. The ladder contains a total of twenty bands: twelve bands ranging in size from 1000 bp to 12,000 bp in 1000-bp increments and eight bands ranging in size from 100 to 1650 bp. The 1650-bp band contains approximately 8% of the mass applied to the gel.

Storage Buffer

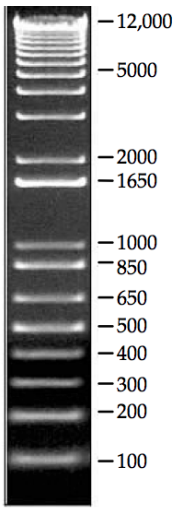
- 10 mM Tris-HCl (pH 7.5)
- 1 mM EDTA
- 50 mM NaCl

Recommended Procedure

We recommend using 10X BlueJuice™ Gel Loading Buffer (Cat. no. 10816-015) at a concentration of 2X for the electrophoresis of DNA standards on agarose gels. Alternately, you may dilute the DNA standard so that the final concentration of NaCl is 20 mM. Apply approximately 0.1 µg of ladder per mm lane width. *Do not heat* before loading.

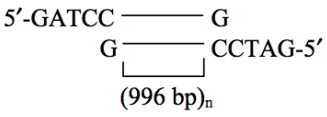
Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.



0.9 µL/lane
0.9% agarose gel stained with ethidium bromide

Structure of Fragments in 1-Kb Increments



Notes

During 1% agarose gel electrophoresis with Tris-acetate (pH 7.5) as the running buffer, bromophenol blue migrates with the 500 bp band.

The 1650 bp band is generated from pUC. The bands smaller than 1000 bp are derived from lambda DNA.

Appendix 1E
Ex Taq DNA Polymerase Manual

10XPCR Buffer,dNTP Mixture for PCR

TaKaRa Ex Taq™

Code No. RR001A
Size: 250 units

Shipping at -20˚C
Stored at -20˚C

Supplied Reagents : 10X *Ex Taq*™ Buffer
dNTP Mixture

Lot No.
Conc. : units/μl
Volume : μl
Expiry Date :

Storage Buffer:

20 mM	Tris-HCl (pH8.0)
100 mM	KCl
0.1 mM	EDTA
1 mM	DTT
0.5%	Tween®20
0.5%	Nonidet P-40®
50%	Glycerol

Unit definition: One unit is the amount of the enzyme that will incorporate10 nmol of dNTP into acid-insoluble products in 30 minutes at 74˚C with activated salmon sperm DNA as the template-primer.

Reaction mixture for unit definition:

25 mM	TAPS (pH 9.3 at 25˚C)
50 mM	KCl
2 mM	$MgCl_2$
1 mM	2-mercaptoethanol
200 μM	each dATP,dGTP,dTTP
100 μM	[α-^{32}P]-dCTP
0.25 mg/ml	activated salmon sperm DNA

Purity: Nicking activity, endonuclease and exonuclease activity were not detected after the incubation of 0.6 μg of supercoiled pBR322 DNA, 0.6 μg of λ DNA or 0.6 μg of λ-*Hin*d III digest with 10 units of this enzyme for 1 hour at 74˚C.

Applications:
For DNA amplification by Polymerase Chain Reaction (PCR).

PCR products : As most PCR products amplified with *TaKaRa Ex Taq* ™ have one A added at 3'-termini, the obtained PCR product can be directly used for cloning into T-vector. Also it is possible to clone the product in blunt-end vectors after blunting and phosphorylation of the end.

PCR test : Good performance of DNA amplification by Polymerase Chain Reaction (PCR) was confirmed by using λ DNA as the template (amplified fragment : 20 kbp).
Good performance of DNA amplification of β-globin gene by PCR was also confirmed by using human genomic DNA as the template (amplified fragment : 17.5 kbp).

PCR condition (an example)
When amplifying 1 kbp DNA fragment

98°C	10 sec	┐		98 °C	10 sec	┐	
55°C	30 sec	┘ 30 cycles	or	68 °C	1 min	┘	30 cycles
72°C	1 min	┘					

Note:Denaturation condition varies depending on an used thermal cycler and tube. It is recommended for 10-30 sec. at 94°C, or 1-10 sec. at 98°C.

General reaction mixture for PCR (total 50 μl)

TaKaRa Ex Taq ™ (5 units/μl)	0.25 μl
10X *Ex Taq* Buffer	5 μl
dNTP Mixture (2.5 mM each)	4 μl
Template	< 500 ng
Primer 1	0.2 ~ 1.0 μM (final conc.)
Primer 2	0.2 ~ 1.0 μM (final conc.)
Sterilized distilled water	up to 50 μl

Supplied 10X *Ex Taq* Buffer

Supplied Size	: 1 ml/vial
Mg^{2+} concentration (10X)	: 20 mM
Storage	: –20˚C

Supplied dNTP Mixture
Mixture of dNTP, ready for use in Polymerase Chain Reaction (PCR) without dilution.

Supplied Size	: 800 μl/vial
Concentration	: 2.5 mM of each dNTP
pH	: pH 7 ~ 9
Form	: Solved in water (sodium salts)
Purity	: ≥ 98% for each dNTP
Storage	: –20˚C

< Cool Start Method >
"Cool Start Method' , enables to minimize the amplification of non-specific band in PCR and achieves more accurate amplification. This is a simpler method without need for special enzyme nor additional reagents.⁺ Higher reaction specificity can be achieved by combining Hot Start PCR techniques with Taq Antibody (Code.9002A) and Cool Start method.

Protocol of Cool Start Method

1) Keep all reagents on ice until use.
2) Prepare the reaction mixture on ice.*·**
   *The adding order of reagents dose not influence on results.
   **The result will not be affected even when the mixture is left on ice 30 min. before thermal cycling.
3) Set a thermal cycler ready to start with the designated program.***
   ***No need to change PCR conditions especially for Cool Start.
4) Set the tubes in a thermal cycler and start thermal cycling immediately.

⁺JAPAN Patent 2576741 for Cool Start Method is owned by SHIMADZU CORPORATION

Note
For research use only. Not for use in diagnostic or therapeutic procedures.

Produced by TAKARA BIOTECHNOLOGY (DALIAN) CO.,LTD.

V2005.06

March 2008

QIAquick® Spin Handbook

QIAquick PCR Purification Kit
For purification of PCR products, 100 bp to 10 kb

QIAquick Nucleotide Removal Kit
For oligonucleotide (17-40mers) and DNA
(40 bp to 10 kb) cleanup from enzymatic reactions

QIAquick Gel Extraction Kit
For gel extraction or cleanup of DNA
(70 bp to 10 kb) from enzymatic reactions

QIAGEN®

Sample & Assay Technologies

Kit Contents

QIAquick PCR Purification Kits Catalog no.	(50) 28104	(250) 28106
QIAquick Spin Columns	50	250
Buffer PB*	30 ml	150 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	55 ml
pH Indicator I	800 µl	800 µl
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

QIAquick Nucleotide Removal Kits Catalog no.	(50) 28304	(250) 28306
QIAquick Spin Columns	50	250
Buffer PN*	30 ml	140 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	55 ml
Collection Tubes (2 ml)	100	500
Loading Dye	110 µl	550 µl
Handbook	1	1

QIAquick Gel Extraction Kits Catalog no.	(50) 28704	(250) 28706
QIAquick Spin Columns	50	250
Buffer QG*	2 x 50 ml	2 x 250 ml
Buffer PE (concentrate)	2 x 10 ml	2 x 50 ml
Buffer EB	15 ml	2 x 15 ml
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

* Buffers PB, PN, and QG contain chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

Storage

QIAquick Spin Kits should be stored dry at room temperature (15–25°C). Under these conditions, QIAquick Spin Kits can be stored for up to 12 months without showing any reduction in performance and quality. Check buffers for precipitate before use and redissolve at 37°C if necessary. The entire kit can be stored at 2–8°C, but in this case the buffers should be redissolved before use. Make sure that all buffers and spin colums are at room temperature when used.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer PB contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

In case liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to the components of the QIAquick system.

Buffer PB

Contains guanidine hydrochloride and isopropanol: harmful, irritant, flammable. Risk and safety phrases*: R10-22-36/38. S23-26-36/37/39-46

Buffer PN

Contains sodium perchlorate and isopropanol: harmful, highly flammable. Risk and safety phrases*: R11-22. S13-16-23-26-36-46

Buffer QG

Contains guanidine thiocyanate: harmful. Risk and safety phrases*: R20/21/22-32. S13-26-36-46

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany
Tel: +49-6131-19240

* R10: Flammable. R11: Highly Flammable. R22: Harmful if swallowed. R20/21/22: Harmful by inhalation, in contact with skin and if swallowed. R32: Contact with acids liberates very toxic gas. R36/38: Irritating to eyes and skin. S13: Keep away from food, drink and animal feedingstuffs. S16: Explosive when mixed with oxidizing substances. S23: Do not breathe vapour/spray. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S36: Wear suitable protective clothing. S36/37/39: Wear suitable protective clothing, gloves and eye/face protection. S46: If swallowed, seek medical advice immediately and show the container or label.

Product Specifications

	QIAquick PCR Purification Kit	QIAquick Nucleotide Removal Kit	QIAquick Gel Extraction Kit
Maximum binding capacity	10 µg	10 µg	10 µg
Maximum weight of gel slice	—	—	400 mg
Minimum elution volume	30 µl	30 µl	30 µl
Capacity of column reservoir	800 µl	800 µl	800 µl
Typical recoveries			
Recovery of DNA	90–95% (100 bp – 10 kb)	80–95% (40 bp – 10 kb)	70–80% (70 bp – 10 kb)
Recovery of oligonucleotides (17–40mers)	0	60–80%	10–20%
Recovered			
Oligonucleotides	—	17–40mers	—
dsDNA	100 bp – 10 kb	40 bp – 10 kb	70 bp – 10 kb
Removed			
<10mers	**YES**	**YES**	**YES**
17–40mers	**YES**	no	no

Introduction

The QIAquick system, designed for rapid DNA cleanup, includes:

- **QIAquick PCR Purification Kits** for direct purification of double- or single-stranded PCR products (100 bp – 10 kb) from amplification reactions and DNA cleanup from other enzymatic reactions.
- **QIAquick Nucleotide Removal Kits** for general cleanup of oligonucleotides and DNA up to 10 kb from enzymatic reactions (e.g., labeling, dephosphorylation, restriction, and tailing).
- **QIAquick Gel Extraction Kits** for extraction of DNA fragments (70 bp – 10 kb) from standard, or low-melt agarose gels in TAE (Tris·acetate/EDTA) or TBE (Tris·borate/EDTA) buffer and DNA cleanup from enzymatic reactions.

QIAquick PCR Kits are also available in multiwell format for preparation of 8 to 96 samples (see page 37 for ordering information).

Enzymatic reaction cleanup using QIAquick Kits

The QIAquick system is suitable for fast cleanup of up to 10 µg of DNA fragments from enzymatic reactions and agarose gels (Table 1). Enzyme contamination of DNA samples can interfere with subsequent downstream applications. QIAquick Spin Kits can be used for highly efficient removal of a broad spectrum of enzymes widely used in molecular biology. In addition, QIAGEN offers the MinElute® Reaction Cleanup Kit, which is specially designed for fast and easy DNA cleanup from all enzymatic reactions. Using proven microspin technology, the MinElute Reaction Cleanup Kit delivers highly concentrated purified DNA by using an elution volume of only 10 µl (see ordering information, page 37).

Table 1. QIAquick DNA Cleanup Guide

	From solutions			From gels
	QIAquick PCR Purification Kit	QIAquick Nucleotide Removal Kit	QIAquick Gel Extraction Kit	QIAquick Gel Extraction Kit
Alkaline phosphatase	**YES**	**YES**	**YES**	**YES**
cDNA synthesis	**YES**	no	no	**YES**
DNase, nuclease digestion	**YES**	**YES**	**YES**	**YES**
Kinase:				
DNA fragments	**YES**	**YES**	**YES**	**YES**
Oligonucleotides	no	**YES**	no	no
Ligation	**YES**	**YES**	**YES**	**YES**
Nick translation	**YES**	**YES**	**YES**	**YES**
PCR	**YES**	no	no	**YES**
Random priming	**YES**	**YES**	**YES**	**YES**
Restriction digestion	**YES**	**YES**	**YES**	**YES**
Tailing:				
DNA fragments	**YES**	**YES**	**YES**	**YES**
Oligonucleotides	no	**YES**	no	no

QIAquick Kits provide high yields of pure nucleic acids, for direct use in applications such as:

- Fluorescent and radioactive sequencing
- Restriction
- Labeling
- Hybridization
- Ligation and transformation
- Amplification
- In vitro transcription
- Microinjection

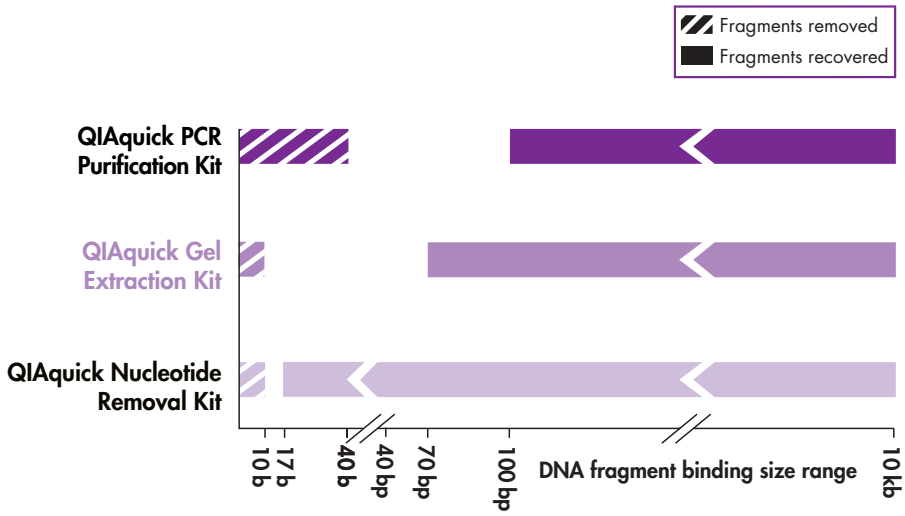


Figure 1. DNA fragment binding-size range. Recoveries of DNA fragments in the size range between "removed" and "recovered" are not defined.

Automated DNA cleanup

The QIAquick PCR Purification Kit and QIAquick Gel Extraction Kit can be fully automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., bind, wash, and elute) enabling purification of high-quality DNA.

The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube .

A detailed protocol for using QIAquick spin columns on the QIAcube is provided with the QIAcube.

Note: It is not necessary to add pH indicator I to Buffer PB when using the QIAcube.

The QIAquick Principle

The QIAquick system combines the convenience of spin-column technology with the selective binding properties of a uniquely designed silica membrane. Special buffers provided with each kit are optimized for efficient recovery of DNA and removal of contaminants in each specific application. DNA adsorbs to the silica membrane in the presence of high concentrations of salt while contaminants pass through the column. Impurities are efficiently washed away, and the pure DNA is eluted with Tris buffer or water (see page 17). QIAquick spin columns offer 3 handling options — as an alternative to processing the spin columns in a microcentrifuge, they can now also be used on any commercial vacuum manifold with luer connectors (e.g., QIAvac 6S or QIAvac 24 Plus with QIAvac Luer Adapters) or automated on the QIAcube.

Adsorption to QIAquick membrane — salt and pH dependence

The QIAquick silica membrane is uniquely adapted to purify DNA from both aqueous solutions and agarose gels, and up to 10 µg DNA can bind to each QIAquick column. The binding buffers in QIAquick Spin Kits provide the correct salt concentration and pH for adsorption of DNA to the QIAquick membrane. The adsorption of nucleic acids to silica surfaces occurs only in the presence of a high concentration of chaotropic salts (1), which modify the structure of water (2).

Adsorption of DNA to silica also depends on pH. Adsorption is typically 95% if the pH is ≤7.5, and is reduced drastically at higher pH (Figure 1). If the loading mixture pH is >7.5, the optimal pH for DNA binding can be obtained by adding a small volume of 3 M sodium acetate, pH 5.0.

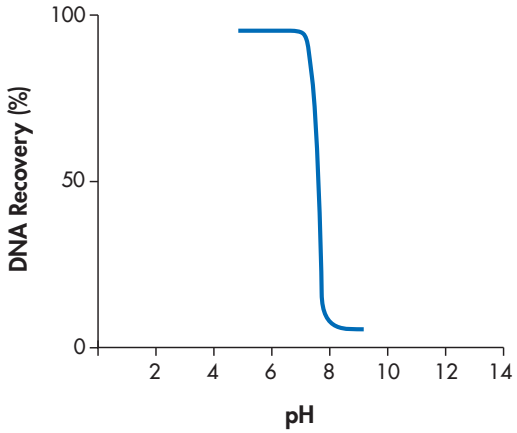


Figure 2. pH dependence of DNA adsorption to QIAquick membranes. 1 µg of a 2.9 kb DNA fragment was adsorbed at different pHs and eluted with Buffer EB (10 mM Tris·Cl, pH 8.5). The graph shows the percentage of DNA recovery, reflecting the relative adsorption efficiency, versus pH of adsorption.

Optimized binding buffers for every DNA cleanup task

All QIAquick Spin Kits contain identical QIAquick spin columns but different binding buffers optimized for each specific application:

- Buffer PB in the QIAquick PCR Purification Kit allows the efficient binding of single- or double-stranded PCR products as small as 100 bp and the quantitative (99.5%) removal of primers up to 40 nucleotides. This kit can therefore be used to remove oligo-dT primers after cDNA synthesis or to remove unwanted linkers in cloning experiments.
- Buffer PN in the QIAquick Nucleotide Removal Kit promotes the adsorption of both oligonucleotides ≥17 bases and DNA fragments up to 10 kb to the membrane.
- Buffer QG in the QIAquick Gel Extraction Kit solubilizes the agarose gel slice and provides the appropriate conditions for binding of DNA to the silica membrane.

All of these buffers are available separately (see ordering information, page 37).

pH indicator

Binding buffer PB and binding and solubilization buffer QG are specially optimized for use with the QIAquick silica membrane. Buffer QG contains an integrated pH indicator, while an optional pH indicator can be added to Buffer PB allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5, which can occur if during agarose gel electrophoresis, the electrophoresis buffer had been used repeatedly or incorrectly prepared, or if the buffer used in an enzymatic reaction is strongly basic and has a high buffering capacity, the binding mixture turns orange or violet (Figure 2). This means that the pH of the sample exceeds the buffering capacity of Buffer PB or QG and DNA adsorption will be inefficient. In these cases, the pH of the binding mixture can easily be corrected by addition of a small volume of 3 M sodium acetate*, pH 5.0, before proceeding with the protocol. In addition, in the QIAquick Gel

Figure 3. Indicator enables easy checking of the optimal pH. Indicator dye in solubilization and binding Buffers QG and PB identifies optimal pH for DNA binding.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

Extraction Kit procedure, the color of the binding mixture allows easy visualization of any unsolubilized agarose, ensuring complete solubilization and maximum yields. The indicator dye does not interfere with DNA binding and is completely removed during the cleanup procedure. Buffers PB and QG do not contain sodium iodide (NaI). Residual NaI may be difficult to remove from DNA samples, and reduces the efficiency of subsequent enzymatic reactions such as blunt-end ligation.

Washing

During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, ethidium bromide, oils, and detergents (e.g., DMSO, Tween® 20) do not bind to the silica membrane but flow through the column. Salts are quantitatively washed away by the ethanol-containing Buffer PE. Any residual Buffer PE, which may interfere with subsequent enzymatic reactions, is removed by an additional centrifugation step.

Elution in low-salt solutions

Elution efficiency is strongly dependent on the salt concentration and pH of the elution buffer. Contrary to adsorption, elution is most efficient under basic conditions and low salt concentrations. DNA is eluted with 50 or 30 µl of the provided Buffer EB (10 mM Tris·Cl, pH 8.5), or water. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water to elute, make sure that the pH is within this range. In addition, DNA must be stored at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent. Elution with TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) is possible, but not recommended because EDTA may inhibit subsequent enzymatic reactions.

DNA yield and concentration

DNA yield depends on the following three factors: the volume of elution buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. 100–200 µl of elution buffer completely covers the QIAquick membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with ≤50 µl requires the buffer to be added directly to the center of the membrane, and if elution is done with the minimum recommended volume of 30 µl, an additional 1 minute incubation is required for optimal yield. DNA will be up to 1.7 times more concentrated if the QIAquick column is incubated for 1 minute with 30 µl of elution buffer, than if it is eluted in 50 µl without incubation (Figure 4, page 14).

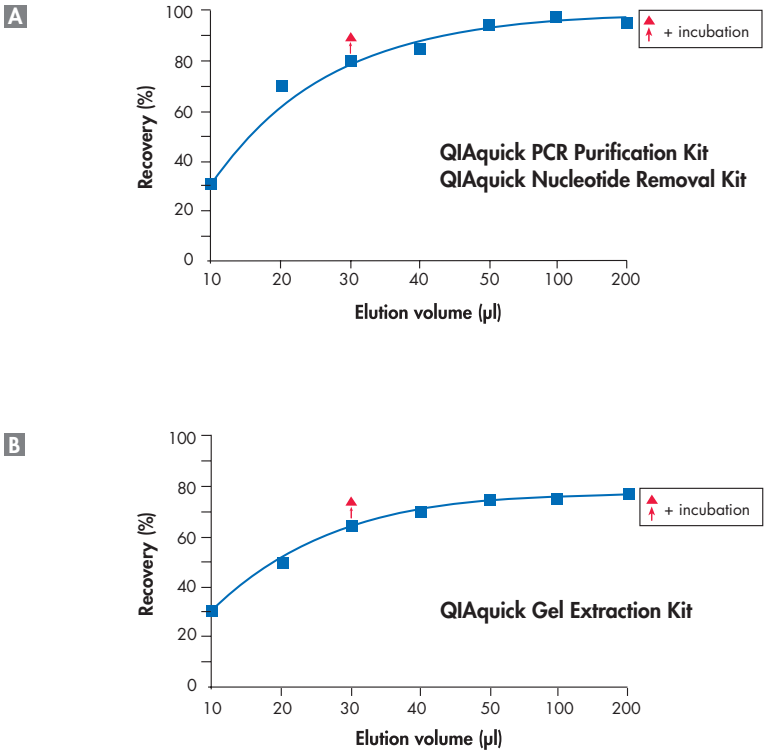


Figure 4. Highly concentrated DNA. Effect of elution buffer volume on DNA yield for A the QIAquick PCR Purification and QIAquick Nucleotide Removal Kit; B the QIAquick Gel Extraction Kit. 5 µg of a 2.9 kb DNA fragment were purified and eluted with the indicated volumes of Buffer EB. 30 µl plus 1 minute incubation on the QIAquick column gives DNA yields similar to 50 µl without incubation, but at a concentration 1.7 times greater.

Loading dye

Loading dye is provided for analysis of purified DNA samples using electrophoresis. It contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type. Loading dye is supplied as a 5x concentrate; thus 1 volume of loading dye should be added to 5 volumes of purified DNA.

Table 2. Migration Distance of Gel Tracking Dyes

%TAE (TBE) agarose gel	Xylene cyanol (light blue)	Bromophenol blue (dark blue)	Orange G (orange)
0.8	5000 bp (3000 bp)	800 bp (400 bp)	150 bp (<100 bp)
1.0	3000 bp (2000 bp)	400 bp (250 bp)	<100 bp (<100 bp)
1.5	1800 bp (1100 bp)	250 bp (100 bp)	<100 bp (<100 bp)
2.0	1000 bp (600 bp)	200 bp (<100 bp)	<100 bp (<100 bp)
2.5	700 bp (400 bp)	100 bp (<50 bp)	<50 bp (<50 bp)

Agarose gel analysis of yield

Yields of DNA following cleanup can be determined by agarose gel analysis. Table 3 shows the total yield obtained following extraction of 1 µg or 0.5 µg starting DNA from an agarose gel with a recovery of 80% or 60% using the QIAquick Gel Extraction Kit. The corresponding amount of DNA in a 1 µl aliquot from 50 µl eluate is indicated. Quantities of DNA fragment corresponding to these 1 µl aliquots are shown on the agarose gel in Figure 4.

Table 3. Amount of DNA in 1 µl aliquots of a 50 µl eluate following QIAquick purification

Starting DNA	Recovery	Total yield (50 µl eluate)	Amount of DNA in 1 µl
1 µg	80%	0.8 µg	16 ng
	60%	0.6 µg	12 ng
0.5 µg	80%	0.4 µg	8 ng
	60%	0.3 µg	6 ng

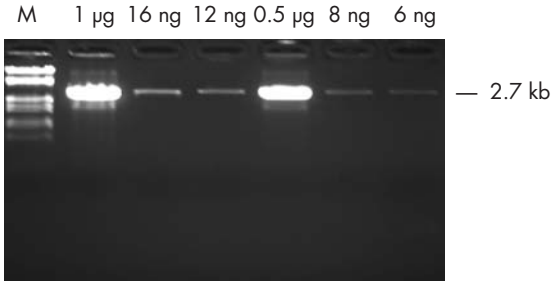


Figure 5. High DNA recovery. Quantities of purified 2.7 kb DNA fragment corresponding to 1/50 of the DNA obtained following purification from 1 µg or 0.5 µg starting DNA with a recovery of 80% or 60% (see Table 1). Samples were run on a 1% TAE agarose gel. **M**: lambda–EcoRI-HindIII markers.

Quantification of DNA fragments

DNA fragments can be quantified by running a sample alongside standards containing known quantities of the same-sized DNA fragment. The amount of sample DNA loaded can be estimated by visual comparison of the band intensity with that of the standards (Figure 5).

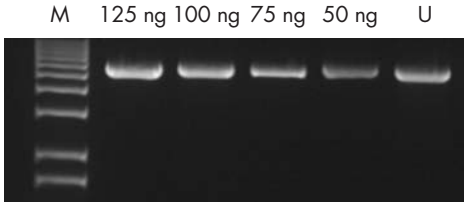
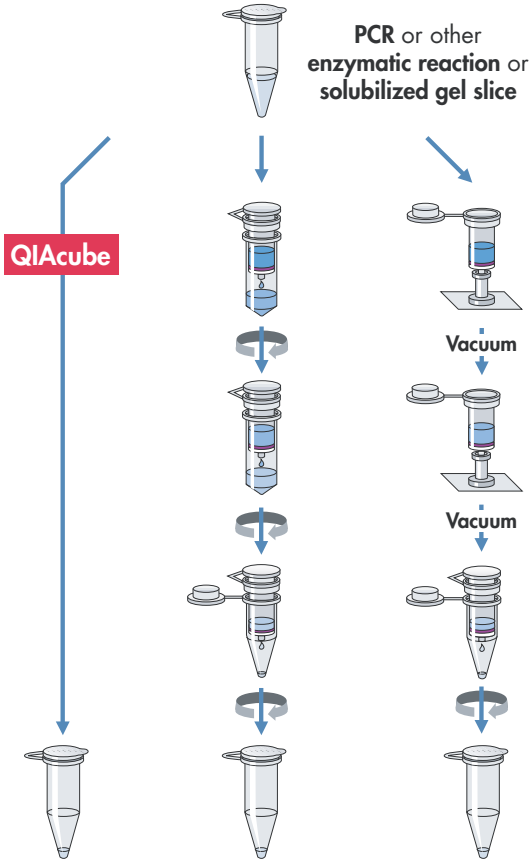


Figure 6. Agarose gel analysis. An unknown amount of a 5.5 kb DNA fragment (**U**) was run alongside known quantities (as indicated in ng) of the same DNA fragment. The unknown sample contained 75–100 ng DNA, as estimated by visual comparison with the standards. **M**: 1 kb DNA ladder.

Applications using QIAquick purified DNA

DNA purified with QIAquick is suitable for any subsequent application, such as restriction, labeling, hybridization, PCR, ligation and transformation, radioactive and fluorescent sequencing, in vitro transcription, or microinjection.

The QIAquick Procedure



Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

For all protocols

- Ethanol (96–100%)*
- Microcentrifuge
- 1.5 or 2 ml microcentrifuge tubes
- 3 M sodium acetate, pH 5.0, may be necessary for PCR purification and gel extraction protocols.
- Optional: Distilled water or TE buffer (10 mM Tris·Cl. 1 mM EDTA, pH 8) for elution of DNA.

Vacuum protocols

- Vacuum manifold (e.g., QIAvac 24 Plus or QIAvac 6S)
- Vacuum pump (e.g., QIAGEN Vacuum Pump, see ordering information).

Gel extraction protocols

- Isopropanol (100%)
- Heating block or water bath set at 50°C

* Do not use denaturated alcohol, which contains other substances such as methanol or methylethylketone.

QIAquick PCR Purification Kit Protocol

using a microcentrifuge

This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions (see page 8). For cleanup of other enzymatic reactions, follow the protocol as described for PCR samples or use the MinElute Reaction Cleanup Kit. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

Important points before starting

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x *g* (13,000 rpm) in a conventional tabletop microcentrifuge at room temperature.
- Add 1:250 volume pH indicator I to Buffer PB (i.e., add 120 µl pH indicator I to 30 ml Buffer PB or add 600 µl pH indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH indicator I indicates a pH of ≤7.5.
- Add pH indicator I to entire buffer contents. Do not add pH indicator I to buffer aliquots.
- If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I.

Procedure

1. **Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.**

   For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).
2. **If pH indicator I has beein added to Buffer PB, check that the color of the mixture is yellow.**

   If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
3. **Place a QIAquick spin column in a provided 2 ml collection tube.**
4. **To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s.**
5. **Discard flow-through. Place the QIAquick column back into the same tube.**

   Collection tubes are re-used to reduce plastic waste.
6. **To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s.**
7. **Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.**

   IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

8. **Place QIAquick column in a clean 1.5 ml microcentrifuge tube.**
9. **To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.**

   IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer.

   Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.
10. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

    Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

QIAquick Gel Extraction Kit Protocol

using a microcentrifuge

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA cleanup from enzymatic reactions (see page 8). For DNA cleanup from enzymatic reactions using this protocol, add 3 volumes of Buffer QG and 1 volume of isopropanol to the reaction, mix, and proceed with step 6 of the protocol. Alternatively, use the MinElute Reaction Cleanup Kit.

Important points before starting

- The yellow color of Buffer QG indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x *g* (13,000 rpm) in a conventional table-top microcentrifuge at room temperature.

Procedure

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**

   Minimize the size of the gel slice by removing extra agarose.

2. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl).**

   For example, add 300 µl of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.

3. **Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.**

   IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

4. **After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose).**

   If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

   The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤7.5. Buffer QG contains a pH indicator which is yellow at pH ≤7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. **Add 1 gel volume of isopropanol to the sample and mix.**

   For example, if the agarose gel slice is 100 mg, add 100 µl isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.

6. **Place a QIAquick spin column in a provided 2 ml collection tube.**
7. **To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.**

   The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.
8. **Discard flow-through and place QIAquick column back in the same collection tube.**

   Collection tubes are reused to reduce plastic waste.
9. **Recommended: Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.**

   This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription, or microinjection.
10. **To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min.**

    Note: If the DNA will be used for salt-sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE, before centrifuging.
11. **Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 17,900 x *g* (13,000 rpm).**

    IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
12. **Place QIAquick column into a clean 1.5 ml microcentrifuge tube.**
13. **To elute DNA, add 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min.**

    IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl.

    Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.
14. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

    Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx . The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and Suggestions
Low or no recovery		
a)	Buffer PE did not contain ethanol	Ethanol must be added to Buffer PE (concentrate) before use. Repeat procedure with correctly prepared Buffer PE.
b)	Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low-salt buffer (e.g., Buffer EB: 10 mM Tris·Cl, pH 8.5) or water. See "Elution in low-salt solutions", page 13.
c)	Elution buffer incorrectly dispensed	Add elution buffer to the center of the QIAquick membrane to ensure that the buffer completely covers the membrane. This is particularly important when using small elution volumes (30 µl).
Gel		
d)	Gel slice incompletely solubilized	After addition of Buffer QG to the gel slice, mix by vortexing the tube every 2–3 min during the 50°C incubation. DNA will remain in any undissolved agarose.
e)	pH of electrophoresis buffer too high (binding mixture turns orange or violet)	The electrophoresis buffer has been repeatedly used or incorrectly prepared, resulting in a sample pH that exceeds the buffering capacity of Buffer QG and leads to inefficient DNA binding. Add 10 µl of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture will turn yellow indicating the correct pH for DNA binding. Even for binding mixtures with only small color changes (slight orange color), add the 10 µl sodium acetate.
f)	Gel slice was too large (>400 mg)	70–80% recovery can only be obtained from ≤400 mg gel slice per QIAquick column. For gel slices >400 mg, use multiple QIAquick columns.

Gel: refers to QIAquick Gel Extraction Kits only.

PCR: refers to QIAquick PCR Purification Kits only.

Other notes refer to all kits.

		Comments and Suggestions
PCR		
g)	Insufficient/no PCR product	Estimate DNA recovery by running 10% of PCR product before and after purification on an agarose gel.
PCR/Gel		
h)	Cloudy and gelatinous appearance of sample mixture after addition of isopropanol	This may be due to salt precipitation, and will disappear upon mixing the sample. Alternatively, the gel slice may not be completely solubilized. In this case, apply the mixture to the QIAquick column, centrifuge, and then add 0.5 ml Buffer QG to the column. Let stand for 1 min at room temperature, and then centrifuge and continue with the procedure. This additional wash will solubilize remaining agarose.
i)	Binding mixture turns orange or violet	The pH in the sample exceeds the buffer capacity of Buffer QG or PB respectively. Add 20 µl of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture will turn yellow indicating the correct pH for DNA binding. Even for samples with slight color changes (orange color), add 10 µl sodium acetate.
DNA does not perform well (e.g., in ligation reactions)		
a)	Salt concentration in eluate too high	Modify the wash step by incubating the column for 5 min at room temperature after adding 750 µl of Buffer PE, then centrifuge.
b)	Eluate contains residual ethanol	Ensure that the wash flow-through is drained from the collection tube and that the QIAquick column is then centrifuged at 17,900 x *g* (13,000 rpm) for an additional 1 min.
Gel		
c)	Eluate contaminated with agarose	The gel slice is incompletely solubilized or weighs >400 mg. Repeat procedure, including the optional Buffer QG column-wash step.
PCR		
d)	Eluate contains primer-dimers	Primer-dimers formed are >20 bp and are not completely removed. After the binding step, wash the QIAquick column with 750 µl of a 35% guanidine hydrochloride aqueous solution (35 g in 100 ml). Continue with the Buffer PE wash step and the elution step as in the protocol.

		Comments and Suggestions
e)	Eluate contains denatured ssDNA, which appears as smaller smeared band on an analytical gel	Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95°C for 2 min, and allow the tube to cool slowly to room temperature. Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 10 mM NaCl. The salt and buffering agent promote the renaturation of DNA strands. However the salt concentration of the eluate must then be considered for subsequent applications.

References

1. Vogelstein, B. and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA ***76***, 615.
2. Hamaguchi, K. and Geiduschek, E.P. (1962) The effect of electrolytes on the stability of deoxyribonucleate helix. J. Am. Chem. Soc. ***84***, 1329.