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PCR and Gel electrophoresis/purification protocol

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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol describes Polymerase chain reaction PCR, Gel electrophoresis and Gel purification.

Materials

A	B	C
Reagent	Manufacturer	Catalog No.
DNA preparation		
QuickExtract DNA Extraction Solution	Biosearch Technologies	76081-768
PCR		
BioMix Red	Meridian Life Sciences	C755F25
Dimethyl sulfoxide (DMSO)	Sigma Aldrich Fine Chemicals Biosciences	D8418
Gel electrophoresis		
Quick-Load Purple 1 kb Plus DNA Ladder	New England Biolabs Inc.	N0550S
SYBR Safe DNA Gel Stain	Invitrogen	S33102
TAE Buffer (Tris-acetate-EDTA) (50X)	Thermo Scientific	B49

A	B
Equipment	Manufacturer
PCR	
Thermal Cycler	Bio-Rad
Gel electrophoresis	
LI-COR Odyssey M Imager	LI-COR
PowerPac HC Power Supply	Bio-Rad
Sub-Cell GT Horizontal Electrophoresis System	Bio-Rad



DNA preparation by QuickExtract™

- 1 Wash cells (from a 96-well plate) with 100 μ L PBS & aspirate
- 2 Add 30 μ L QuickExtract™ and scrape well bottom with pipette tip to detach cells
- 3 Transfer cells to labelled PCR tubes
Vortex for 15 secs
- 4 Incubate at 65°C for 6 mins
Vortex for 15 secs
- 5 Incubate at 98°C for 2 mins
- 6 Store at -20°C
- 7





Figure 1. Procedure for using QuickExtract DNA Extraction Solution ^[1]

Primer preparation

- 8 Resuspend IDT primers with H₂O to 100 µM
E.g. 20 nmol of primer (marked on tube label) - Add 200 µL H₂O
- 9 Dilute primer to 10 µM in a new microcentrifuge tube
E.g. 20 µL + 180 µL H₂O
- 10 Store at -20°C



PCR reaction setup

- 11 Prepare Master Mix – Number of reactions + 1 (as extra)



Master Mix using BioMix Red - for one 20 μ L reaction

A	B
BioMix Red	10 μ L
DMSO	0.6 μ L
Forward primer (10 μ M)	1 μ L
Reverse primer (10 μ M)	1 μ L
RNase/DNase-free H ₂ O	6.4 μ L

Master Mix using BioMix Red - for one 20 μ L reaction

A	B
Q5 High-Fidelity 2X Master Mix	10 μ L
Forward primer (10 μ M)	1 μ L
Reverse primer (10 μ M)	1 μ L
RNase/DNase-free H ₂ O	7 μ L

- 12 For each PCR tube: 1 μ L DNA sample + 19 μ L Master Mix

- 13 Bio-Rad Thermal Cycler – Select/Edit Protocol



PCR Cycle

A	B	C
Step	Temperature	Time
Enzyme Activation	94°C	3 mins
Denaturation	94°C	30 secs

A	B	C
Annealing	50-65°C	30 secs
Primary Extension	72°C	1 min/kb
Repeat from step 2 (34x)		
Secondary Extension	72°C	5 mins
Hold	4°C	∞

Note: Annealing temperature should be 5-10°C lower than T_m of primers

Gel electrophoresis preparation

14 **Make 1x TAE buffer**

Add 36 mL 50X TAE Buffer

Add diH₂O up to 1800 mL

15 **Gel preparation** on Bio-Rad Sub-Cell GT Electrophoresis Cell system



Figure 2. Bio-Rad Sub-Cell GT Cell ^[2]



- 15.1 Volume:
Big tray: ~ 300 mL
Small tray: ~ 150 mL
- 15.2 Gel percentage: 1.5-2%
E.g. For 2% gel: 2 g agarose + 100 mL 1X TAE buffer
- 15.3 Heat in microwave until completely dissolve
- 15.4 Add SYBR™ Safe DNA Gel Stain
E.g. 15 µL for 150 mL of gel
- 15.5 Pour onto the tray
Note: Remove big bubbles
- 15.6 Put in the comb (15/20 well)
- 15.7 Let it cool until solidify

Running gel electrophoresis

- 16 Make sure the gel is solidified completely
- 17 Carefully remove comb from gel
- 18 Put tray into Bio-Rad Sub-Cell GT Cell
Note: Make sure the wells are placed near the end of the negative (black) terminal
- 19 Add 1X TAE buffer to the Sub-Cell GT Cell if needed
Note: Make sure the gel is submerged in TAE buffer completely
- 20 Carefully load DNA ladder & PCR products

Notes:
 - Submerge pipette tips into the well before dispensing PCR product





- Prevent touching the wall of wells, which might break the gel
- Recommend dispensing liquid by pressing to the first stop only to prevent creating air bubbles, which could lead to loss of PCR product

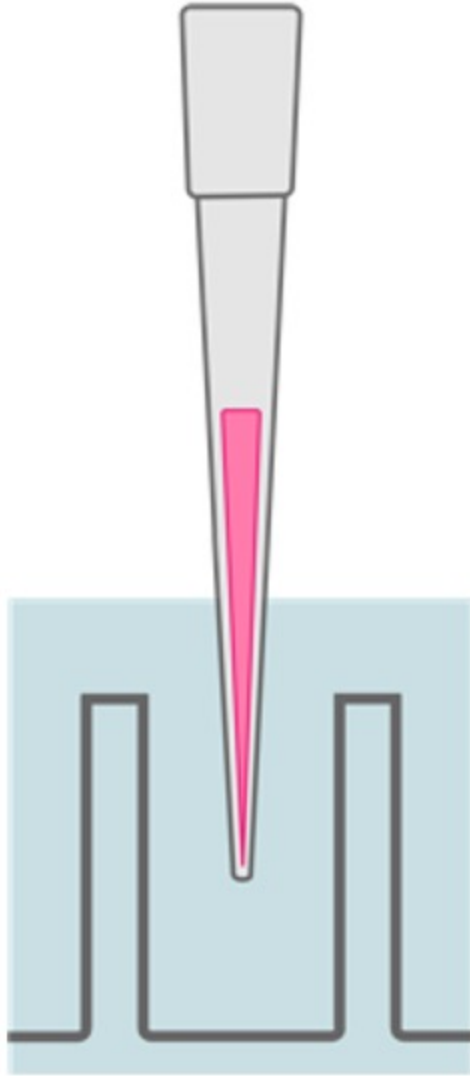


Figure 3.
Submerging pipette
tips in the well ^[3]

- 21 Set 100-140V on the Bio-Rad Power Supply

Notes:

Voltage depends on gel size

Make sure the gel is connected to the power supply properly - Black to Black; Red to Red

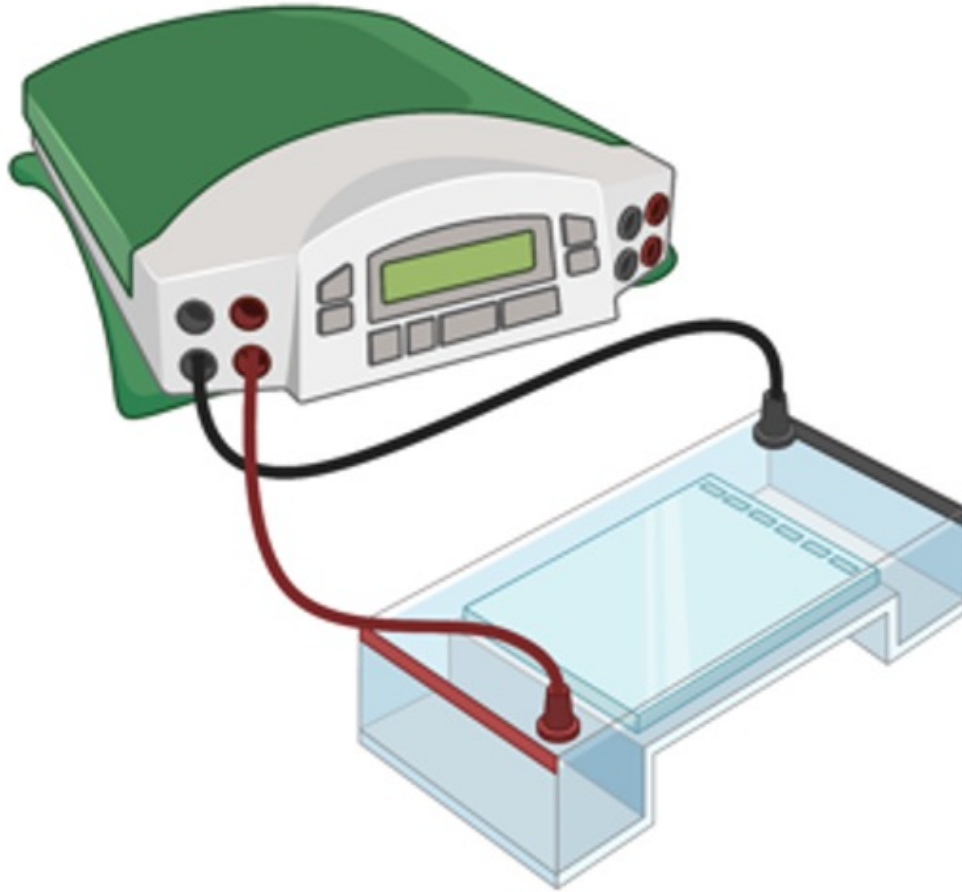


Figure 4. Gel connected to power supply ^[3]

- 22 START

Gel imaging - LI-COR Odyssey M Imager

23 Position the gel in LI-COR Odyssey M Imager



Figure 5. LI-COR Odyssey M Imager ^[4]

24 Open LI-COR Acquisition Software
Select:

- **Scan**

- Username: _____

Imager: **Odyssey M** → **Connect**

- **Gel** → **Connect**

- Draw Scan Area → **Next**

- **488 SYBR Safe** → **Save**

- Focus offset (mm): **2.00** → **Scan**

25 Wait for scan to finish

26 Export image to desired destination



- 27 **Note:** Remember to clean the imager glass with EtOH and Kimwipe before and after each use
- 28 Quick-Load® Purple 1 kb Plus DNA Ladder

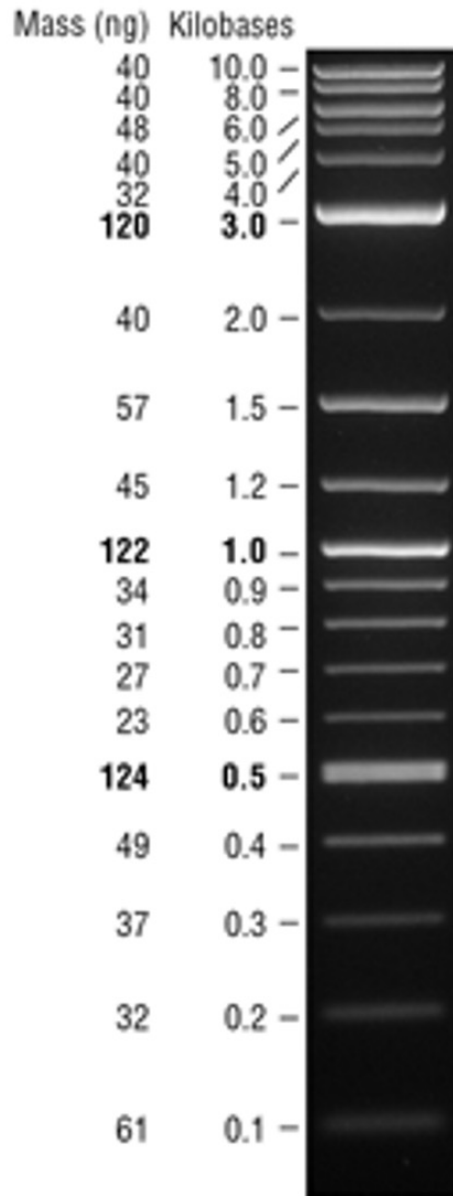


Figure 6. New England BioLabs Quick-Load® Purple 1 kb Plus DNA Ladder on a 1.0% TBE agarose gel ^[5]

PCR for gel purification

29 Run a 50 µL reaction instead of 20 µL

30 Prepare Master Mix – Number of reactions + 1 (as extra)



Master Mix using BioMix Red – for one 50 µL reaction

	A	B
	BioMix Red	25 µL
	DMSO	1.5 µL
	Forward primer (10 µM)	2.5 µL
	Reverse primer (10 µM)	2.5 µL
	RNase/DNase-free H ₂ O	16 µL

Master Mix using Q5 – for one 50 µL reaction

	A	B
	Q5 High-Fidelity 2X Master Mix	25 µL
	Forward primer (10 µM)	2.5 µL
	Reverse primer (10 µM)	2.5 µL
	RNase/DNase-free H ₂ O	17.5 µL

31 For each PCR tube: 2.5 µL DNA sample + 47 µL Master Mix

32

Bio-Rad Thermal Cycler – Select/Edit Protocol



	A	B	C
	Step	Temperature	Time

A	B	C
Enzyme Activation	94°C	3 mins
Denaturation	94°C	30 secs
Annealing	50-65°C	30 secs
Primary Extension	72°C	1 min/kb
Repeat from step 2 (39x)		
Secondary Extension	72°C	5 mins
Hold	4°C	∞

Note: Annealing temperature should be 5-10°C lower than T_m of primers

Gel extraction - modified from Qiagen MinElute Gel Extraction Kit^[6]

- 33 Run gel electrophoresis as instructed above
- 34 Excise the desired gel band under UV light
- 35 Transfer the gel slice into a 1.5 mL tube
- 36 Add 0.6 mL Qiagen Buffer QG
- 37 Incubate at 50°C until gel has completely dissolved
Note: Mix the tube every 1-2 mins to help dissolve the gel
- 38 Add 100 µL 100% isopropanol & mix by inverting
- 39 Transfer sample to a MinElute spin column (stored at 4°C)
- 40 Centrifuge at 8000 rpm for 1 min & discard the filtrate





- Put back the spin column to the same collection tube
- 41 Repeat previous two steps until all sample has passed through the column
- 42 Add 650 μ L Buffer PE to spin column
- 43 Centrifuge at 8000 rpm for 1 min & discard the filtrate
Put back the spin column to the same collection tube
- 44 Centrifuge at 13000 rpm for 1 min & discard the filtrate+collection tube
Place the column in a new 1.5 mL tube
- 45 Add 35-50 μ L Buffer EB
Note: Make sure it is added to the center of the membrane
- 46 Incubate at 42°C for 3 mins
- 47 Centrifuge at 13000 rpm for 1 min
- 48 Repeat previous three steps to increase product yield
Note: Reload with the purified DNA product instead of adding new Buffer EB
- 49



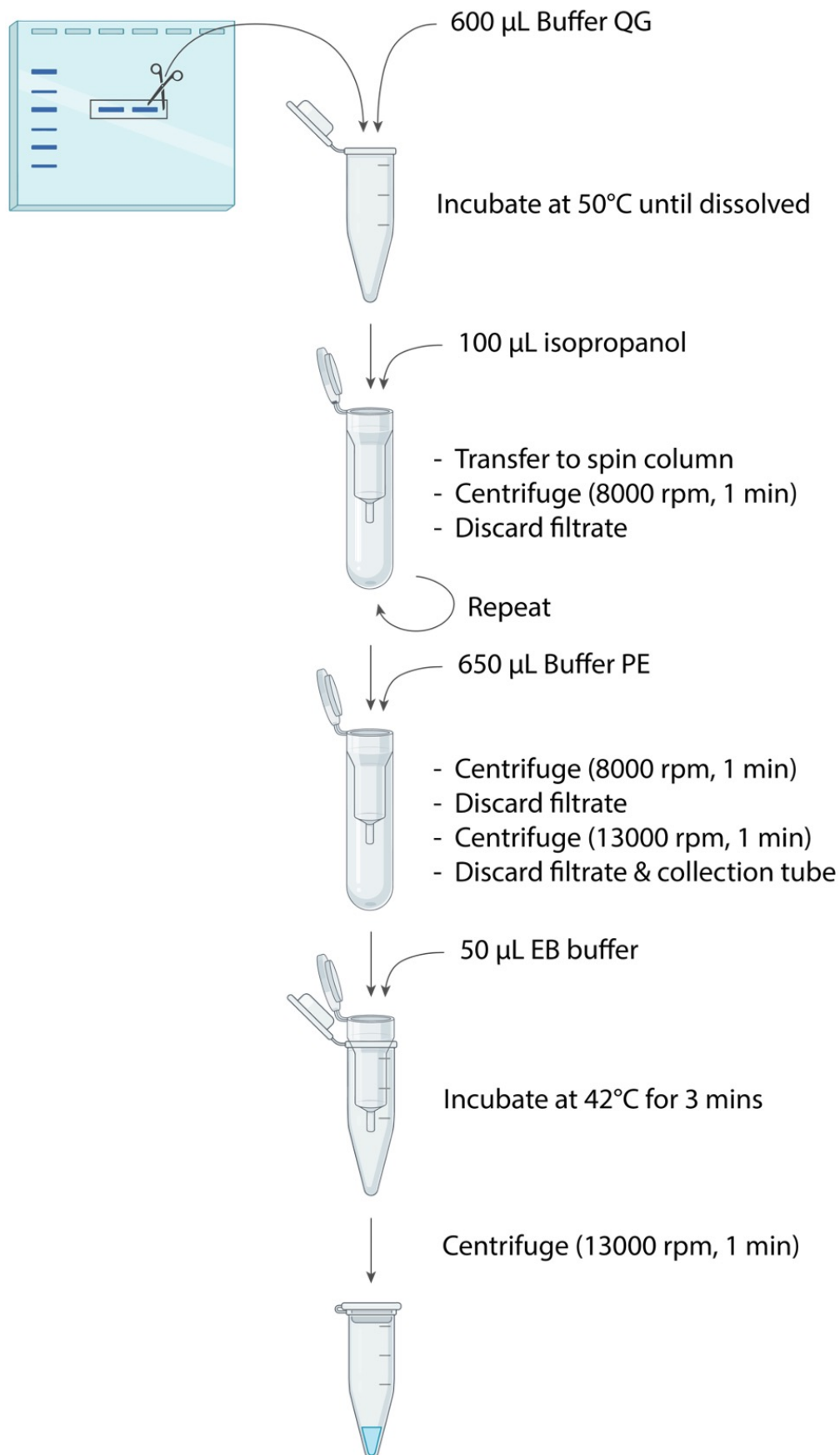


Figure 7. *Gel extraction workflow* ^[3]

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