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PCR and Gel electrophoresis/purification protocol

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ABSTRACT

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

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Keywords: PCR, Gel electrophoresis, 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 Sceiences	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 µL PBS & aspirate

2 Add 30 µL 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 H_2O to 100 µM

E.g. 20 nmol of primer (marked on tube label) - Add 200 µL H_2O

9 Dilute primer to 10 µM in a new microcentrifuge tube

E.g. 20 µL + 180 µL H_2O

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 µL reaction

	A	B
	BioMix Red	10 µL
	DMSO	0.6 µL
	Forward primer (10 µM)	1 µL
	Reverse primer (10 µM)	1 µL
	RNase/DNase-free H2O	6.4 µL

Master Mix using BioMix Red - for one 20 µL reaction

	A	B
	Q5 High-Fidelity 2X Master Mix	10 µL
	Forward primer (10 µM)	1 µL
	Reverse primer (10 µM)	1 µL
	RNase/DNase-free H2O	7 µL

12 For each PCR tube: 1 µL DNA sample + 19 µL 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
	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 Tm of primers

Gel electrophoresis preparation

14 **Make 1x TAE buffer**
Add 36 mL 50X TAE Buffer
Add diH_2O up to 1800 mL

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

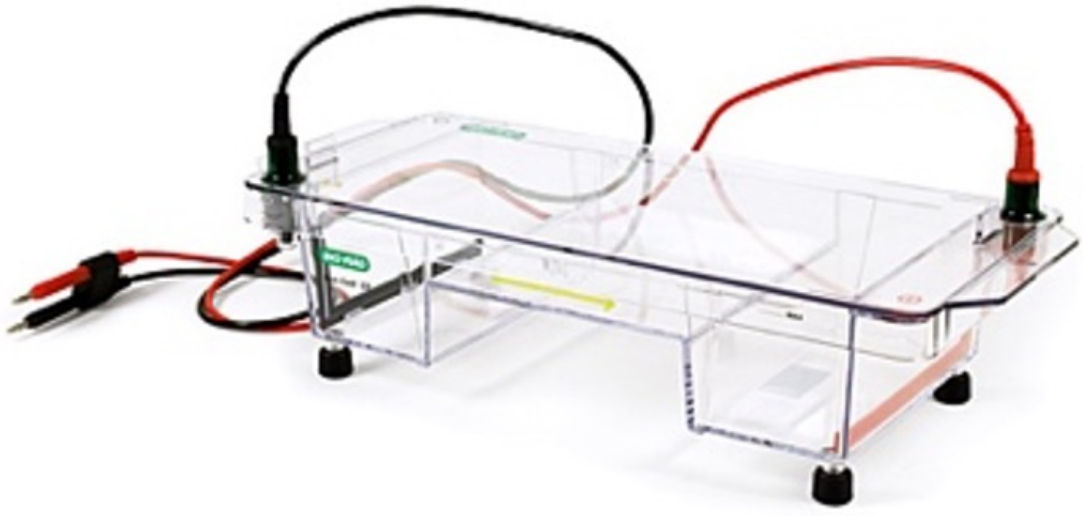

Figure 2. Bio-Rad Sub-Cell GT Cell [2]

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

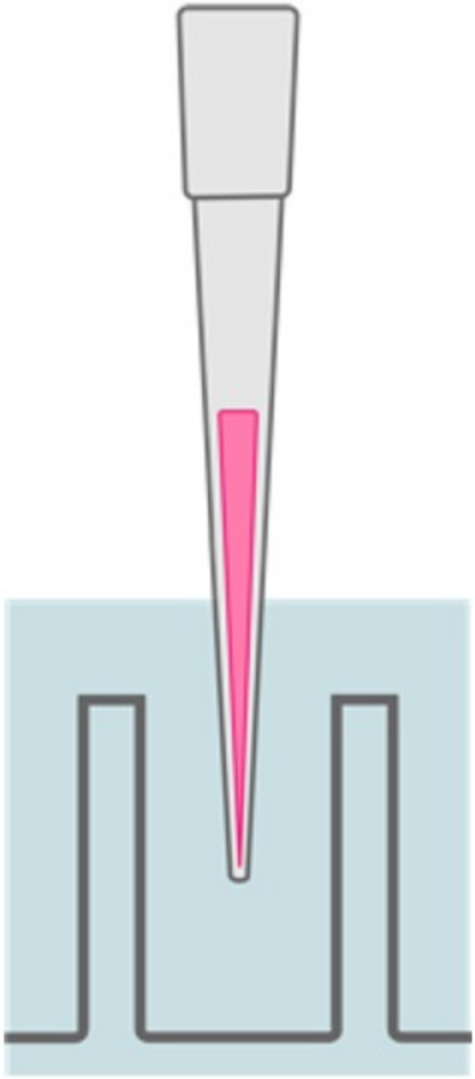

Figure 3. Submerging pipette tips in the well [3]

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

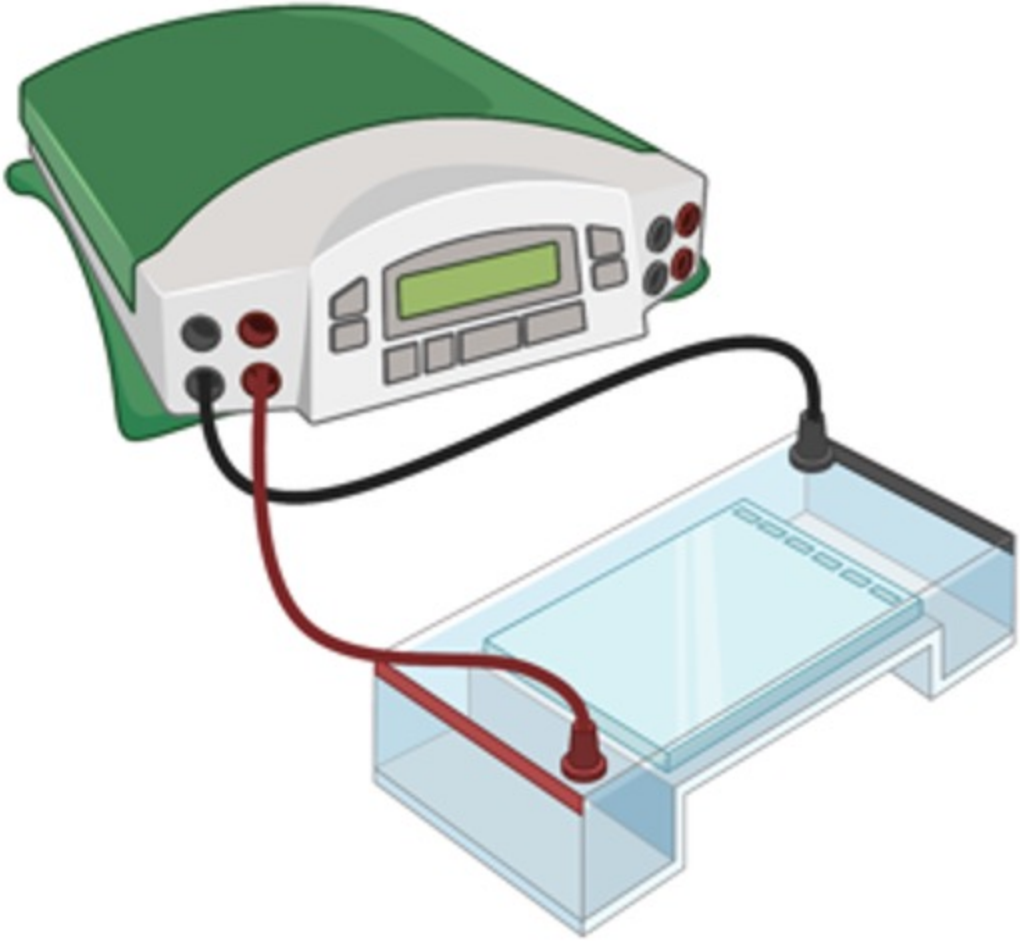

Figure 4. Gel connected to power supply [3]

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

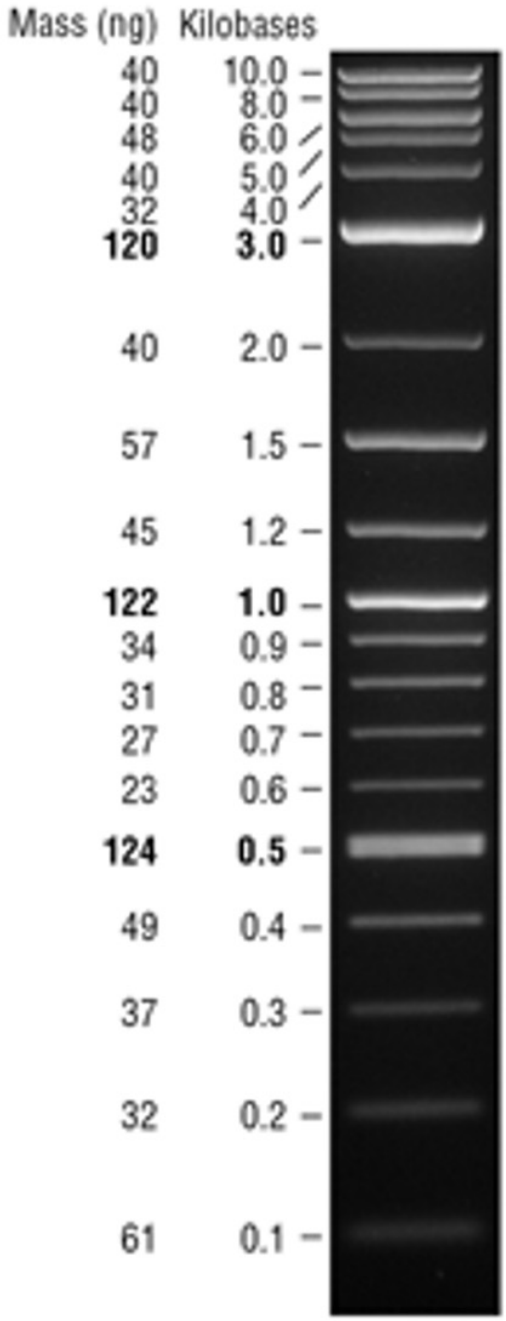

Figure 6. New England BioLabs Quick-Load® Purple 1 kb Plus DNA Ladder on a 1.0% TBE agarose gel [5]

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 H2O	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 H2O	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
	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)		

	A	B	C
	Secondary Extension	72°C	5 mins
	Hold	4°C	∞

Note: Annealing temperature should be 5-10°C lower than Tm 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 µL Buffer PE to spin solumn

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 µL 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

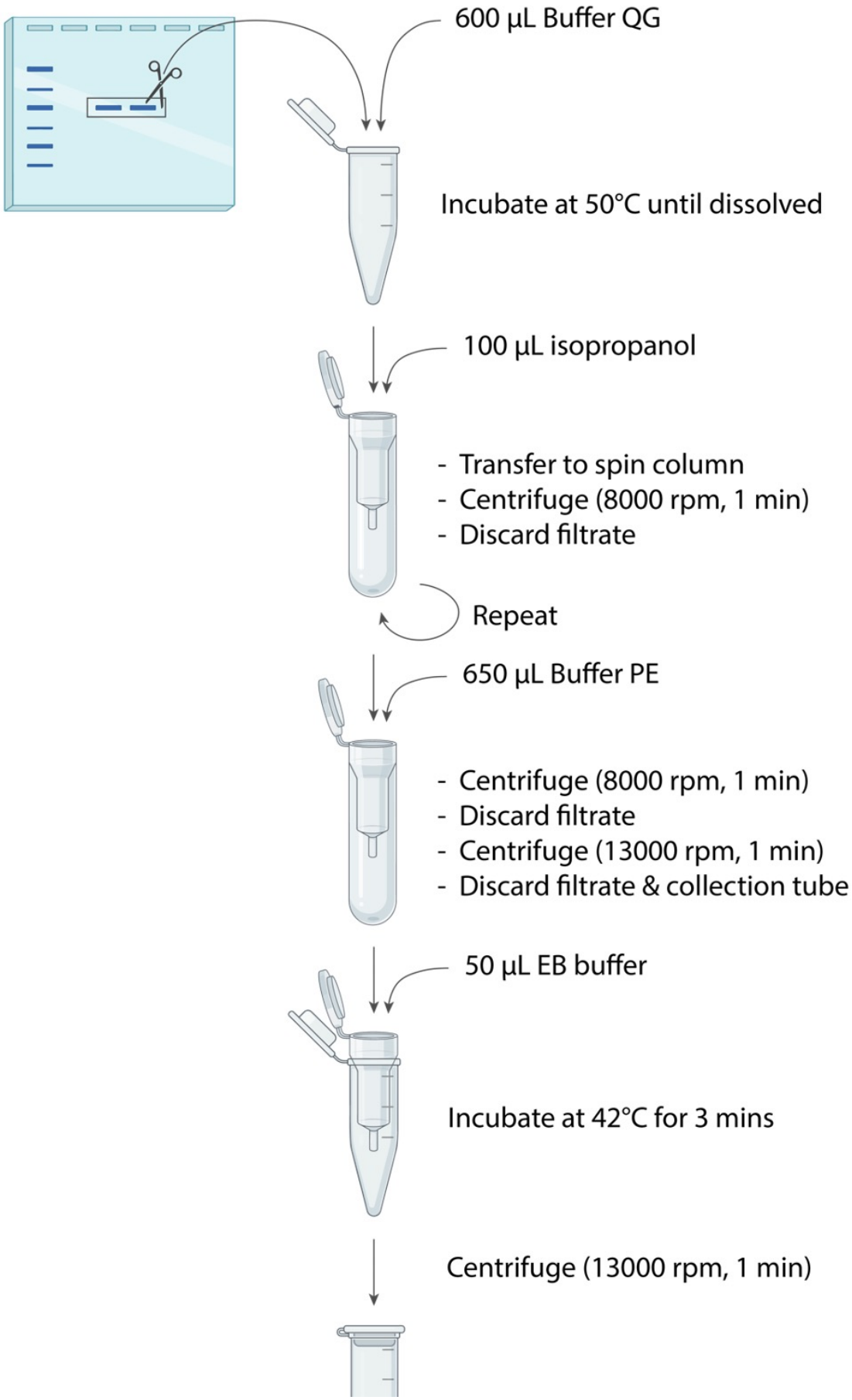
600 µL Buffer QG
Incubate at 50°C until dissolved
100 µL isopropanol
- Transfer to spin column
- Centrifuge (8000 rpm, 1 min)
- Discard filtrate
Repeat
650 µL Buffer PE
- Centrifuge (8000 rpm, 1 min)
- Discard filtrate
- Centrifuge (13000 rpm, 1 min)
- Discard filtrate & collection tube
50 µL EB buffer
Incubate at 42°C for 3 mins
Centrifuge (13000 rpm, 1 min)

Figure 7. *Gel extraction workflow* [3]