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Introduction

The E.Z.N.A.™ SQ Tissue DNA Kit is designed for isolating genomic DNA from fresh, frozen, and fixed animal tissue samples and cell cultures. The method can also be used for preparation of genomic DNA from sperm and fecal samples. The kit allows single or multiple, simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation are eliminated.DNA purified using the E.Z.N.A™ SQ Tissue DNA method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

The E.Z.N.A.® SQ Tissue DNA Kit uses a highly efficient solution based system to provide a convennient, fast, reliable and non-toxic method to isolate high molecular weight genomic DNA from various samples. Samples are first lyed with WBL buffer. Cellular proteins are removed by precipitation and high molecular weight genomic DNA will remain in solution. High quality genomic DNA the is purified by isopropanol precipitation.

Storage and Stability

All components of the E.Z.N.A.® SQ Tissue DNA Kit should be stored at 22°C-25°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. Under cool ambient conditions, a precipitate may form in the Buffer WTL. In case of such an event, heat the bottle at 55°C to dissolve. Store Buffer WTL at room temperature.

*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

DNA Yields from various Starting Materials

Species and Material	Amount of Starting material	Typical Yield
Mouse Tail	5mm (5-10mg)	10-60µg
	10mm (10-20mg)	25–120µg
	15 mm (15-30mg)	40-200µg
Human or Animal Solid Tissue	1-2 mg	0.5-1.5µg
	10 mg	10-12µg
	30 mg	30-35µg
	300 mg	400-450µg
Cultured Cells	100 x 10^6 cells	10-15µg

Kit Contents

These systems contain enough reagents for 20, 100 and 500 isolations of genomic DNA from 10mg animal tissue.

Product	D6032-00	D6032-01	D6032-02
Total amount of tissue	200mg	1 g	5 g
Buffer WTL	7 ml	35 ml	160 ml
Buffer PCP	2.5 ml	12 ml	55 ml
Buffer EB	2.5 ml	15 ml	60 ml
RNase A	30 µl	150 µl	750 µl
User Manual	1	1	1

A. DNA Purification Protocol for 0.1-2.5mg Animal Tissue

NOTE: The procedures below have been optimized for use with FRESH or FROZEN animal tissue samples. In addition, ≤ 2 x 10^6 leukocytes or cultured cells may be used with this procedure.

1. Dissect 0.1-2.5mg fresh or frozen tissue sample and place into a 1.5 ml tube containing 150µl WTL Buffer, homogenize throughly using a microfuge tube pestle (Product # SS1015-39).
2. Incubate lysate at 60°C for 20 minutes. If maximum yield is required, add 1µl Proteinase K solution (20mg/ml), Incubate lysate at 60°C for 3 hours to overnight. Invert the tube occasionally during incubation.
3. Add 1µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.
4. Cool the sample to room temperature. Add 50µl PCP Buffer to the cell lysate.
5. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate on ice for 5 minutes.
6. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 6.
7. Transfer the supernatant to a new sterile 1.5 ml centrifuge tube that containing 150µl of 100% isopropanol. If the DNA yield is expected to be low, add total 1µg carrier DNA or Glycogen solution (OBI Product # G-5006) to the 150µl isopropanol.
8. Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 13, 000 x g for 5 minutes at room temperature.
9. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 150µl of 70% ethanol and invert the tube a few times to wash the DNA pellet.
10. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**
11. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.
12. Add 35 µl of Buffer EB and vortex for 1 minutes to mix.
13. Incubate sample at 65ºC for 10 min. Some sample may need to incubate at 65ºC for 1 hour to rehydrate DNA. Store DNA at 2-8ºC.

B. DNA Purification Protocol for 3-10mg Animal Tissue

1. Dissect 3-10mg fresh or frozen tissue sample and place into a 1.5 ml tube containing 300µl WTL Buffer, homogenize throughly using a microfuge tube pestle (Product # SS1015-39).

2. Incubate lysate at 60°C for 20 minutes. If maximum yield is required, add 2µl Proteinase K solution (20mg/ml), Incubate lysate at 60°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 2µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 100µl PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate on ice for 5 minutes.

7. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 7.

8. Transfer the supernatant to a new sterile 1.5 ml centrifuge tube that containing 300µl of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 13, 000 x g for 1 minutes at room temperature.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 300µl of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

14. Add 60 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 10 min. Some sample may need to incubate at 65°C for 1 hour to rehydrate DNA.

16. Store DNA at 2-8°C.

C. DNA Purification Protocol for 10-20mg Animal Tissue

1. Dissect 10-20mg fresh or frozen tissue sample and place into a 1.5 ml tube containing 600µl WTL Buffer, homogenize throughly using a microfuge tube pestle (Product # SS1015-39).

2. Incubate lysate at 60°C for 20 minutes. If maximum yield is required, add 3µl Proteinase K solution (20mg/ml), Incubate lysate at 60°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 3µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 200µl PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 7.

8. Transfer the supernatant to a new sterile 1.5 ml centrifuge tube that containing 600µl of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 14, 000 x g for 1 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 600µl of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

14. Add 100 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 10 min. Some sample may need to incubate at 65°C for 1 hour to rehydrate DNA. Store DNA at 2-8°C.

D. DNA Purification Protocol for 30mg Animal Tissue

1. Dissect 30mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 2.0 ml tube containing 900µl WTL Buffer.

2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 5µl Proteinase K solution (20mg/ml), Incubate lysate at 60°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 5µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 300µl PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at max speed (≥13,000 x g) for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 7.

8. Transfer the supernatant to a new sterile 2.0 ml centrifuge tube that containing 900µl of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 13, 000 x g for 1 minutes at room temperature.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 900µl of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 13, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

14. Add 150 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA.

16. Store DNA at 2-8°C. For long term storage, store at -20°C.

E. DNA Purification Protocol for 30-50mg Animal Tissue

1. Dissect 30-50mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 15 ml tube containing 1.5ml WTL Buffer.

2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 10µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 8µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 500µl PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 7.

8. Transfer the supernatant to a new sterile 15 ml centrifuge tube that containing 1.5 ml of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 1.5ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

14. Add 250 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for ovemight to rehydrate DNA. Store DNA at 2-8°C.

F. DNA Purification Protocol for 50-100mg Animal Tissue

1. Dissect 50-100mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 15 ml tube containing 3.0 ml WTL Buffer.

2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 15µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 15µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature. Add 1ml PCP Buffer to the cell lysate.

5. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

6. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.

7. Transfer the supernatant to a new sterile 15 ml centrifuge tube that containing 3 ml of 100% isopropanol.

8. Gently mix the solution by inverting the tube 30-40 times.

9. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.

10. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 3 ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.

11. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

12. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

13. Add 150 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

14. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA.

15. Transfer the DNA to a 1.5 ml tube and store DNA at 2-8°C.

G. DNA Purification Protocol for 100-200mg Animal Tissue

1. Dissect 100-200mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 15 ml tube containing 6.0 ml WTL Buffer.

2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 30µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 30µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 2ml PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.

8. Transfer the supernatant to a new sterile 15 ml centrifuge tube that containing 6 ml of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 6 ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.
14. Add 500 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA.

16. Transfer the DNA to a 1.5 ml tube and store DNA at 2-8°C.

H. DNA Purification Protocol for 300mg Animal Tissue

1. Dissect 300mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 50 ml tube containing 9.0 ml WTL Buffer.
2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 50µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.
3. Add 50µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.
4. Cool the sample to room temperature.
5. Add 3ml PCP Buffer to the cell lysate.
6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.
7. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.
8. Transfer the supernatant to a new sterile 50 ml centrifuge tube that containing 9 ml of 100% isopropanol.
9. Gently mix the solution by inverting the tube 30-40 times.
10. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.
11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 9 ml of 70% ethanol and invert the tube a few times to wash the DNA pellet.
12. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**
13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.
14. Add 500 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.
15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA.
16. Transfer the DNA to a 1.5 ml tube and store DNA at 2-8°C.

I. DNA Purification Protocol for 300-500mg Animal Tissue

1. Dissect 300-500mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 50 ml tube containing 18.0 ml WTL Buffer.
2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 100µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.
3. Add 100µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.
4. Cool the sample to room temperature.
5. Add 6ml PCP Buffer to the cell lysate.
6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 10 minutes.
7. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.
8. Transfer the supernatant to a new sterile 50 ml centrifuge tube that containing 18 ml of 100% isopropanol.
9. Gently mix the solution by inverting the tube 30-40 times.
10. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.
11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 18 ml 70% ethanol and invert the tube a few times to wash the DNA pellet.
12. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**
13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.
14. Add 1ml of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.
15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA.
16. Transfer the DNA to a 1.5 ml tube and store DNA at 2-8°C.

J. DNA Purification Protocol for 500-700 mg Animal Tissue

1. Dissect 500-700mg fresh or frozen tissue sample, grind tissue with a ceramic mortar and pestle or tissue grinder under approximately 10 ml of liquid nitrogen and place sample into a 50 ml tube containing 21.0 ml WTL Buffer.

2. Incubate lysate at 60°C for 30- 60 minutes. If maximum yield is required, add 120µl Proteinase K solution (20mg/ml), Incubate lysate at 55°C for 3 hours to overnight. Invert the tube occasionally during incubation.

3. Add 120µl RNase A solution to the sample and mix by inverting tubes few times. Incubate the sample at 37°C for 15 minutes.

4. Cool the sample to room temperature.

5. Add 7ml PCP Buffer to the cell lysate.

6. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

7. Centrifuge at 3000 x g for 10 minutes at room temperature. The precipitated protein will form a tight. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat this step.

8. Transfer the supernatant to a new sterile 50 ml centrifuge tube that containing 21 ml of 100% isopropanol.

9. Gently mix the solution by inverting the tube 30-40 times.

10. Centrifuge at 3, 000 x g for 10 minutes at room temperature. DNA will be visible as a small white pellet.

11. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 21 ml 70% ethanol and invert the tube a few times to wash the DNA pellet.

12. Centrifuge at 3, 000 x g for 5 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point so pour slowly and watch the pellet.**

13. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

14. Add1.4 ml of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

15. Incubate sample at 65°C for 60 min. Some sample may need to incubate at 25°C for overnight to rehydrate DNA..

16. Transfer the DNA to a 1.5 ml tube and store DNA at 2-8°C.

K. DNA Purification Protocol for Cultured cells

1. This protocol is designed for isolating genomic DNA from 0.5-1 million cultured cells. For larger or smaller amount of starting cell number, please use protocol in E.Z.N.A® SQ Tissue DNA Kit.

2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a 1.5 ml microcentrifuge tube. For adherent cells, trypsinize the cell before harvesting.

3. Centrifuge at 14,000 x g for 10 seconds to pellet the cells. Remove the cells and leave behind about 10µl residue liquid.

4. Vortex the cells to resuspend the cells in the residue liquid. Make no cell clumps visible at this point.

5. Add 150µl of WTL Buffer to the resuspended cells an mix by pipetting. The solution should become very viscous. If the cell clumps are still visible after mixing, incubate the solution at 37°C until the clumps are not visible.

6. Optional) Add 1 µl RNase A solution to the cell lysate. Mix the sample by inverting the tube 20-25 times. Incubate the mixture at 37°C for 15-30 minutes.

7. Cool the sample to room temperature. Add 50µl PCP Buffer to the cell lysate. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes.

8. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 8.

9. Transfer the supernatant to a new sterile 1.5 ml centrifuge tube that containing 150µl of 100% isopropanol. Gently mix the solution by inverting the tube 30-40 times. Centrifuge at 14, 000 x g for 1 minutes at room temperature. DNA will be visible as a small white pellet.

10. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 150µl of 70% ethanol and invert the tube few times to wash the DNA pellet.

11. Centrifuge at 14, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. Pellet may be very loose at this point so pour slowly and watch the pellet.

12. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

13. Add 50 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

14. Incubate sample at 65°C for 10 min. Some sample may need to incubate at 65°C for 1 hour to rehydrate DNA. Store DNA at 2-8°C.

L. DNA Purification from Bacterial cultures

1. Harvest 1 ml overnight culture (1-3 billion cells) by centrifugation at ≥ 13,000 x g for 1 minute at room temperature.

2. Discard the supernatant with pipet.

3A For gram negative bacteria, add 600µl of WTL Buffer and gently pipet up and down until cells are throughly suspended.

3B. For gram positive bacteria, add 600µl of TE Buffer (10 mM Tris-HCl, pH 8.0; 1mM EDTA) and gently pipet up and down until cells are throughly suspended. Add 5µl lysozyme solution (100mg/ml) and vortex the tube to mix throughly. Incubate at 37°C for 30 minutes. Invert the tube few times during the incubation. Centrifuge the tube at ≥ 13,000 x g for 1 minute at room temperature to pellet the cells. Remove the supernatant. Add 600µl of WTL Buffer and gently pipet up and down until cells are throughly suspended.

4. Incubate the sample at 8°0C for 5 minutes to lysis the cells.

5. Add 5µl RNase A to the cell lysate. Mix the sample by invert the tubes 20 times and incubate at 37C for 15-20 minutes.

6. Cool the sample to the room temperature, add 200µl PCP buffer to the cell lysate.

7. Vortex vigorously at high speed for 30 seconds to mix. Some protein clumps may be visible after vortexing. Incubate in ice for 5 minutes (For species with high polysaccharide contents, 15-60 minutes incubation may be required).

8. Centrifuge at max speed for 3 minutes at room temperature. The precipitated protein will form a tight pellet. If the pellet is not tight or visible, incubate the tube in ice for 5 minutes and repeat step 7.

9. Transfer the supernatant to a new sterile 1.5 ml centrifuge tube that containing 600µl of 100% isopropanol.

10. Gently mix the solution by inverting the tube 20-40 times.

11. Centrifuge at 13, 000 x g for 1 minutes at room temperature.DNA will be visible as a small white pellet.

12. Pour of the supernatant and drain the tube briefly on a clean absorbent paper. Add 600µl of 70% ethanol and invert the tube few times to wash the DNA pellet.

13. Centrifuge at 14, 000 x g for 2 minutes at room temperature. Carefully pour off the ethanol. Pellet may be very loose at this point so pour slowly and watch the pellet.

14. Invert the tube on a clean adsorbent paper and air dry the pellet for 10-15 minutes.

15. Add 50 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

16. Incubate sample at 65°C for 10 min. Some sample may need to incubate at 65°C for 1 hour or overnight at room temperature to rehydrate DNA.

17. Store DNA at 2-8°C

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. Dilute the DNA in TE buffer and calculate concentration as:

[DNA] = (Absorbance$_{260}$*) x (0.05 µg/µl) x (Dilution factor)*

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A_{260}/A_{280}) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields range from 4 µg to 12 µg DNA per 250 µl whole blood, depending on source of sample, its age, and the method of storage. Yields are generally 5-fold higher with Buffy Coat samples.