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Introduction

The E.Z.N.A.™ SQ DNA Kit II is designed for isolating high molecular weight genomic DNA from fresh, frozen, and anticoagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, bone marrow or cultured cells. The procedure can be easily scaled up and down, allowing purification from different amount of starting material. The whole procedure can be performed in single tube so it can reduce the waste the chance for potential cross contamination. This kit allows single or multiple, simultaneous processing of samples in under 90 minutes. 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 DNA Kit II method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.™ SQ DNA Kit II uses a highly efficient solution based system to provide a convenient, fast, reliable and non-toxic method to isolate high molecular weight genomic DNA from whole blood or buffy coat. Plasma membrane are first lysed with NL1 buffer, cell nuclei and mitochondria are then pelleted by centrifugation. The pellet is resuspended and lysed by a Buffer NL2 buffer which contains chaotropic salt and proteinase. This step effectively removes most contaminate such as proteins. High quality genomic DNA is then purified by isopropanol precipitation.

Storage and Stability

All components of the E.Z.N.A.™ SQ DNA Kit II should be stored at 22°C-25°C. Under cool ambient conditions, a precipitate may form in the Buffer NL2. In case of such an event, heat the bottle at 55°C to dissolve.

Expiration Date: All E.Z.N.A.™ SQ DNA Kit II components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C

Yield and Size of Purified DNA

DNA yield depends on the number of nucleated cell numbers presented in the sample. Yields from whole blood may vary widely. The following table shows the typical yields obtained from different samples. The purified DNA size can be up to 200kb.

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*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
Human Whole Blood (Yield varies depending on the quantity of white blood cells present)	50 µl	0.3-0.6 µg
	100 µl	1-5 µg
	200 µl	3-10 µg
	300 µl	5-15 µg
	500 µl	7-23 µg
	600 µl	10-30 µg
Mouse Whole Blood	50 µl	0.2-0.6 µg
	100 µl	0.5-1.0 µg
	200 µl	2 -5 µg
	300 µl	4–7 µg
Cultured Cells	2 x 10^6 cells	10-15 µg

Kit Contents

Product	D0714-50	D0714-250
Volume of Blood can be processed per kit	50 ml	250 ml
NL Buffer	140 ml	700 ml
XL Buffer	30 ml	150 ml
EB Buffer (hydration buffer)	50 ml	250 ml
Proteinase K	6 mg	35 mg
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Before Starting

Important	**Proteinase K** must be diluted with **EB Buffer** as follows before use D0714-50 Add 0.3ml EB Buffer D0714-250 Add 1.4 ml EB Buffer
	Prepare the **XL Buffer/Proteinase K** mixture For each 1 ml whole blood, mix 500µl XL buffer with 5µl of constituted proteinase K solution.

Storage of Blood Samples

The procedure can use whole blood treated with EDTA, heparin, or citrate with either fresh or frozen condition. Fresher blood yield better results. For short term storage (for up to 2 weeks), it is recommended to collect blood in the tube contains EDTA as anticoagulant. For long term storage, sample should be collected in the tube contains EDTA as anticoagulant and store at -70°C.

A. DNA Purification Protocol for 100-500µl whole blood

NOTE: The buffer volume of the following protocol is for isolating 200µl whole blood sample. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 100 µl to 500 µl in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the EB buffer volume for 100µl blood). Frozen blood should be thawed quickly in a 37°C water bath with gently agitation and stored on ice before starting the procedure.

Reagent volumes required for processing 100-500µl whole blood samples

Reagent	Blood Volume				
	100µl	200µl	300µl	400µl	500µl
NL Buffer	250µl	500µl	750µl	1000µl	1250µl
XL Buffer/Proteinase K	50µl	100µl	150µl	200µl	250µl
100% isopropanol	50µl	100µl	150µl	200µl	250µl
70% ethanol	50µl	100µl	150µl	200µl	250µl
EB Buffer	100µl	200µl	200µl	200µl	200µl

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 1.5 ml or 2 ml microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- 100% Isopropanol
- 70% ethanol

1. Add 200µl whole blood (or bone marrow) to a nuclease-free 1.5 ml microcentrifuge tube containing 500µl NL Buffer. Mix by inverting the tube 5 times.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

3. Add 100µl XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized .

   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.

4. Centrifuge at 10,000 x for 5 seconds to bring down any liquid drop from tube lid.

5. Incubate at 65°C for 5 minutes in a water bath or heating block.

   Note: The sample should change color from red to oliver green during proteinase digestion

6. Add 100µl isopropanol to the lysate.

7. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate become visible as threads or clumps.

8. Centrifuge at 14,000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.

9. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 100µl of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.

10. 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.**

11. Invert the tube on a clean absorbent paper towel and air dry the pellet for 10-15 minutes.

12. Add 200 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

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

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

B. DNA Purification Protocol for 1-3 ml whole blood

NOTE: The buffer volume of the following protocol is for isolating 2 ml whole blood sample. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 1 ml to 3 ml l in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the EB buffer volume for 3 ml blood). Frozen blood should be thawed quickly in a 37°C water bath with gently agitation and stored on ice before starting the procedure.

Reagent volumes required for processing 100-500µl whole blood samples

Reagent	Blood Volume		
	1 ml	2 ml	3 ml
NL Buffer	2.5 ml	5 ml	7.5 ml
XL Buffer/Proteinase K	0.5 ml	1 ml	1.5 ml
100% isopropanol	0.5 ml	1 ml	1.5 ml
70% ethanol	0.5 ml	1 ml	1.5 ml
EB Buffer	0.2 ml	0.2 ml	0.3 ml

Materials to be supplied by user

- Microcentrifuge capable of 2,000 x g
- Nuclease-free 15 ml centrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 2 ml whole blood (or bone marrow) to a nuclease-free 15 ml centrifuge tube containing 5 ml NL Buffer. Mix by inverting the tube 5 times.

2. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

3. Add 1 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.

4. Incubate at 65°C for 5 minutes in a water bath or heating block.

Note: The sample should change color from red to olive green during proteinase digestion

5. Add 1 ml isopropanol to the lysate.

6. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate become visible as threads or clumps.

7. Centrifuge at 2000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.

8. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 1ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.

9. Centrifuge at 2000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

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

11. Add 200 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

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

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

C. DNA Purification Protocol for 4 -14 ml whole blood

NOTE: The buffer volume of the following protocol is for isolating 12 ml whole blood sample. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 4 ml to 14 ml l in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the EB buffer volume for 3 ml blood). Frozen blood should be thawed quickly in a 37°C water bath with gently agitation and stored on ice before starting the procedure.

Reagent volumes required for processing 100-500µl whole blood samples

Reagent	Blood Volume				
	4 ml	5 ml	6 ml	7 ml	8 ml
NL Buffer	10 ml	12.5ml	15 ml	17.5 ml	20 ml
XL Buffer/Proteinase K	2 ml	2.5 ml	3 ml	3.5 ml	4 ml
100% isopropanol	2 ml	2.5 ml	3 ml	3.5 ml	4 ml
70% ethanol	2 ml	2.5 ml	3 ml	3.5 ml	4 ml
EB Buffer	0.4ml	200µl	200µl	200µl	200µl

Reagent	Blood Volume					
	9 ml	10 ml	11 ml	12 ml	13 ml	14 ml
NL Buffer	22.5 ml	25 ml	27.5 ml	30 ml	32.5 ml	35 ml
XL Buffer /Proteinase K	4.5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
100% isopropanol	4.5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
70% ethanol	4.5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
EB Buffer	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

Materials to be supplied by user

- Microcentrifuge capable of 2000 x g
- Nuclease-free 50 ml centrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 12 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 30 ml NL Buffer. Mix by inverting the tube 5 times.

2. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

3. Add 5 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.

4. Incubate at 65°C for 5 minutes in a water bath or heating block.

   Note: The sample should change color from red to olive green during proteinase digestion

5. Add 5 ml isopropanol to the lysate.

6. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate become visible as threads or clumps.

7. Centrifuge at 2000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.

8. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 5 ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.

9. Centrifuge at 2000 x g for 3 minutes at room temperature. Carefully pour off the ethanol. **Pellet may be very loose at this point, so pour slowly and watch the pellet.**

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

11. Add 1 ml of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

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

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

D. DNA Purification Protocol for 20 ml whole blood

Materials to be supplied by user

- Microcentrifuge capable of 2000 x g
- Nuclease-free 50 ml centrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. Add 10 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 25 ml NL Buffer. Mix by inverting the tube 5 times.

2. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Make sure the pellet remains in the tube.

3. Add another 10 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 25 ml NL Buffer. Mix by inverting the tube 5 times.

4. Centrifuge at 2000 x g for 5 minutes at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

5. Add 5 ml XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized.
   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.

6. Incubate at 65°C for 5 minutes in a water bath or heating block.
   NOTE: The sample should change color from red to olive green during proteinase digestion

7. Add 5 ml isopropanol to the lysate.

8. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate become visible as threads or clumps.

9. Centrifuge at 2000 x g for 5 minute at room temperature. DNA will be visible as a small white pellet.

10. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 5 ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.

11. Centrifuge at 2000 x g for 3 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 absorbent paper towel and air dry the pellet for 10-15 minutes.

13. Add 1 ml of DNA rehydration solution (Buffer EB) and vortex for 1 minute 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.

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

E. DNA Purification Protocol for 100-500 µl Buffy Coat

NOTE: The buffer volume of the following protocol is for isolating 2 ml whole blood sample. This procedures can be scaled up and down for use with FRESH or FROZEN blood samples 1 ml to 3 ml l in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the EB buffer volume for 3 ml blood). Frozen blood should be thawed quickly in a 37°C water bath with gently agitation and stored on ice before starting the procedure.

Reagent volumes required for processing 100-500µl Buffy coat

Reagent	Buffy Coat Volume				
	100µl	200µl	300µl	400µl	500µl
NL Buffer	250µl	500µl	750µl	1000µl	1250µl
XL Buffer/Proteinase K	100µl	200µl	300µl	400µl	500µl
100% isopropanol	100µl	200µl	300µl	400µl	500µl
70% ethanol	100µl	200µl	300µl	400µl	500µl
EB Buffer	200µl	200µl	200µl	200µl	200µl

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 ml microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

The buffy coat fraction of whole blood is enriched with WBC, and usually gives at least 5-fold more DNA than the same volume of blood. To prepare buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x g for 10 min at room temperature. Three layers should be obtained, with plasma in the upper layer, leucocytes in the middle layer (buffy coat), and erythrocytes in bottom layer. Carefully aspirate the plasma, making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette and used directly in the E.Z.N.A.® SQ Blood DNA Kit II , or frozen at -70°C for storage.

1. Add 200 µl buffy coat preparation to a nuclease-free 1.5 ml microcentrifuge tube containing 500µl NL Buffer. Mix by inverting the tube 5 times.

2. Centrifuge at 14,000 x g for 30 seconds at room temperature. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

3. Add 200µl XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized .

   Important: When process multiple samples, vortex each tube immediately after addition of XL/Proteinase K mixture.

4. Centrifuge at 10,000 x for 5 seconds to bring down any liquid drop from tube lid.

5. Incubate at 65°C for 5 minutes in a water bath or heating block.
   Note: The sample should change color from red to olive green during proteinase digestion

6. Add 200µl isopropanol to the lysate.

7. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate becomes visible as threads or clumps.

8. 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.**
9. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 200µl of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.

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

11. Add 200 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minute to mix.

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

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

F. DNA Purification Protocol for 1-2 x 10^6 Cultured Cells

Materials to be supplied by user

- Microcentrifuge capable of 14,000 x g
- Nuclease-free 2.0 ml microcentrifuge tubes
- Water Bath preset at 37°C and 65°C
- Isopropanol
- 70% ethanol

1. This protocol is designed for isolating genomic DNA from 1-2 million cultured cells.

2. Harvest the cells and transfer them with salt balanced buffer (such as PBS) to a 2.0 ml microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

3. Centrifuge at 300 x g for 5 minutes to pellet the cells. Remove and discard supernatant. Leave the tube inverted on a absorbent paper for 2-3 minutes. Make sure the pellet remains in the tube.

4. Add 300µl of NL Buffer to the resuspended cells and mix by pipetting up and down until the pellet is resuspended. The lysate should be cloudy.

5. Add 300µl XL Buffer/Proteinase K mixture to the tube containing the nuclei pellet. Vortex immediately for 10 seconds.

6. Incubate at 65°C for 5 minutes in a water bath or heating block.
Note: The sample should change color from red to olive green during proteinase digestion

7. Add 600µl isopropanol to the lysate.

8. Gently mix the solution by inverting the tube 20-3 times or until the DNA precipitate become visible as threads or clumps.

9. 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.**

10. Pour out the supernatant and drain the tube briefly on a clean absorbent paper towel. Add 600µl of 70% ethanol and invert the tube a 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 towel and air dry the pellet for 10-15 minutes.

13. Add 200 µl of DNA rehydration solution (Buffer EB) and vortex for 1 minutes to mix.

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

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

G: DNA Purification from large volume (up to 10 ml) Clotted Blood

1. Transfer the clotted blood include any liquid residual into a 50 ml centrifuge tube.

2. Homogenize the sample with a rotor-stator homogenizer until the sample is uniformly homogenous

3. Add 25 ml NL buffer and mix by invert the tube 5-7 times.

4. Centrifuge at 2000 x g for 5 minutes in a swing-out rotor.

5. Discard the supernatant and leave the tube inverted on a clean absorbent paper for 2 minutes. Make sure that the pellet remain in the tube.

6. Add 5ml XL Buffer and 50 µl Proteinase K solution (25mg/ml), close the cap and vortex immediately until the pellet is completely homogenized.

   Note: When processing multiple samples, vortex each tube immediately after addition of XL Buffer/ Proteinase K . Do not wait until buffer has been added to all samples before vortexing. Although the pellet can be easily homogenized with few pulses of high-speed vortexing, however, traces of pellet with a jelly-like consistency (often barely visible) may remain. If these traces are seen, vortex sample for another 30 seconds.

7. Incubate the tube at 65°C for 30 minutes in a water bath or heating block.
Note: The sample should change color from red to olive green during proteinase digestion.

8. Vortex again for 10 seconds, inspect the tube to make sure the homogenization is complete.

9. Add 5 ml of isopropanol and mix throughly by invert the tube 20-30 times.

DNA precipitate will become visible as threads or a clump.

10. Centrifuge at 2000 x g for 5 minutes to pellet the DNA.

11. Discard the supernatant and briefly invert the tube onto a clean absorbent paper, make sure the DNA pellet remains inside the tube.

12. Add 5 ml 70% ethanol and vortex for 10 seconds.

13. Centrifuge at 2000 x g for 5 minutes.

14. Discard the supernatant and invert the tube onto a clean absorbent paper for 5 minutes, make sure the DNA pellet remains inside the tube.

15. Air dry the DNA pellet for 10 minutes or until all of the liquid evaporates. Do not over dry the pellet because it is very difficult to dissolve the over-dried DNA pellet.

16. Add 1 ml EB Buffer or TE Buffer, vortex 5 seconds at lower speed. Dissolve the DNA by incubating 1 hour at 65C or overnight at room temperature.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Low DNA yield	Blood Sample contains too few white blood cells	Draw new blood samples
	Blood sample is too old.	Try to use fresh blood if possible.
	XL Buffer/Proteinase K is not prepared correctly.	Ensure that the XL Buffer/Proteinase K is not prepared correctly.
	Proteinase K is dissolved in wrong buffer	Use EB to dissolve the proteinase
	Incomplete sample lysis	Mix the sample throughly after addition of NL Buffer.
	DNA pellet was lost during isopropanol precipitation	Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps.
Low A_{260}/A_{280} ratio	Proteinase digestion was not complete	Make sure to prepare the XL/proteinase K properly and fresh.
	Poor cell lysis due to incomplete mixing with Buffer NL	Repeat the procedure, this time making sere to vortex the sample with Buffer NL immediately and completely.
	Hemoglobin remains	Repeat the procedure, this time making sure enough volume of NL buffer is used .
No DNA	DNA pellet was lost during isopropanol precipitation	Be very careful not to lose the DNA when removing isopropanol or ethanol during precipitation and wash steps.
DNA Pellet is difficult to dissolve	DNA pellet was over dried	Rehydrate the DNA by incubating the DNA pellet with EB Buffer at 65°C for 1 hour and then leave the sample at room temperature or 4°C for overnight.
	DNA pellet was not mixed well during rehydration step.	Shake a few times during the rehydration step.
Gel-like traces of pellet remaining after resuspension of pellet in XL/Proteinase mixture	After addition of the XL/proteinase K, the sample was left too long before the vortexing	Immediately mix the sample after the addition of XL/proteinase K.