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Introduction

The E.Z.N.A.® SQ Blood 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 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 Blood DNA Kit II method is ready for applications such as PCR*, Southern blotting, and restriction digestion.

Principle

E.Z.N.A.® SQ Blood 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 Buffer NL, cell nuclei and mitochondria are then pelleted by centrifugation. The pellet is resuspended and lysed by Buffer XL which contains chaotropic salt and proteinase. This step effectively removes most contaminant 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 Blood DNA Kit II should be stored at 22-25°C. OB Protease should be stored at 15-25°C.Under cool ambient conditions, a precipitate may form in the Buffer XL. 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

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

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.

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
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 Number	D0714-50	D0714-250	D0714-1000
processed Blood Volumes	50 ml	250 ml	1000 ml
Buffer NL	140 ml	4 x 175 ml	11 x 260 ml
Buffer XL	30 ml	150 ml	3 x 200 ml
Buffer EB (hydration buffer)	50 ml	250 ml	5 x 220 ml
OB Protease	300 µl	1.4 ml	5.6 ml
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Before Starting

IMPORTANT	**Prepare the Buffer XL/OB Protease mixture:** For each 1 ml whole blood, mix 500 µl Buffer XL with 5 µl OB Protease. This mixture should be prepare under 10 minutes. without the sample, OB Protease will lost the activities.

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.

Materials to be supplied by user

? Microcentrifuge capable of 14,000 x g

? Nuclease-free 1.5 ml or 2 ml microcentrifuge tubes (for 100-500 ul blood)

? Nuclease-free 15 ml microcentrifuge tubes (for 0.5-3 ml blood)

? Nuclease-free 50 ml microcentrifuge tubes (for 3-10 ml blood)

? Water Bath preset at 37°C and 65°C

? 100% Isopropanol

? 70% ethanol

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

NOTE: The buffer volume of the following protocol is for isolating 200 µl 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 Buffer EB volume for 100 µl 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 100-500 µl whole blood samples.

Reagent	Blood Volume				
	100 µl	200 µl	300 µl	400 µl	500 µl
Buffer NL	250 µl	500 µl	750 µl	1000 µl	1250 µl
Buffer XL	50 µl	100 µl	150 µl	200 µl	250 µl
OB Protease	0.5 µl	1 µl	1.5 µl	2 µl	2.5 µl
100% isopropanol	50 µl	100 µl	150 µl	200 µl	250 µl
70% ethanol	50 µl	100 µl	150 µl	200 µl	250 µl
Buffer EB	100 µl	200 µl	200 µl	200 µl	200 µl

1. **Add 200 µl whole blood to a nuclease-free 1.5 or 2 ml microcentrifuge tube containing 500 µl Buffer NL(2.5 x volume of blood).** Mix by inverting the tube 5 times.

2. **Centrifuge at 14,000 x g for 30 seconds at room temperature.**

3. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube.**

4. **Add 100 µl Buffer XL/OB Protease (0.5 volume of blood) mixture to the tube containing the nuclei pellet.** Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Tips: OB Protease and Buffer XL can be premix together. Add 1ul OB Protease to 100 ul Buffer XL. This Mixture should be prepared in 10 minutes. without the samples, OB Protease will lose activity.

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

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

6. **Incubate at 65°C for 10-30 minutes in a water bath or heating block.**

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

7. **Add 100 µl isopropanol (0.5 volume of blood) to the lysate.**

8. **Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.**

9. **Centrifuge at 14,000 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 100 µl of 70% ethanol and vortex the tube for 10 seconds 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 absorbent paper towel and air dry the pellet for 10-15 minutes.

13. **Add 50-200 µl of DNA rehydration solution (Buffer EB)** and vortex for 1 minute 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.

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 in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the Buffer EB 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 1-3 ml whole blood samples

Reagent	Blood Volume		
	1 ml	2 ml	3 ml
Buffer NL	2.5 ml	5 ml	7.5 ml
Buffer XL	0.5 ml	1ml	1.5 ml
OB Protease	5 ul	10 ul	15 ul
100% isopropanol	0.5 ml	1 ml	1.5 ml
70% ethanol	0.5 ml	1 ml	1.5 ml
Buffer EB	0.2 ml	0.2 ml	0.3 ml

1. **Add 2 ml whole blood (or bone marrow) to a nuclease-free 15 ml centrifuge tube containing 5 ml Buffer NL(2.5 volume of blood).** Mix by inverting the tube 5 times.

2. **Centrifuge at 2,000 x g for 5 minutes at room temperature.**

3. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube.**

4. **Add 1 ml Buffer XL/OB Protease mixture (0.5 x volume of blood) to the tube containing the nuclei pellet**. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Tips: OB Protease and Buffer XL can be premix together. Add 10ul OB Protease to 1000 ul Buffer XL. This Mixture should be prepared in 10 minutes. without the samples, OB Protease will lose activities.

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

5. **Incubate at 65°C for 10-30 minutes in a water bath or heating block.**

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

6. **Add 1 ml isopropanol(0.5 volume of blood) to the lysate.**

7. **Gently mix the solution by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.**

8. **Centrifuge at 2000 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 1ml of 70% ethanol and vortex the tube for 10 seconds to wash the DNA pellet.**

10. **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.

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

12. **Add 200-500 µl of DNA rehydration solution (Buffer EB)**.

13. **Incubate sample at 65°C for 10 min.** Some sample 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.

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 in volume by scaling reagent volume up and down in proportion to the volume of sample used. (Except the Buffer EB 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 4-14 ml whole blood samples

Reagent	Blood Volume				
	4 ml	5 ml	6 ml	7 ml	8 ml
Buffer NL	10 ml	12.5ml	15 ml	17.5 ml	20 ml
Buffer XL	2 ml	2.5 ml	3 ml	3.5 ml	4 ml
OB Protease	20 ul	25 ul	30 ul	35 ul	40 ul
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
Buffer EB	0.4ml	200 µl	200 µl	200 µl	200 µl

Reagent	Blood Volume					
	9 ml	10 ml	11 ml	12 ml	13 ml	14 ml
Buffer NL	22.5 ml	25 ml	27.5 ml	30 ml	32.5 ml	35 ml
Buffer XL	4.5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
OB Protease	45 ul	50 ul	50 ul	50 ul	50 ul	50 ul
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
Buffer EB	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml

1. **Add 12 ml whole blood (or bone marrow) to a nuclease-free 50 ml centrifuge tube containing 30 ml (2.5 x volume of blood) Buffer NL.** Mix by inverting the tube 5 times.

2. **Centrifuge at 2000 x g for 5 minutes at room temperature.**

3. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube.**

4. **Add 5 ml Buffer XL/OB Protease mixture to the tube containing the nuclei pellet**. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Tips: OB Protease and Buffer XL can be premix together. Add 50ul OB Protease to 5 ml Buffer XL. This Mixture should be prepared in 10 minutes. without the samples, OB Protease will lose activities.

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

5. **Incubate at 65°C for 15-30 minutes in a water bath or heating block.**
   Note: The sample should change color from red to oliver green during proteinase digestion

6. **Add 5 ml isopropanol to the lysate.** Gently mix by inverting the tube 20-30 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 70% ethanol and vortex the tube for 10 seconds to wash.**

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).

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

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

2. **Centrifuge at 2000 x g for 5 minutes at room temperature.** Remove and discard supernatant.

3. **Pipet 25 ml Buffer NL into the same 50 ml centrifuge tube. Add another 10 ml whole blood and mix by inverting the tube 5 times.**

4. **Centrifuge at 2000 x g for 5 minutes at room temperature.**

5. **Discard the supernatant and leave the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube.**

6. **Add 5 ml Buffer XL/OB Protease mixture to the tube containing the nuclei pellet**. Vortex immediately for 10 seconds or until the pellet is completely homogenized.

   Tips:OB Protease and Buffer XL can be premix together. Add 50ul OB Protease to 5 ml Buffer XL. This Mixture should be prepared in 10 minutes. without the samples, OB Protease will lose activities.

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

7. **Incubate at 65°C for 15-30 minutes in a water bath or heating block.**

8. **Add 5 ml isopropanol to the lysate.** Gently mix by inverting the tube 20-30 times or until the DNA precipitate become visible as threads or clumps.

9. **Centrifuge at 2000 x g for 5 minute at room temperature.**

10. **Pour out the supernatant and 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). Incubate sample at 65°C for 10 min.** Some sample 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.

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

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.® Blood SQ DNA Protocol, or frozen at -70°C for storage.

Reagent volumes required for processing 100-500 µl Buffy coat

Reagent	Buffy Coat Volume				
	100 µl	200 µl	300 µl	400 µl	500 µl
Buffer NL	250 µl	500 µl	750 µl	1000 µl	1250 µl
Buffer XL	100 µl	200 µl	300 µl	400 µl	500 µl
OB Protease	1 ul	2 ul	3 ul	4 ul	5 ul
100% isopropanol	100 µl	200 µl	300 µl	400 µl	500 µl
70% ethanol	100 µl	200 µl	300 µl	400 µl	500 µl
Buffer EB	200 µl	200 µl	200 µl	200 µl	200 µl

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

2. **Centrifuge at 14,000 x g for 30 seconds at room temperature.**

3. **Remove and discard supernatant.** Leave the tube inverted on a absorbent paper for 2 minutes. Make sure the pellet remains in the tube.
4. **Add 200µl Buffer XL/OB Protease mixture to the tube containing the nuclei**

pellet. Vortex immediately for 10 seconds or until the pellet is completely homogenized .

Tips:OB Protease and Buffer XL can be premix together. Add 50ul OB Protease to 5 ml Buffer XL. This Mixture should be prepared in 10 minutes. without the samples, OB Protease will lose activities.

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

5. **Centrifuge at 10,000 x for 5 seconds to collect any liquid drop from lid.**

6. **Incubate at 65°C for 10-30 minutes in a water bath or heating block.**

7. **Add 200 µl isopropanol to the lysate.**

8. **Gently mix the solution by inverting the tube 20-30 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 200µl of 70% ethanol and vortex the tube for 10 seconds to wash the DNA 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).** Incubate sample at 65ºC for 10 min. Some samples may need to incubate at 65ºC for 1 hour to rehydrate DNA.

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

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

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

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

2. **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.

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

4. **Add 300 µl Buffer XL/OB Protease mixture.** Vortex immediately for 10 seconds.

5. Incubate at 65°C for 10-30 minutes in a water bath or heating block.

6. **Add 600 µl isopropanol to the lysate.**

7. **Gently mix the solution by inverting the tube 20-30 times** or until the DNA precipitate become visible as threads or clumps.

8. **Centrifuge at 14,000 x g for 2 minutes at room temperature.** Carefully pour off the ethanol.

9. 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.

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 adsorbent paper towel and air dry the pellet for 10-15 minutes.**

12. **Add 100-200 µl of DNA rehydration solution (Buffer EB). Incubate sample at 65ºC for 10 min.** Some samples may need to incubate at 65ºC for 1 hour to rehydrate DNA.

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

G. DNA Purification from up to 10 ml Clotted Blood

1. **Transfer 10 ml the clotted blood including any liquid residual into a 50 ml centrifuge tube.** Homogenize the sample with a rotor-stator homogenizer until the sample is uniformly homogenous.

2. **Add 25 ml Buffer NL and mix by inverting the tube 5-7 times.** Centrifuge at 2000 x g for 5 minutes in a swing-out rotor.

3. **Discard the supernatant and add another 10 ml Buffer NL to the pellet**. Vortex to resupsend the pellet. Centrifuge at 2000 x g for 5 minutes in a swing-out rotor.

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

5. **Add 5ml Buffer XL and 50 µl OB Protease solution (20mg/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 Buffer XL/OB Protease. 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.

6. **Incubate the tube at 65°C for 30 minutes in a water bath or heating block.**

7. **Vortex for 10 seconds, inspect the tube to make sure the homogenization is complete.** Centrifuge at 2000xg for 5 to remove undigested particles.

8. **Transfer the supernatant into a new tube. Add 5 ml of isopropanol and mix throughly by inverting the tube 20-30 times.** Centrifuge at 2000 x g for 5 minutes to pellet the DNA.

9. Discard the supernatant and add 5 ml 70% ethanol and vortex for 10 seconds. Centrifuge at 2000 x g for 5 minutes.

10. Discard the supernatant and invert the tube onto a clean absorbent paper for 10 minutes.

11. Add 1 ml Buffer EB or TE Buffer, vortex 5 seconds at lower speed. Dissolve the DNA by incubating 1 hour at 65^{0}C 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.
	Buffer XL/OB Protease is not prepared correctly.	Ensure that the Buffer XL/OB Protease is not prepared correctly.
	OB Protease is dissolved in wrong buffer	Use EB to dissolve the proteinase
	Incomplete sample lysis	Mix the sample throughly after addition of Buffer NL.
	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/OB Protease properly and fresh.
	Poor cell lysis due to incomplete mixing with Buffer NL	Repeat the procedure, this time making sure to vortex the sample with Buffer NL immediately and completely.
	Hemoglobin remains	Repeat the procedure, this time making sure enough volume of NL bufefr 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 Buffer EB 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/Proteinse mixture	After addition of the XL/OB Protease, the sample was left too long before the vortexing	Immediately mix the sample after the addition of XL/OB Protease.