

## **SUPPLEMENTAL ON-LINE MATERIAL**

### ***PrepMan Ultra Sample Preparation Reagent***

1. Place sample in Eppendorf Tube.
2. Add 1 metal pellet to sample.
3. Add 100 µl Prepman Ultra Sample Preparation Reagent.
4. Place tubes with specimens in shaker.
5. Shake for 3 minutes at speed of 30.
6. Vortex samples for 30 seconds.
7. Heat samples for 10 minutes @ 100°C in heating block.
8. Remove bead with magnetic stick.
9. Centrifuge sample for 3 minutes in a microcentrifuge at 13 000 rpm for 3 minutes.
10. Transfer supernatant to new Eppendorf tube.

### ***G1N350 GenElute Mammalian Genomic DNA Miniprep Kit Catalog Number***

#### **Section 1: Release DNA from cultured cell, tissues(including rodent tails), fresh whole blood or white blood cells.**

1. Lyse insect
2. Transfer to 1.5-2 ml microcentrifuge tube.
3. Add 180µl lysis solution for tissue and 20µl Proteinase K. Vortex or invert to mix.
4. Incubate @ 55°C until fully digested (4 hours).
5. Add 20µl Rnase. Mix and incubate at room temperature for 2 minutes.
6. Add 200µl lysis solution. Vortex or pipette to mix. Incubate @ 70°C for 10 minutes.

### **Section: 2 Bind DNA to column**

7. Add 200µl ethanol. Vortex or invert to mix.
8. Transfer to binding column. Spin > 6 500 x g for 1 minute.

### **Section: 3 Wash to remove contaminants**

9. Transfer column to new collection tube. Add 500µl wash solution to column. Spin @ >6500 x g for 1 minute [\*Ethanol must be added to Wash solution before first use]
10. Transfer column to new collection tube. Add second 500µl Wash solution to column.
11. Spin @ >12 000 x g for 3 minutes to dry the column.

### **Section 4: Elute purified DNA**

12. Transfer column to new collection tube
13. Add 200µl Elution Solution. Spin @ 6500 x g for 1 minute
14. Repeat elution in same tube.

## ***DNAzol® Reagent INVITROGEN Genomic DNA Isolation Reagent***

### **Section 1: Homogenization of tissue**

1. Homogenize 25-50 mg of tissue in 1ml of DNAzol Reagent.

### **Section 2: Centrifugation (optional)**

2. Sediment the homogenate for 10 minutes at 10 000 x g at 4°C or room temperature. Following centrifugation, transfer the resulting viscous supernatant to a fresh tube. This step removes insoluble tissue fragments, RNA, and excess polysaccharides from the lysate/homogenate. It is required only for the isolation of DNA from tissues such as liver, muscles, and most plant tissues containing a large amount of cellular and/or extracellular material. this process is recommended in order to minimize RNA carry-over into the DNA.

### **Section 3: DNA precipitation**

3. Precipitate DNA from the lysate/homogenate by the addition of 0.5ml of 100% ethanol per 1 ml of DNAzol Reagent used for the isolation. Mix samples by inversion and store them at room temperature for 1-3 minutes. DNA should quickly become visible as a cloudy precipitate. Remove the DNA precipitate by spooling with a pipette tip. Swirl the DNA onto the tip and attach it to the tube wall near the top of the tube by gently sliding the DNA off the tip (alternatively transfer the DNA to a clean tube). Carefully decant the supernatant, leaving the DNA pellet near the top of the tube. Place the tubes upright for 1 minute and aspirate the remaining lysate/homogenization before precipitation with ethanol, the resulting sheared DNA will not spool. The same is true for small quantities of DNA (<15 ug). In this case, centrifugation @4 000 x g for 1-2 minutes at room temperature or 4°C will pellet the DNA.

### **Section 4: Wash**

4. Wash the DNA precipitate twice with 0.8-1.0 ml of 75% ethanol. At each wash, suspend the DNA in ethanol by inverting the tubes 3-6 times. Store the tubes vertically for 0.5-1 minute to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting or decanting.

### **Section 5: DNA Solubilization**

5A. Air dry the DNA by storing in an open tube for 5-15 seconds after removing the ethanol. (If the DNA is exposed to air for more than a few seconds, it will be much more difficult to dissolve). Dissolve the DNA in 8mM NaOH by slowly passing the pellet through a pipette tip. Use of the 8mM NaOH assures full solubilization of the DNA precipitate. Add an adequate amount of the 8 mM NaOH to approach a DNA concentration of 0.2 - 0.3 µg/µl. Typically add 0.2-0.3 ml of 8 mM NaOH to the DNA isolated from  $10^7$  cells or 10-20 mg of animal tissue. DNA will not be fully solubilized in TE or water. (The resolubilization of DNAzol -isolated

DNA is low in Tris buffers. Therefore the use of 8 mM NaOH is highly recommended.) DNA is stable in 8 mM NaOH for several months at 4°C and greater than one year at -20°C.

5B. The DNA preparations isolated from tissues such as liver, muscles, and plants may contain some insoluble material (mostly polysaccharides). Remove the insoluble material by centrifugation at 12 000 x g for 10 minutes.

Protocol followed up to this point.

5C. Weak alkaline solutions are neutralized by CO<sub>2</sub> from the air. Once a month, prepare 8 mM NaOH from 2-4 M NaOH stock solution that is less than six months old.

5D. After DNA is solubilized in 8 mM NaOH, adjust the DNA solution to a desired pH by the addition of HEPES. Use the following amounts of 0.1M or 1M HEPES (free acid) per 1 ml of 8mM NaOH:

Final pH	0.1 M HEPES (ul)	Final pH	1M HEPES (ul)
8.4	86	7.2	23
8.2	93	7.0	32
8.0	101		
7.8	117		
7.5	159		

## Section 6: Quantitation of DNA and Results

6A. Mix an aliquot of solubilized DNA with 1ml of 8mM NaOH and measure A<sub>260</sub> and A<sub>280</sub> of the resulting solution. Calculate the DNA content assuming that one A<sub>260</sub> unit equals 50µg of double-stranded DNA per ml.

6B. For calculations of cell number in analysed samples or an expected yield of DNA, assume that the amount of DNA per 10<sup>6</sup> diploid cells of human, rat, mouse origin equals 7.1 µg, 6.5µg and 5.8µg respectively.

6C. Typical yield for animal tissues ( $\mu\text{g}$  DNA/mg tissue): liver, kidney, or lungs 3-5  $\mu\text{g}$ ; skeletal muscle, heart or brain 1-3  $\mu\text{g}$ .

6D. The A260/A280 ratio of the isolated DNA is within the 1.6-1.9 range and with a molecular weight ranging from 20 to 100 kb. The molecular weight of the isolated DNA depends upon its shearing by mechanical forces applied during lysis/homogenization or during solubilization of the DNA precipitate.

6E. The isolated DNA contains partially degraded RNA. If a reduction of the RNA content to less than 3% is necessary, perform the centrifugation step as described in Step 2 of the protocol. In Southern Analysis, RNA can be digested by supplementing the restriction mix with RNase A (1  $\mu\text{g}/\text{ml}$ ).

### ***Charge Switch gDNA Micro Tissue Kit***

**A. Before Starting :** Perform the following before beginning

- Set a water bath at 55°C.
- Prepare Lysis Mix: For each sample, mix 1 ml of ChargeSwitch® Lysis Buffer (L15) and 10  $\mu\text{l}$  of Proteinase K to prepare the Lysis Mix. When isolating DNA from multiple samples, scale up the volume of reagents used and prepare a master Lysis Mix. Note: The ChargeSwitch® Lysis Buffer may appear slightly cloudy. If cloudy, shake the bottle before use until the solution becomes clear.

**B. Preparing the Tissue Lysate:** Follow the procedure below to prepare a lysate from the tissue sample.

1. Place the tissue sample into a sterile microcentrifuge tube.
2. Add 1 ml of Lysis Mix (see above) to the tube. Ensure that the tissue is completely immersed in the Lysis Buffer.

3. Vortex for 10-15 seconds to mix.
4. Incubate the sample overnight at 55°C until lysis is complete.

**B. Preparing the Tissue Lysate:** Follow the procedure below to prepare a lysate from the tissue sample.

5. Add 5 µl of RNase A to the lysate. Pipette up and down gently 5 times or until a homogeneous solution is obtained. Important: Use a 1 ml pipette tip set to 900 µl to mix the sample. Make sure that the tip is submerged, and pipette up and down gently to avoid forming bubbles as this may result in shearing of the DNA.
6. Incubate at room temperature for 5 minutes.
7. Proceed to Binding DNA.

**C. Binding DNA:** Follow the procedure below to bind the DNA to the ChargeSwitch® Magnetic Beads.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer. Make sure that all of the solution containing the beads is at the bottom of the tube.
2. Add 200 µl of ChargeSwitch® Purification Buffer (N5) to the digested tissue sample (from Step 6, previous page) and pipette up and down gently twice to mix. Note: Adding the ChargeSwitch® Purification Buffer lowers the pH of the sample, and optimizes the binding conditions.
3. Add 40 µl of ChargeSwitch® Magnetic Beads (from Step 1) to the sample and pipette up and down gently 5 times to mix.
4. Incubate at room temperature for 1 minute to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
5. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight

pellet.

6. Without removing the tube from the MagnaRack™, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.

7. Proceed immediately to Washing DNA.

#### **D. Washing DNA:**

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 6, previous page). There should be no supernatant in the tube.

2. Add 1 ml of ChargeSwitch® Wash Buffer (W12) to the tube and pipette up and down gently twice to resuspend the magnetic beads. Important: Use a 1 ml pipette tip set to 900 µl to mix the sample. Make sure that the tip is submerged, and pipette up and down gently to avoid forming bubbles.

3. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.

4. Without removing the tube from the MagnaRack™, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 9).

5. Repeat Steps 1-4.

6. Proceed to Eluting DNA.

#### **E. Eluting DNA:**

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 5, previous page). There should be no supernatant in the tube.

2. Add 150 µl of Charge Switch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the tube and pipette up and down gently 10 times to resuspend the magnetic beads. Important: Do not use

water for elution. The DNA will not elute due to the poor buffering capacity of water.

3. Incubate at room temperature for 5 minutes. Tip: For maximum yield, mix the suspension of beads (by pipetting up and down gently) half way through the incubation. Incubating the sample at 55°C may also improve yield.

4. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.

5. Without removing the tube from the MagnaRack™, carefully remove the supernatant containing the DNA to a sterile microcentrifuge tube. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 9). Note: If the eluate containing the DNA is discoloured, repeat Steps 5-6.

6. Discard the used magnetic beads. Do not reuse the beads.

#### **F. Storing DNA:**

- Store the purified DNA at -20°C or use immediately for the desired downstream application.
- Avoid repeatedly freezing and thawing DNA. Store the purified DNA at 4°C for short-term use or aliquot the DNA and store at -20°C for long-term storage.

#### ***Wizard Genomic DNA Purification Kit***

D. Add 3µl of RNase Solution to the nuclear lysate and mix the sample by inverting the tube 2-5 times. Incubate the mixture for 15-30 minutes at 37°C. Allow the sample to cool to room temperature for 5 minutes before proceeding.

E. To the room temperature sample, add 200µl of Protein Precipitation Solution and vortex vigorously at high speed for 20 seconds. Chill sample on ice for 5 minutes.

F. Centrifuge for 4 minutes at 13,000–16,000 × g. The precipitated protein will form a tight white pellet.



G. Carefully remove the supernatant containing the DNA (leaving the protein pellet behind) and transfer it to a clean 1.5ml microcentrifuge tube containing 600 $\mu$ l of room temperature isopropanol. Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

H. Gently mix the solution by inversion until the white thread-like strands of DNA form a visible mass.

I. Centrifuge for 5 minute at 13,000–16,000  $\times$  g at room temperature. The DNA will be visible as a small white pellet. Carefully decant the supernatant.

J. Add 600 $\mu$ l of room temperature 70% ethanol and gently invert the tube several times to wash the DNA. Centrifuge for 1 minute at 13,000-16,000  $\times$  g at room temperature.

K. Carefully aspirate the ethanol using either a drawn Pasteur pipette or a sequencing pipette tip. The DNA pellet is very loose at this point and care must be used to avoid aspirating the pellet into the pipette.

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

M. Add 100 $\mu$ l of DNA Rehydration Solution and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube.

N. Store the DNA at 2 - 8°C.

### ***ZR Insect/Tissue DNA Kit***

1. Add specimen to a ZR Bashing Bead Lysis Tube.

2. Secure in a bead fitter with a 2.0 ml tube holder assembly and process at maximum speed for 10 minutes.

3. Centrifuge the ZR Bashing bead Lysis Tube in microcentrifuge at  $>10\ 000\ \times\ g$  for 1 minute.
4. Transfer up to 400 $\mu$ l of supernatant to a Zymo-Spin IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7 000rpm for 1 minute. Snap off the base of the Zymo-Spin IV Spin Filter prior to use.
5. Add 1200  $\mu$ l of Genomic Lysis buffer to the filtrate in the collection tube from Step 4 and mix.
6. Transfer 800 $\mu$ l of the mixture from Step 5 to a Zymo-spin IC Column in a Collection Tube and centrifuge at  $10\ 000\ \times\ g$  for 1 minute.
7. Discard flow through from the Collection tube and repeat step 6.
8. Add 200 $\mu$ l DNA Pre-Wash Buffer to the Zymo-Spin IC Column and centrifuge at  $10\ 000\ \times\ g$  for 1 minute.
9. Add 500  $\mu$ l g-DNA Wash Buffer to the Zymo-Spin IC Column and centrifuge at  $10\ 000\ \times\ g$  for 1 minute.
10. Transfer the Zymo-Spin IC Column to a clean 1.5 ml microcentrifuge tube and add 30  $\mu$ l DNA Elution Buffer directly to the column matrix. Centrifuge at  $10\ 000\ \times\ g$  for 30 seconds to elute the DNA.

### *Nucelospin Tissue XS*

#### **Section 3: Lysis**

3. Add 80  $\mu$ l buffer B3, vortex 2 x 5 sec and incubate at 70°C for 5 min. Vortex briefly at the end of the incubation and adjust the thermal heating block temperature to 90°C for the last step of the protocol. Let the lysate cool down to ambient temperature. A white precipitate may form in the lysate upon addition of B3, especially if very small samples are used. Precipitates

will dissolve during the incubating step at 70°C. If insoluble particles are visible, centrifuge for 5 min at high speed (e.g. 11,000 x g) and transfer the supernatant to a new microcentrifuge tube.

#### **Section 4: Adjust DNA binding conditions**

4. Add 80 µl ethanol (96-100%) to the lysate and mix by vortexing 2 x 5 sec. Spin down briefly to clear the lid.

#### **Section 5: Bind DNA**

5. For each sample, place one NucleoSpin® Tissue XS column into a 2 ml collecting tube. Apply the sample to the column. Centrifuge for 1 min at 11,000 x g. Discard the flow-through and place the column into a new 2 ml collecting tube (provided). If the sample is not drawn completely through the matrix, repeat the centrifugation step at 11,000 x g.

#### **Section 6: Wash silica membrane**

6. 1st wash: Add 50 µl buffer B5 to the membrane. Centrifuge for 1 min at 11,000 x g. It is not necessary to discard the flow-through. Reuse the collecting tube.

2nd wash: Add 50 µl buffer B5 directly onto the membrane. Centrifuge for 2 min at 11,000 x g.

#### **Section 7 Elute DNA**

7. Place the NucleoSpin® Tissue XS column into a 1.5 ml microcentrifuge tube (not supplied) and apply 20 µl BE directly onto the centre of the silica membrane of the column. Centrifuge 1 min at 11,000 x g.

## **Section 8: Optional: Removal of residual ethanol**

8. Incubate elution fraction with open lid for 8 min at 90°C.

### ***ZyGEM DNA extraction using prepGEM insect***

The following methods is recommended for extracting DNA from insects or insect parts using prepGEM™ insect.

- All manipulations should be performed in a clean-room or PCR hood
- Use only certified DNA-free tubes and reagents
- Wash forceps and scalpels and dissection surfaces in 0.05% Hypochlorite bleach. Rinse thoroughly with DNA-free water.

### **Procedure Outline**

#### **Sample Preparation**

- Insects, or parts of insects should be placed in a thin-walled PCR tube for extraction. Approximately 1-2 mm<sup>3</sup> of total tissue should be used.
- for older tissue the sample should be crushed

#### **Extraction Method**

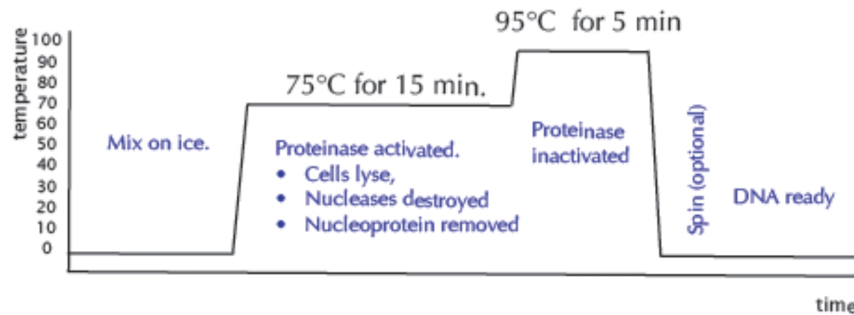
1. Add to the material 35µl PCR grade water, 4 µl 10xBuffer (BLACK), 1µl prepGEM™. For small insects, these volumes can be reduced further. Solutions can be made as a master mix and stored on ice.

2. Incubate at 75°C for 15 minutes.

3. Incubate at 95°C for 5 minutes.

*A thermal cycler can be used for these steps*

4. Aspirate supernatant. Centrifugation is undesirable for automation and should not normally be needed. However, with some material, two minutes at 13,000 x g may assist clarifying the extract.



### ***Genomic DNA from Yeast (nexttec™)***

- **Equilibration of nexttec™ clean columns**
  1. Add 350µl of Prep buffer onto each nexttec™ Clean Column, incubate for at least 5 minutes at room temperature and centrifuge at 350 x g for 1 minute to remove excess buffer.
  2. Discard the waste collection tube, place the nexttec™ Clean Column into a new DNA collection tube, store equilibrated columns closed at +2°C to +8°C and use within 1 week.
- **Preheat a thermoblock to 30°C and a thermomixer (e.g. Eppendorf) to 60°C**
- **Dissolve Lyticase**
  1. Dissolve the lyophilised enzyme in a buffer containing 100mM potassium phosphate buffer (pH 7.5), 100 mM NaCl, 50% glycerol to a concentration of 25µ/µl, store solution at -20°C.
- **Lysis**

1. Transfer yeast cultures ( $1 \times 10^8$  to  $1 \times 10^9$  cells) to reaction tubes, centrifuge (1 min, 6000 x g), remove and discard supernatant
2. Add 50  $\mu$ l buffer Y4, 5  $\mu$ l Rnase A, 5  $\mu$ l Lyticase and 0.5  $\mu$ l DTT to each sample, resuspend cells by vortexing.
3. Incubate the samples (30°C, 30 min) in a thermoblock, centrifuge the suspensions (3 min, 5000 x g), remove and discard supernatant
4. Add 132.5 $\mu$ l Buffer Y1, 5 $\mu$ l Buffer Y2, 12.5 $\mu$ l Buffer Y3 and 1.5 $\mu$ l DTT to each pellet, vortex and incubate with shaking (60°C, 1200 rpm, 2h) in a thermomixer.

- **Purification of DNA**

1. Transfer 120 $\mu$ l of the lysate onto each equilibrated nexttec<sup>TM</sup> Clean Column and incubate the column for 3 minutes at room temperature.
2. Centrifuge at 700 x g for 1 minute, discard nexttec<sup>TM</sup> Clean Column.

**Chelex** (<http://www.wsu.edu/~drown/Protocol/Chelex.pdf>) BioRad Chelex ® Ultra 100 Molecular Biology Grade Resin, BioRad Laboratories, South Africa. Catalogue Number 142-1253

1. Remove pre-made tubes filled with 300 $\mu$ L 10% Chelex from refrigerator. You will need for each sample, plus 1 extra for a control. Handle the container with gloves and shake out the number you need into your gloved hand. Do not put extra tubes back into jar.
2. Label each Chelex tube to correspond to your sample listed on your Chelex worksheet. Label the tubes on the cap. Put as much information as you can manage, but keep labels legible. Adding initials and a date is always a good idea. Avoid labelling on the side of tube as this writing can be washed off during the incubation stage.
3. Turn on heating block. Set to 95°C. Fill holes with ddH<sub>2</sub>O water.

4. Dip forceps into ethanol, then wave forceps through flame of an alcohol burner to ignite. When you are certain that the flame on the forceps has extinguished, repeat 2 times.
5. Using the sterile forceps, remove a small piece of tissue from your sample, uncap the tube of chelex, place sample in the appropriately labelled tube and close lid. The piece of tissue should be big enough to be visible, but not so big as to be easily visible. Imagine cutting a 0.2mm section of a standard staple.
6. Repeat (step 5) with each sample in a new Chelex tube, being sure to sterilize forceps 3 times between samples (step 4). When finished, make a negative Chelex control by dipping your sterilized forceps into a tube of Chelex slurry. (It may be necessary to wipe excess tissue from forceps with a kimwipe prior to flame sterilization) dipping your sterilized forceps into a tube of Chelex slurry. (It may be necessary to wipe excess tissue from forceps with a kimwipe prior to flame sterilization).
7. When finished with all tubes, vortex samples in chelex slurry for 10-15 seconds. Be sure lids are snapped on tightly before beginning.
8. Spin samples briefly (10-15 sec) at high speed in a microcentrifuge. This step is to ensure that the sample is inside the slurry of Chelex.
9. Incubate samples for 20 minutes at 95°C. The block temperature may drop slightly when doing this step. This drop is normal. Check tubes while incubating to ensure that lids have not popped off.
10. Vortex samples again for 10-15 seconds (Be careful as steam may pop lid off of centrifuge tube. Hold lids down).
11. Spin tubes again at high speed in microcentrifuge to ensure that all contents are in the bottom of the microcentrifuge tube.

12. Samples are ready to use (or not, see below). ONLY USE SUPERNATANT FOR PCR REACTIONS. CHELEX BEAD WILL INACTIVATE TAQ!

### ***Phenol/ Chloroform***

1. Grind freeze-dried mycelia with a mortar and pestle. Avoid cross contamination if working with several samples.
2. Add around 0.2 - 0.3 ml of ground mycelia to each Eppendorf tube.
3. Add 800ul (400  $\mu$ l) of DNA extraction buffer (DEB)

### **DEB**

200 mM Tris-HCl (pH 8.0)

250 mM NaCl

25 mM EDTA (pH 8.0)

0.5 % SDS

4. Incubate @ 65°C for 1 hour.
5. Add 500  $\mu$ l (250  $\mu$ l) of phenol and vortex (phenol is toxic!)
6. Add 300  $\mu$ l (150  $\mu$ l) of chloroform and vortex (chloroform is harmful!)
7. Centrifuge at 10 000 rpm for 60 minutes at 4°C.
8. Transfer upper aqueous layer to new tube (dispose of the organic phase into the organic waste bottle)
9. Add 200  $\mu$ l phenol and 200  $\mu$ l chloroform, vortex and centrifuge for 5 minutes @ 13 000 rpm.
10. Transfer upper aqueous layer to new tube.
11. Add 400 $\mu$ l chloroform, vortex and centrifuge for 5 minutes @ 13 000 rpm.
12. Repeat steps 10 and 11 once or twice. White interface should disappear.



13. Transfer upper aqueous layer to new tube.
14. Add (0.1 x volume transferred in step 13 to new tube) of 3 M NaAc (pH 5 to pH 5.5) and 2 volumes of absolute ethanol.
15. Invert tubes several times. Chromosomal DNA will form a little ball.
16. Place in freezer overnight.
17. Centrifuge for 30 minutes at 4°C and remove EtOH.
18. Wash with 500µl 70% EtOH. Centrifuge for 5 minutes and remove EtOH.
19. Dry DNA pellets for 5 minutes under vacuum and resuspend them in 50µl sterile dH<sub>2</sub>O
20. Add 4 µl RNase (1 mg/ml) to DNA solution and incubate for a 1.5 hours @ 37°C.
21. Run a 1% agarose gel with an aliquot (5-10µl) of the DNA sample and estimate/determine DNA concentration.