



PROTOCOL OF DNA EXTRACTION

- 1- Phenol/chloroform extraction and ethanol precipitation (INPBARCELONA)
 - 1.1 Phenol extraction of Genomic DNA from frozen tissue
 - 1.2 Phenol extraction of Genomic DNA from fixed paraffin-embedded tissue
 - 1.3. Phenol extraction of Genomic DNA from formalin-fixed tissue or tissue treated with Formic acid
- 2- DNeasy® Tissue (Qiagen)
 - 2.1 Purification of Genomic DNA from frozen tissues
 - 2.2 Purification of Genomic DNA from paraffin-embedded tissues
 - 2.3 Purification of Genomic DNA from formalin-fixed tissues
- 3- QIAmp DNA Micro
 - 3.1. Purification of Genomic DNA from frozen tissues
 - 3.2. Purification of Genomic DNA from paraffin-embedded tissues
 - 3.3. Purification of Genomic DNA from formalin-fixed tissues

BrainNet Europe II

Project Co-ordinator:

Prof. Dr. Hans Kretzschmar

Contact:

www.brainnet-europe.org

Network of Excellence funded by the EC in the 6th Framework Program "Life Science"



Content

PROTOCOL OF DNA EXTRACTION..1	
1. PHENOL/CHLOROFORM EXTRACTION AND ETHANOL PRECIPITATION (INPBarcelona protocol).....2	
1.1. Phenol extraction of Genomic DNA from frozen tissue	2
1.2 Phenol extraction of Genomic DNA from fixed paraffin-embedded tissue ...	5
1.3 Phenol extraction of Genomic DNA from formalin-fixed or treated with formic acid	9
2. DNEASY® TISSUE (QIAGEN)	12
2.1 Purification of Genomic DNA from frozen tissue.....	12
2.2 Purification of Genomic DNA from paraffin-embedded tissue	13
2.3 Purification of Genomic DNA from formalin-fixed tissue	15
3- QIAMP DNA MICRO.....	17
3.1 Purification of Genomic DNA from frozen tissue.....	17
3.2 Purification of Genomic DNA from paraffin-embedded tissue	19
3.3 Purification of Genomic DNA from formalin-fixed tissues	21
QUALITY AND QUANTIFICATION OF DNA	23
1- Quantification of DNA by agarose gel	23
2. Quantification of DNA by Agilent Bioanalyzer	24

DNA Preservation



1. PHENOL/CHLOROFORM EXTRACTION AND ETHANOL PRECIPITATION (INPBarcelona protocol)

1.1. Phenol extraction of Genomic DNA from frozen tissue

Frozen sample: the frozen sample must be cut in sterile petri plaques with 5ml of SSC 1x, EDTA 10mM washing solution just filtered in 0.45-0.22µm filters. The cut sample will be collected in a falcon tube.

MATERIALS

- Frozen samples
- Washing solution (SSC 1x, EDTA 10mM)
- Digestion solution (TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM)
- 0.45-0.22µm filters
- SDS 10% sterile
- Proteinase K (10mg/ml)
- 70% ethanol, bottle kept at -20°C
- 100% ethanol, bottle kept at -20°C
- Water bath, 37°C, 56°C and 65°C
- Sterile 1.5ml microcentrifuge tubes (eppendorf)
- Sterile 15 ml falcon centrifuge tubes
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- Sodium acetate 3M
- Rotator
- Vortex
- Centrifuge, 15 ml tubes
- Microcentrifuge, 1.5 ml tubes
- Sterile Pasteur pipet
- Micropipet
- Sterile water
- TE 1x buffer, pH 8.0

DNA Preservation



PROCEDURE

A. Cell lysis (day 1)

1. Add SSC 1x, EDTA 10mM washing solution just filtered until 7-10 ml final volume. Mix by inversion or using sterile Pasteur pipet.
2. Centrifuge at 800 x g for 10 min at RT.
3. Remove and discard as much SN as possible without disturbing the visible pellet using sterile Pasteur pipet.
4. Repeat from step 1.
5. Filter 5 ml/sample TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM digestion solution using 0.45-0.22µm filters.
6. Transfer the visible pellet to a new tube (2 ml eppendorf if possible or 15 ml falcon tube if not) using half the volume of digestion solution required. Use the second half to transfer the rest of the pellet. The volume of digestion solution required depends on the quantity of sample to be processed (e.g., 1.2 ml of digestion solution/100mg of tissue).
7. Add 5% of the final volume used of SDS 10% sterile digestion solution. Mix the tubes gently with fingertips (e.g., final volume of digestion solution=400µL, 5% of 400L =20µL of SDS10%).
8. Add 2.5% of the final volume used of Proteinase K digestion solution (10mg/ml). Mix the tubes gently with fingertips (e.g., final volume of digestion solution=400µL, 2.5% of 400L =10µL of Proteinase K (10mg/ml)).
9. Put the tubes in a +56°C water bath in agitation. During the first three hours, mix the tubes again to facilitate the lysis. Incubate o/n.

B. Phenol/Chloroform extraction and ethanol precipitation (day 2) (Under vertical laminar flow cabinet)

1. Add V/V of phenol/chloroform/isoamyl alcohol. Mix by inversion for 1 minute, or until the sample becomes homogenous. If 15 ml falcon tubes are used, mix with vortex.
2. Centrifuge at maximum speed for 2 min at 4°C. If 15 ml falcon tubes are used, centrifuge at 2,000 x g for 10 min at 4°C.
3. Remove the inner phase (corresponding to phenol) carefully with a micropipet and discard.
4. Repeat from step 1. If the sample looks white, repeat three times from step 1 instead of two.

DNA Preservation



5. Add V/V of chloroform/isoamyl alcohol. Mix by inversion for 1 minute, or until the sample becomes homogenous. If 15 ml falcon tubes are used, mix with vortex.
6. Centrifuge at maximum speed for 2 min at 4°C. If 15 ml falcon tubes are used, centrifuge at 2,000 x g for 10 min at 4°C.
7. Remove the inner phase (corresponding to chloroform) carefully with a micropipette and discard.
8. Repeat from the step 5.
9. Remove the upper phase (corresponding to DNA) carefully with micropipet and transfer to a new tube.
10. Precipitate the DNA adding 1/10V of Sodium acetate 3M. Mix by inversion.
11. Add 2V/V cold ethanol 100% (from a bottle kept at -20°C).
12. Mix by inversion until the DNA becomes visible. Sometimes the DNA is not visible; only a few white thread-like strands of DNA or a few bubbles are visible. This is the DNA obtained.
13. Leave the tubes at -20°C minimum for 2 hours. If 15 ml falcon tubes are used, leave them at -80°C for 30 min or at -20°C o/n.

C. DNA rehydration

1. Centrifuge for 30 min at maximum speed at 4°C. If 15 ml falcon tubes are used, centrifuge at 2,000 x g for 10 min at 4°C.
2. A white pellet is visible or white shadows in the walls of the tube. This is the precipitated DNA. Discard SN and add 500µL of cold ethanol 70% (from a bottle kept at -20°C). If 15 ml falcon tubes are used, add 5-10 ml of cold ethanol 70%.
3. Centrifuge for 5 min at maximum speed. If 15 ml falcon tubes are used, centrifuge at 2000 x g for 10 min at 4°C.
4. Discard SN and leave the ethanol to dry; open tubes at +65°C for 10 min or at RT until complete evaporation of the ethanol.
5. Add TE 1x the quantity of DNA and leave for rehydration at +37°C in a bath for 2 hours or at +4°C o/n.

D. Quantification of DNA. See: Quality of DNA.

DNA Preservation



1.2 Phenol extraction of Genomic DNA from fixed paraffin-embedded tissue

From entire fixed paraffin-embedded tissue block; incubate at 60-65°C in a sterile Petri plaque to melt paraffin, and then remove it. Cut the block using a sterile surgical blade and place in a 1.5 ml microcentrifuge tube. From 30µm thick tissue sections; place 3-6 tissue sections in a sterile 1.5 ml microcentrifuge tube. The ideal is 2-4 tubes with 3-6 tissue sections each. From tissue section on paraffin section-mounted microscope slides; 15-20 slides with 12 µm thick sections are required.

MATERIALS

- Fixed paraffin-embedded tissue samples
- Xylenes
- 100%, 96% and 70% ethanol, RT (bottle and battery)
- Washing solution (SSC 1x, EDTA 10mM)
- Digestion solution (TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM)
- 0.45-0.22µm filters
- SDS 10% sterile
- Proteinase K (10mg/ml)
- 70% ethanol, bottle kept at -20°C
- 100% ethanol, bottle kept at -20°C
- Water bath, 37°C, 56°C and 65°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Sterile 15 ml falcon centrifuge tubes
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- Sodium acetate 3M
- Sterile water
- Rotator
- Vortex
- Centrifuge, 15 ml tubes
- Microcentrifuge, 1.5 ml tubes
- Sterile Pasteur pipet

DNA Preservation



- Micropipet
- TE 1x buffer, pH 8.0

PROCEDURE

A.I Remove paraffin from the tissue section or entire paraffin block (day 1)

1. Add 1 ml xylene, vortex and leave it to mix on the rotator o/n.
2. Centrifuge for 10 min at maximum speed. Remove the SN by aspiration.
3. Add 1 ml xylene, vortex briefly and leave it to mix on the rotator for 10 min. Centrifuge for 10 min at maximum speed. Remove the SN by aspiration.
4. Repeat step 3 twice.
5. Add 1 ml of 100% ethanol, vortex briefly and leave it to mix on the rotator for 10 min. Centrifuge for 10 min at maximum speed. Remove the SN by aspiration.
6. Repeat step 5 twice.
7. Add 1 ml of 96% ethanol, vortex briefly and leave it to mix on the rotator for 10 min. Centrifuge for 10 min at maximum speed. Remove the SN by aspiration.
8. Repeat step 7 twice.
9. Add 1 ml of 96% ethanol, vortex briefly and leave it to mix on the rotator for 10 min. Centrifuge for 10 min at maximum speed. Remove the SN by aspiration.
10. Repeat step 9 twice.
11. Dry the ethanol; open tubes at +65°C for 10 min or at RT until ethanol is completely evaporated.

A.II Remove paraffin from section mounted microscope slides and B. Cell lysis (day 1)

1. Put the slides in a glass basket to remove the paraffin, using a conventional alcohol battery.
2. The steps to follow are: 3 x 15 min of xylene; 3 x 5 min 100% ethanol; 3 x 5 min 96% ethanol; 1 x 5 min 70% ethanol; and, finally, 1 x 5 min sterile water.
3. The slide can remain in the last step of sterile water until separation of the normal from the tumoral tissue sections.

DNA Preservation



4. Filter 200-300 μ L of TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM digestion solution using 0.45-0.22 μ m filters and place in a 1.5 ml microcentrifuge tube. The slides should be scraped using sterile surgical blades.
5. Add 5% of the final volume used of digestion solution of SDS 10% sterile. Mix the tubes gently with fingertips (e.g., final volume of digestion solution=400 μ L, 5% of 400L =20 μ L of SDS10%).
6. Add 2.5% of the final volume used of digestion solution of Proteinase K (10mg/ml). Mix the tubes gently with fingertips ((e.g., final volume of digestion solution=400 μ L, 2.5% of 400L =10 μ L of Proteinase K (10mg/ml).
7. Put the tubes in a +56°C water bath in agitation. During the first three hours, mix the tubes again to facilitate the lysis. Incubate 1-3 days and add Proteinase K each day while the digestion is running.

From here, go directly to section C.

B. Cell lysis (day 2)

1. Filter 2 ml/sample TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM digestion solution using 0.45-0.22 μ m filters.
2. Add 200-400 μ L of digestion solution. The volume of digestion solution required depends on the quantity of sample to be processed.
3. Add 5% of the final volume used of digestion solution of SDS 10% sterile. Mix the tubes gently with fingertips (e.g., final volume of digestion solution=400 μ L, 5% of 400L =20 μ L of SDS10%).
4. Add 2.5% of the final volume used of digestion solution of Proteinase K (10mg/ml). Mix the tubes gently with fingertips (e.g., final volume of digestion solution=400 μ L, 2.5% of 400L =10 μ L of Proteinase K (10mg/ml).
5. Put the tubes in a +56°C water bath in agitation. During the first three hours, mix the tubes again to facilitate the lysis. Incubate 1-3 days and add Proteinase K each day while the digestion is running.

DNA Preservation



C. Phenol/Chloroform extraction and ethanol precipitation (days 3 or 4) (Under vertical laminar flow cabinet)

1. Add V/V of phenol/chloroform/isoamyl alcohol. Mix by inversion for 1 min, or until the sample becomes homogenous.
2. Centrifuge at maximum speed for 2 min at 4°C.
3. Remove the inner phase carefully with micropipet (corresponding to phenol).
4. Repeat from step 1. If the sample looks white, repeat from step 1 three times instead of two.
5. Add V/V of chloroform/isoamyl alcohol. Mix by inversion for 1 minute, or until the sample becomes homogenous.
6. Centrifuge at maximum speed for 2 min at 4°C.
7. Remove the inner phase carefully with micropipet (corresponding to chloroform).
8. Repeat from step 5.
9. Remove the upper phase carefully with micropipet (corresponding to DNA) and transfer to a new tube.
10. Precipitate the DNA adding 1/10V of sodium acetate 3M. Mix by inversion.
11. Add 2V/V cold ethanol 100% (from a bottle kept at -20°C).
12. Mix by inversion until the DNA becomes visible. Sometimes the DNA is not visible; a few white thread-like strands of DNA or a few bubbles are visible. This is the DNA obtained.
13. Leave the tubes at -20°C minimum for 2 hours.

D. DNA rehydration

1. Centrifuge for 30 min at maximum speed at 4°C.
2. A white pellet is visible, or white shadows in the walls of the tube. This is the precipitated DNA. Discard SN and add 500µL of cold ethanol 70% (from a bottle kept at -20°C).
3. Centrifuge for 5 min at maximum speed.
4. Discard SN and leave the ethanol to dry in open tubes at +65°C for 10 min or at RT until ethanol is completely evaporated.
5. Add TE 1x the quantity of DNA seen in step B.13 and allow rehydration at +37°C in a bath for 2 hours or at +4°C o/n.

DNA Preservation



E. Quantification of DNA. See Quality of DNA.

1.3 Phenol extraction of Genomic DNA from formalin-fixed or treated with formic acid

From tissue fixed with formol or treated with formic acid; cut at least 1gr of tissue using a sterile surgical blade and sterile petri plaque and place in a 15 ml falcon tube.

MATERIALS

- Tissue fixed with formol or treated with formic acid samples
- Washing solution (SSC 1x, EDTA 10mM)
- Digestion solution (TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM)
- 0.45-0.22µm filters
- SDS 10% sterile
- DTT (Dithithreitol powder)
- Proteinase K (10mg/ml)
- Dialysis membranes
- Acetic acid
- Saline solution
- 70% ethanol, bottle kept at -20°C
- 100% ethanol, bottle kept at -20°C
- Water bath, 37°C, 56°C and 65°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Sterile 15 ml falcon centrifuge tubes
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- Sodium acetate 3M
- Sterile water
- Vortex
- Centrifuge, 15 ml tubes
- Microcentrifuge, 1.5 ml tubes
- Sterile Pasteur pipet

DNA Preservation



- Micropipet
- TE 1x buffer, pH 8.0

PROCEDURE

A. Remove formol or formic acid from the tissue (days 1, 2 and 3)

1. Add SSC 1x, EDTA 10mM washing solution just filtered until 7-10 ml final volume. Mix by inversion or using sterile Pasteur pipet.
2. Centrifuge at 800 x g for 10 min at RT.
3. Remove and discard as much SN as possible without disturbing the visible pellet, using sterile Pasteur pipet.
4. Repeat from step 1.
5. Add 3-5 ml of saline solution.
6. Boil dialysis membrane in sterile bi-distilled water with a few drops of acetic acid.
7. Make a knot in one end of the membrane and place the cut tissue in saline solution in the dialysis membrane. Make a knot in the other end of the membrane.
8. Submerge the membrane in a pot full of saline solution. Leave in agitation 48 hours at RT.

B. Cell lysis (days 3 and 4)

1. Filter 5 ml/1gr of sample of TRIS 10mM pH 10.5, EDTA 1mM, NaCl 0.15 mM digestion solution using 0.45-0.22µm filters. Add 55µL SDS 10%, 40mg DTT and 10mg Proteinase K to the digestion solution.
2. Digest the sample with 5 ml of digestion solution/gr of sample using 15mL falcon tube. Mix by inversion.
3. Put the tubes in a +56°C water bath in agitation. During the first three hours, mix the tubes again to facilitate the lysis. Incubate 1-3 days and add Proteinase K each day.

DNA Preservation



C. Phenol/Chloroform extraction and ethanol precipitation (day 2) (Under vertical laminar flow cabinet)

1. Add V/V of phenol/chloroform/isoamyl alcohol. Vortex for 1 min, or until the sample becomes homogenous.
2. Centrifuge at 2000 x g for 10 min at 4°C.
3. Remove the inner phase carefully with sterile Pasteur pipet (corresponding to phenol) and discard.
4. Repeat from step 1. If the sample looks white, repeat three times from step 1 instead of two.
5. Add V/V of chloroform/isoamyl alcohol. Vortex for 1 min, or until the sample becomes homogenous.
6. Centrifuge at 2,000 x g for 10 min at 4°C.
7. Remove the inner phase carefully with sterile Pasteur pipet (corresponding to chloroform) and discard.
8. Repeat from step 5.
9. Remove the upper phase carefully with sterile Pasteur pipet (corresponding to DNA) and transfer to a new tube.
10. Precipitate the DNA adding 1/10V of sodium acetate 3M. Mix by inversion.
11. Add 2V/V cold ethanol 100% (from a bottle kept at -20°C).
12. Mix by inversion until the DNA becomes visible. Sometimes the DNA is not visible; a few white thread-like strands of DNA are visible or a few bubbles are seen. This is the DNA obtained.
13. Leave at -80°C for 30 min or at -20°C o/n.

D. DNA rehydration

1. Centrifuge at 2000 x g for 30 min at 4°C.
2. A white pellet is visible, or white shadows in the walls of the tube. This is the precipitated DNA. Discard SN and add 5-10 ml of cold ethanol 70%.
3. Centrifuge at 2000 x g for 10 min at 4°C.
4. Discard SN and leave the ethanol to dry in open tubes at +65°C for 10 min or at RT until ethanol is completely evaporated.
5. Add TE 1x the quantity of DNA observed and allow rehydration at +37°C in a bath for 2 hours or at +4°C o/n.

DNA Preservation



E. Quantification of DNA. See Quality of DNA.

2. DNEASY® TISSUE (QIAGEN)

2.1 Purification of Genomic DNA from frozen tissue

MATERIALS

- Water bath 56°C
- Heating block 70°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- Ethanol 96-100%

PROCEDURE

A. Digestion of frozen tissue (day 1)

Cut up to 25 mg tissue into small pieces, place in a 1.5 ml microcentrifuge tube, and add 180 µl Buffer ATL. Ensure the correct amount of starting material is used. It is advisable to cut the tissue into small pieces to enable more efficient lysis. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1-3 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

B. Precipitation of DNA (day 2)

1. Vortex for 15 s. Add 200 µl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C. It is essential that the sample and Buffer AL be mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.
2. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol be mixed to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the Dneasy Mini-spin column.

DNA Preservation



3. Pipet the mixture from step b into the Dneasy Mini spin column placed in a 2 ml collection tube. Centrifuge at $\geq 6000 \times g$ for 1 min. Discard flow-through and collection tube.
4. Place the Dneasy Mini spin column in a new 2 ml collection tube, add 500 μl Buffer AW1, and centrifuge for 1 min at $\geq 6,000 \times g$. Discard flow-through and collection tube.
5. Place the Dneasy Mini-spin column in a new 2 ml collection tube, add 500 μl Buffer AW2, and centrifuge for 3 min at $\geq 20,000 \times g$ to dry the Dneasy membrane. Discard flow-through and collection tube. This centrifugation step ensures that no residual ethanol is carried over during the following elution.
6. Place the Dneasy Mini-spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipet 200 μl Buffer AE directly onto the Dneasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at $\geq 6,000 \times g$ to elute. Elution with 100 μl (instead of 200 μl) increases the final DNA concentration in the elute, but also decreases the overall DNA yield.

2.2 Purification of Genomic DNA from paraffin-embedded tissue

MATERIALS

- Water bath 56°C
- Heating block 37°C and 70°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- Xylene
- Ethanol 96-100%

DNA Preservation



PROCEDURE

The Dneasy procedure has been successfully used to purify DNA from fixed tissues. However, the length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used. Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended, as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.

This protocol describes the removal of paraffin by extraction with xylene.

A. Removal of paraffin and digestion of paraffin-embedded tissues (day 1)

1. Place a small section (not more than 25 mg, 2 or 3 cuts of 20 μm) of paraffinembedded tissue in a 2 ml microcentrifuge tube.
2. Add 1200 μl xylene. Vortex vigorously.
3. Centrifuge at full speed for 5 min at room temperature.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200 μl ethanol (96-100%) to the pellet to remove residual xylene, and mix gently by vortexing.
6. Centrifuge at full speed for 5 min at room temperature.
7. Remove supernatant by pipetting. Do not remove any of the pellet.
8. Repeat steps 5-7 once.
9. Incubate the open microcentrifuge tube at 37°C for 10-15 min until the ethanol has evaporated.
10. Re-suspend the tissue pellet in 180 μl Buffer ATL
11. Add 20 μl proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1-3 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

B. Precipitation of DNA (day 2)

1. Vortex for 15 s. Add 200 μl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C. It is essential that the sample and Buffer AL be mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

DNA Preservation



2. Add 200 μ l ethanol (96-100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol be mixed to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the Dneasy Mini spin column.
3. Pipet the mixture from step 2 into the Dneasy Mini spin column placed in a 2 ml collection tube. Centrifuge at $\geq 6000 \times g$ for 1 min. Discard flow-through and collection tube.
4. Place the Dneasy Mini spin column in a new 2 ml collection tube, add 500 μ l Buffer AW1, and centrifuge for 1 min at $\geq 6000 \times g$. Discard flow-through and collection tube.
5. Place the Dneasy Mini spin column in a new 2 ml collection tube, add 500 μ l Buffer AW2, and centrifuge for 3 min at $\geq 20000 \times g$ to dry the Dneasy membrane. Discard flow-through and collection tube. This centrifugation step ensures that no residual ethanol is carried over during the following elution.
6. Place the Dneasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipet 50-100 μ l Buffer AE directly onto the Dneasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at $\geq 6,000 \times g$ to elute.

2.3 Purification of Genomic DNA from formalin-fixed tissue

MATERIALS

- Water bath 56°C
- Heating block 37°C and 70°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- PBS, pH 7,2 (50 mM potassium phosphate; 150 mM NaCl)
- Ethanol 96-100%

DNA Preservation



PROCEDURE

The Dneasy procedure has been successfully used to purify DNA from fixed tissues. However, the length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used. Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.

A. Removal of formalin and digestion of formalin-fixed tissues (day 1)

1. Wash tissue sample twice with PBS to remove fixative.
2. Discard PBS and resuspend the tissue pellet in 180 μ l Buffer ATL.
3. Add 20 μ l proteinase K, mix by vortexing, and incubate at 56°C until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1-3 h. If it is more convenient, samples can be lysed overnight; this will not affect them adversely.

B. Precipitation of DNA (day 2)

1. Vortex for 15 s. Add 200 μ l Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70°C. It is essential that the sample and Buffer AL be mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.
2. Add 200 μ l ethanol (96-100%) to the sample, and mix thoroughly by vortexing. It is important that the sample and the ethanol be mixed to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the Dneasy Mini-spin column.
3. Pipet the mixture from step 2 into the Dneasy Mini-spin column placed in a 2 ml collection tube. Centrifuge at $\geq 6000 \times g$ for 1 min. Discard flow-through and collection tube.
4. Place the Dneasy Mini-spin column in a new 2 ml collection tube, add 500 μ l Buffer AW1, and centrifuge for 1 min at $\geq 6000 \times g$. Discard flow-through and collection tube.

DNA Preservation



5. Place the Dneasy Mini-spin column in a new 2 ml collection tube, add 500 μ l Buffer AW2, and centrifuge for 3 min at $\geq 20000 \times g$ to dry the Dneasy membrane. Discard flow-through and collection tube. This centrifugation step ensures that no residual ethanol is carried over during the following elution.
6. Place the Dneasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipet 50-100 μ l Buffer AE directly onto the Dneasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at $\geq 6000 \times g$ to elute.

3- QIAMP DNA MICRO

3.1 Purification of Genomic DNA from frozen tissue

MATERIALS

- Water bath 56°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- Ethanol 96-100%

PROCEDURE

A. Digestion of frozen tissue (day 1)

1. Transfer a tissue sample of less than 10 mg in weight to a 1.5 ml microcentrifuge tube, and add 180 μ l Buffer ATL.
2. Add 20 μ l proteinase K, and mix by pulse-vortexing for 15 s.
3. Incubate at 56°C until the tissue is completely lysed. For small amounts of tissue, lysis is complete in 4-6 h, but best results are achieved after overnight lysis.

DNA Preservation



B. Precipitation of DNA (day 2)

1. Add 200 μ l Buffer AL, close the lid, and mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL be thoroughly mixed to yield a homogeneous solution.
2. Add 200 μ l ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s. Incubate for 5 min at RT.
3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
4. Carefully transfer the entire lysate to the QIAmp MinElute Column without wetting the rim, close the lid, and centrifuge at $\geq 6000 \times g$ for 1 min. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAmp MinElute Column is empty.
5. Carefully open the QIAmp MinElute Column and add 500 μ l Buffer AW1 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6000 \times g$. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
6. Carefully open the QIAmp MinElute Column and add 500 μ l Buffer AW2 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6000 \times g$. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
7. Centrifuge at full speed (20000 $\times g$) for 3 min to dry the membrane completely.
8. Place the QIAmp MinElute Column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the flow-through. Carefully open the lid of the QIAmp MinElute Column and apply 20-100 μ l Buffer AE directly or distilled water to the center of the membrane.
9. Close the lid and incubate at room temperature for 1 min. Centrifuge for 1 min at full speed (20,000 $\times g$) to elute. Incubating the QIAmp MinElute Column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

DNA Preservation



3.2 Purification of Genomic DNA from paraffin-embedded tissue

MATERIALS

- Water bath 56°C
- Heating block 37°C
- Sterile 1.5 ml microcentrifuge tubes (Eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- Xylene
- Ethanol 96-100%

PROCEDURE

The Dneasy procedure has been successfully used to purify DNA from fixed tissues. However, the length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used. Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.

This protocol describes the removal of paraffin by extraction with xylene.

A. Removal of paraffin and digestion of Paraffin-Embedded Tissues (day 1)

1. Place a small section (not more than 25 mg, 2 or 3 cuts of 20 μm) of paraffin-embedded tissue in a 2 ml microcentrifuge tube.
2. Add 1200 μl xylene. Vortex vigorously.
3. Centrifuge at full speed for 5 min at room temperature.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200 μl ethanol (96-100%) to the pellet to remove residual xylene, and mix gently by vortexing.
6. Centrifuge at full speed for 5 min at room temperature.
7. Remove supernatant by pipetting. Do not remove any of the pellet.
8. Repeat steps 5-7 once.

DNA Preservation



9. Incubate the open microcentrifuge tube at 37°C for 10-15 min until the ethanol has evaporated.
10. Re-suspend the tissue pellet in 180 µl Buffer ATL.
11. Add 20 µl proteinase K, and mix by pulse-vortexing for 15 s. Incubate at 56°C until the tissue is completely lysed. For small amounts of tissue, lysis is complete in 4-6 h, but best results are achieved after overnight lysis.

B. Precipitation of DNA (day 2)

1. Add 200 µl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL be thoroughly mixed to yield a homogeneous solution.
2. Add 200 µl ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s. Incubate for 5 min at room temperature.
3. Briefly, centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
4. Carefully transfer the entire lysate to the QIAmp MinElute Column without wetting the rim, close the lid, and centrifuge at $\geq 6,000 \times g$ for 1 min. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until QIAmp MinElute Column is empty.
5. Carefully open the QIAmp MinElute Column and add 500 µl Buffer AW1 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6,000 \times g$. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
6. Carefully open the QIAmp MinElute Column and add 500 µl Buffer AW2 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6,000 \times g$. Place the QIAmp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
7. Centrifuge at full speed (20,000 $\times g$) for 3 min to dry the membrane completely. Place the QIAmp MinElute Column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the flow-through. Carefully open the lid of the QIAmp MinElute Column and apply 20-40 µl Buffer AE directly or distilled water to the center of the membrane.

DNA Preservation



8. Close the lid and incubate at room temperature for 1 min. Centrifuge for 1 min at full speed (20,000 x g) to elute. Incubating the QIAmp MinElute Column loaded with Buffer AE or water for 5 min at RT before centrifugation generally increases DNA yield.

3.3 Purification of Genomic DNA from formalin-fixed tissues

MATERIALS

- Water bath 56°C
- Heating block 37°C
- Sterile 1.5 ml microcentrifuge tubes (eppendorf)
- Vortex
- Microcentrifuge, 1.5 ml tubes
- Micropipet
- PBS, pH 7,2 (50 mM potassium phosphate; 150 mM NaCl)
- Ethanol 96-100%

PROCEDURE

The Dneasy procedure has been successfully used to purify DNA from fixed tissues. However, the length of DNA purified from fixed tissues is usually <650 bp, depending on the type and age of the sample and the quality of the fixative used. Use of fixatives such as alcohol and formalin is recommended. Fixatives that cause cross-linking, such as osmic acid, are not recommended as it can be difficult to obtain amplifiable DNA from tissue fixed with these agents.

A. Removal of formalin and digestion of formalin-fixed tissues (day 1)

1. Wash tissue sample twice with PBS to remove fixative.
2. Discard PBS and resuspend the tissue pellet in 180 µl Buffer ATL.
3. Add 20 µl proteinase K, and mix by pulse-vortexing for 15 s.
4. Incubate at 56°C until the tissue is completely lysed. For small amounts of tissue, lysis is complete in 4-6 h, but best results are achieved after overnight lysis.

DNA Preservation



B. Precipitation of DNA (day 2)

1. Add 200 μ l Buffer AL, close the lid, and mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL be thoroughly mixed to yield a homogeneous solution.
2. Add 200 μ l ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortexing for 15 s. Incubate for 5 min at room temperature.
3. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
4. Carefully transfer the entire lysate to the QIAamp MinElute Column without wetting the rim, close the lid, and centrifuge at $\geq 6,000 \times g$ for 1 min.
5. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until QIAamp MinElute Column is empty.
6. Carefully open the QIAamp MinElute Column and add 500 μ l Buffer AW1 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6,000 \times g$. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
7. Carefully open the QIAamp MinElute Column and add 500 μ l Buffer AW2 without wetting the rim. Close the lid and centrifuge for 1 min at $\geq 6,000 \times g$. Place the QIAamp MinElute Column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.
8. Centrifuge at full speed (20,000 $\times g$) for 3 min to dry the membrane completely. Place the QIAamp MinElute Column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute Column and apply 20-40 μ l Buffer AE directly or distilled water to the center of the membrane.
9. Close the lid and incubate at room temperature for 1 min. Centrifuge for 1 min at full speed (20,000 $\times g$) to elute. Incubating the QIAamp MinElute Column loaded with Buffer AE or water for 5 min at RT before centrifugation generally increases DNA yield.

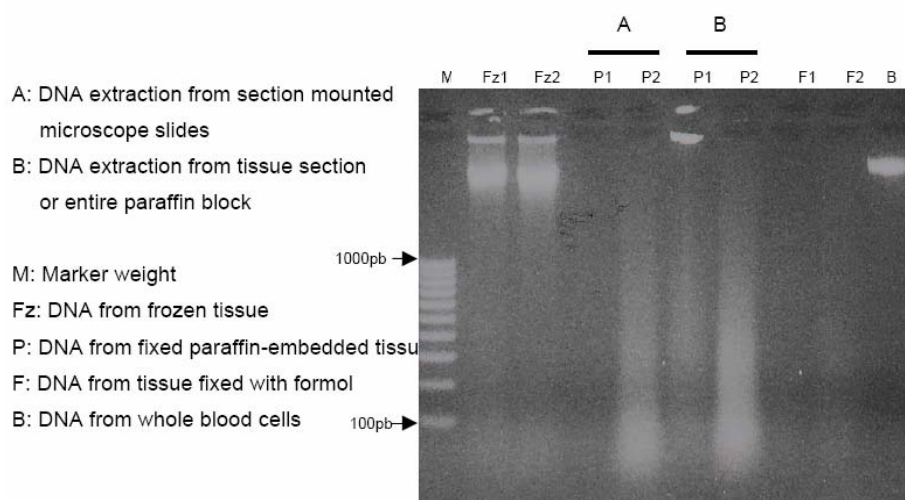
DNA Preservation

QUALITY AND QUANTIFICATION OF DNA

1- Quantification of DNA by agarose gel

Using 1 μ L of DNA sample obtained, together with 1, 2 and 3 μ L of known concentration DNA control run in a 0.8% agarose gel. Visual comparison can be made after ethidium bromide staining and quantification of DNA concentration can be obtained.

Figure 1. Agarose gel. DNA samples extracted from different conserved tissues are run in 0.8% agarose gel.



DNA Preservation

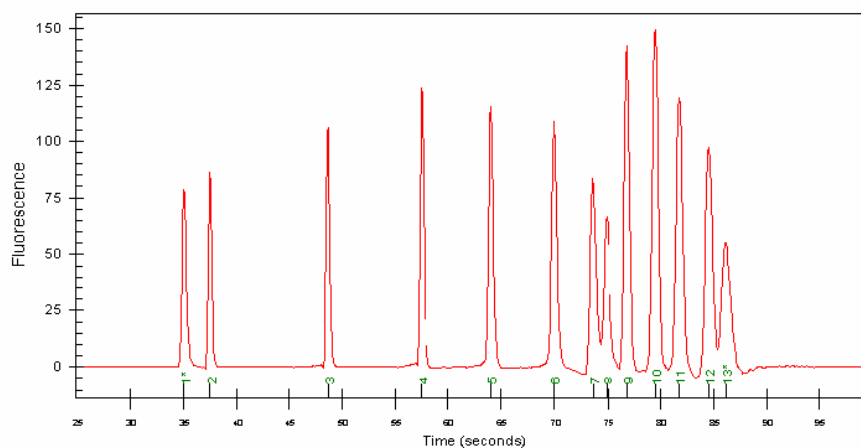
2. Quantification of DNA by Agilent Bioanalyzer

The purpose of bio sizing assays is to calculate the size and /or concentration of nucleic acid fragments. Results are calculated after all data for an individual well have been read.

The data analysis procedures consist of the following steps:

- Raw data is read and stored by the system for all the individual wells.
- A software algorithm filters the data and plots the resulting electropherograms of all wells.
- Peaks are identified for all wells and tabulated by migration time.
- A DNA ladder--a mixture of DNA fragments of different sizes--is run first from the ladder well.

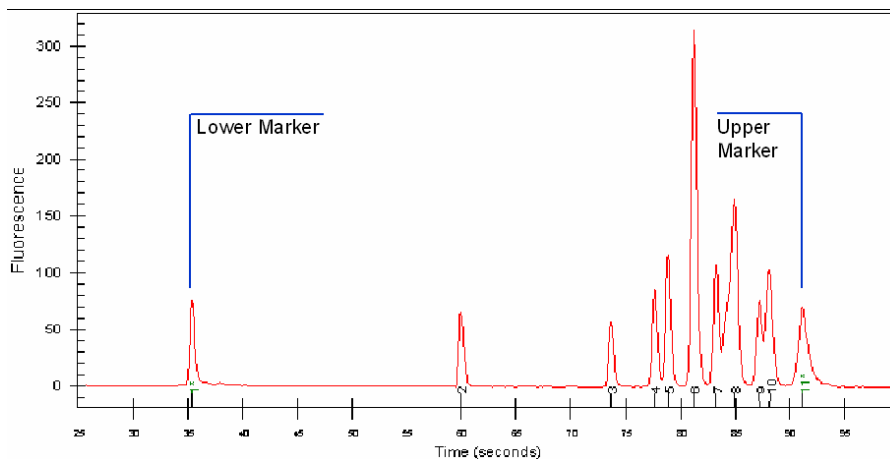
Figure 2. DNA ladder. The concentration and sizes of the individual base pairs are present in the assay.



DNA Preservation

- A standard curve of migration time against DNA size is plotted by using linear interpolation.
- Two DNA fragments are run with each of the samples, bracketing the DNA sizing analysis. Called lower and upper markers, these are internal standards and are used to align the ladder analysis with the individual sample analysis.

Figure 3. Lower and upper markers



- The standard curve (plotting migration time against DNA size), in conjunction with the markers, is then used to calculate DNA fragment sizes for each well from the migration times measured.
- To calculate the concentration of the individual DNA fragment of all sample wells, the upper marker is used.

DNA Preservation

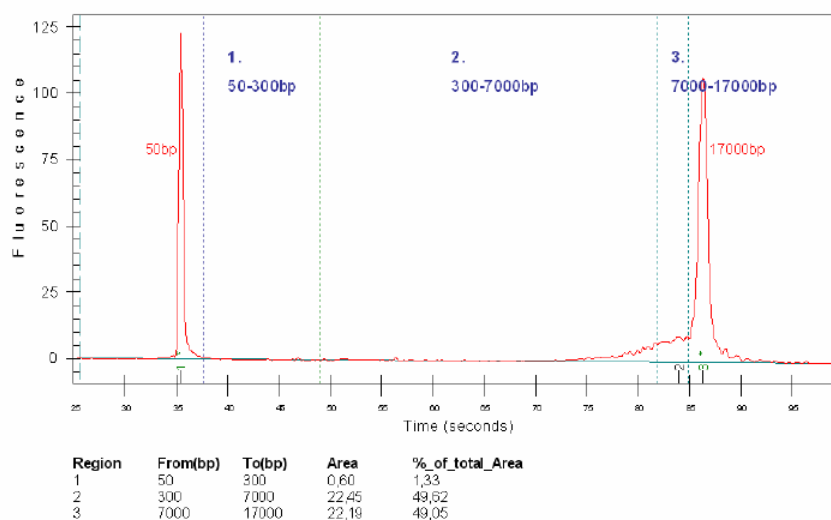
Analyzing DNA smear assays

DNA smear assays are designed to analyze broad bands as they occur with fragmented genomic DNA or double stranded cDNA. These assays allow for definitions of regions in terms of base pairs that enable characterization of dsDNA smears. The total area to be evaluated is determined by two dotted lines; the lower and upper time markers. These time markers are displayed between the lower and upper DNA markers of the sample.

To analyze the quality of DNA, we used three different regions (Figure 4):

- region 1: from 50bp to 300bp. We considered this region to be degraded DNA. Most of the DNA in this region is extracted from formalin-fixed tissue, and is unusable.
- region 2: from 300bp to 7,000bp. We considered this region to be acceptable quality DNA for performing mutation analysis by PCR. The DNA extracted from paraffin-embedded tissue is located in this region.
- region 3: from 7,000bp to 17,000bp. The DNA in this region is considered to be good quality, allowing for analyses other than PCR. In this region we found DNA extracted from frozen tissue.

Figure 4. Analysis of DNA smear assays. The results related to the defined region are displayed in the Results table.



DNA Preservation



BrainNet Europe II

Project Co-ordinator:

Prof. Dr. Hans Kretzschmar

Contact:

www.brainnet-europe.org

Network of Excellence funded by the
EC in the 6th Framework Program
"Life Science"



Author

Prof. Dr. Isidro Ferrer

Hospital de Bellvitge/
Universitat de Barcelona

Phone: +34 (93) 403 - 5808

Mail: 8082ifa@comb.es

Copyright:

© by author(s) 01.01.2008

Reproducing, redistributing, or making
commercial use of this information is
expected to adhere to the terms and
conditions asserted by the copyright
holder.

All rights reserved

Edited: 28.02.2008

Filename DNA Preservation