STORE

SOP Title:

Genomic DNA isolation from FFPE tissue blocks

The STORE processing methods were shown to be fit-for purpose for DNA, RNA and protein extraction from FFPE material. The STORE characterisation methods were shown to be fit for-purpose for Quality Control of the extracted DNA, RNA and proteins.

HUMAN CANCER STUDIES GROUP

SOP reference MB06

Standard Operating Procedure for

Genomic DNA isolation from FFPE tissue blocks

Version number ……2……

Date created ……24/07/2008

Date of review ……24/07/2009

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Summary

This protocol explains the procedure for the isolation of genomic DNA from FFPE tissue blocks either with or without prior microdissection

Safety Considerations

Protective clothing and gloves should be worn throughout.

Materials

- xylene
- methanol
- ethanol (absolute, 96%, 70%)
- NaSCN (Sigma, S-7757)
- Haematoxyline (only for DNA isolation from microdissected samples)
- QIAmp DNA mini kit
- Proteinase K (Qiagen) or from another supplier (20 mg/ml)
- Nuclease-free water
- 0.22 µm syringe filters

Preparation of reagents

NaSCN (Sodium thiocyanate, 1M)

Dissolve 8.1 g NaSCN in 100 ml nuclease-free water. Sterilize the solution by filtering through a 0.22 µm syringe filter.

Proteinase K (if used from other supplier than Quiagen)

Dissolve 100 mg proteinase K in 5 ml nuclease-free water and sterilize the solution by filtering trhough a 0.22 µm syringe filter.

A Treatment of sections supposed to be microdissected prior to DNA isolation

To obtain a sufficient yield of DNA, place at least ten 10 µm sections plus one thin reference section on uncoated glass slides and dry them overnight at room temperature. Deparaffinise the slides 3 times for 7 minutes in xylene at room temperature and wash them two times for 7 minutes with methanol at room temperature. Wash slides for 7 minutes each in absolute, 96% and 70% ethanol. Wash slides with water from the tab before staining mit haematoxyline for 5 min. Wash for 5 min with water from the tab.

Select the area of interest under the microscope using the reference slide and scrape the selected area from the 10 µm sections using a fresh scalpel (keep sections wet with water) and transfer them into a microcentrifuge tube. Spin the tube at maximum speed for 5 min and discard the liquid.

B Treatment of whole sections prior to DNA isolation

Cut at least five 10 µm sections if the embedded tissue is large and at least ten 10 µm sections if the embedded tissue is very small. Transfer the sections directly into a microcentrifuge tube and add 1 ml of xylene before vortexing. Incubate the tube for 7 min at room temperature and spin for 5 min at maximum speed. Remove the supernatant and repeat for two times. Add 1ml methanol to the tube, incubate at room temperature for 5 minutes and spin down for 5 min at maximum speed. Repeat the methanol washing and spinning step. Wash the pellet with absolute, 96% and 70% ethanol by 5 min incubation at room temperature, spinning down at maximum speed for 5 min and discarding the supernatant. Vortex the tube before every spinning-step. Add 1 ml distilled water and vortex the tube, spin down at maximum speed and remove the supernatant.

C DNA isolation (adopted from VUMC, Microarray Facility, Amsterdam)

Day 1 – Deparaffinization (RecoverAll Ambion protocol)

- Add Xylene 1 ml, vortex briefly to mix and incubate the tube for 3 min at 50°C in heat-block or water bath to melt the paraffin.
- Centrifuge the sample for 2 min at room temp and maximum speed to pellet the tissue.
- (Optional) If the sample does not form a tight pellet, recentrifuge for an additional 2 min. If a tight pellet still does not form, then proceed with caution to the next step.
- Remove the xylene without disturbing the pellet. Discard the xylene. If the pellet is loose, you may need to leave some xylene in the tube to avoid removing any tissue pieces.
- Add 1 ml Ethanol 100% and vortex to mix. Centrifuge for 2 min at maximum speed to pellet the tissue and remove the supernatant.
- Add 1 ml Ethanol 100% and vortex to mix. Centrifuge for 2 min at maximum speed to pellet the tissue and remove the supernatant.
- Briefly centrifuge again to collect any remaining drops of ethanol in the bottom of the tube. Remove as much residual ethanol as possible without disturbing the pellet.
- Air dry the pellet for 10- 15 min.
- Add 1ml 1 M NaSCN to each micro centrifuge tube and shake by vortexing. Incubate the tube at 38°C overnight. (MW: 81.06 gr/l; 4.9 gr in 60 mls dH20=60 samples)

Day 2- Protease digestion.

- Spin the tube for 5 min at maximum speed (the tissue will not form a pellet). Remove the supernatant.
- Wash 2x with 1 mL PBS and spin down for 5 min. at full speed, discard the PBS by pipetting
- Add 60 ul ATL buffer (QIAmp DNA micro-kit, Qiagen)
- Add 40 ul prot K (20 mg/mL) and vortex for 15 sec! Make sure all tissue is in the liquid
- Incubate o/n at 55°C in heat-block or waterbath and vortex regularly!

When the isolation procedure is started in the morning, add another volume of 20 ul prot K. at the end of the day. When started in the afternoon, add another volume of 20 ul prot K in the morning of day 3 and continue in the afternoon. Check if all tissue is digested!

Day 3- DNA isolation

- Incubate at 98°C for 10 min
- Add 100 ul ATL buffer (QIAmp DNA micro-kit, Qiagen)

- Add 200 ul AL buffer (QIAmp DNA micro-kit, Qiagen) and mix very well by vortexing!
- Add 200 ul Ethanol 100% and mix very well by vortexing!
- Incubate 5 min. at RT
- Spin down the eppendorf cups (maximum speed for 3 min).
- Transfer the lysate to the QIAamp MinElute Column *
- The column can contain max. 600 ul of sample. Apply 300 ul of sample per time. Repeat step marked with * until all of the sample is loaded on the column
- Spin down for 1 min at 8.000 rpm and place the column in a new tube *
- Place the QI Column in a clean 2 ml collection tube, discard the flow-through *
- Add 500 ul AW1 buffer (QIAmp micro-kit) to the column
- Spin down for 1 min at 8.000 rpm and place the column in a new tube
- Add 500 ul AW2 buffer (QIAmp micro-kit) to the column
- Spin down for 1 min at 8.000 rpm and place the column in a new tube
- Spin down for 3 min at full speed to dry the membrane
- Place the QI column in a properly marked eppendorf cup and discard collection tube
- Add 20 to 30 ul (depending on sample size) of AE buffer (QIAmp micro-kit) to the column and incubate 5 min. at RT
- Spin down for 3 min at 8.000 rpm
- Dispose the column, close the eppendorf cup and add a parafilm and store the DNA at 4°C