

# ***Automated dispersion and sample handling in the Umeå Medical Biobank***

Work package 4.1 of the National Biobanking Program

*Annual report 2004*

## ***Background***

The Umeå Medical Biobank is collecting population-based blood samples from the Västerbotten Intervention Program (VIP (population-based health-promoting project), the MONICA-project (a population-based WHO study on risk factors for cardiovascular) and from the population-based regional mammography screening program. The blood samples are intended for studies on the impact of environmental and genetic background on diseases. The sample collection is one of the largest and oldest population-based biobanks in the world. The biobank is also administrating and handling samples for 20 smaller sample collections collected by a large number of scientists (i.e. a biobanking facility according to the “safety deposit box” principle).

An optimisation of the use of the limited material and that maintains a high quality of samples and data was made possible through a financial allowance from the Wallenberg Consortium North (WCN) for a dispersion robot. The automation of the DNA and plasma handling has greatly enhanced the throughput of sample retrieval for research projects.

## ***Objectives for the optimal usage of the collected blood samples***

*To develop new systems of:*

1. Measurement and dispersion of DNA for genetic analysis
2. Dispersion of plasma for protein studies
3. Collecting buffy coat
4. DNA extraction
5. RNA extraction

## ***Deliverables (1&2)***

The automation of DNA and plasma handling consists of a dispersion robot, Beckman Coulter FX and a plate reader, BMG Fluostar. Routine applications and methods for DNA and plasma were transferred to the robot platform. For every new method of dispersion and dilution a validation of the protocol is performed. For DNA, the validation of volumes is based on absorbance measurements and for plasma the weight is measured. Repeated studies of contamination between wells are performed using fluorescence with Orange G. The same method is used for the control of the homogeneity of resuspended DNA. The overall result showed good accuracy and mixture and no signs of contamination has been detected. A continuous control of log files is made, where transfer of sample, volume and positions is recorded.

During 2004, we have been processing projects dealing with the dilution and dispersion of DNA, plasma and erythrocytes. A protocol for dispersion of erythrocytes using the robot has not been successfully developed, because of the very high viscosity of erythrocyte samples.

A survey of the stations for sample handling at the Medical Biobank has been done with focus on the estimated risk of errors and contamination. In appendix 1 an overview is presented where identification and estimation of risks are visualised.

## ***Deliverables (3)***

In the method for collecting blood samples, the blood is centrifuged and divided into a plasma-, buffy coat- and erythrocyte fractions. No further division of buffy coat is made before the storage in -80C freezers. A division of buffy coat at the sampling occasion would be preferable but this is difficult when most sampling is done at the health care centres. An alternative is to withdraw an aliquot when thawing the buffy coat sample and refreeze the remaining sample. The effect on DNA integrity after repeated freeze-thawing was investigated on a group of samples. No effect on yield and integrity of DNA was detected after rounds of freezing and thawing.

## ***Deliverables (4)***

A comparison of extraction methods was carried out during late 2002 pointed towards two systems, Flexigene and Gentra. Apart from the manual extraction methods an automated version of DNA extraction is available for both systems.

The Flexigene system was chosen as being most suitable looking at costs and premises.

#### *Deliverables (5)*

An investigation to compare results of sampling RNA using the PAXgene Blood RNA Tube method with that of ordinary buffy coat sampling was initiated during 2004. A group of patients were asked to donate blood to 4 different sampling tubes, apart from PAX also EDTA, heparin and citrate. Aliquots of buffy coat was either extracted right away or frozen 6 months before RNA isolation. RNA has been isolated and analyses of expression levels of selected genes is ongoing using real-time PCR and should be completed during spring 2005. A small set of samples from one individual (PAX, EDTA, citrate) were analysed for expression levels of the matrix metalloproteinases MMP-2, MMP-9 and their tissue inhibitors, TIMP-1 and TIMP-2. The result is presented in appendix 3 and shows quite different results depending on analysed genes.

#### *Projects with processing completed during 2004*

Last year 35 administrated projects were activated in form of retrieved samples for extraction and/or dispersion. The total amount of samples were 9100 and a number of aliquots thereof.

NCI-EPIC breast cancer DNA, 250 samples  
MORGAM DNA, 375 samples  
Fia2 plasma, MA cohort 210 samples  
Fia2 plasma, Vab/MO cohort 1452 samples  
RA DNA, 1325 samples  
Metabolic syndrome/ cancer erythrocytes, 83 samples  
NCI-EPIC prostata DNA, 77 samples  
Metabolic syndrome/prostate cancer, plasma, 824 samples  
Castro2, plasma, 538 samples  
DIVE diabetes plasma, 1377 samples  
Esophagus cancer, 75 samples  
Pancreas cancer, 158 samples  
Metabolic syndrome, colon cancer, erythrocytes, 643 samples  
Colorectal cancer DNA, 262 samples  
Lycksele diabetes, 172 samples  
Smaller projects and controls

#### *Projects being processed*

Diabetes DNA, 1400 samples  
Prostate cancer DNA, 824 samples  
Prostate cancer plasma, 824 samples  
Metabolic syndrome/colon cancer DNA, 856 samples  
“ plasma, 856 samples  
“ erythrocytes, 856 samples  
Colorectal cancer CCPRB Jer-2 DNA, 850 samples  
Breast cancer NCI-NY DNA, 2000 samples  
Breast cancer Jer-2 DNA, 2000 samples  
Amyloidosis DNA and/or plasma about 1000 samples  
TRIM diabetes, 500 plasma samples  
Osteoporosis plasma, 450 samples  
Ovarian cancer, 450 DNA and plasma samples  
Endometrial cancer, 525 DNA and plasma samples

To this a substantial list of projects will be activated after approval from expert groups and the ethical review board.

*Financial report for work package 4.1*

Salary for molecular biologist, Kerstin Enquist 2004-01-01-2004-12-31	437.268 Skr
Material, service agreement, BiomekFX	154.830 Skr
Additional material	7.704 Skr
Travel and accommodation	
Participation in the ISBER meeting in Perugia 17-20 okt	
WCN course in Epidemiology, Arlanda 1-3 June	
Work package meeting 26 Oct, Arlanda	29.810 SKr
Secretarial assistance, Margaretha Tagewall	40.195 SKr
Overhead	65.126 SKr
Total	734.934 SKr

*Publications 2004*

***NSHDS, Northern Sweden Health and Disease Study***

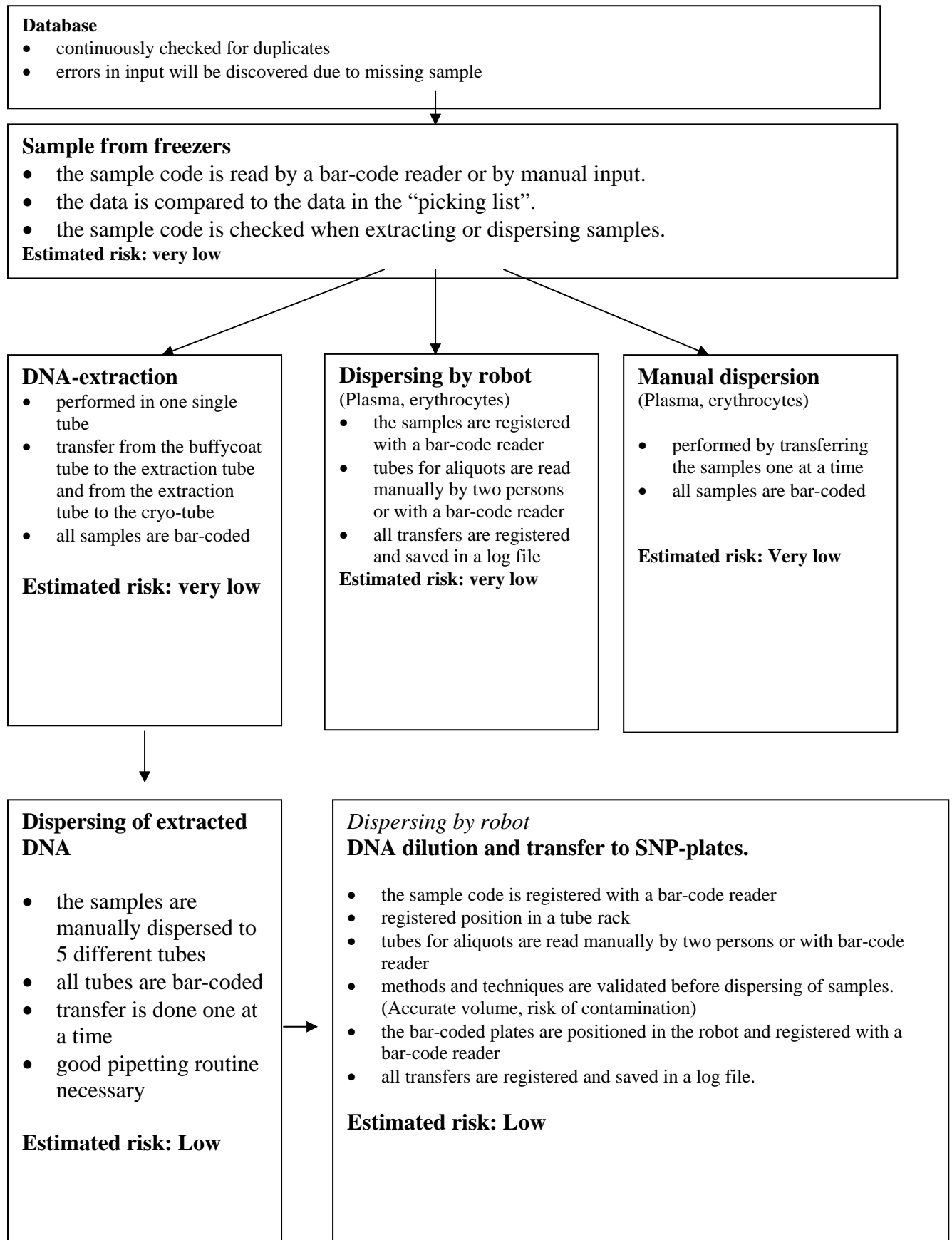
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## Appendix I

### Estimated risk of errors and contamination when handling samples at the Medical Biobank



## APPENDIX II

### Division of buffy coat before extraction of DNA.

#### Background

Up till now the extraction of DNA has been performed using the entire sample of buffy coat, generated from 10 ml of whole blood. The average yield from one buffy coat sample is almost 250  $\mu\text{g}$ . This amount of DNA is well enough for 10000 SNP analysis using 10 ng per genotyping assay. The amount of buffy coat is limited and the extraction of the whole sample makes analysis of other components in the white blood cell fraction impossible and as well as correction of any errors like sample mix-up and bad extraction methods.

Advantages of retaining half of the buffy coat sample in the original tube would be that:

- New analysis of components in WBC would be made possible
- If problems with the extraction method would occur, additional methods could be used
- Sample mix-up would be possible to correct
- Lost sample could be recovered
- New (and better) extraction methods could be used on remaining sample
- Automation of DNA extraction and handling would be facilitated

Disadvantages would be:

- A higher total cost for the extraction
- The insecurity of eventual negative effects of freezing and thawing on DNA quality

To investigate whether DNA yield and quality is affected when buffy coat samples are thawed and frozen multiple times a study was performed as follows.

#### Material and methods

##### Study no. 1

One portion of buffy coat from a pooled material from EDTA tubes was divided in 12 aliquots containing 125  $\mu\text{l}$  each. One tube was extracted immediately while the rest were re-frozen in  $-80^{\circ}\text{C}$ . The DNA was extracted with GenoM48 prep robot using silica coated magnetic beads. The frozen aliquots were thawed 9 days later and one portion was extracted while the remaining ones was frozen once again. This scheme was repeated 9 times. DNA yield was measured at A260 nm and purity at A260/A280. The integrity and size of the extracted DNA was visualised on an agarose gel. As a control a buffy coat sample was split in 10 aliquots and thawed only once before extraction. Fig.1 show the consistent of yield after the repeated freeze-thaw cycles and the corresponding agarose gel is seen in fig.2.

This study showed no negative effects on yield, purity or molecular weight of the extracted DNA, whether it was thawed one or ten times.

Fig.1

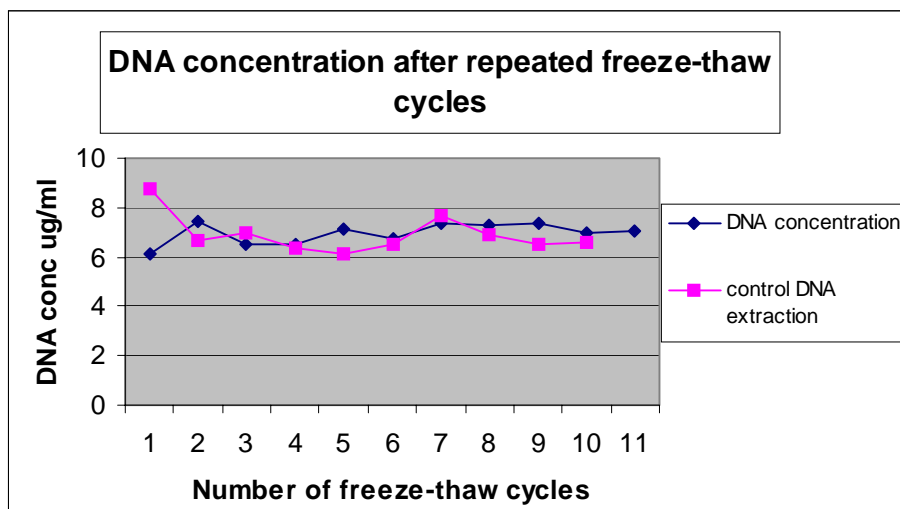
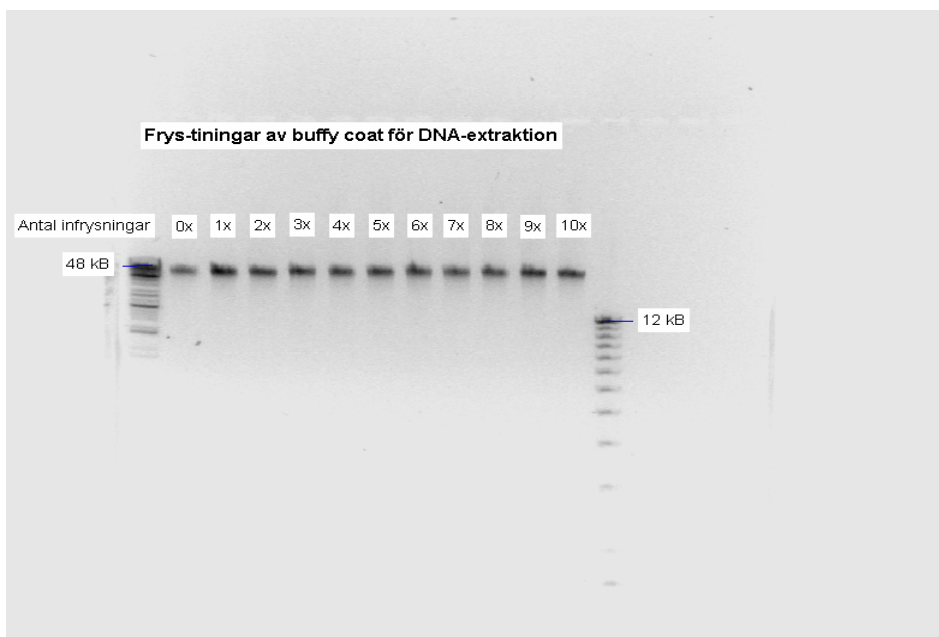


Fig.2

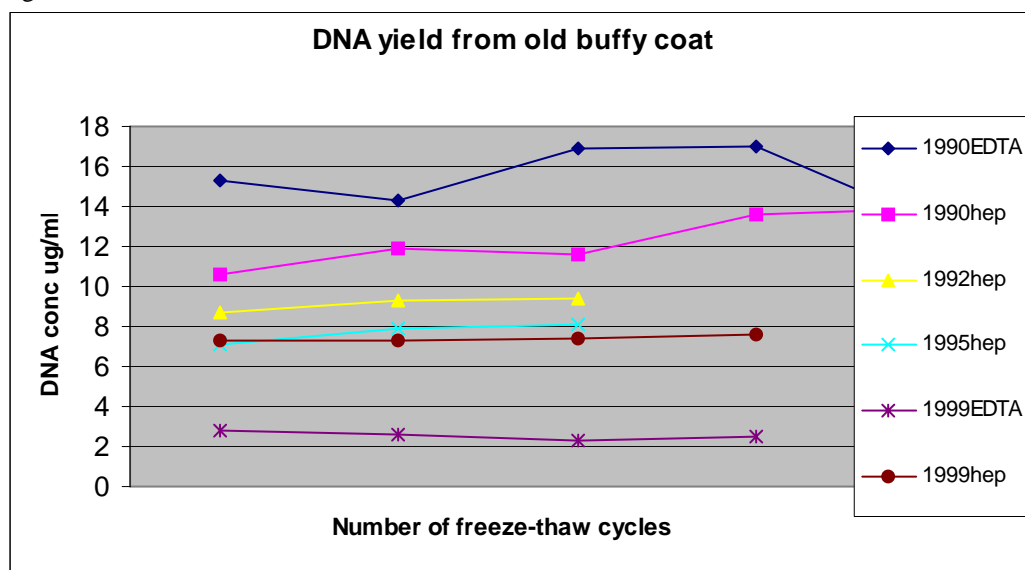


### Study no. 2

Buffy coat from 6 old and unidentified biobank samples were thawed and aliquoted in 4 to 6 tubes and a set of repeated freeze-thaw cycles was performed. The samples were collected both in EDTA and heparin tubes during 1990 to 1999. The extraction was made using the GenoM48 robot.

DNA yield and purity was measured at  $A_{260nm}$  (Fig.3). The molecular weight was checked by electrophoresis and no degradation of the DNA was visible. (Data not shown)

Fig.3



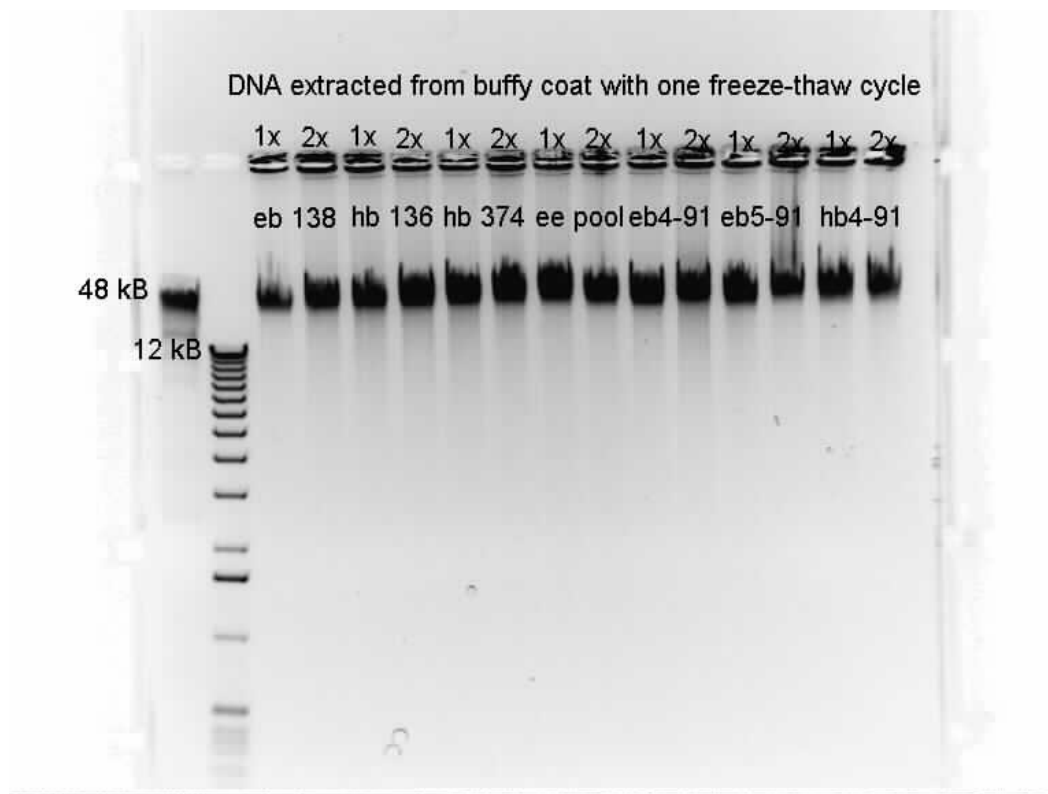
### *Study no.3*

Blood from 7 different sources (erythrocytes and buffy coat), was divided in two portions. One part was extracted immediately and the other was re-frozen in  $-80^{\circ}\text{C}$  in two weeks before DNA extraction was performed. (Fig.4)

The extraction method used was Qiagens Flexigene, which is the method that we are still using.

No difference in yield and purity was seen, comparing DNA extracted at the before and after an additional freeze-thawing cycle.

Fig.4



### *Study no.4*

Three buffy coat samples (2 EDTA ,1 heparin) were divided in three parts. One aliquot was extracted right after the division and the other two were frozen in  $-80^{\circ}\text{C}$ . After one week the two frozen aliquots were thawed and one was extracted. The third was re-frozen for another week before DNA extraction.

DNA was measured at 260 nm and checked on agarose gel. The concentration seemed to increase with additional freeze-thaw cycles rather than the opposite, probably due to inconsistent mixing of buffy coat before the division. No deterioration in purity was detected nor any decrease of DNA molecular weight.

(Fig. 5 lower lane)

### *Study no 5*

To investigate the rate of degradation of DNA in stored buffy coat samples, two EDTA and two heparin samples were thawed. They were incubated at room temperature for 3 hours before the mixing and aliquotation into four parts was performed

**No.I** was extracted using the Flexigene DNA kit while the other aliquots were frozen in  $-80^{\circ}\text{C}$ .

**No.II** was thawed after 24 hours and incubated at room temperature for three hours before DNA extraction  
**No.III** was treated similar to No II but incubated 24 hours before extraction.  
**No. IV** was treated similar to No.II but was incubated at RT 7 days before extraction.

The DNA concentration (Fig.6) was verified by gel electrophoresis. Degradation of DNA was most pronounced in heparin buffy coat. Especially at longer incubation periods. (Fig.5 upper lane)  
 Buffy coat sample collected in EDTA tubes is more resistant to degradation even though 7 days at room temperature does effect the DNA quality.

Fig.5

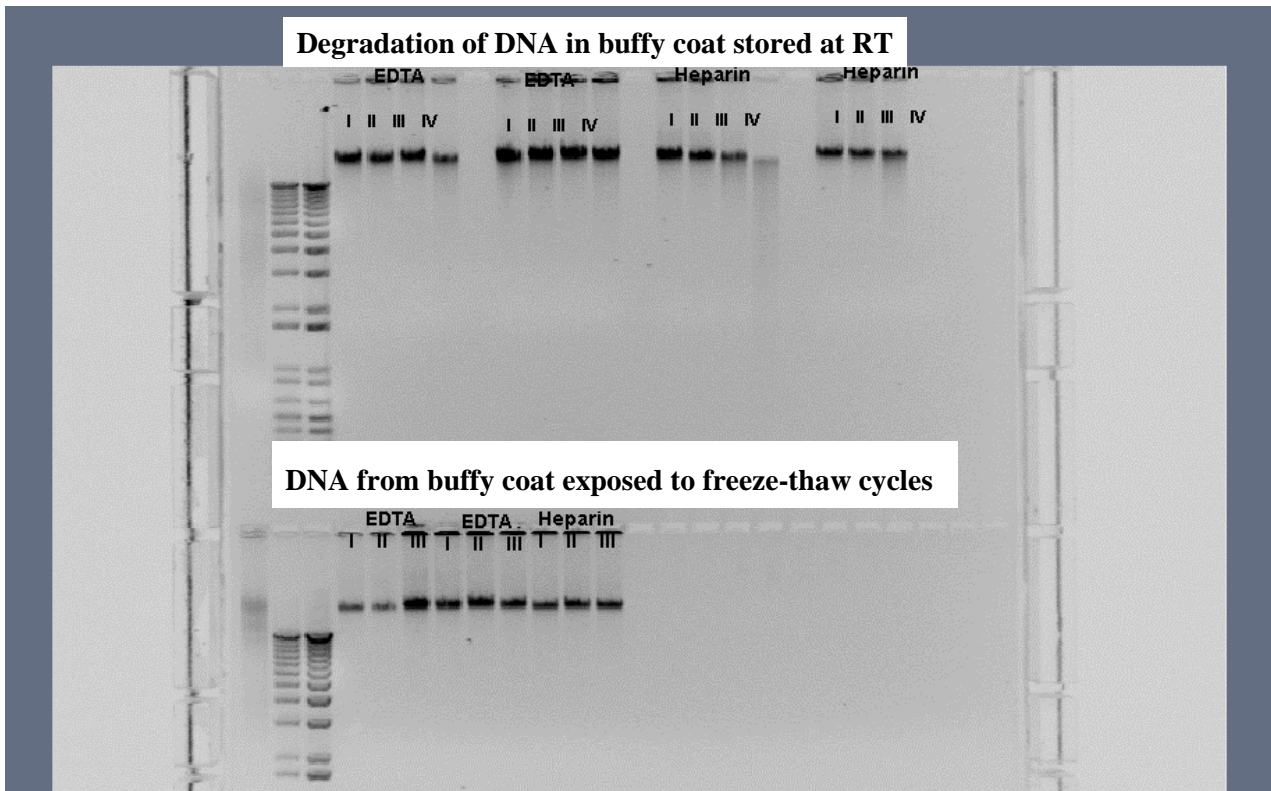
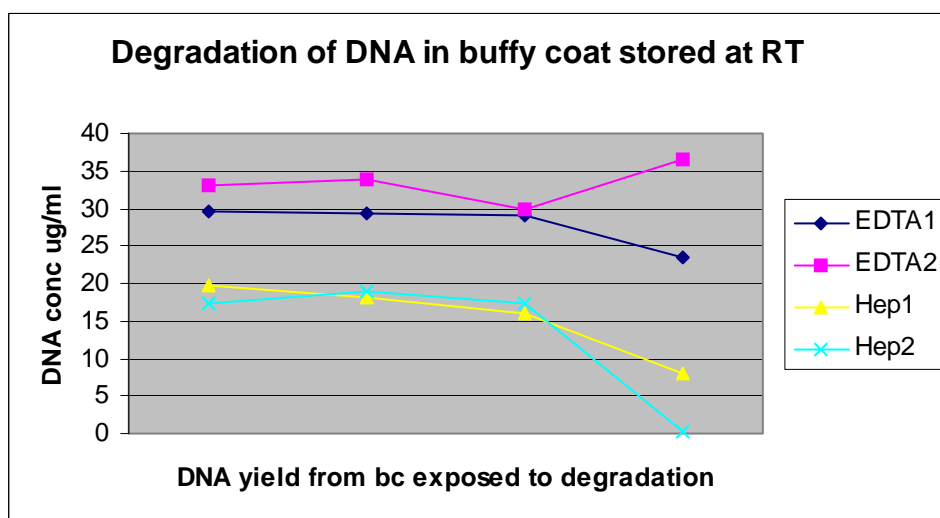


Fig.6



## **Conclusion**

In the experiments presented above, no indication of negative effects on DNA yield and purity has been detected after repeated freeze-thaw cycles of buffy coat. The degradation test in room temperature show that the DNA molecule is quite stable.