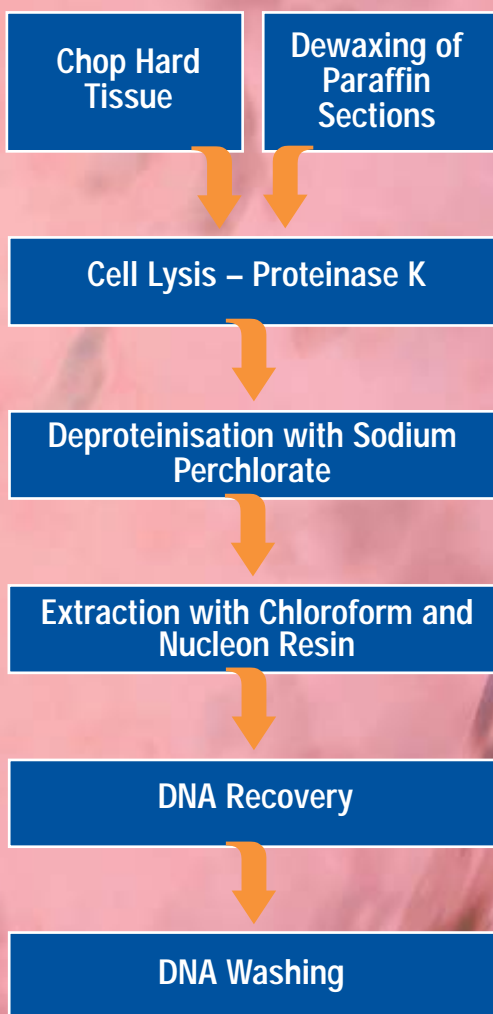


Principle
steps in the
Nucleon®
Hard Tissue &
Paraffin Section
protocol



GENOMIC DNA FROM HARD TISSUE AND PARAFFIN SECTIONS

- High speed (<40 minutes)
- High yield (>40µg/1cm tail)
- High purity ($A_{260/280}$ 1.8-1.9)
- High MWt DNA (≥ 50 kb)
- Suitable for a range of hard tissues e.g. mouse tail, skin, xiphisternum and paraffin-embedded tissue

Introduction

The Nucleon Hard Tissue (HT) DNA extraction system provides the user with an effective and consistent method for extraction of DNA from hard tissues and paraffin-embedded tissues that require a Proteinase K digestion for effective lysis.

Principle

Following tissue preparation, cells are lysed and extracted with sodium perchlorate. DNA is then extracted by a single partition against chloroform in the presence of Nucleon proprietary resin. This resin covalently binds proteins (Figure 1) and subsequently

forms a semi-solid stratum during partitioning (Figure 2), which traps proteinaceous material at the interface and in the organic phase. This ensures excellent recovery of DNA (Table 1).

Applications

DNA recovered by the Nucleon HT DNA extraction system is suitable for restriction digests (Figure 3), PCR[†] (Figure 4), blotting and hybridisation. The proprietary resin does not rely on binding and subsequent elution of DNA and so minimises DNA loss and shearing.

Figure 1 Mechanism of Nucleon Resin protein binding

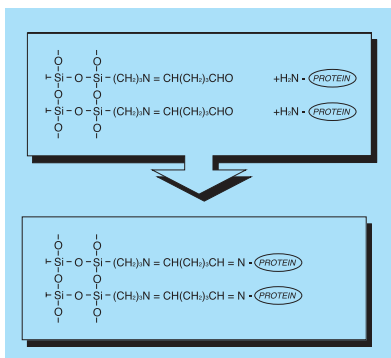


Figure 2 Formation of semi-solid stratum after adding Nucleon Resin and chloroform

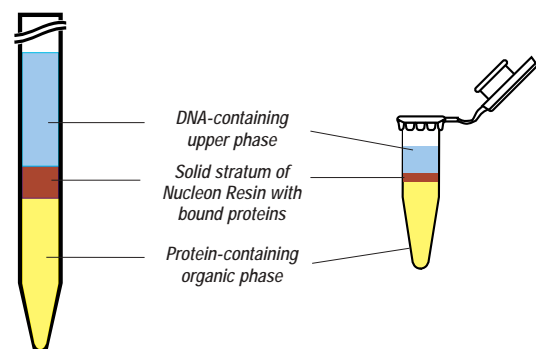
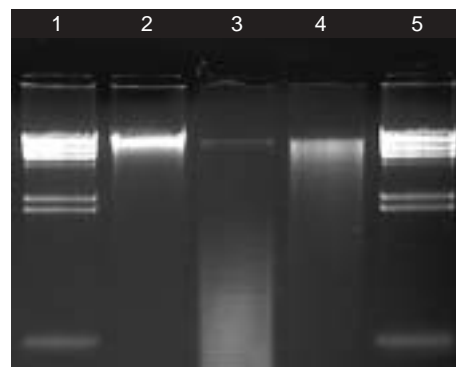


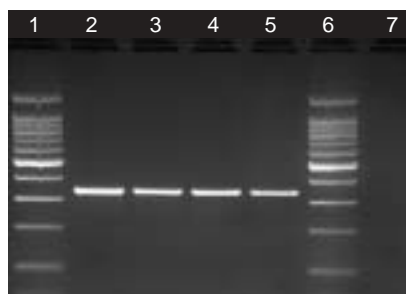
Figure 3 Genomic DNA isolation and restriction enzyme digests



DNA, isolated from hard tissues using the Nucleon protocol, was digested with *AluI* and *MspI* restriction enzymes and analysed on a 1.0% agarose gel.

- 1) *HindIII* λ digest marker
- 2) undigested DNA purified from mouse tail
- 3) DNA purified from mouse tail and digested with *AluI*
- 4) DNA purified from mouse tail and digested with *MspI*
- 5) *HindIII* λ digest marker

Figure 4 PCR amplification



DNA, isolated from hard tissues, using the Nucleon protocol, was used as a template for PCR amplification using a mouse DNA methyltransferase gene model. The products were separated on a 1.0% agarose gel.

- 1) 100bp ladder
- 2) mouse tail DNA (prep 1)
- 3) mouse tail DNA (prep 2)
- 4) mouse tail DNA (prep 3)
- 5) positive control (mouse Balb/C DNA)
- 6) 100bp ladder
- 7) negative control

Table 1 Recoveries of DNA from various hard tissues

Sample	Yield of DNA
Mouse tail	> 40 μ g per 1cm
Skin	1.4 μ g/mg
Xiphisternum	1.6-1.9 μ g/mg

Ordering Information

SL8509 Nucleon HT kit for 50 preps of up to 25mg of hard tissue or paraffin-embedded sections

Other kits available in the Nucleon range:

SL8501 Nucleon BACC1 kit for 50 extractions of up to 1ml whole blood or cell cultures

SL8502 Nucleon BACC2 kit for 50 extractions of between 3 to 10ml of whole blood or cell cultures

SL8512 Nucleon BACC3 kit for 50 extractions of up to 10ml of whole blood or cell cultures

SL8508 Nucleon ST kit for 50 preps of up to 250mg of soft tissue

SL8510 Nucleon PhytoPure® kit for 50 extractions of 0.1g of plant tissue

SL8511 Nucleon PhytoPure kit for 50 extractions of 1.0g of plant tissue

44100 Nucleon Genomic DNA Extraction kit for 50 preps of 10ml whole blood

44200 Nucleon Genomic DNA Extraction kit for 50 preps of 1cm mouse tail

44201 Nucleon Genomic DNA Extraction kit for 200 preps of 1cm mouse tail

44300 Nucleon Genomic DNA Extraction kit for 50 extractions of 0.1g of plant tissue

Other kits available in the Nucleopure™ range for use with the Nucleopure Automated System for mouse tail DNA extraction:

35100 Nucleopure Mouse Tail kit for up to 960 extractions

Other kits available in the Nucleoplex™ range for use with the Nucleoplex Automated System for BAC/Plasmid DNA extraction:

33100 Nucleoplex Plasmid DNA extraction kit for up to 192 extractions

33200 Nucleoplex BAC DNA extraction kit for up to 192 extractions

33201 Nucleoplex BAC DNA extraction kit for up to 192 extractions (plasticware not included)