

**Real-time DNA quantification**

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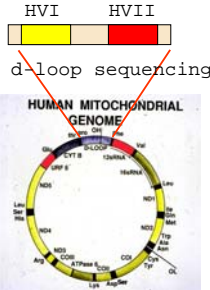
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**DNA analysis in Sweden**

Routine forensic analysis  
 - National Forensic Laboratory in Linköping  
 All STR-analyses  
 ~ 15 000 cases/year

mtDNA analysis  
 - Uppsala university  
 ~ 30 cases/year

Research at Uppsala university  
 - mtDNA  
 - Novel technologies  
 - Quantification




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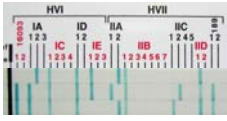
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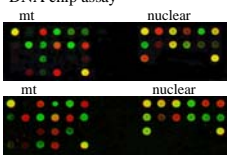
**Some research projects**

HVI/HVII linear arrays



Evaluation of linear arrays for mtDNA typing - Sandy Calloway, Roche

DNA chip assay



Development of a microarray assay - 21 mtDNA and 12 nDNA markers - more

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**Pyrosequencing**

**Sequencing-by synthesis**

- dNTPs are added one at a time
- Incorporation
- PPi released → converted to ATP by ATP-sulfurylase
- Luciferase use ATP to generate light
- dNTPs degraded by Apyrase → new cycle
- Light is proportional to:
  - released pyrophosphate, PPi
  - incorporated nucleotides

Added nt : A T G A T  
Sequence : A T GG - T

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**Pyrosequencing**

**D-loop**

- 2 PCR-fragment
- 8 pyro reaktionen
- ~ 400 bp

**Coding region analysis**

- increased discrimination
- 15 fragments
- ~ 1500 bp

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**DNA Quantification**

Valuable DNA in evidence materials  
Use the minimal amount of DNA required

Routine mtDNA analysis  
- mtDNA copy number information

No commercial assay available

**Quantification of mitochondrial DNA and nuclear DNA**

Real-time 5' exonuclease detection assay - TaqMan™  
Simultaneous mtDNA and nDNA quantification

Andreasson et al. 2002. Real-Time DNA Quantification of Nuclear and Mitochondrial DNA in Forensic Analysis. *BioTechniques* 33:402-411

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**TaqMan™**

Reporter  
Quencher

- 5' exonuclease activity  
- cleavage of probe
- Increased reporter fluorescent emission intensity

- Ct = The cycle at which the sample emission rises above the baseline (threshold cycle)
- High DNA amounts - low Ct

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**Real-time DNA Quantification assay**

Nuclear target - Retinoblastoma 1 gene  
Single copy gene - conserved region  
Exon 25 - 79 bp product  
FAM-labeled 28 bp probe

Mitochondrial target - tRNA Lys gene  
143 bp product  
VIC-labeled 29 bp probe

Establish standard curves  
nDNA/Genomic DNA dilutions  
mtDNA/mtDNA clone dilutions

ABI 7700 instrument

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**The Clone**

A plasmid with a tRNA<sup>Lys</sup> fragment spanning from nt 8049 to 8661

Plasmid concentration:  
1. Spectrophotometric measurement  
2. Limited dilution PCR (# of positive PCRs at a 1 copy dilution, Poisson distribution used to estimate # expected positives.

**Research Report** Similar estimates for both methods

**Real-Time DNA Quantification of Nuclear and Mitochondrial DNA in Forensic Analysis**

Andréasson et al. 2002. Real-Time DNA Quantification of Nuclear and Mitochondrial DNA in Forensic Analysis. *BioTechniques* 33:402-411

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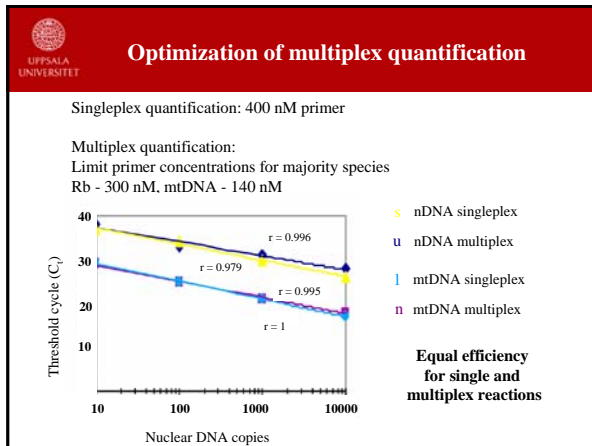
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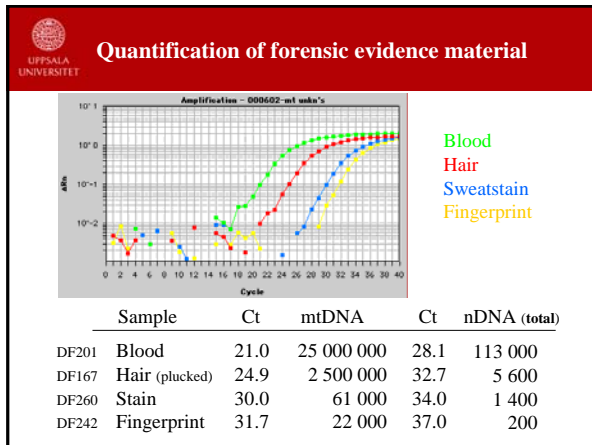
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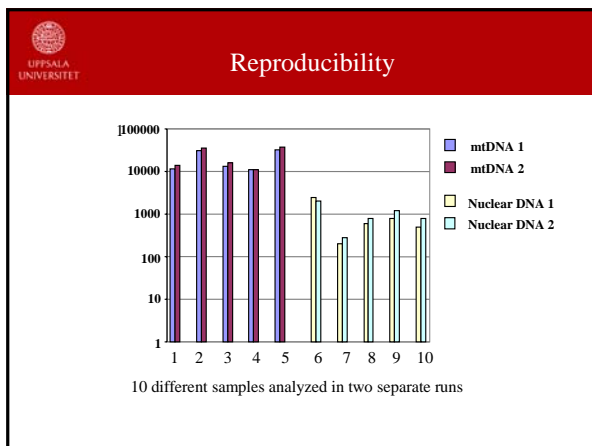
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**Applications for our duplex assay**

**DNA content in samples prior to typing:**  
 Quantified 500+ samples for mtDNA and nDNA  
 - Majority shed hairs - mtDNA sequence analysis in routine

**Evaluation of DNA content in various forensic materials:**

Head hairs - Plucked and shed	WGA methods - PEP - Genomiphi™
Body hairs - Plucked	Extraction methods - Ancient DNA
Accessories	
Fingerprints	

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**Quantification of DNA on accessories**

Category	mtDNA (copies/item)	nDNA (copies/item)
Rings	1720	0
Watches	342133	2487
Necklaces	104376	318
Glasses	25133	5487
Charms	27918	128
Bracelets	2048	208
Earrings	47919	1440

Quantification of samples taken from accessories in 7 different categories

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**DNA content in fingerprints**

Treatment	mtDNA/cm <sup>2</sup>	nDNA/cm <sup>2</sup>	PCR	HVII Sequence	
	Ninhydrin	200	0	-	-
	Ninhydrin	0	0	-	-
	Ninhydrin	50	0	+	+
	Ninhydrin	50	0	+	-
	Ninhydrin	700	20	+	+
	Ninhydrin	0	0	-	-
	Magnetic black powder	150	0	+	-
	Magnetic black powder	12700	150	+	+
	Magnetic black powder	30400	120	+	+
	Magnetic black powder	10000	40	+	+
	Magnetic black powder	1200	20	+	+
	Magnetic black powder	4500	30	+	+
	Magnetic black powder	3000	10	+	+
	Black powder	10000	80	+	+
	Black powder	6700	30	+	+
	Black powder	15000	50	+	-
	Black powder	71000	850	+	+
	Black powder	9200	10	+	+

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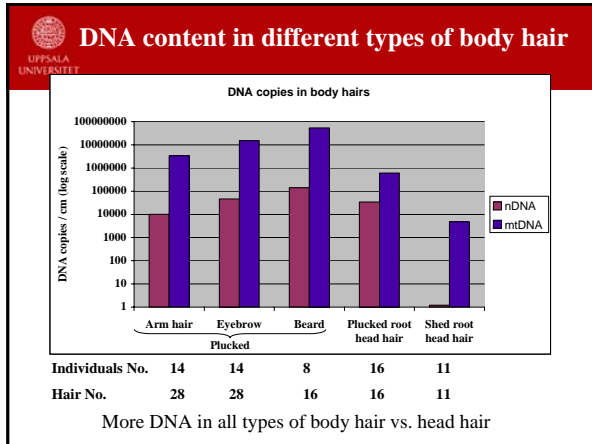
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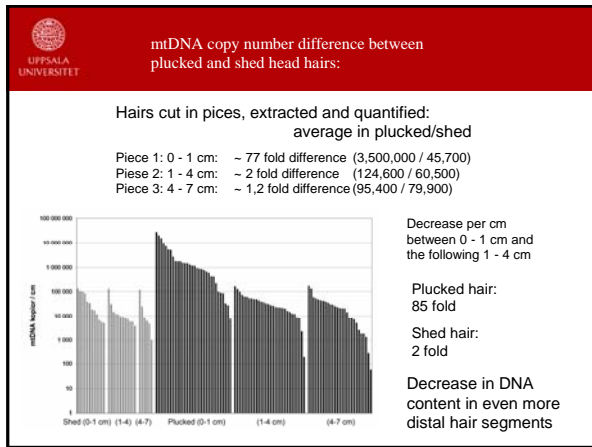
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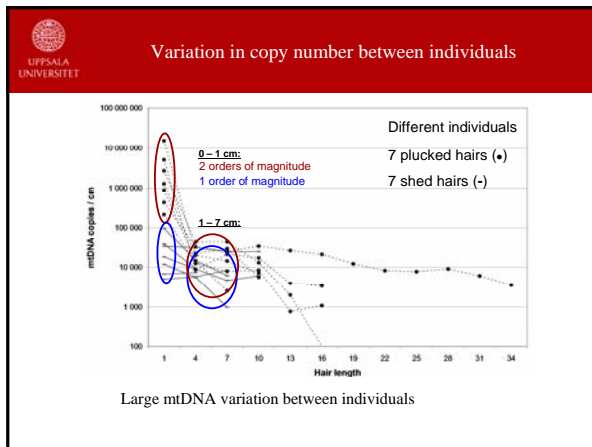
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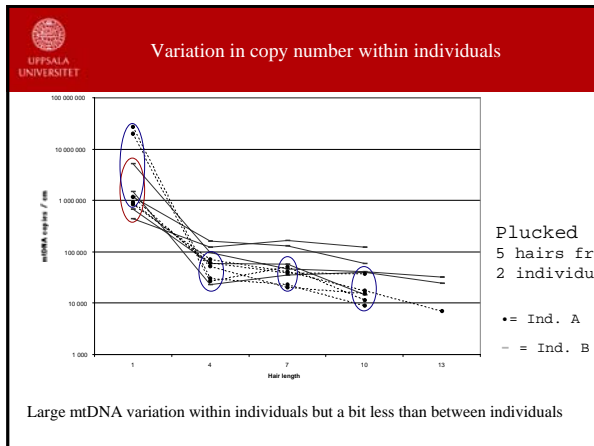
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**Summary of average DNA contents in the material evaluation study**

Material	N	Average mtDNA/cm
Plucked head hair, the root	13	1 200 000
Shed head hair, the root	13	63 000
Plucked head hair, 1-4 cm	9	19 000
Shed head hair, 1-4 cm	7	5 800
Plucked head hair, 7-10 cm	7	8 700
Shed head hair, 7-10 cm	2	3 700
Beard (plucked)	16	53 300 000
Eye Brow (plucked)	28	15 200 000
Arm hair (plucked)	28	3 400 000
		Average mtDNA/item
Earrings	2	4 700 000
Other accessories (6 categories)	14	130 000
		Average mtDNA/cm <sup>2</sup>
Finger prints (magnetic and carbon)	12	15 000

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**nDNA contents**

nDNA copy numbers (average):

- Plucked and shed head hair
  - 0 - 1 cm, the root part: Plucked: 25,800 / Shed: 0
  - 1 - 4 cm, 4 - 7 cm, 7 - 10 cm etc.: 0
- Body hairs (per hair)
  - Arm hair: 13,700
  - Eyebrow: 38,100
  - Beard: 78,000
- Fingerprints visualised using different technologies (per sample)
  - Magnetic black powder: 90
  - Black powder: 170
- Accessories (per sample)
  - Rings: 180
  - Watches: 1,800
  - Necklaces/Bracelets: 290
  - Glasses: 4,200
  - Charms Earrings: 80
  - Total: 144,400

*Note: No additives in this reaction*

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**Some conclusions:**

Large variation of DNA content in hairs  
 - between and within individuals

More mtDNA in plucked hairs than shed hairs  
 - largest DNA content difference observed in the first cm (0 - 1 cm)  
 - different growth phases of the hairs

Decrease in DNA content in more distal hair segments

Body hairs; beard contained most nDNA

Fingerprints; black powder treated prints contained slightly more mtDNA and nDNA compared to magnetic black powder

Accessories; large variation  
 - earrings contained the highest DNA amounts  
 - rings and charms contained the lowest DNA amounts

Nuclear and mitochondrial DNA quantification of various forensic materials  
 H. Andriantsoa<sup>1</sup>, M. Nilsson<sup>1,2</sup>, B. Budowle<sup>3</sup>, H. Larsson<sup>1</sup>, M. Alsterlund<sup>1</sup>

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**Evaluation of two Whole Genome Amplification methods**

WGA could increase DNA content in forensic samples

Genomiphi™ - Multiple Displacement Amplification using the phage 29 enzyme - kit

PEP - Primer Extension Pre-amplification method

- uses a random 15-mer primer extended by Taq polymerase

1000 - 30 genomic DNA copies/reaction

Genomiphi is more efficient in samples with lower DNA copies  
 - 2000 fold amplification

More efficient on nDNA

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**Comparison of Genomiphi and PEP**

Quantification of low DNA amounts after PEP and Genomiphi reactions

Genomiphi is 4-fold more efficient than PEP on nDNA  
 PEP is more efficient on mtDNA - a 400-fold increase in yield

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## CONCLUSIONS

- Highly sensitive
- Multiplex quantification - save material (2 µl sample used)
- Quick and easy method
- Estimation of optimal target/optimal extract volume to PCR
- Detection of inhibitors
- Evaluation of different forensic materials
- Evaluation of WGA strategies, DNA extraction protocols

**Further applications:**

- Optimization of different PCR protocols, purification procedures or to evaluate material storage conditions
- Evaluation of DNA degradation by quantifying targets of different lengths

Two other quant assays.....

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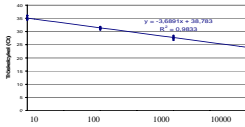
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## Simultaneous nDNA quantification and sex determination

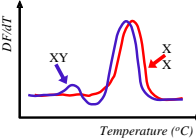
Real-time SYBR Green I assay  
DNA intercalating dye  
Fluorescent when bound to minor groove of dsDNA

Real-time PCR data  
- Standard curve / nDNA Quantification



Dissociation curve analysis for sex determination

Amelogenin gene 3 bp deletion  
70 bp product X chromosome  
73 bp product Y chromosome




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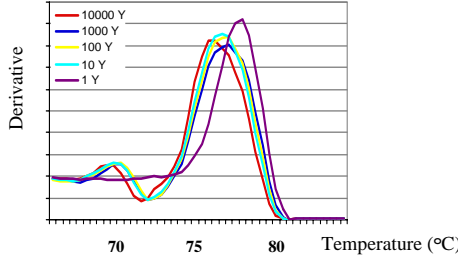
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## Limitation in sex determination



Limitation in sex determination < 10 nDNA copies

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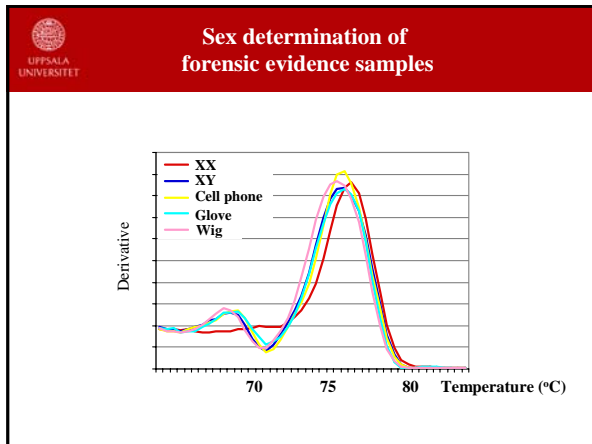
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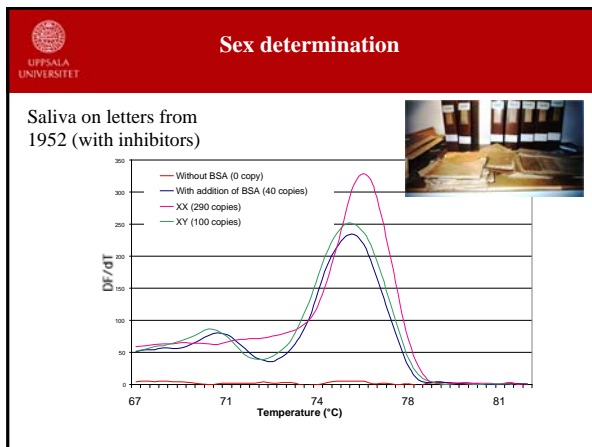
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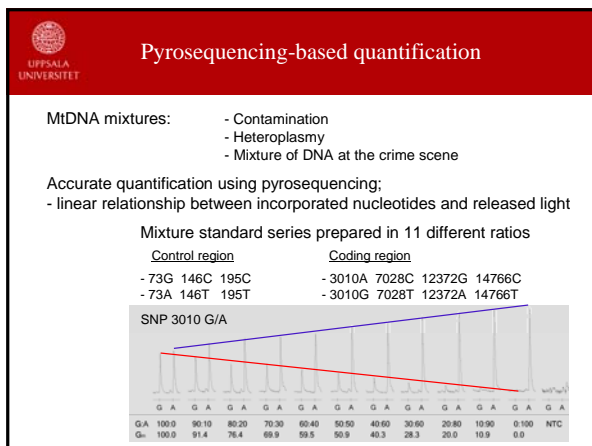
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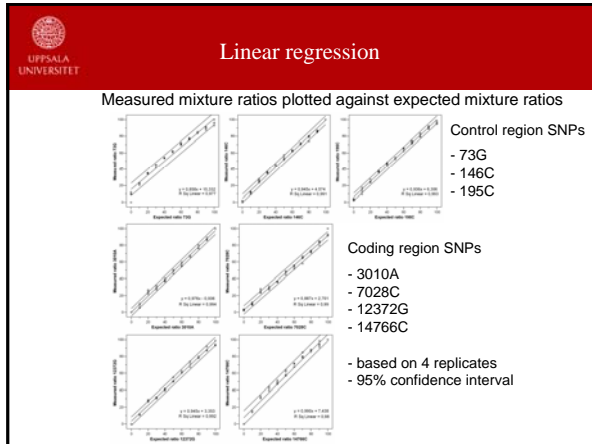
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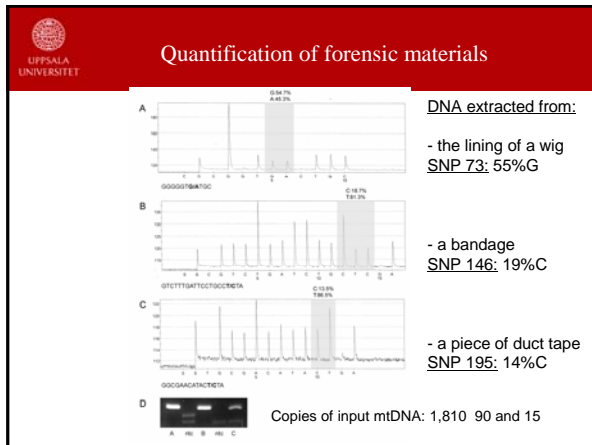
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**Acknowledgements**

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