



# Evaluation of DNA damage in biosamples

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# Conflict of interest

- Founder of Lifeind ehf. (BioCule) a startup company developing this technology in a microgel platform.

# Northern Lights Assay (NLA) and biosamples



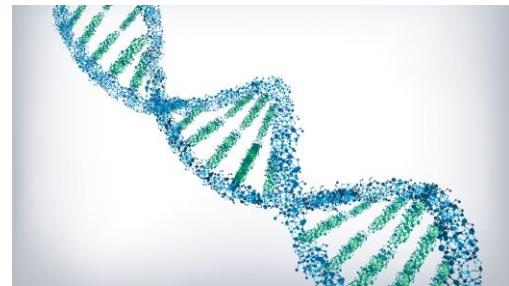
Guðmundsson *et al.*  
Nucleic Acid Research, 2018

Big surprise!

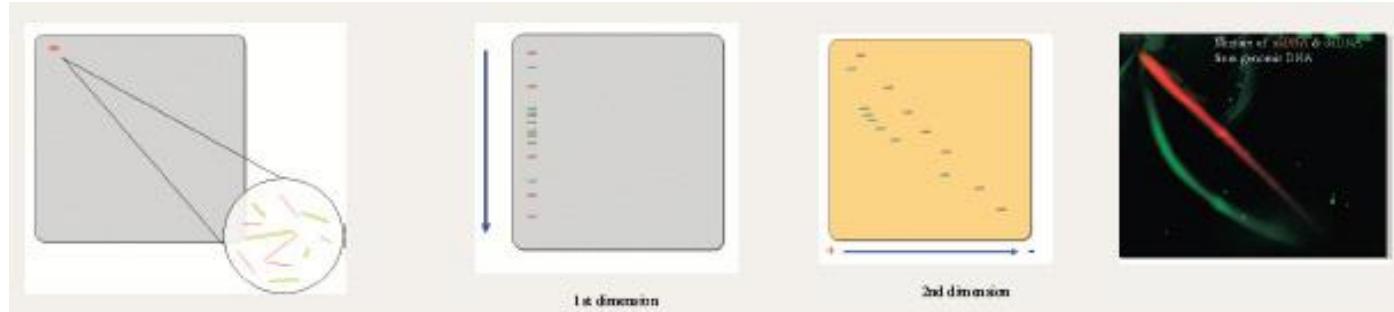
Scientists have managed to grow human vocal cords in tissue culture. The results speak for themselves!

# What do we want to know about DNA in biosamples?

- Base sequence
- Structure
- DNA damage:
  - Biological
  - Isolation
  - Storage
- Template for complex molecular procedures



## 2D Strandness-Dependent Electrophoresis (2D-SDE)

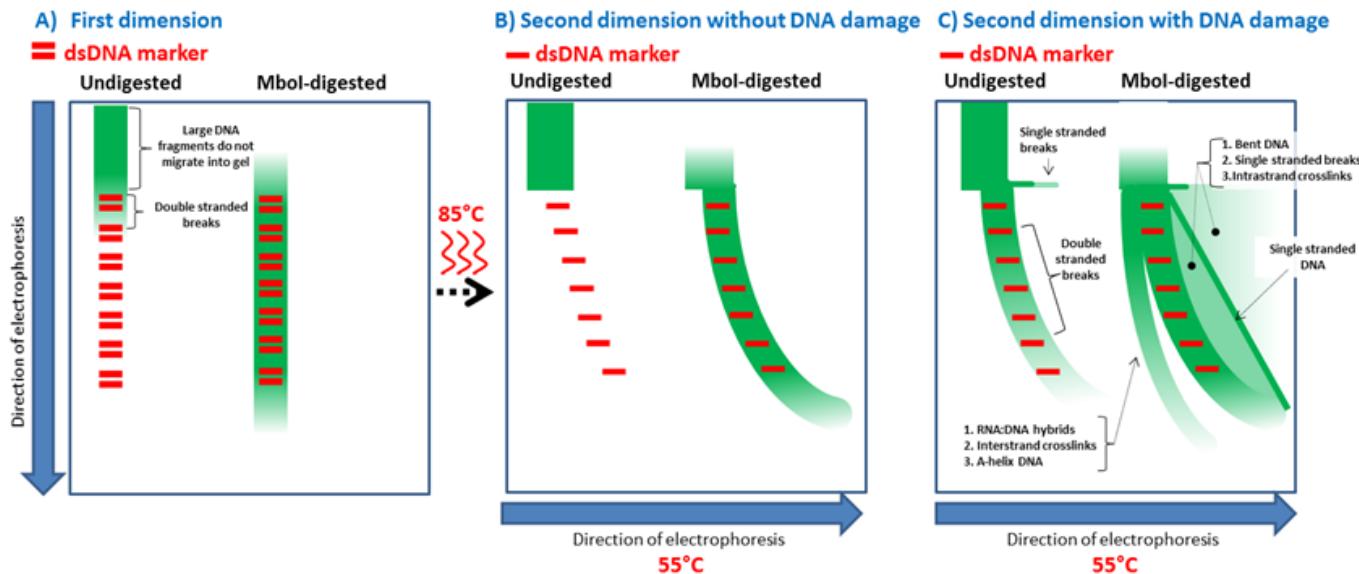


- In the first dimension nucleic acid fragments are separated according to length and strandness (double-stranded DNA, single-stranded DNA or DNA•RNA hybrids)
- In the second dimension fragments are separated only according to length
- After 2D separation, different arcs can be seen representing different strandness of the nucleic acids within the original sample

Gunnarsson *et al.* Analytical Biochemistry, 2006

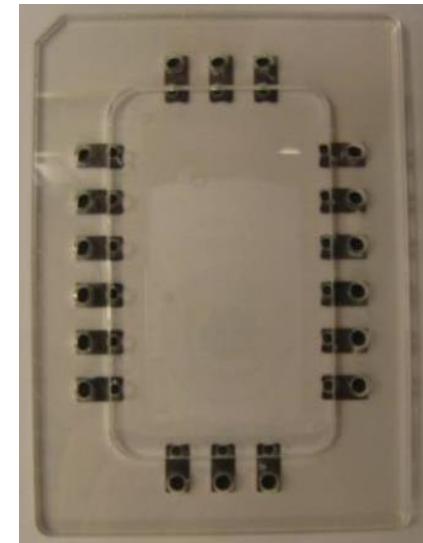
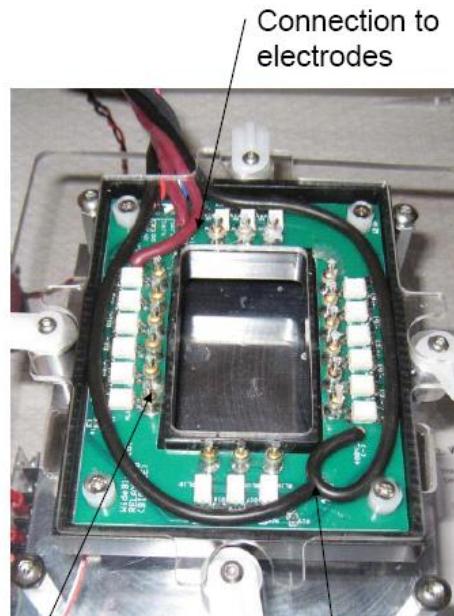
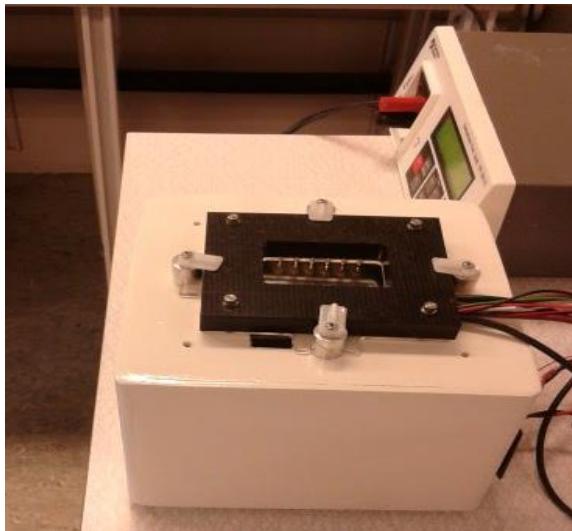
Gunnarsson *et al.* Nature Protocols, 2006

# Northern Lights Assay



A method to characterize complex DNA and RNA samples

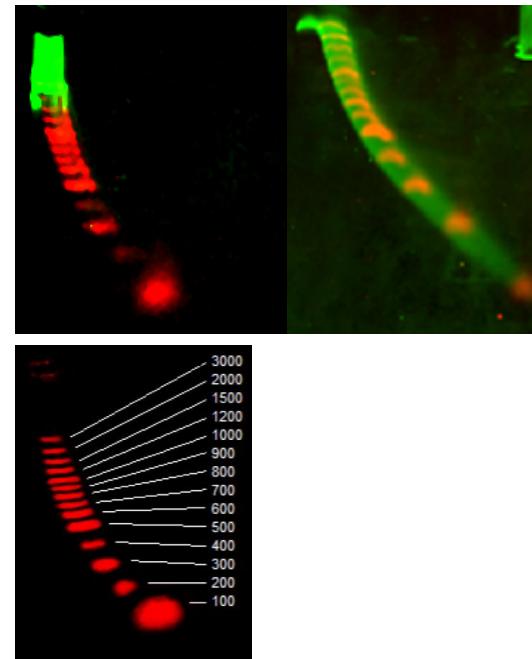
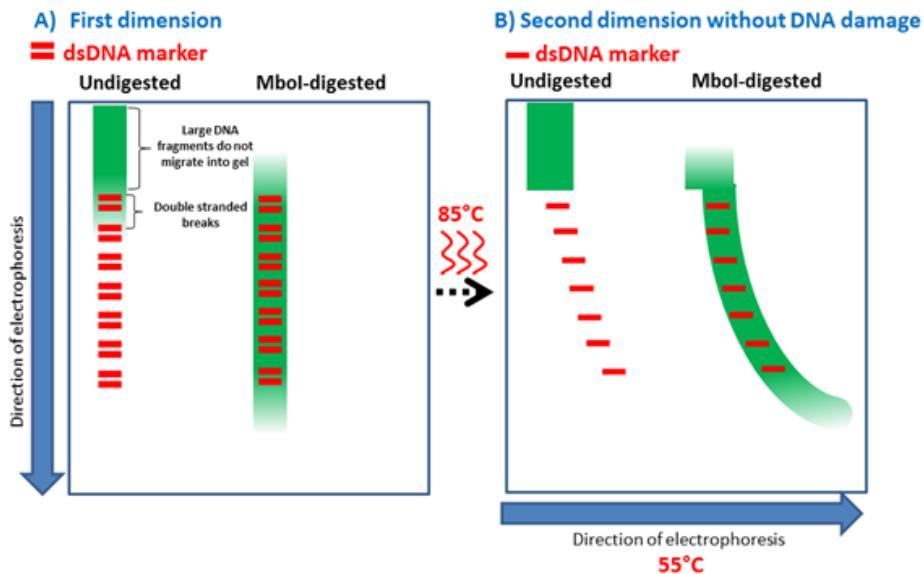
# Apparatus



- Analysis is quick (20 min.) and sensitive (30 ng)

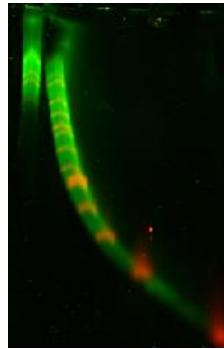
Thormar *et al.* *Biotechniques*, 2018

# Northern Lights Assay

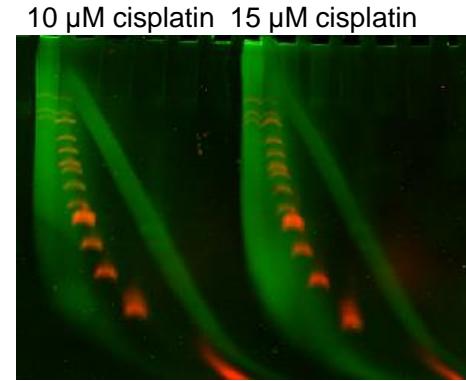
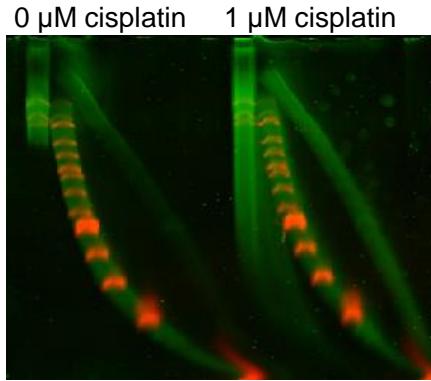


# Analysis of crosslinks induced by cisplatin in human genomic DNA

Original  
Untreated DNA

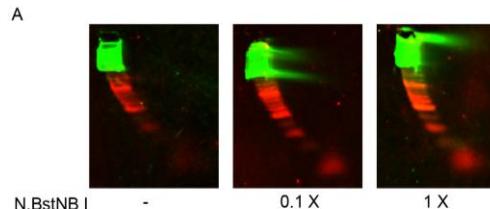


DNA samples treated with different concentrations of cisplatin for 18 hours at 37° C

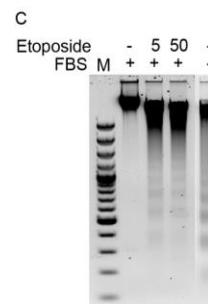
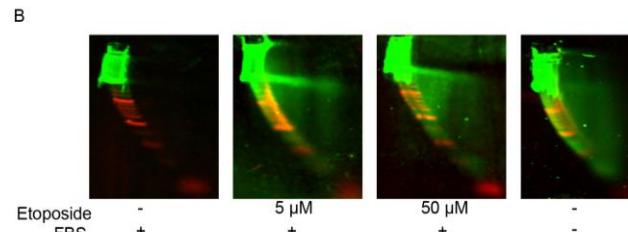


# Detection of single- and double-stranded DNA breaks

Human genomic DNA sample treated with nicking enzyme *N.BstNB I*

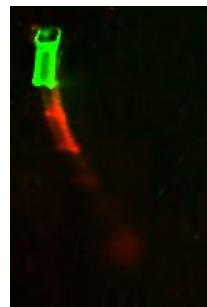


HUVEC cell cultures grown without FBS or treated with etoposide



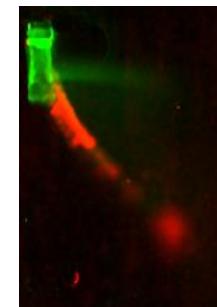
## Treatment of BRCA2<sup>-/-</sup> with a PARP inhibitor (olaparib)

MCF-7 BRCA<sup>+/+</sup>

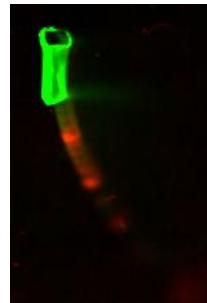


Untreated  
Control

CAPAN1 BRCA2<sup>-/-</sup>

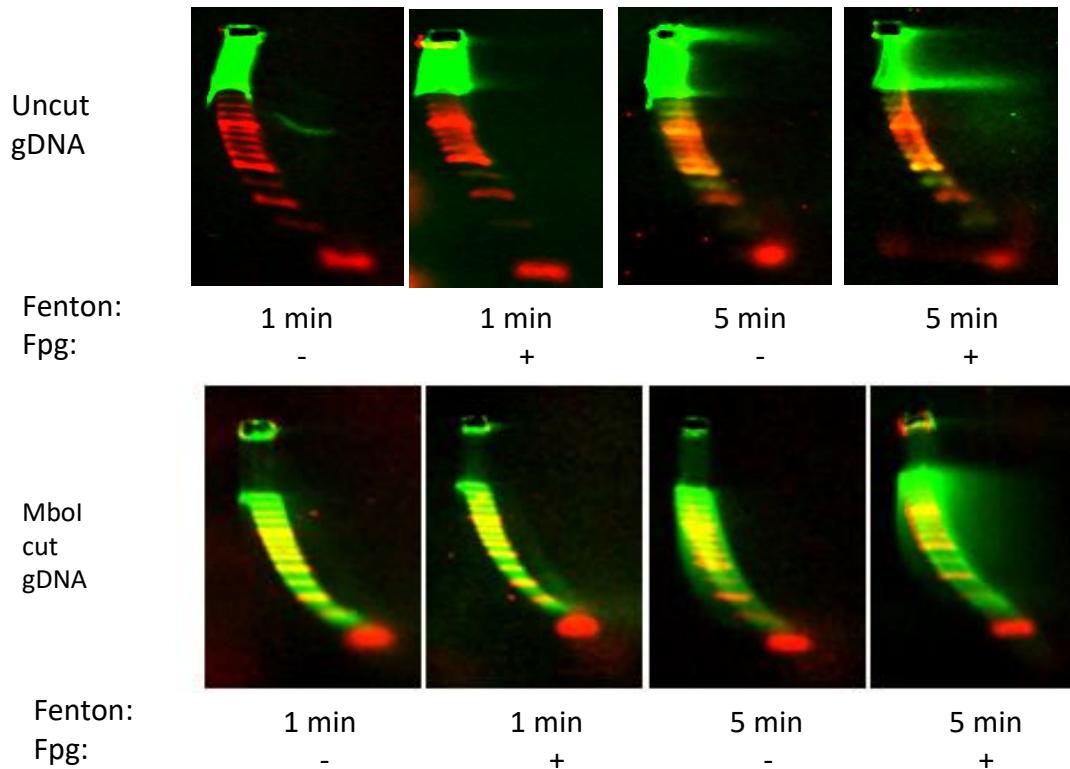


Olaparib  
50 µM  
48 h



Synthetic lethality

# Enzyme treatment to detect modified bases

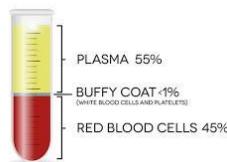
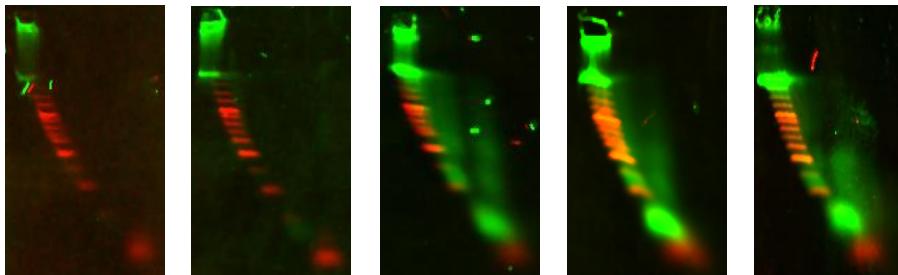


Example: Treatment with Fpg enhances detection of oxidative lesions.

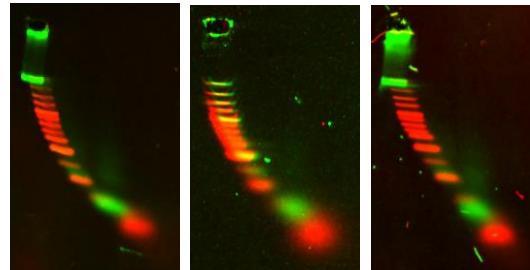
Fpg is a glycosylase for oxidized bases and AP-lyase.

ICL and single-stranded breaks

## Northern Lights Assay of cfDNA in plasma in healthy subjects



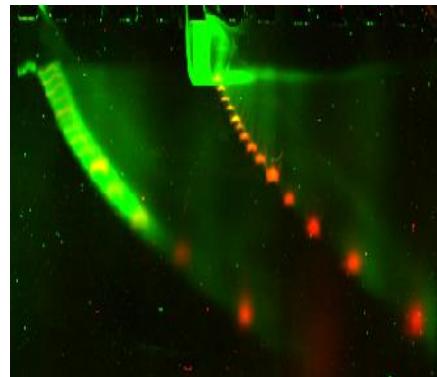
NLA of cfDNA in plasma in patients with severe leukopenia



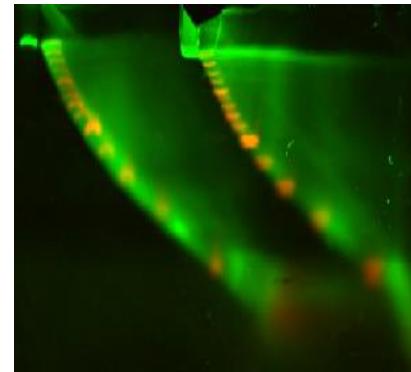
## DNA in saliva is heavily damaged



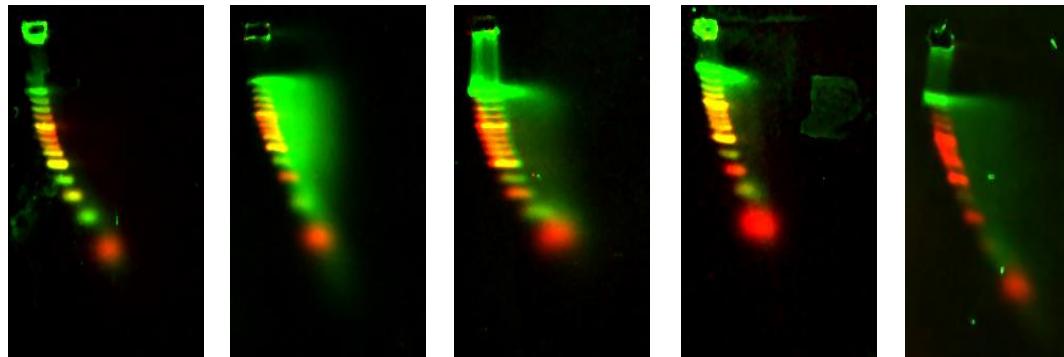
Healthy control



Patient with Sjögren disease

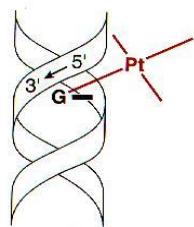


## NLA of cfDNA from urine of healthy subjects

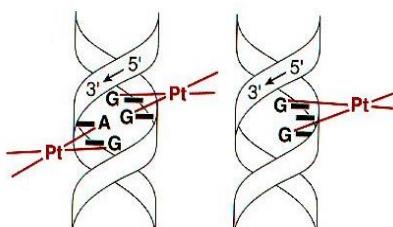


Variability in amount of apoptosis fragments as well as in double-stranded and single-stranded breaks, non-specific degradation and denaturation.

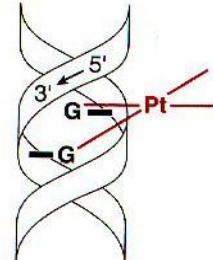
# Interstrand crosslinks in urinary sediment cells after treatment with crosslinking medication



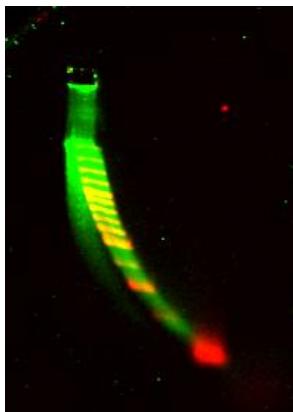
Monoadduct



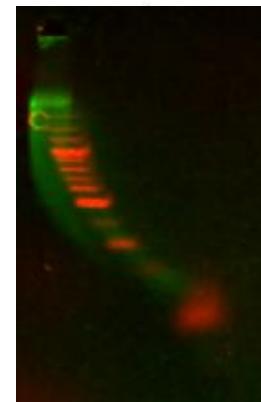
Intrastrand cross-link



Interstrand cross-link



Oxaliplatin



Carboplatin

## Efficiency of molecular procedures



# Optimization of complex PCR

- 1D-agorse electrophoresis looked good
- 2D-SDE revealed accumulation of single-stranded DNA
- Optimization resulted in expected double-stranded products

1D-Agarose



2D-SDE



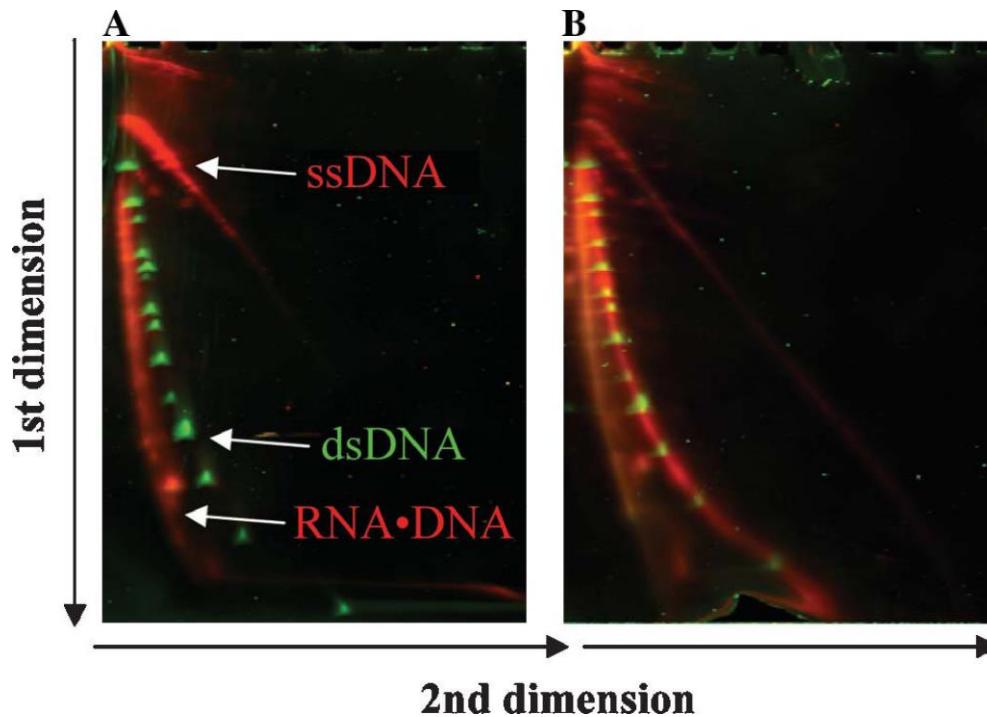
1D-Agarose



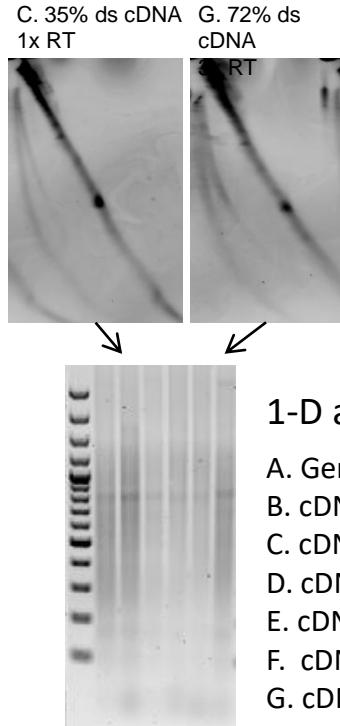
2D-SDE



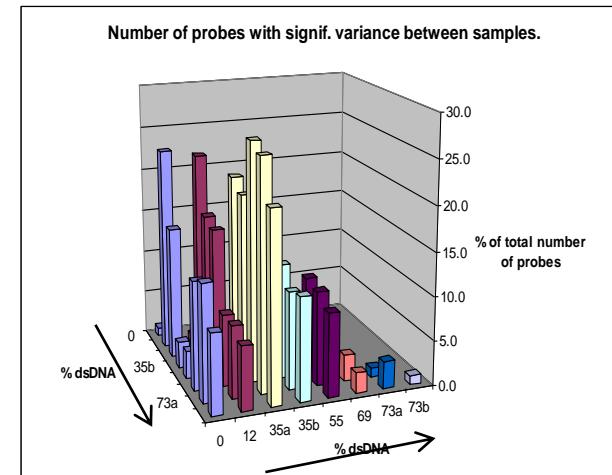
# Monitoring efficiency of cDNA synthesis



# Quality of cDNA synthesis

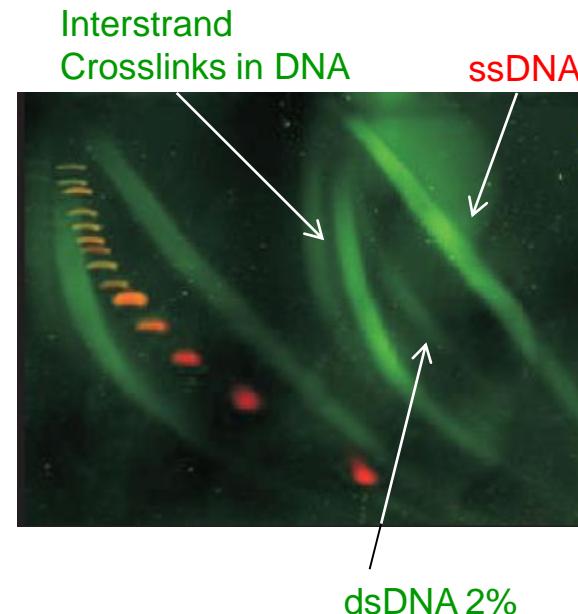
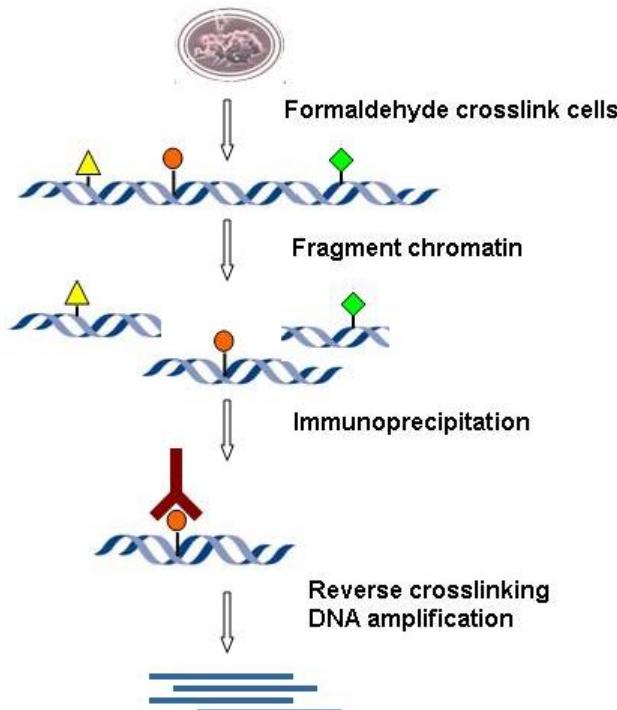


Probe variance is significantly lower with increased amount of dsDNA

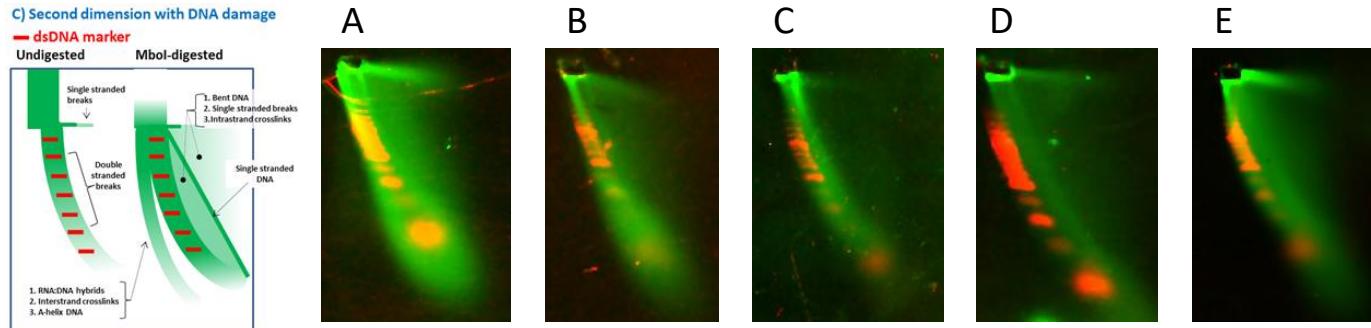


Thormar *et al.* Clinical Chemistry, 2013

# CHIP-Seq samples analyzed with NLA



# DNA in FFPE samples and sequencing



Fold coverage	No reads	194X	1231X	2245X	3278X
Amplicons missing	All	38/212	8/212	5/212	5/212

# In conclusion the Northern Lights Assay can detect:

- Interstrand and intrastrand crosslinks/adducts
- Single-stranded breaks (nicks) and double-stranded breaks
- Various lesions causing bending in DNA
- Damage causing denaturation
- Oxidative lesions (pretreatment with fpg)

In summary most lesions in DNA

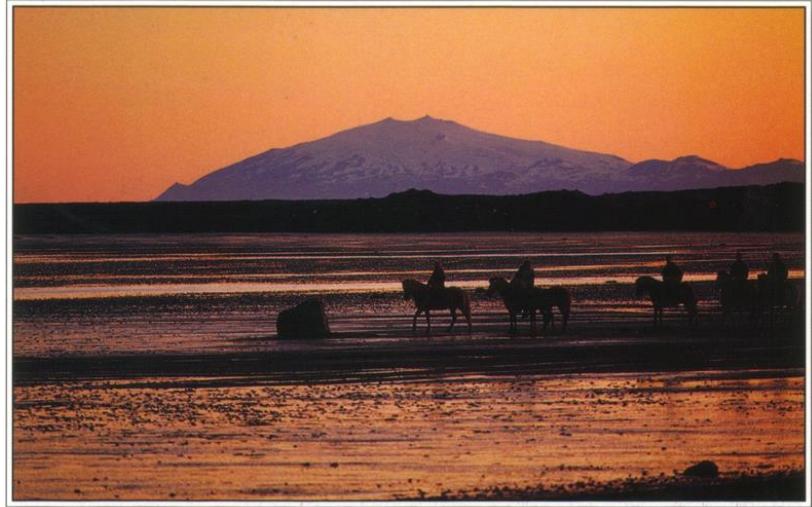
Detecting DNA damage in biosamples:

Biological and response to therapy

Storage

Isolation

Template and products of complex procedures



# Thank you

- Acknowledgements:
  - Hans G. Thormar
  - Bjarki Guðmundsson
  - Guðmundur Heiðar Gunnarsson
  - Elsa Jónsdóttir
  - And many others
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