



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)

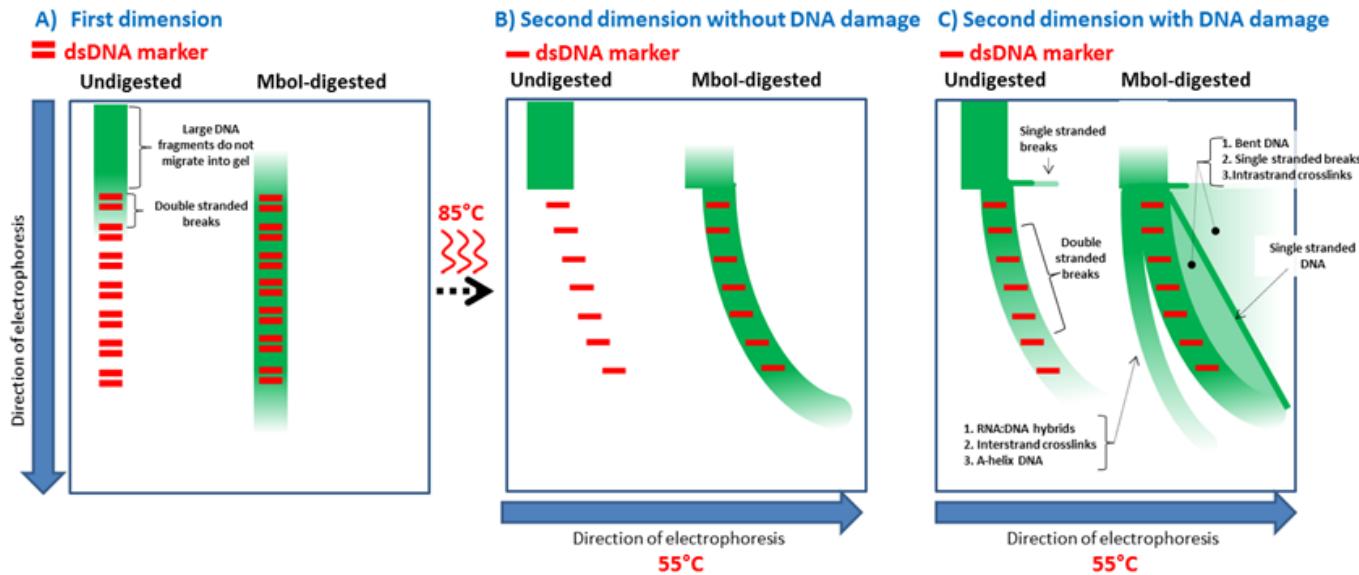


- In the first dimension nucleic acid fragments are separated according to length and strandness (double-stranded DNA, single-stranded DNA or DNA•RNA hybrids)
- In the second dimension fragments are separated only according to length
- After 2D separation, different arcs can be seen representing different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

Gunnarsson *et al.* Analytical Biochemistry, 2006

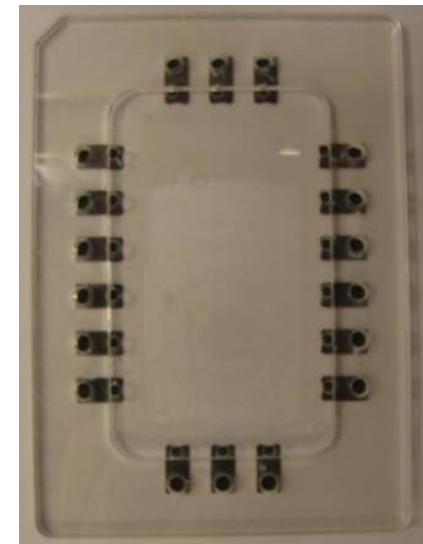
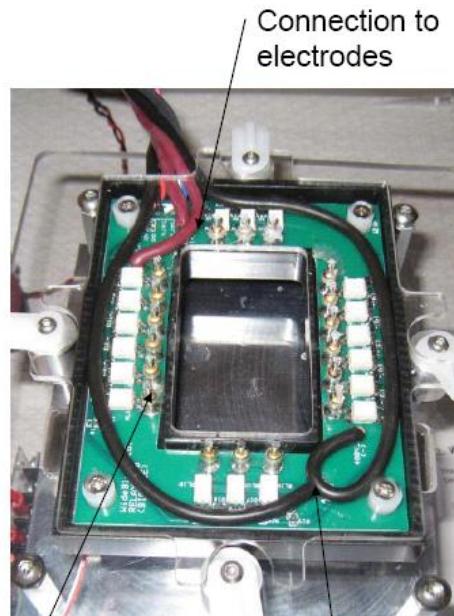
Gunnarsson *et al.* Nature Protocols, 2006

Northern Lights Assay



A method to characterize complex DNA and RNA samples

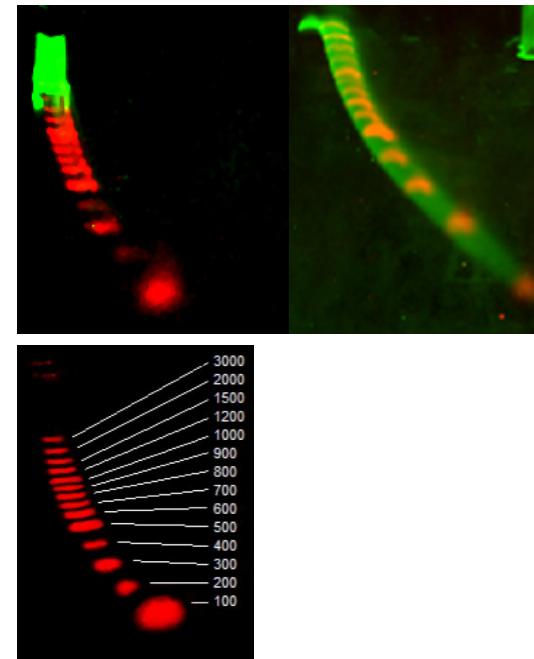
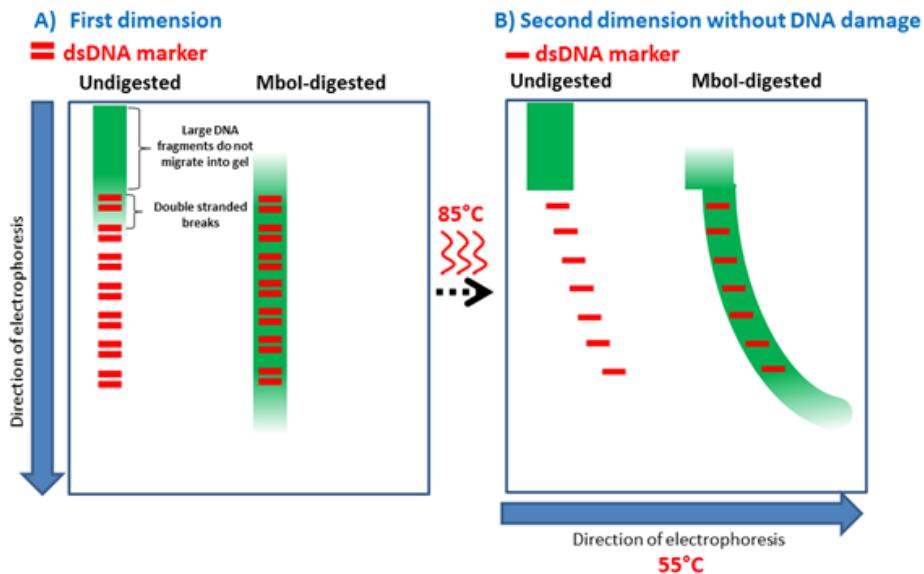
Apparatus



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

Thormar *et al.* *Biotechniques*, 2018

Northern Lights Assay

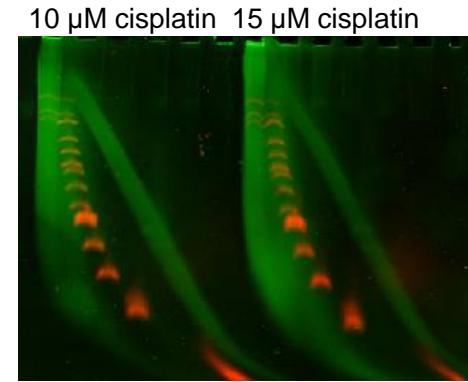
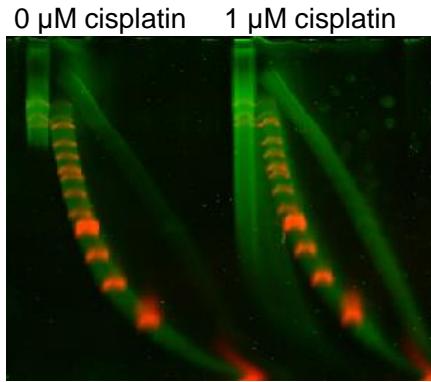


Analysis of crosslinks induced by cisplatin in human genomic DNA

Original
Untreated DNA

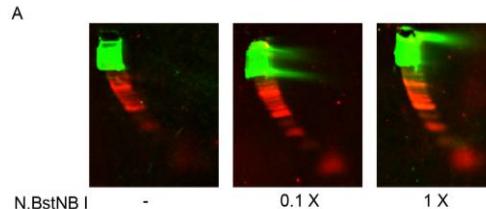


DNA samples treated with different concentrations of cisplatin for 18 hours at 37° C

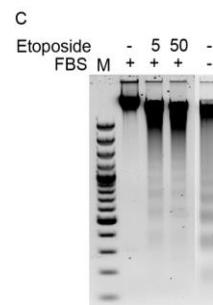
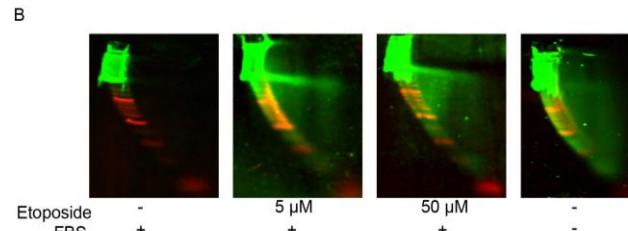


Detection of single- and double-stranded DNA breaks

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

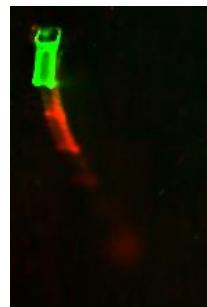


HUVEC cell cultures grown without FBS or treated with etoposide



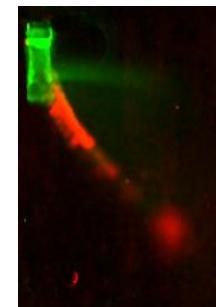
Treatment of BRCA2^{-/-} with a PARP inhibitor (olaparib)

MCF-7 BRCA^{+/+}

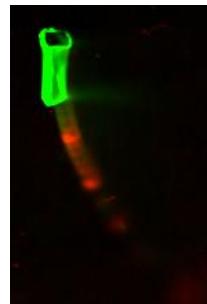


Untreated
Control

CAPAN1 BRCA2^{-/-}

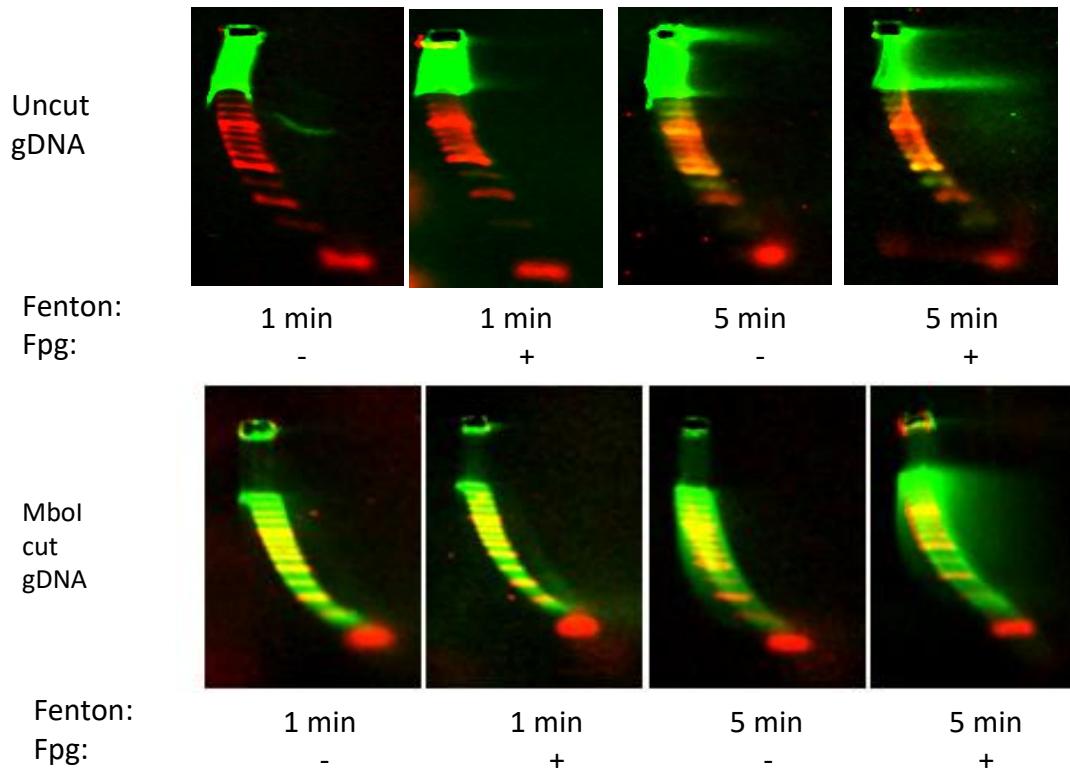


Olaparib
50 µM
48 h



Synthetic lethality

Enzyme treatment to detect modified bases

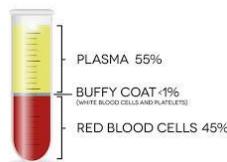
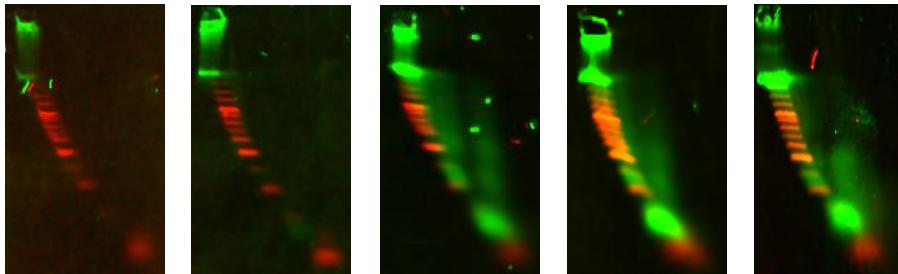


Example: Treatment with Fpg enhances detection of oxidative lesions.

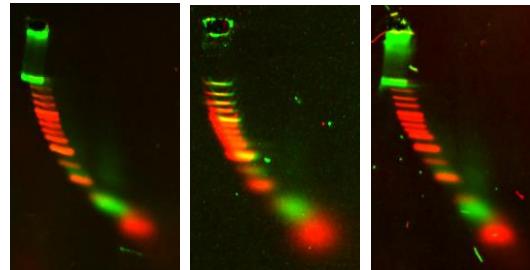
Fpg is a glycosylase for oxidized bases and AP-lyase.

ICL and single-stranded breaks

Northern Lights Assay of cfDNA in plasma in healthy subjects



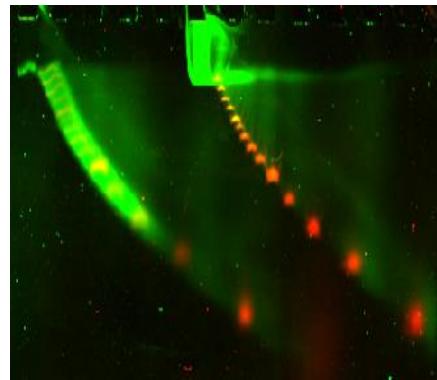
NLA of cfDNA in plasma in patients with severe leukopenia



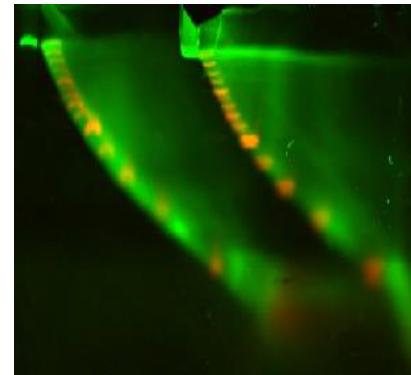
DNA in saliva is heavily damaged



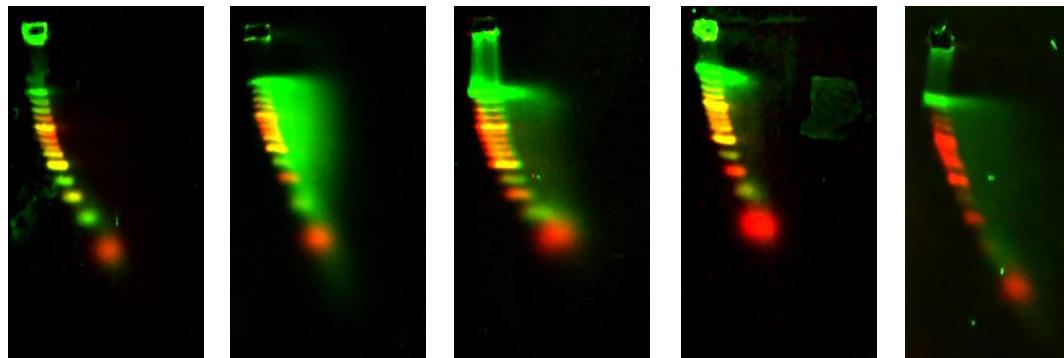
Healthy control



Patient with Sjögren disease

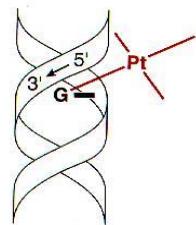


NLA of cfDNA from urine of healthy subjects

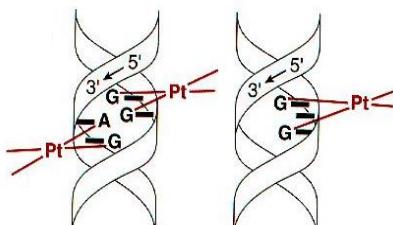


Variability in amount of apoptosis fragments as well as in double-stranded and single-stranded breaks, non-specific degradation and denaturation.

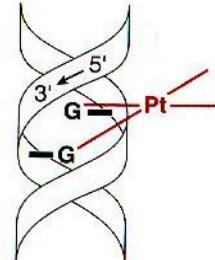
Interstrand crosslinks in urinary sediment cells after treatment with crosslinking medication



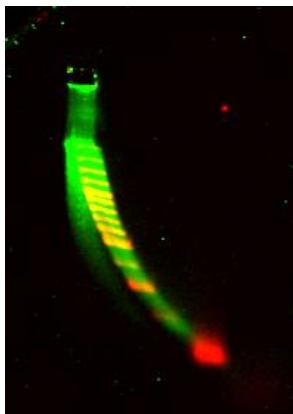
Monoadduct



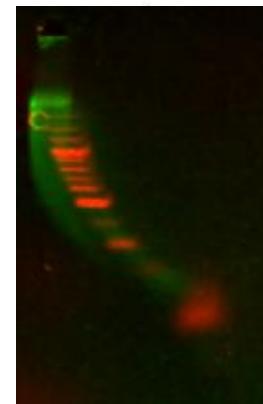
Intrastrand cross-link



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



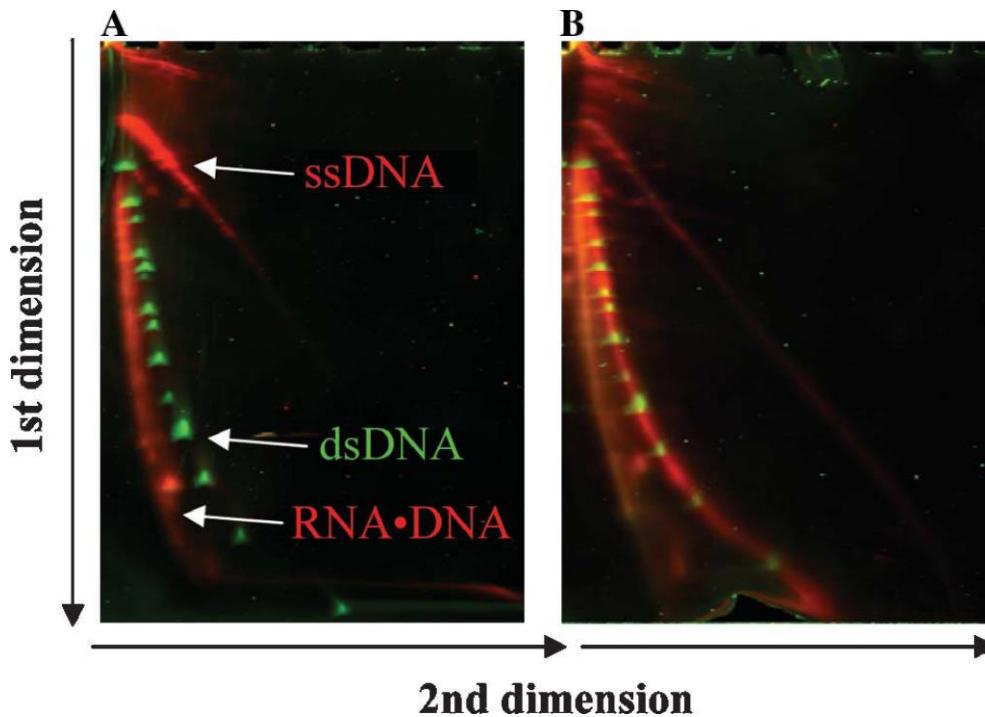
1D-Agarose



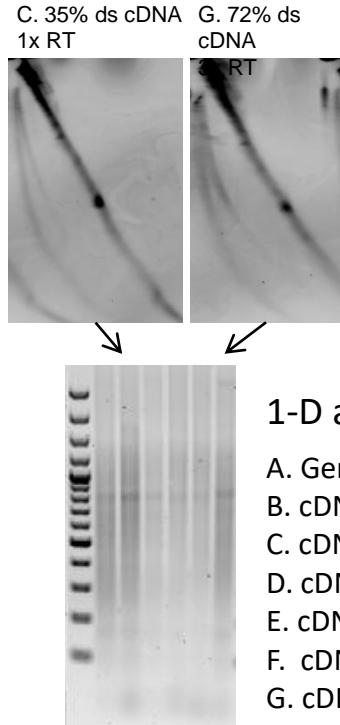
2D-SDE



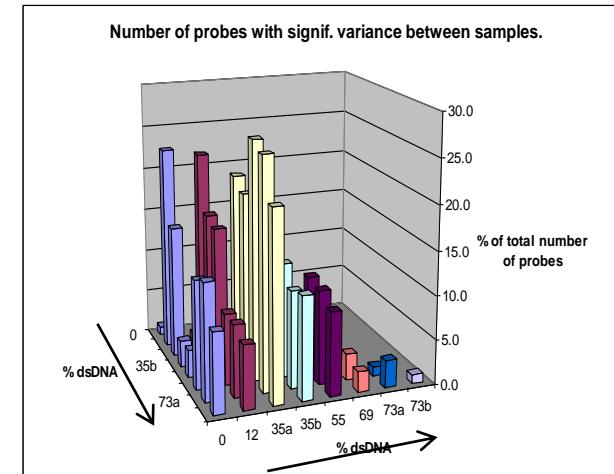
Monitoring efficiency of cDNA synthesis



Quality of cDNA synthesis

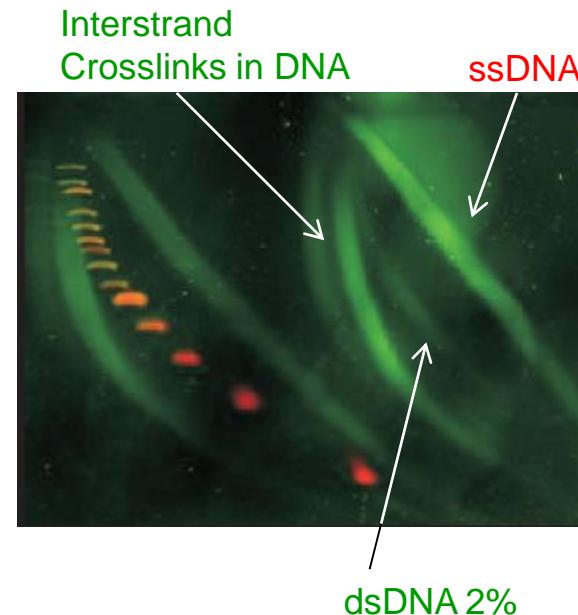
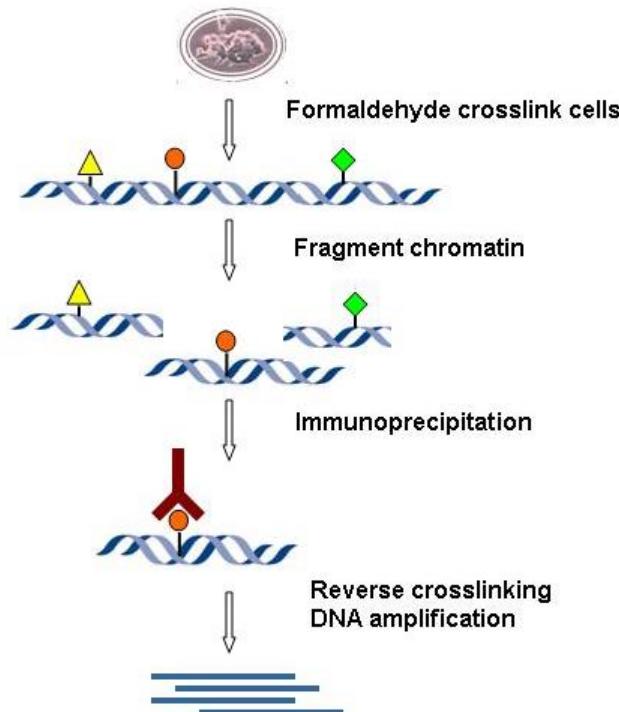


Probe variance is significantly lower with increased amount of dsDNA

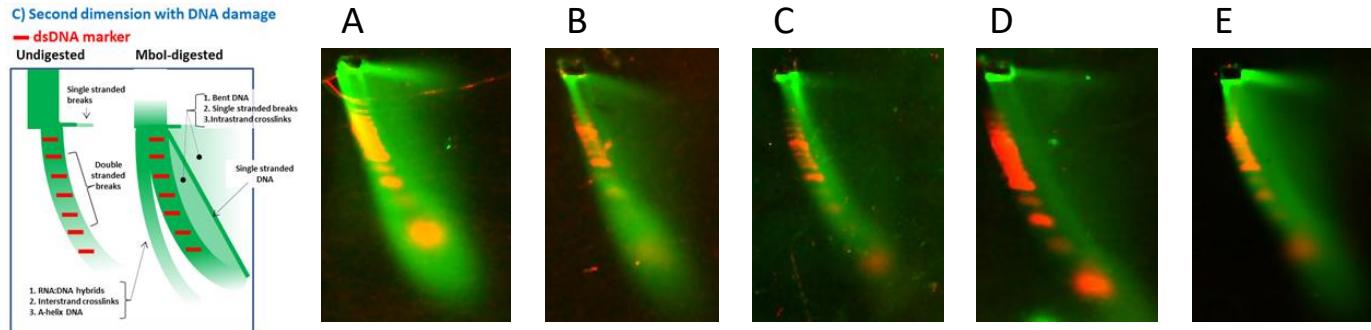


Thormar *et al.* Clinical Chemistry, 2013

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

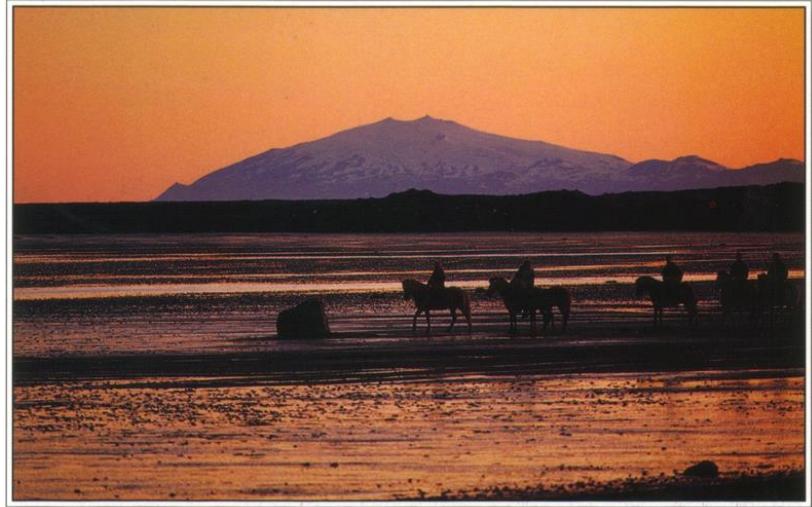
Detecting DNA damage in biosamples:

Biological and response to therapy

Storage

Isolation

Template and products of complex procedures



Thank you

- Acknowledgements:
 - Hans G. Thormar
 - Bjarki Guðmundsson
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