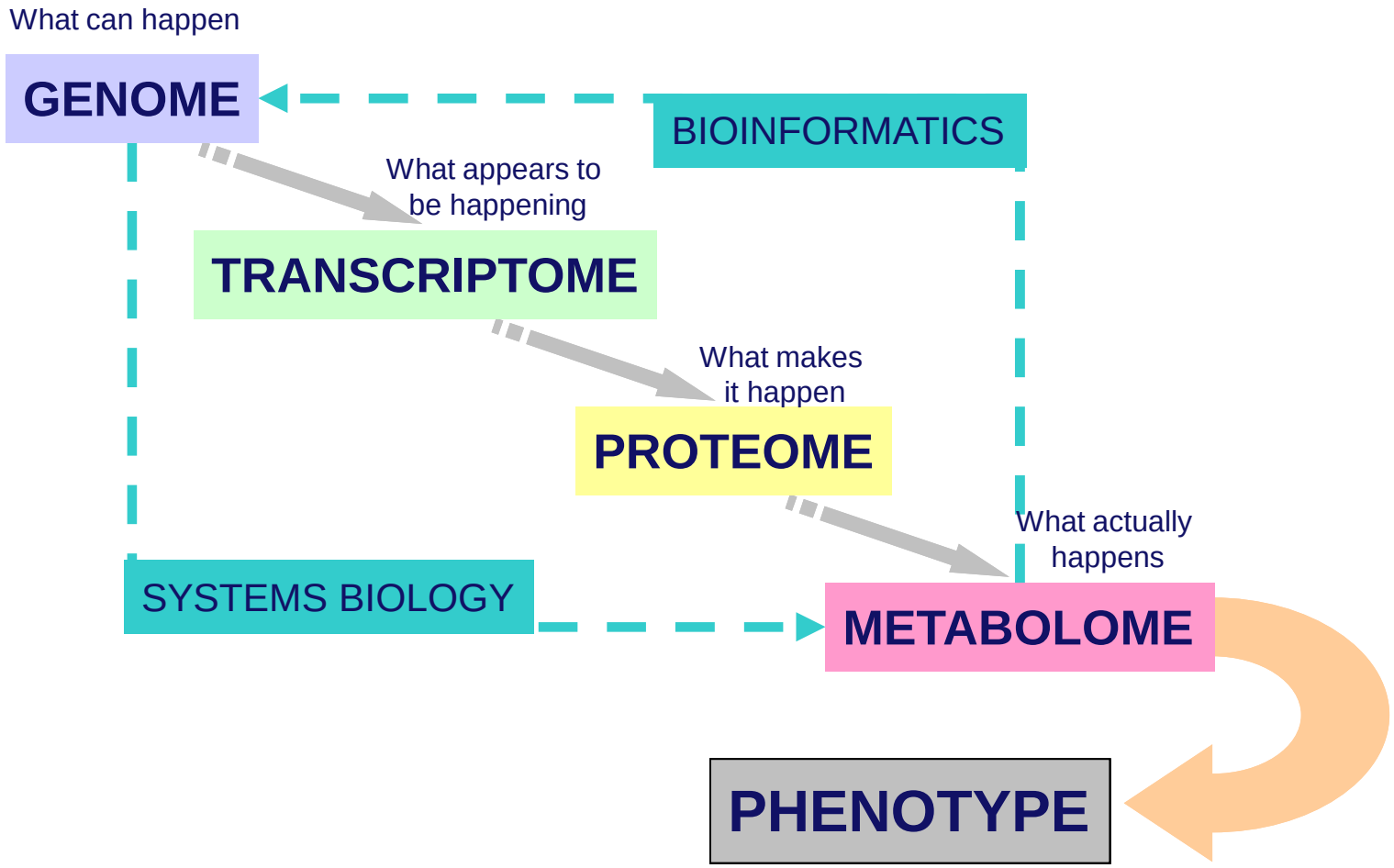
LEIDEN UNIVERSITY MEDICAL CENTER

Molekuláris vizsgálatok paraffinos szövetmintából: korlátok és lehetőségek

Group of Molecular Cytogenetics and Cellular Oncology

Károly Szuhai, M.D., Ph.D

The OMICs



Adapted from: Ahn et al., PLoS Med, 2006.

Biomarker Discovery and Validation

GENOME
DNA

- **Specific tests**
  - Mutation detection
  - Clonality test
  - FISH (cell based)
  - Pathogen detection
- **Genome-wide screening**
  - Array-CGH/SNP-array
  - Whole genome sequencing

TRANSCRIPTOME
RNA

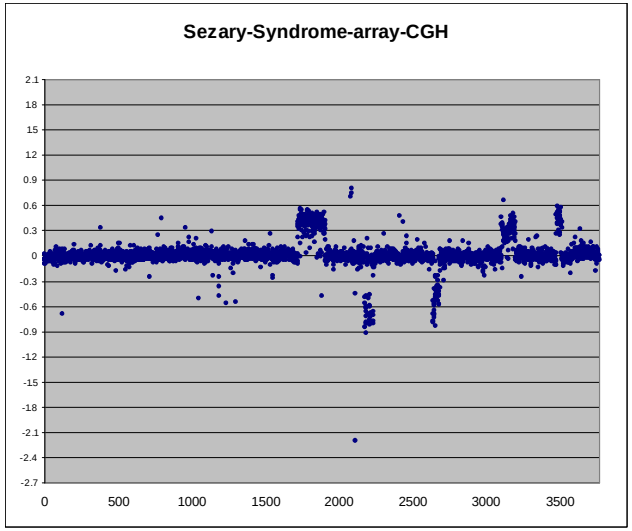
- **Specific tests**
  - Chimera detection
  - Mutation detection
  - MRD
- **Transcriptome-wide**
  - Expression profiling
    - *Array-chip*
  - Next generation sequencing
    - *Expression*
    - *Mutation*
    - *Splice variants*

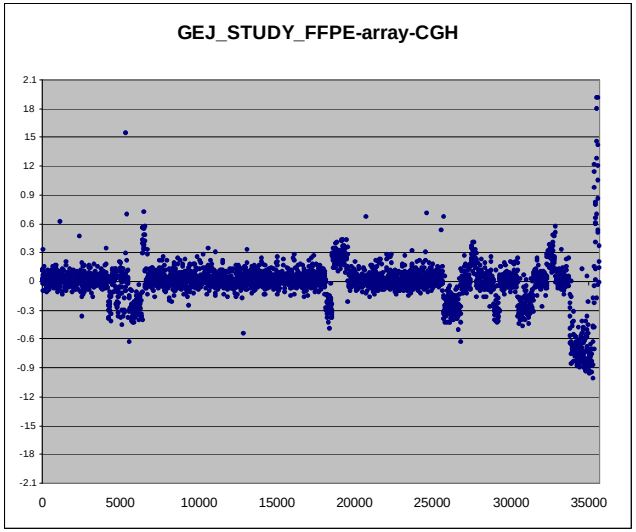
PROTEOME
Proteins

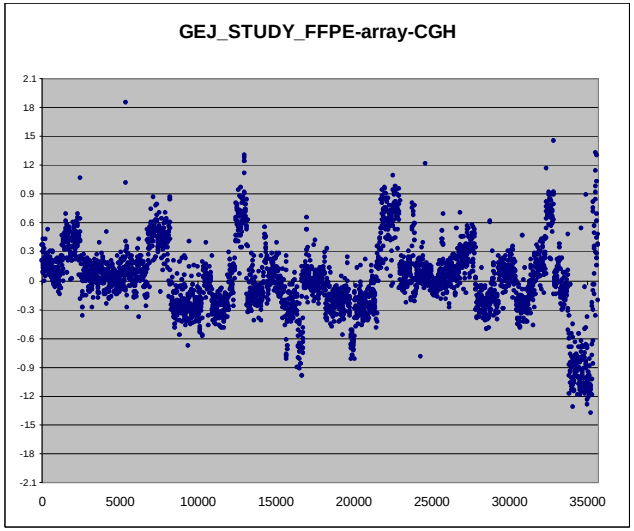
- **Specific tests**
  - Immunohistochemistry
- **Proteome-wide**
  - MALDI
  - MALDI-TOF, …
  - Functional screening
    - *kinome profiling*
  - TMA
    - *research*
    - *discovery tool*

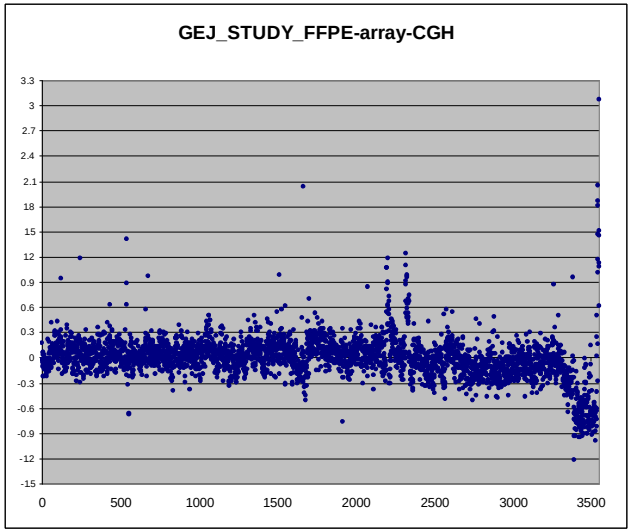
Personalized medicine

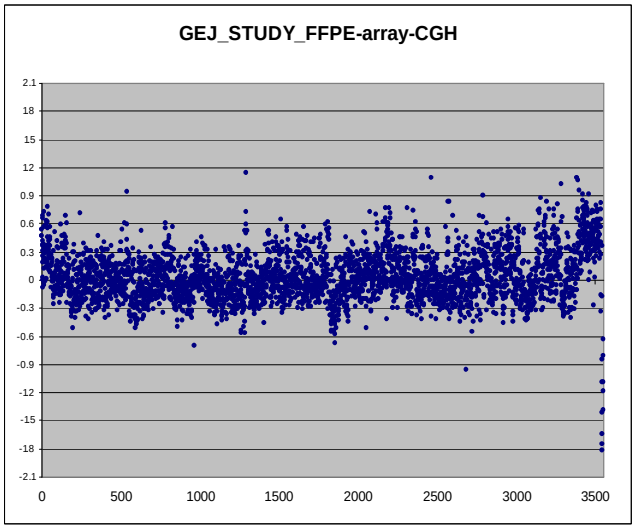
FFPE-DNA & array-CGH I

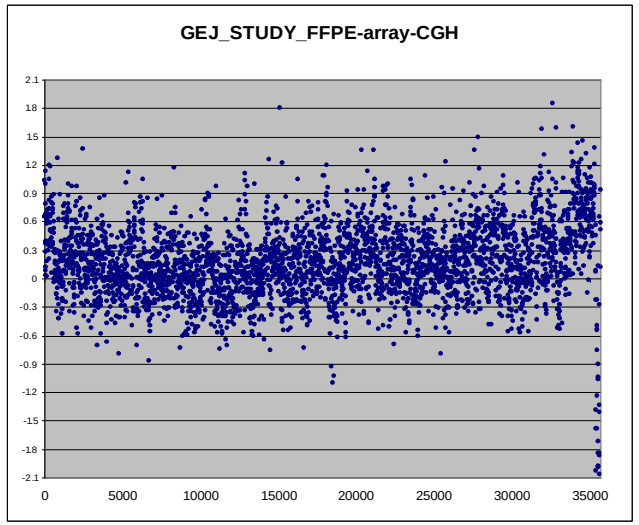












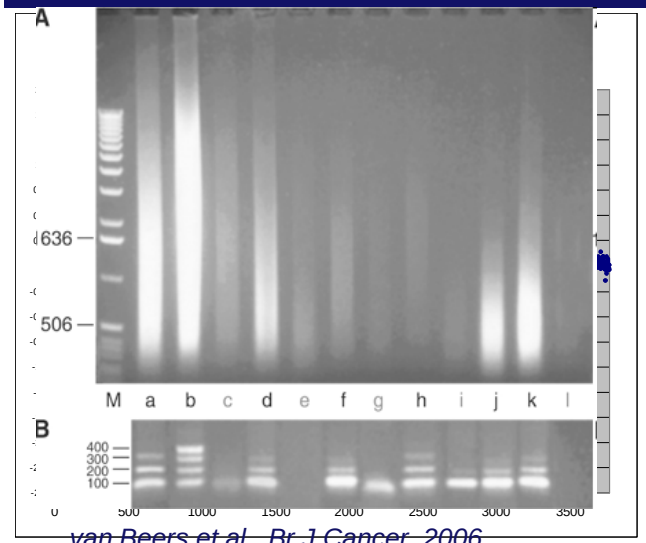
van Dekken et al., Genes Chromosomes Cancer, 2006

Knijnenburg et al., Cytometry, 2007, Oosting et al., Gen Res, 2007

Vermeer et al., Cancer Res, 2008

33 out of 43 FFPE-GEJ tumor samples informative

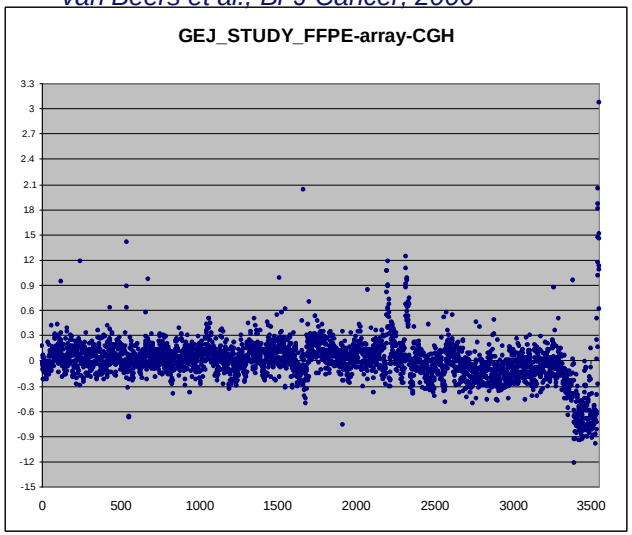
FFPE-DNA & array-CGH I

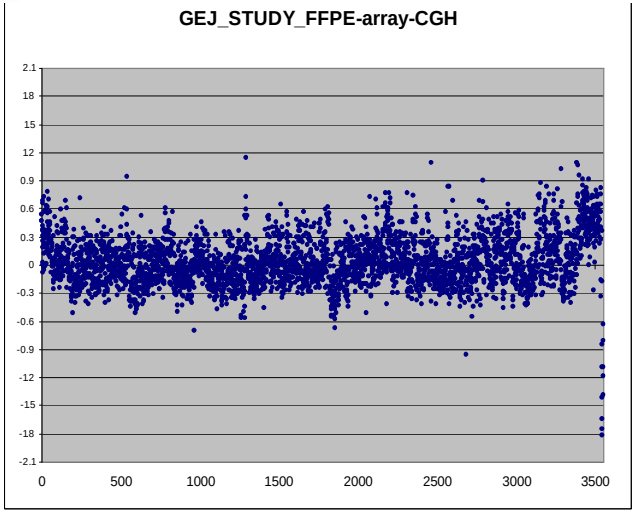


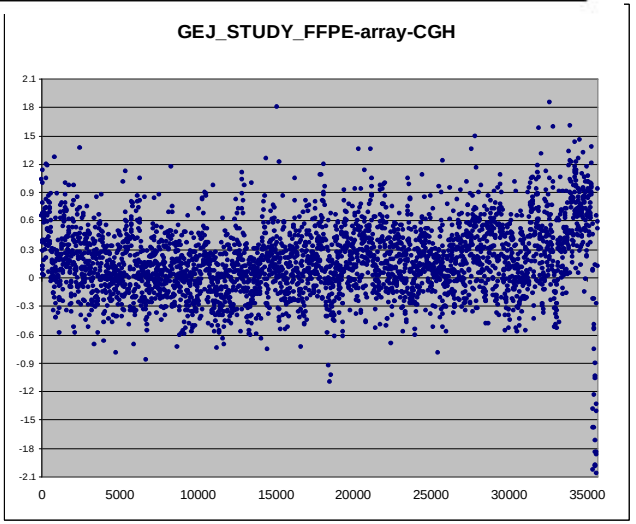
van Beers et al., Br J Cancer, 2006

(B) Prospective correlation of 93 breast tumour FFPE DNA samples aCGH success with performance of their prior multiplex PCR

Largest product in multiplex PCR	Success (%)	Good aCGH	Failed aCGH	Not done	N
400 bp (100%)	100	2	0		2
300 bp	100	5	0		5
200 bp	97	38	1		39
100 bp	16	6	31		37
No product	ND	0	0	10	10
Totals		51	32	10	93







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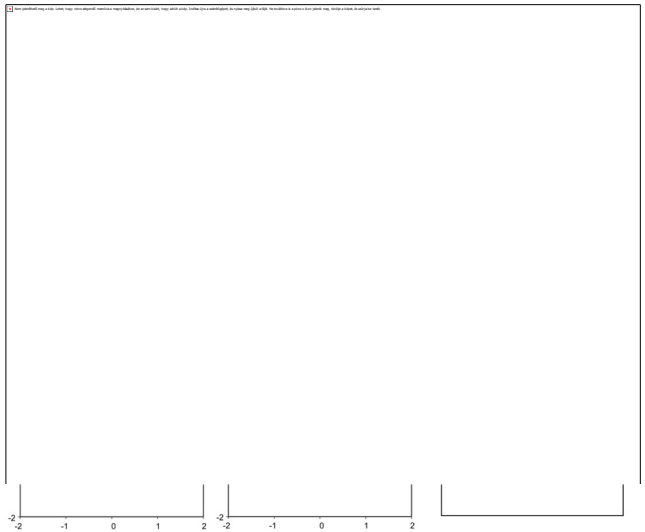
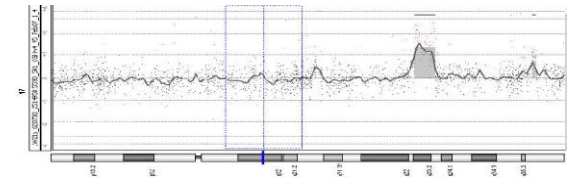
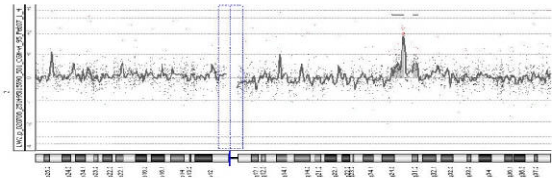
FFPE-DNA & array-CGH II



Pearson, Bland-Altman correlation

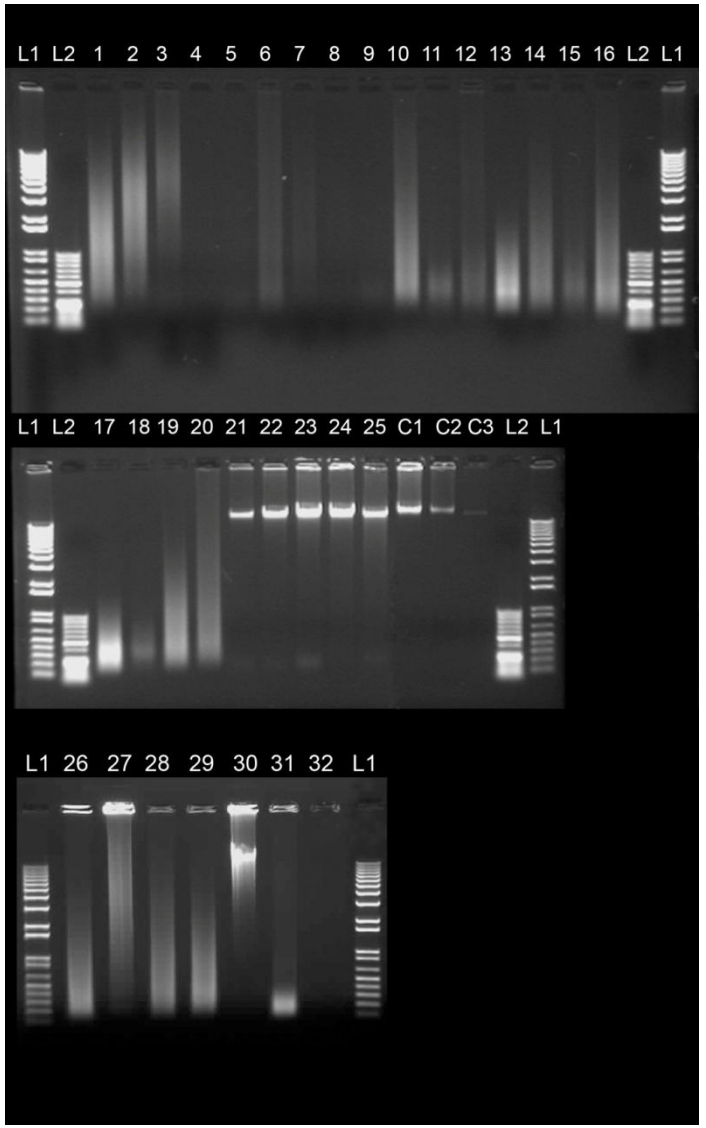




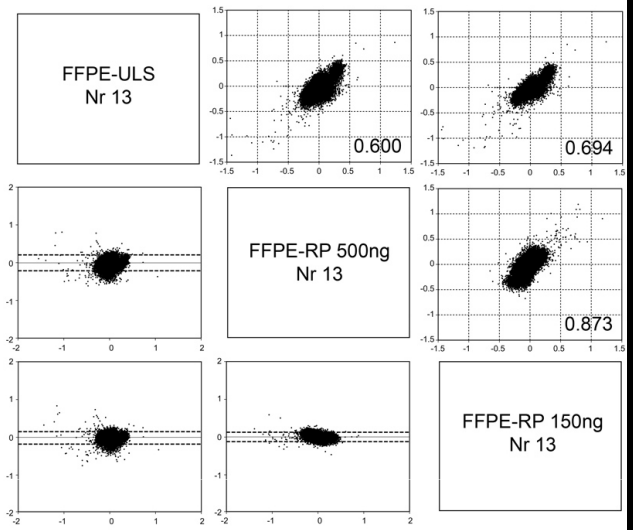
de Jong et al., submitted

Verbeke et al., submitted

- Rare chondrosarcoma
  - Dediff., clear cell, mesench.
- Primary angiosarcoma of bone cases >10 EuroBoNet institutions
- Decalcified samples
  - EDTA
  - formic acid
- 200 ng DNA loaded per sample (Nanodrop)
- Agilent 4x44k oligo array
- FFPE chemical labeling kit from Agilent/Kreatech
  - 500ng DNA
- FFPE RP kit (Invitrogen)
  - 500 and 150 ng



FFPE-DNA & array-CGH II



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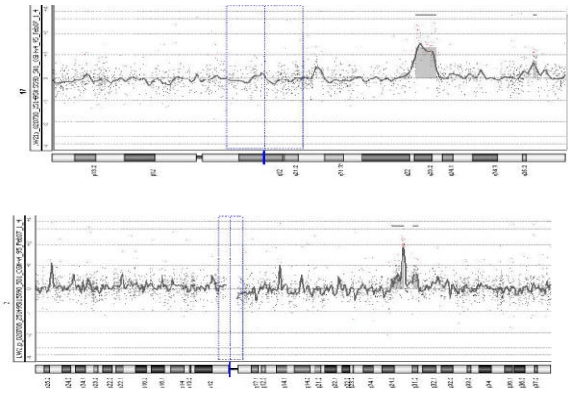
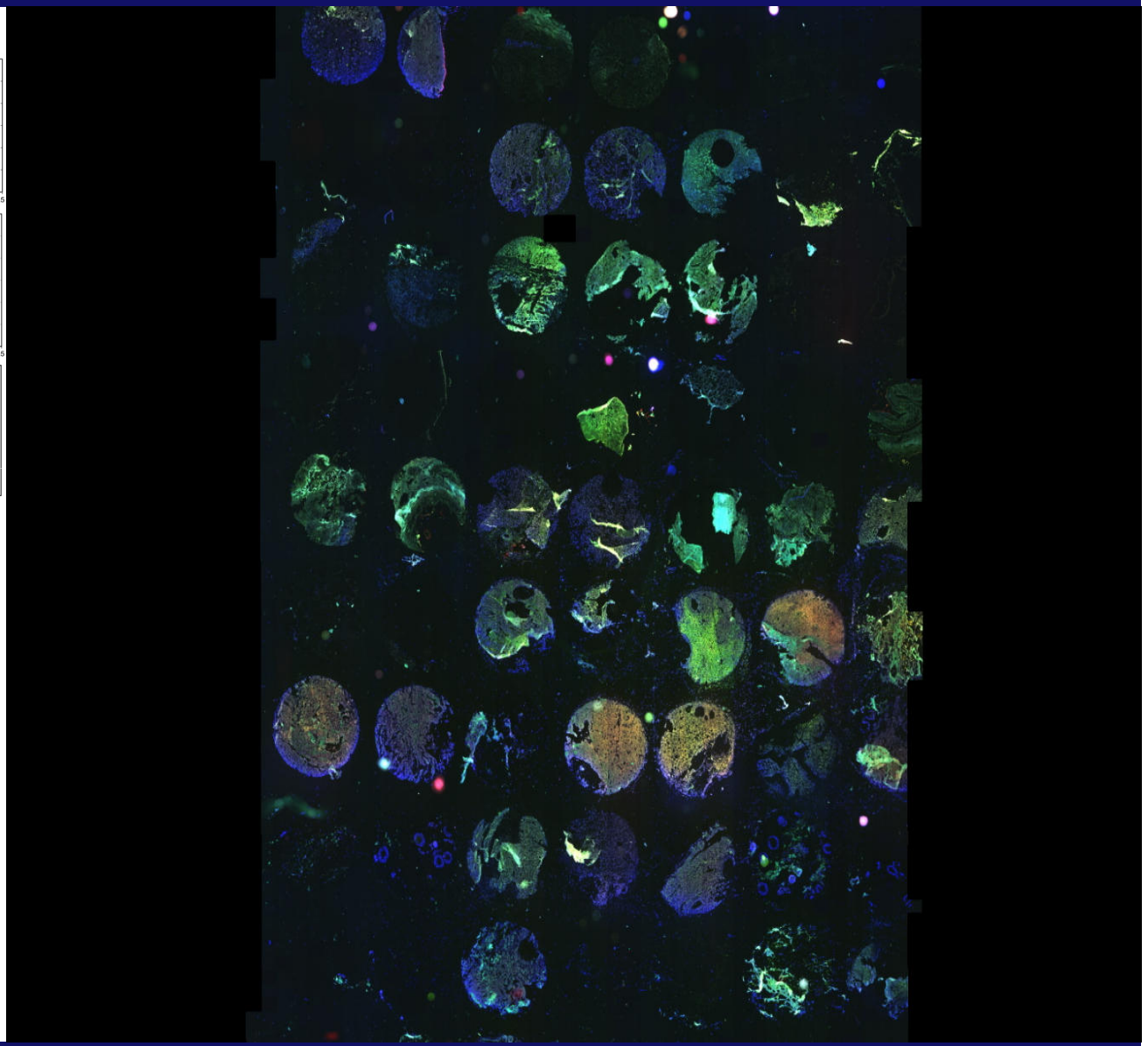


de Jong et al., submitted

Verbeke et al., submitted



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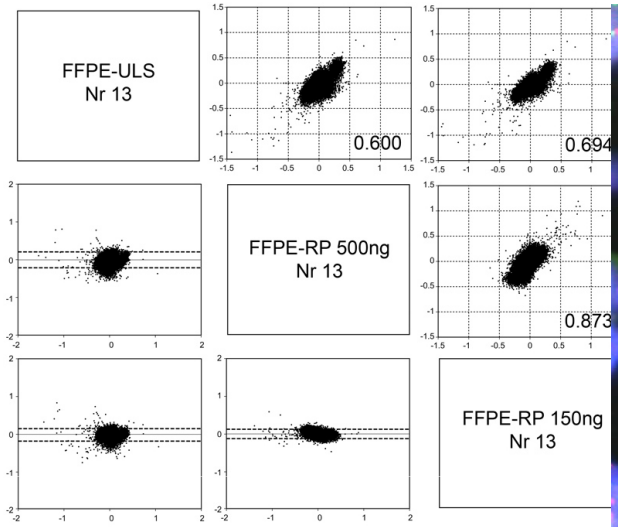

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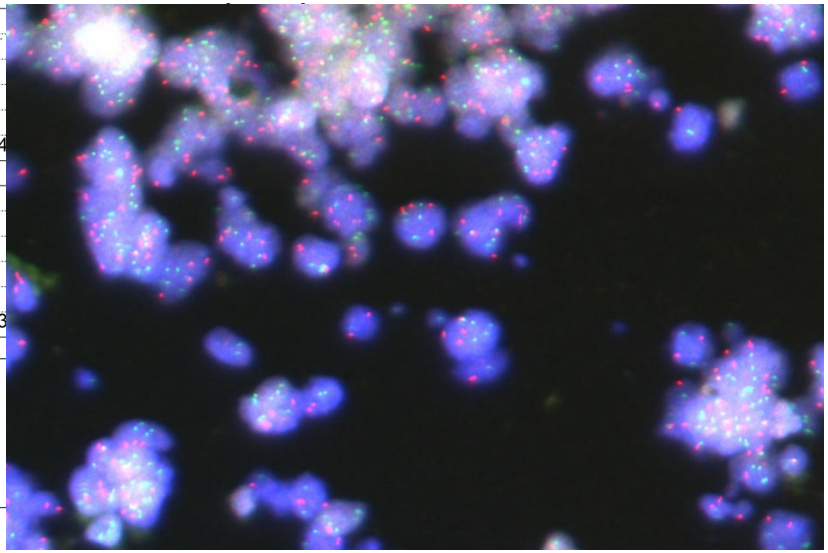

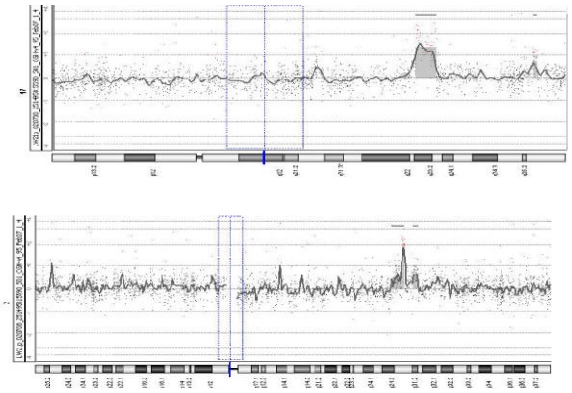

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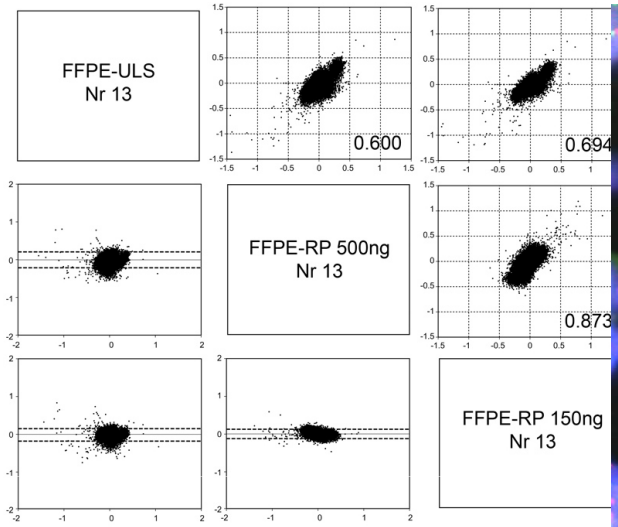
FFPE-DNA & array-CGH II



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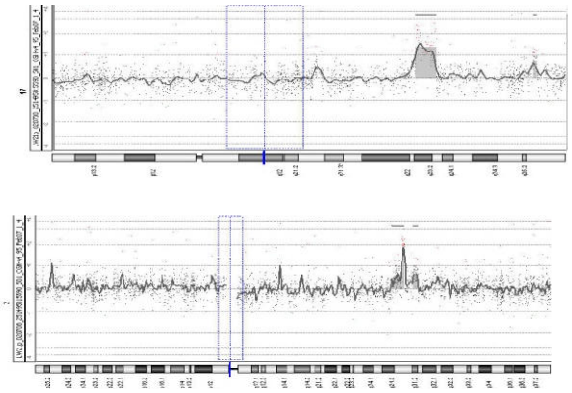

de Jong et al., submitted

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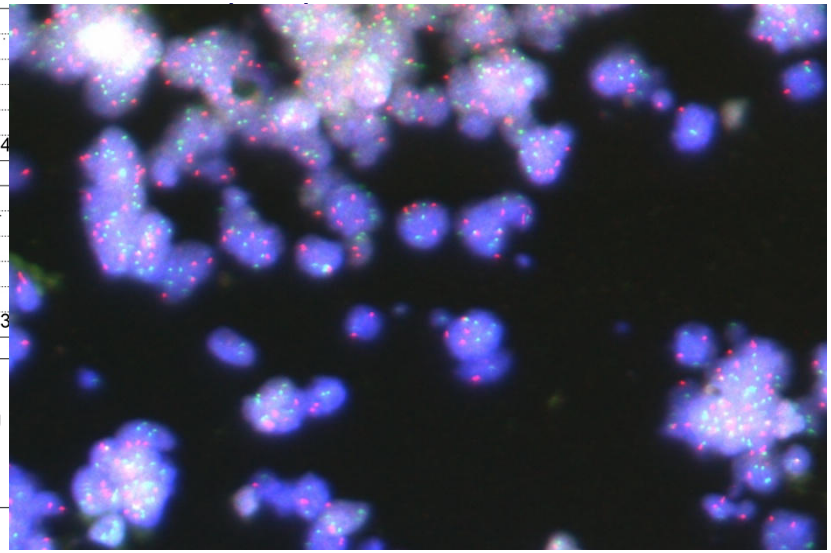
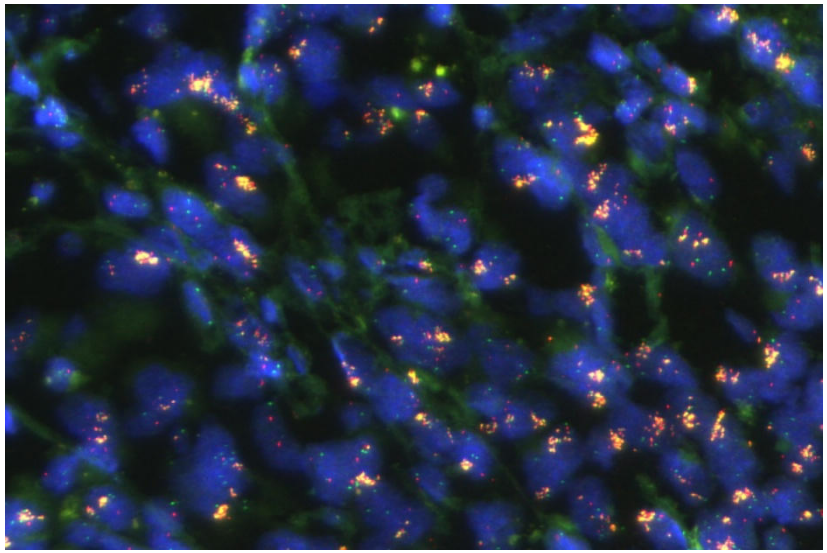
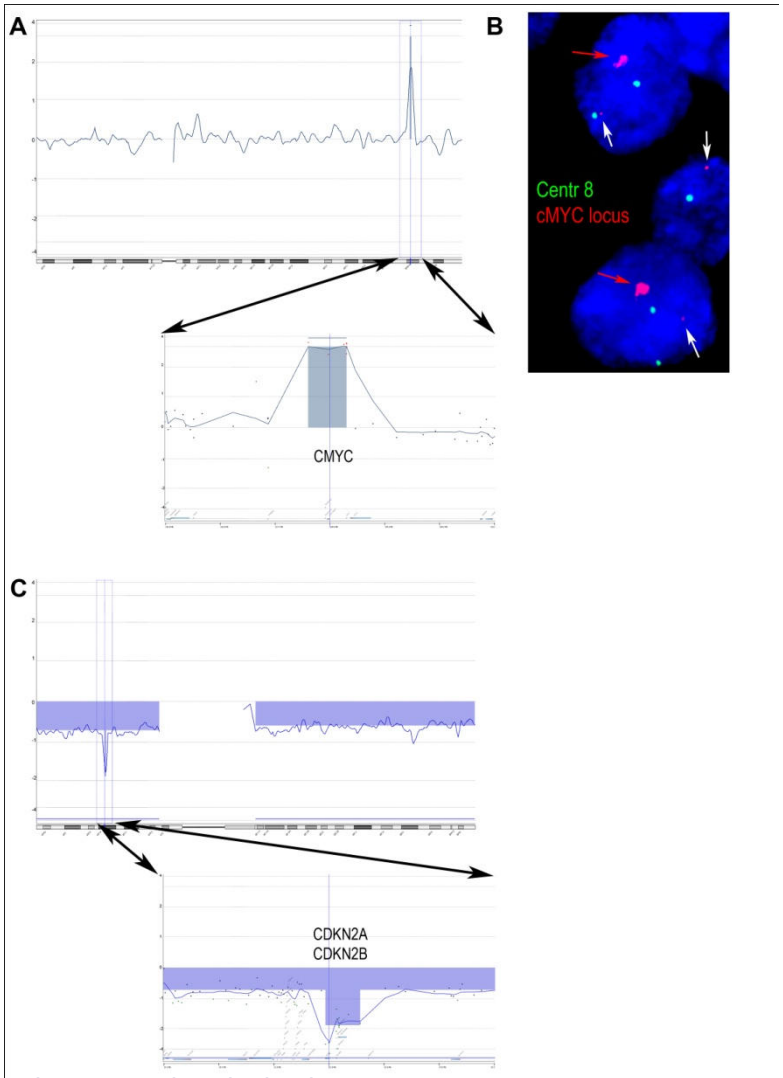




FFPE-DNA & array-CGH III



de Jong et al., submitted

Meijer et al., submitted

- **Detection sensitivity**
  - ~700kb amplified region at *CMYC* locus
  - 600kb homozygous deletion region at *CDKN2a/p16* locus
- **DNA obtained after extended digestion in combination with double purification**
- **Suitable for novel FFPE labeling kits**

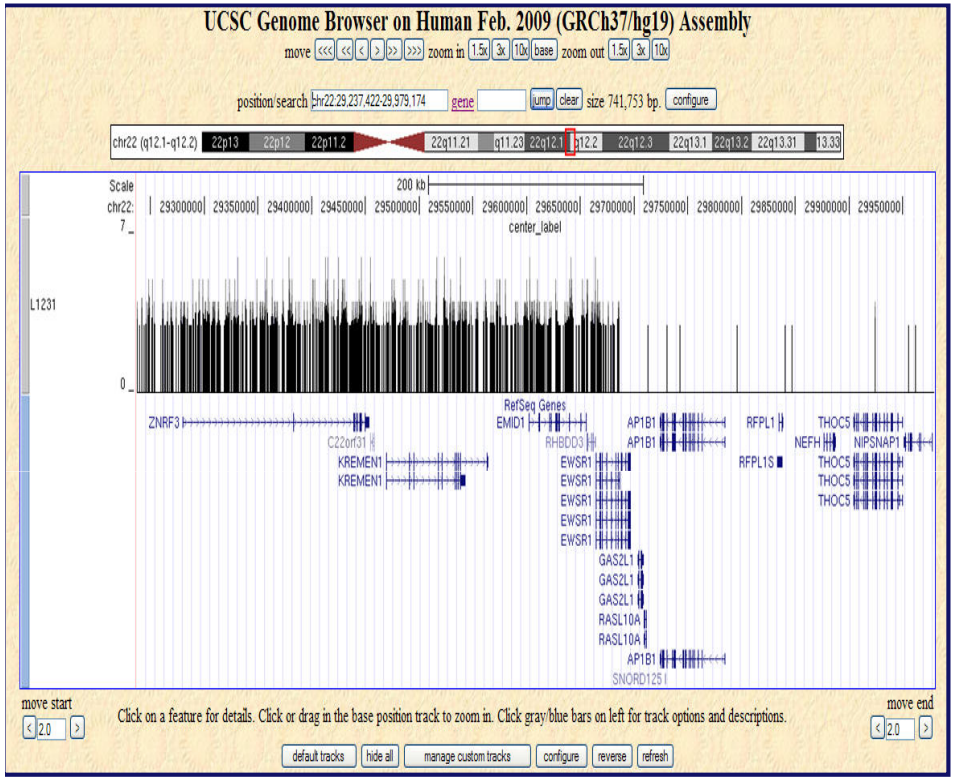
FFPE Next-generation Sequencing: Helicos

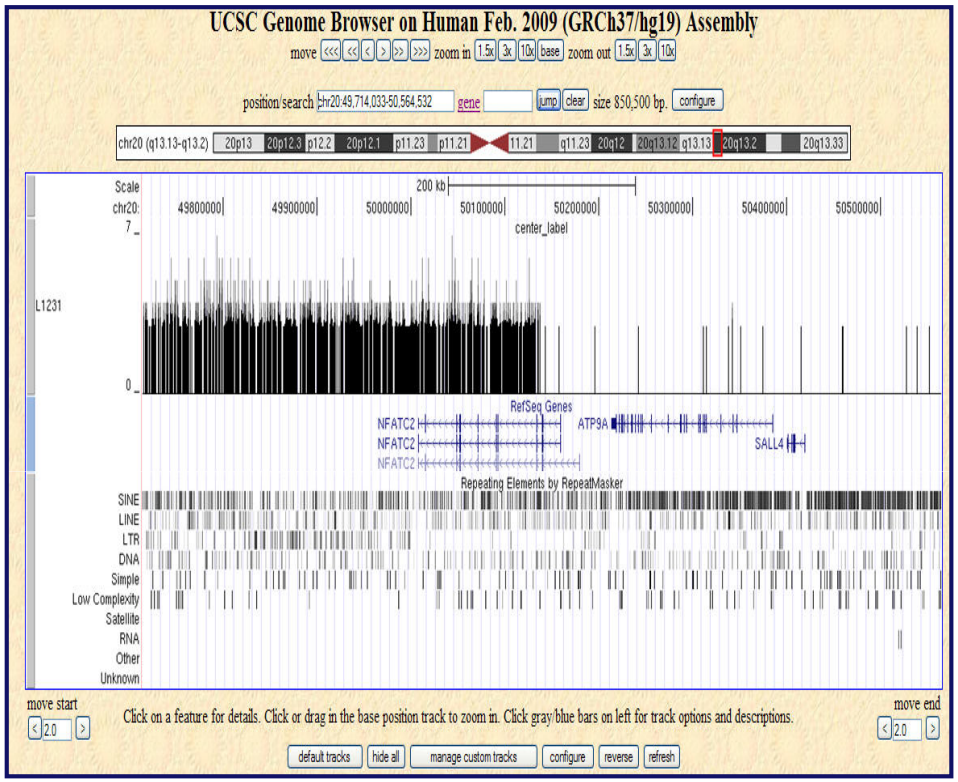
- **100 ng non-amplified DNA used for direct sequencing**
  - Optimal fragment size ~100bps
  - Degraded FFPE DNA is a **pro**
- **Varian Ewing sarcoma sample tested with cloned breakpoints**
- **After QC and blasting 12.2 million fragment reads**
  - at unique locations
  - 97.000 with > 15 read/loci
    - *likely to be repeat elements*
- **2.4 million unique loci**
  - >3 and <15 read / 500bp bin

www.lgtc.nl/equipment.php

Szuhai et al., Clin Cancer Res, 2009

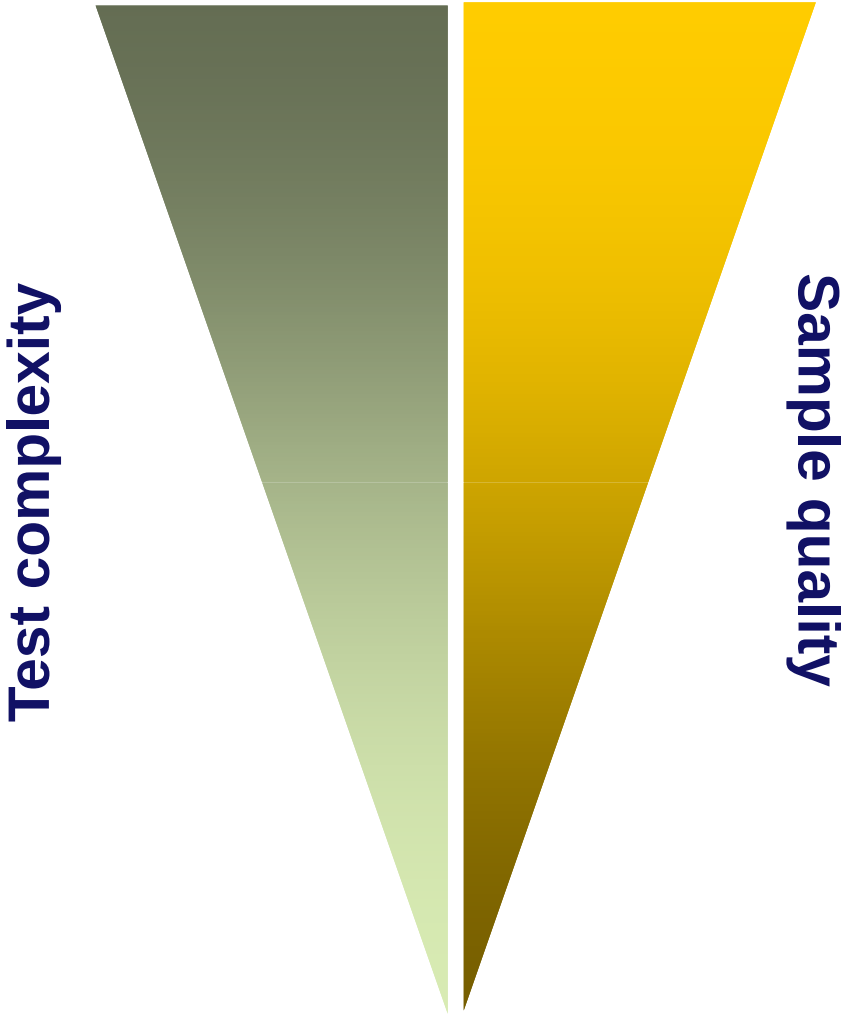
Helicos Read: Chromosomes 20 & 22





- **One channel Helicos run ~2.4 million reads ~~ 2.4 million array**
- **Breakpoints localized within 1000 bps after a single run within EWSR1 and NFATc2 genes**

Summary



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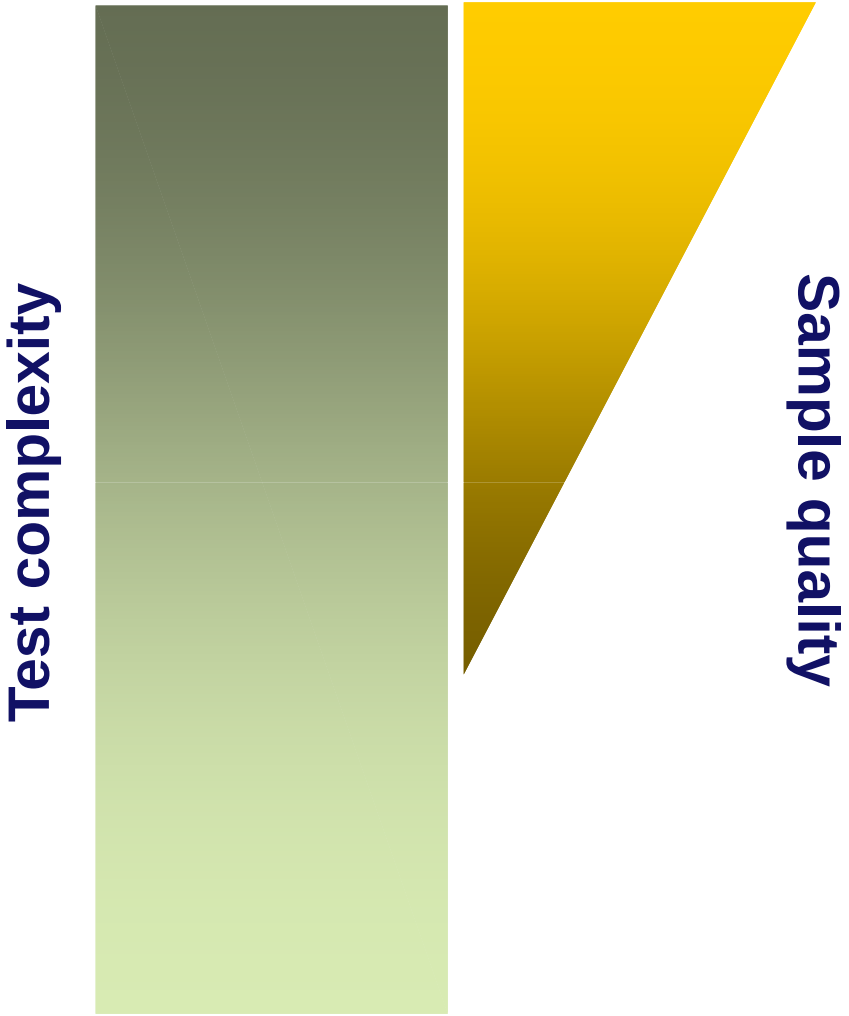
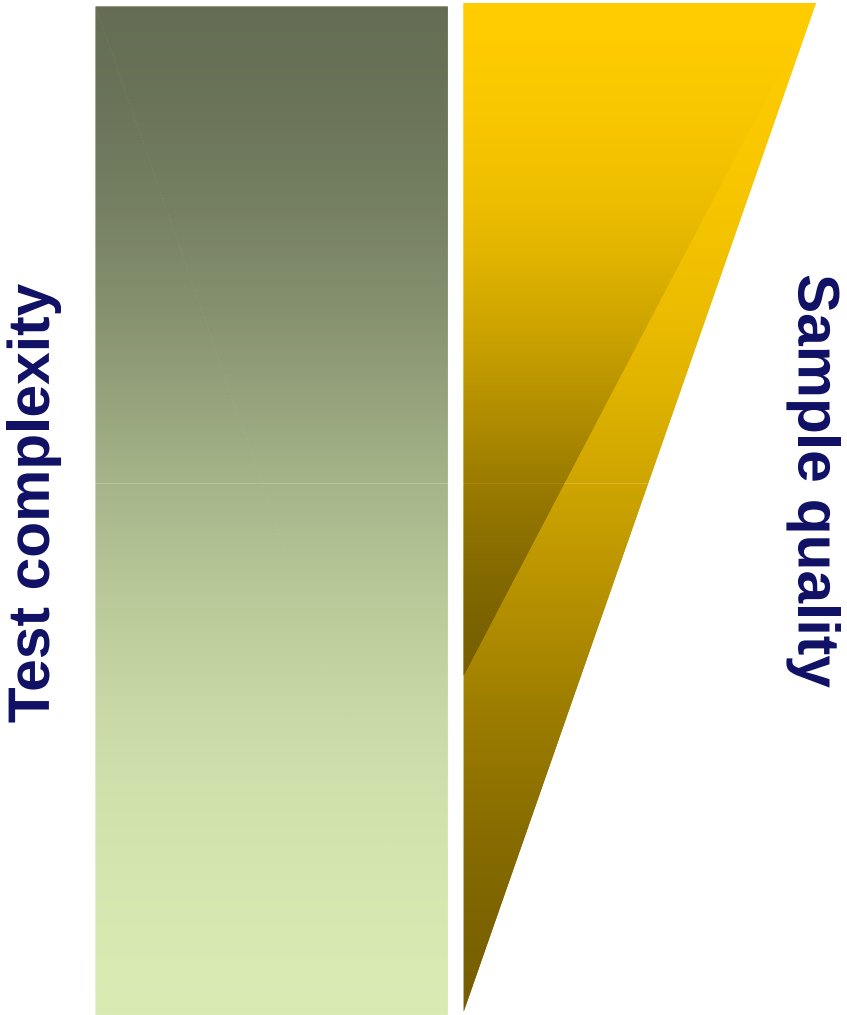


- **Sample**
  - Detailed sample annotations
    - *treatment, type of surgery, time*
  - Standardized protocols
    - *Cut out*
    - *Fixation*
      - (time, fixative, volume, sampling)
    - *Storage*
- **Isolation process**
  - Extensive retriaval and purification
    - *DNA, RNA and protein*
    - *Double QC (size & concentration)*
- **Methodolocigal improvements**
  - Novel applications/platforms
  - Improved/adapted labeling
  - …

- *biospecimens.cancer.org*
- *www.impactsnetwork.eu*
- *www.eurobonet.eu*

Summary



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Virchows Arch
DOI 10.1007/s00428-010-0917-5

ORIGINAL ARTICLE

Multicentre validation study of nucleic acids extraction from FFPE tissues

Serena Bonin · Falk Hlubek · Jean Benhattar · Carsten Denkert · Manfred Dietel · Pedro L. Fernandez · Gerald Höfler · Hannelore Kothmaier · Bozo Kruslin · Chiara Maria Mazzanti · Aurel Perren · Helmuth Popper · Aldo Scarpa · Paula Soares · Giorgio Stanta · Patricia J. T. A. Groenen

OPEN ACCESS Freely available online

PLoS one

Determinants of RNA Quality from FFPE Samples

Silke von Ahlfen[1], Andreas Missel[1], Klaus Bendrat[2], Martin Schlumpberger[1]*

Tissue Handling and Specimen Preparation in Surgical Pathology

Issues Concerning the Recovery of Nucleic Acids From Formalin-Fixed, Paraffin-Embedded Tissue

Stephen M. Hewitt, MD, PhD; Fraser A. Lewis, PhD; Yanxiang Cao, PhD; Richard C. Conrad, PhD; Maureen Cronin, PhD; Kathleen D. Danenberg; Thomas J. Goralski, PhD; John P. Langmore, PhD; Rajiv G. Raja, PhD; P. Mickey Williams, PhD; John F. Palma, PhD; Janet A. Warrington, PhD

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Judith Bovee
Pancras Hogendoorn
Daniëlle Meijers
Jan Oosting
Sofie Verbeke

Erasmus MC, Rotterdam

Herman van Dekken

LGTC
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Yavuz Ariyurek
Johan den Dunnen
Matthew Hestand