IMPAKT BREAST CANCER CONFERENCE

IMProving cAre and Knowledge through Translational research

Basic techniques in translational research

IMPAKT training course

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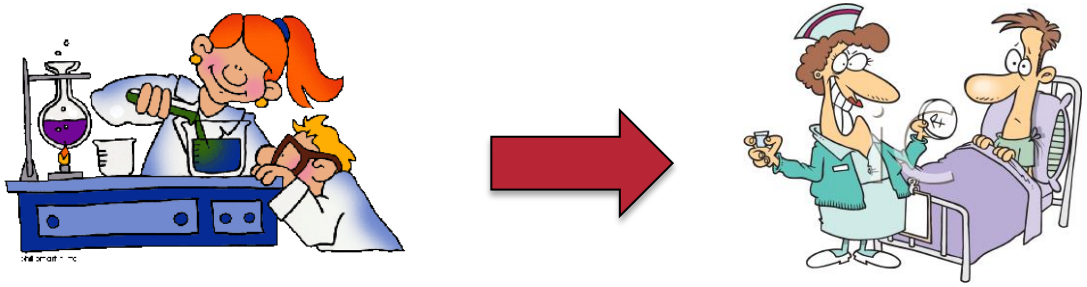
I have no conflict of interest to disclose

From bench to bedside

Translational research is meant to bridge the gap between **basic research** and **the clinic** in order to facilitate the transition of knowledge and discovery into therapeutics and lead to improvement of healthcare.



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Overview

1/ Sample types used in translational research/ collection

2/ Biological material - extraction

- quality and quantity assessment

3/ Basic technologies to dissect cancer complexity

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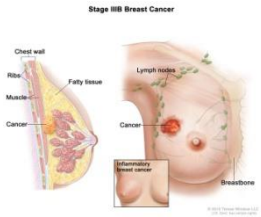
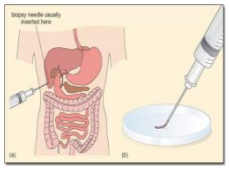
Sources of biological material

Tissues
(FFPE/frozen)

- Primary breast cancer



- Metastasis



Blood derived

- Plasma/serum
- Circulating Tumor Cells
- Circulating DNA

Cancer cell lines, mouse models…

Basic rules: High quality samples

Sample collection and preservation

- **Stabilize** tissue in a suitable fixative (Formalin, RNAlater®, ...)

or **freeze** the tissue **immediately** (in liquid nitrogen or -80° C)

- Process blood within 1h after blood draw for serum and plasma preparation
- Whole blood should be frozen **immediately**

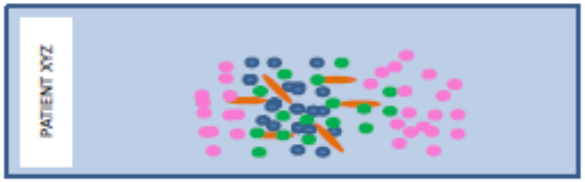
SPEED is the key

Be aware of the cellular composition of the samples

- DNA/RNA does not only come from the tumor epithelial cells
- Many other cell types are present in the tumor microenvironment (fibroblasts, lymphocytes, adipocytes…)
- Normal/stromal cells might influence the signal

Cellular composition evaluation

- Evaluation on an H&E slide
- Tumor area or tumor cellularity?
- Semi-quantitative



- Tumor epithelial cell
- Lymphocyte
- Fibroblast
- Normal epithelial cell

Tumor area = 40%

Tumor cellularity:

Tumor epithelial cells = 30%

Normal epithelial cells = 30%

Lymphocytes = 30%

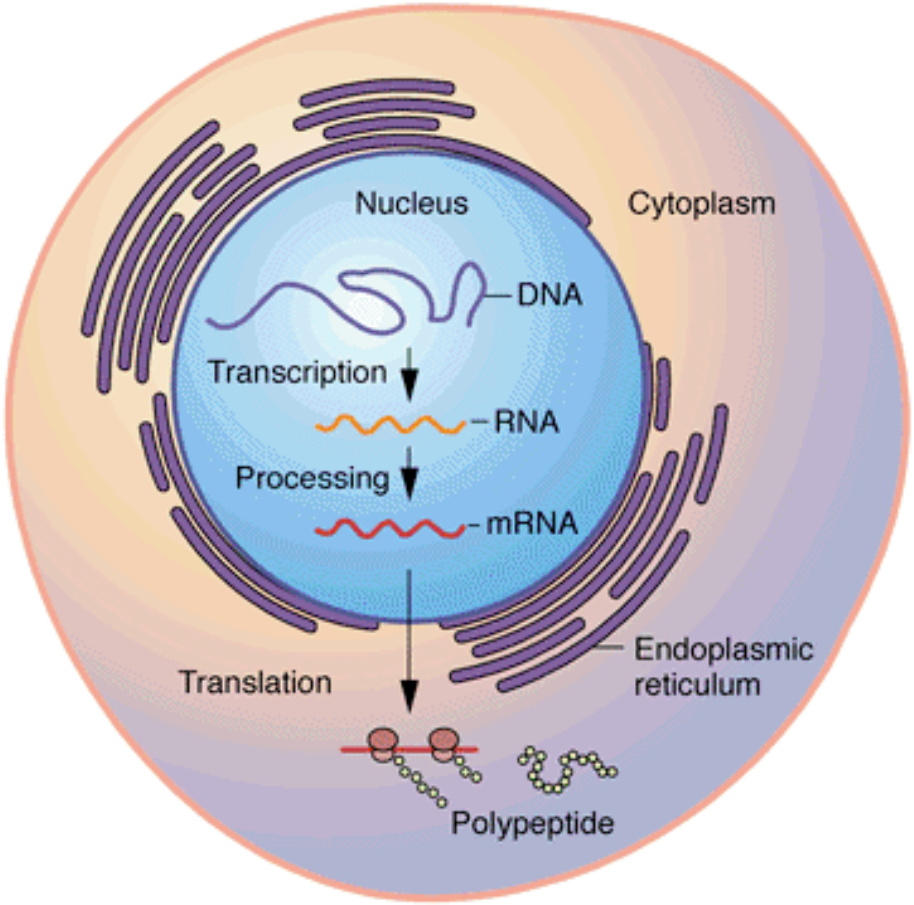
Fibroblasts = 10%

Adipocytes = 0%

DCIS = 0%

Courtesy of C.Desmedt

From genes to proteins

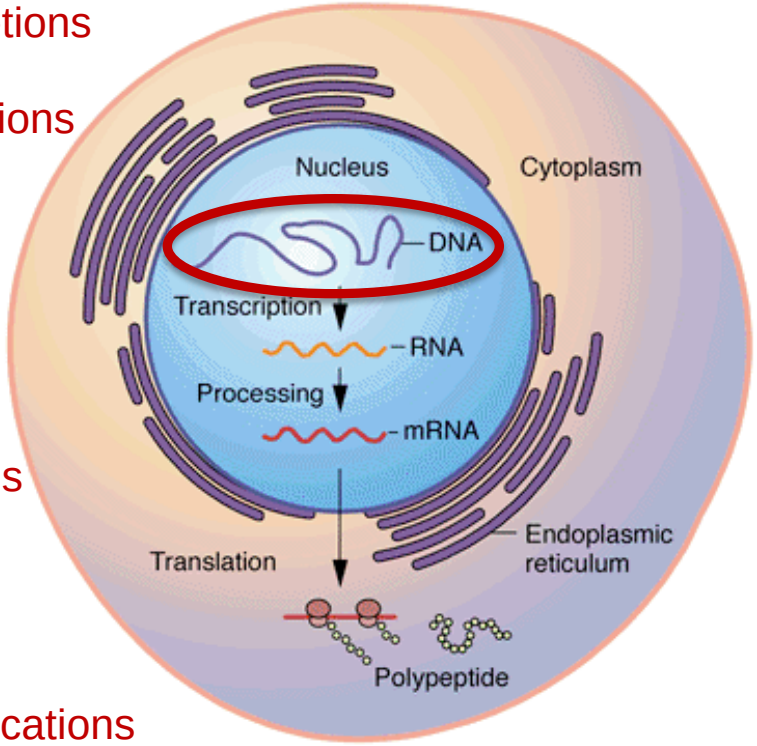


At the DNA level

Chromosomal aberrations

Deletions

Gene fusions



Mutations

SNP

Amplifications

Copy Number Variation

Next generation sequencing

DNA methylation profiling

At the RNA level

lncRNA

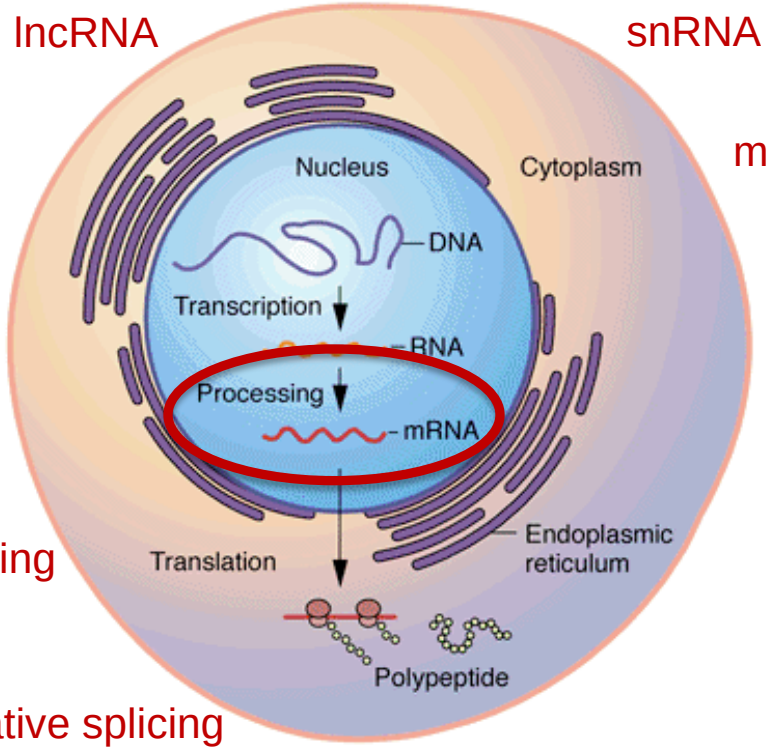
snRNA

miRs

mRNAs

RNA editing

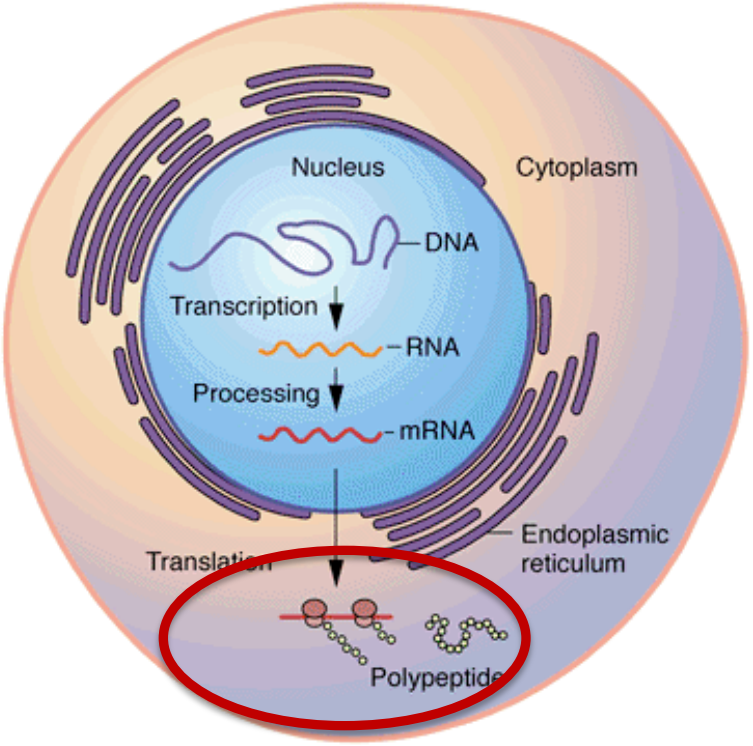
Alternative splicing



Next generation sequencing

Gene expression profiling

At the protein level



Protein expression

Post translational modifications

Nucleic acids extraction methods

- First extraction in 1869
- **Specific** techniques depending on samples types

(blood, FFPE, frozen…)

- Common steps for DNA/RNA:

Cell lysis → proteins/lipids removal → RNase/DNase treatment → DNA/RNA purification

- Phenol-chloroform method (ex: Trizol)

- Column-based methods (ex: Qiagen kits, Roche kits…)

Top 5 sources of RNAse contamination

1) Skin
2) Endogenous cellular RNAses
3) Unsterile material (tips, tubes,…)
4) Water and buffers
5) Lab surfaces

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DNA/RNA quantification and quality control

Nucleic acids quantification

1/ Spectrophotometer

- Most widely used method (ex: Nanodrop)
- Simple and accurate method to determine DNA/RNA concentration
- DNA and RNA show maxima absorbance at 260nm
- Assessment of nucleic acids purity:

Ratio A260/280: protein contamination

Ratio A260/230: organic compounds contamination

→ Both ratios should be close to 2 for pure DNA/RNA

- No information about the integrity of the sample

2/ Fluorometer

- Qubit® technology or Quant-iT™ PicoGreen®
- Incorporation of fluorescent dye in selected molecule

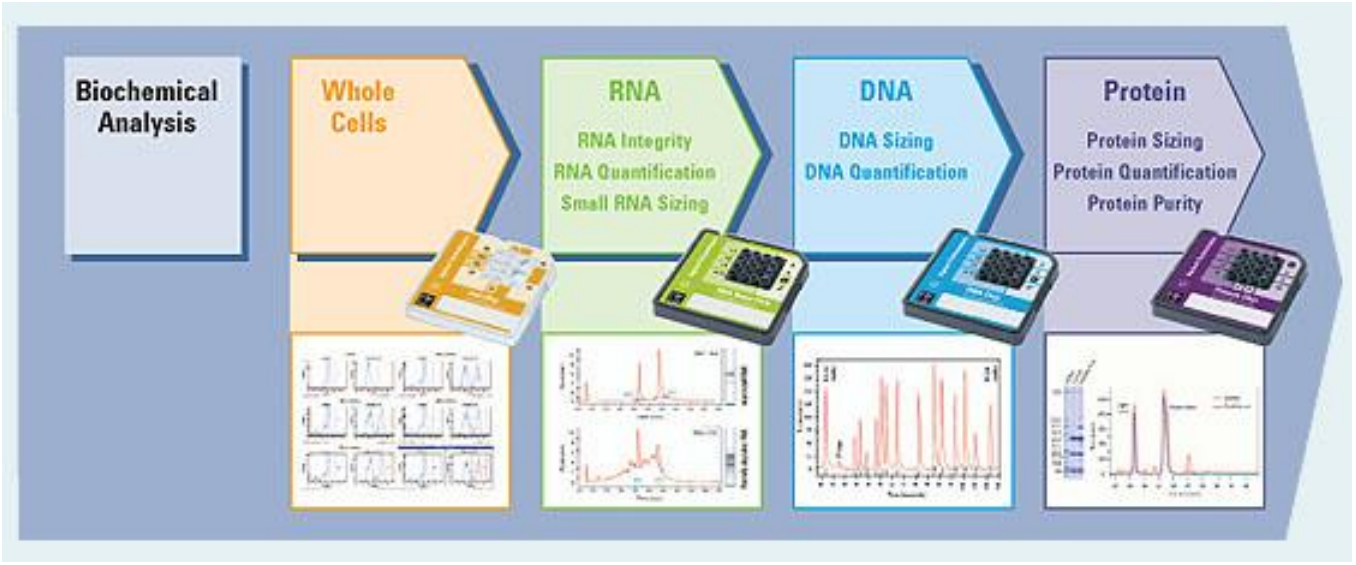
→ Quantification of dsDNA, RNA, proteins

Fluorescence emitted only when dye bound to the specific molecule

- More sensitive and accurate than UV light absorbance
- Saving on precious samples
- Standard technique used to quantify DNA for NGS applications

Nucleic acids quality control using Agilent Bioanalyzer

Microfluidics-based chip platform for sizing, quantification and quality control



Use of Agilent Bioanalyzer for RNA

- Total RNA: - mRNA represent only 1-3%

  - ribosomal RNA makes up >80% total RNA

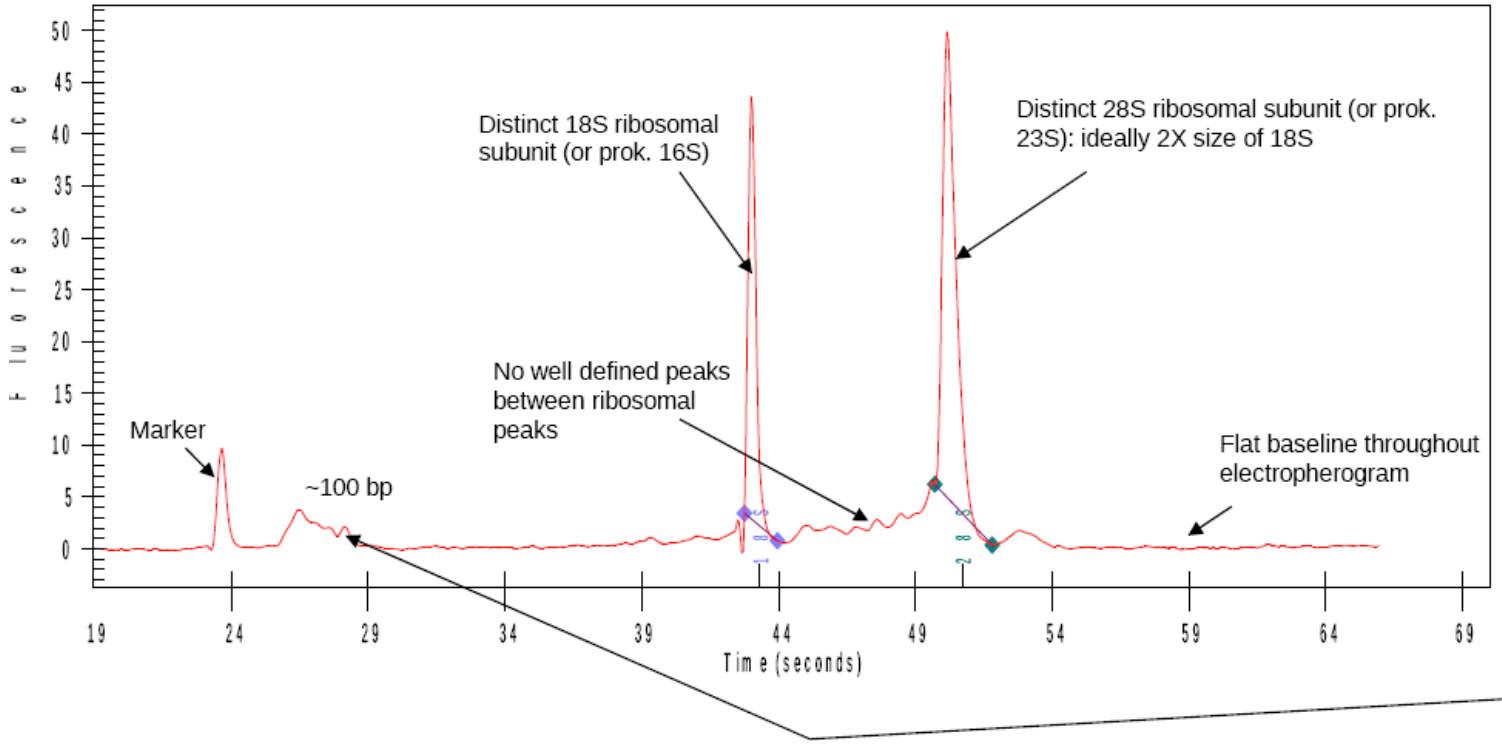
- 2 most abundant RNAs= ribosomal RNAs 18S and 28S
- Ribosomal ratio 28S/18S =2 for intact RNA
- RNA Integrity Number (**RIN**) calculated for each sample

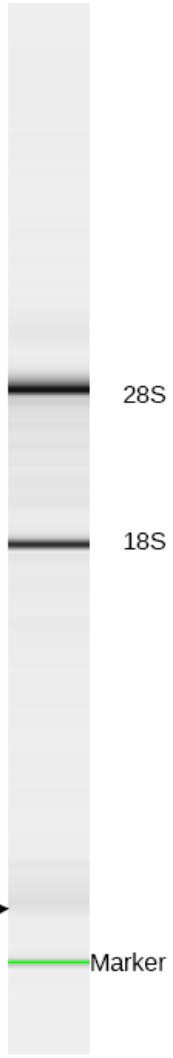
  (1: degraded RNA → 10: intact RNA)

- Developed to remove individual interpretation in RNA quality control

Intact total RNA

RIN~10

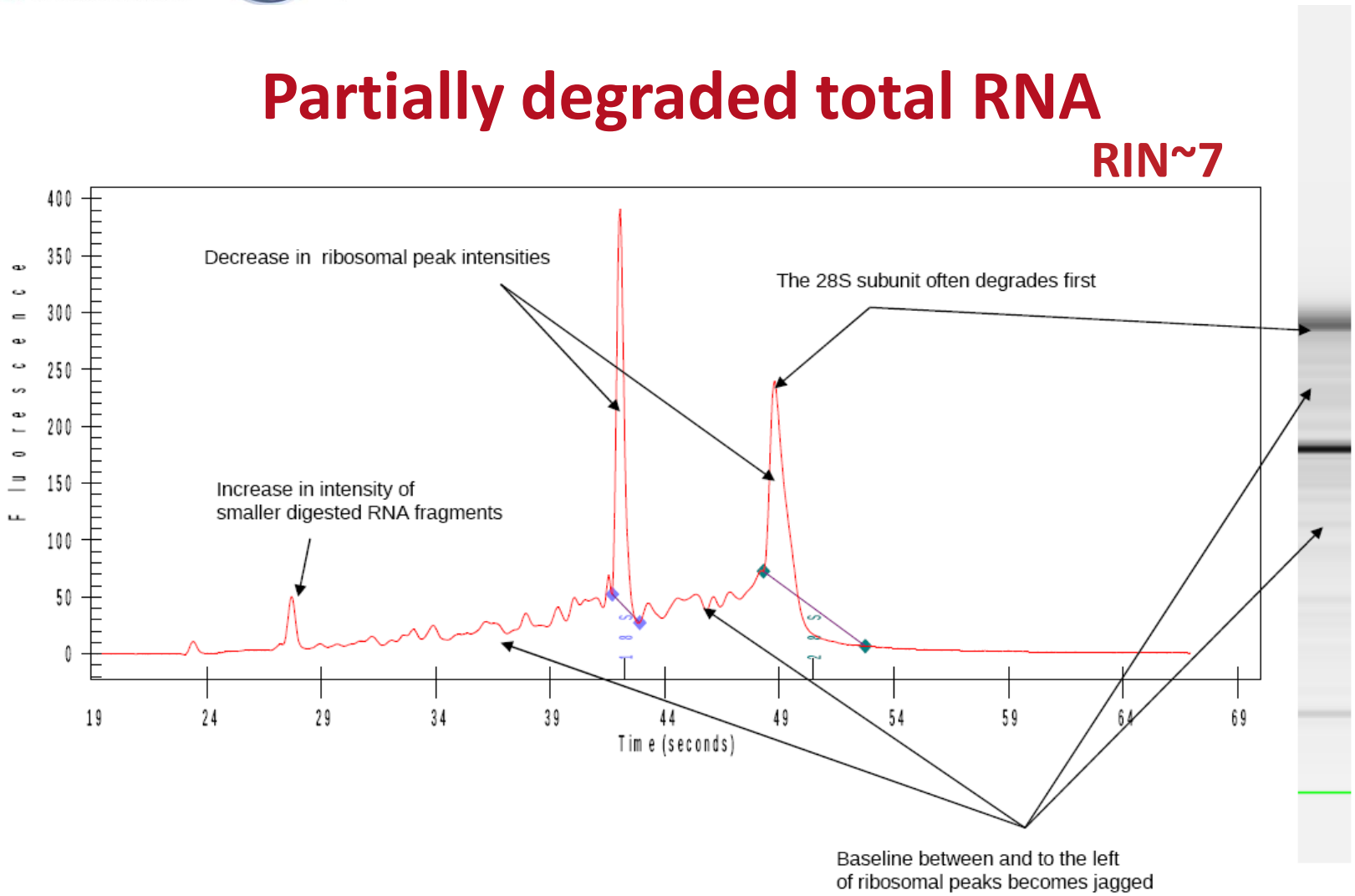




Small peaks are sometimes present after the marker at 24 – 29 seconds. These are represented by 5S and 5.8S subunits, tRNAs, and small RNA fragments about 100bp. These are especially noted when using phenol and trizol extraction methods. They can be removed by treating total RNA through Qiagen columns which removes small RNAs.

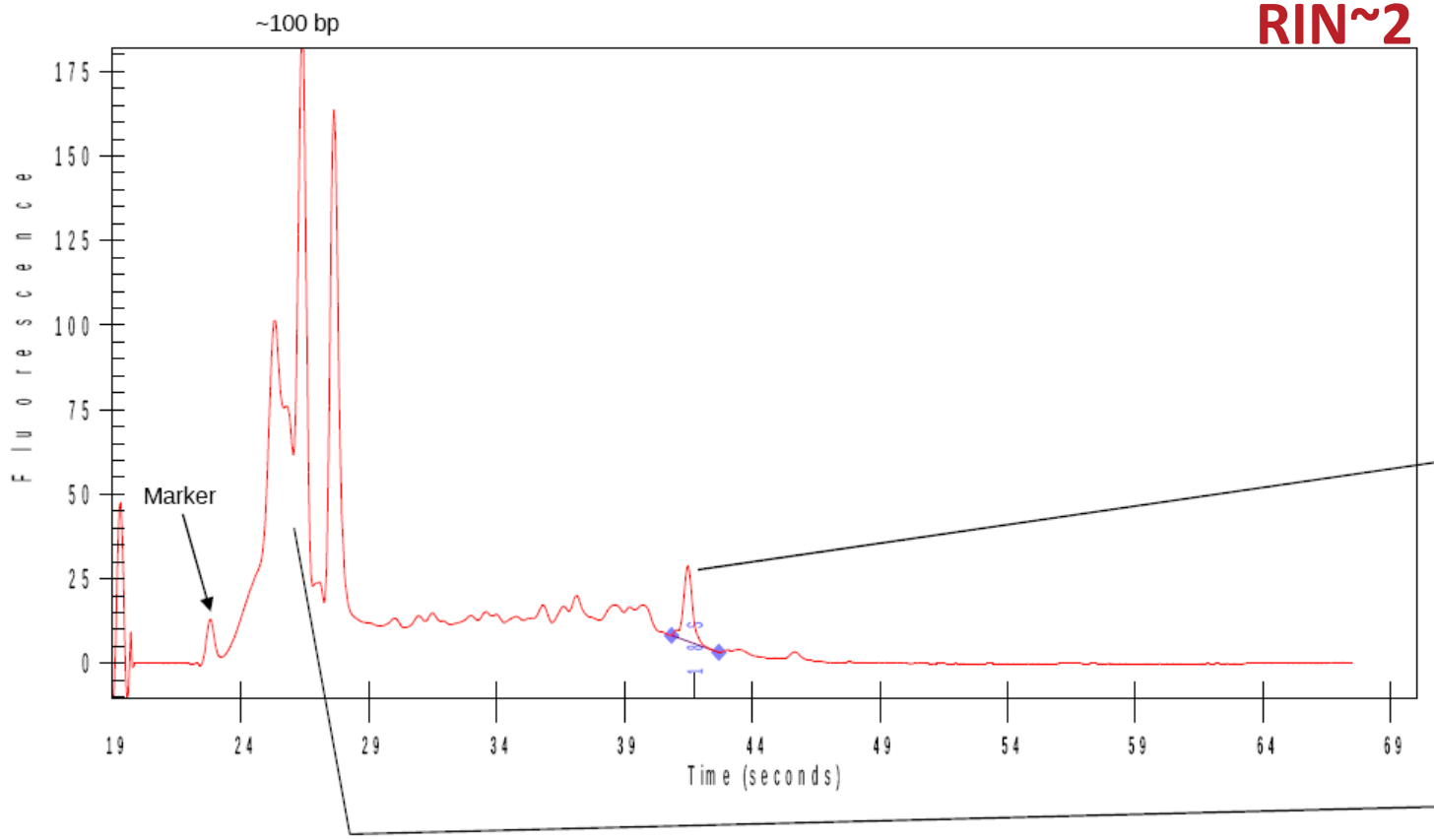
Partially degraded total RNA

RIN~7



Totally degraded total RNA

RIN~2



Use of Agilent Bioanalyzer for DNA

Especially useful for NGS applications:

- Assess quality of starting material
- Monitor size distribution after fragmentation and adapter ligation steps
- Quantify yield and detection of artifacts post-PCR amplification
- Detect small quantities of DNA in amplification-free protocols

Some basic applications in TR

At the DNA level:

1/ PCR:

- **Specific** exponential DNA amplification from few to millions of copies
- High sensitivity, specificity and robustness
- **Great spectrum of research and diagnostic applications:**

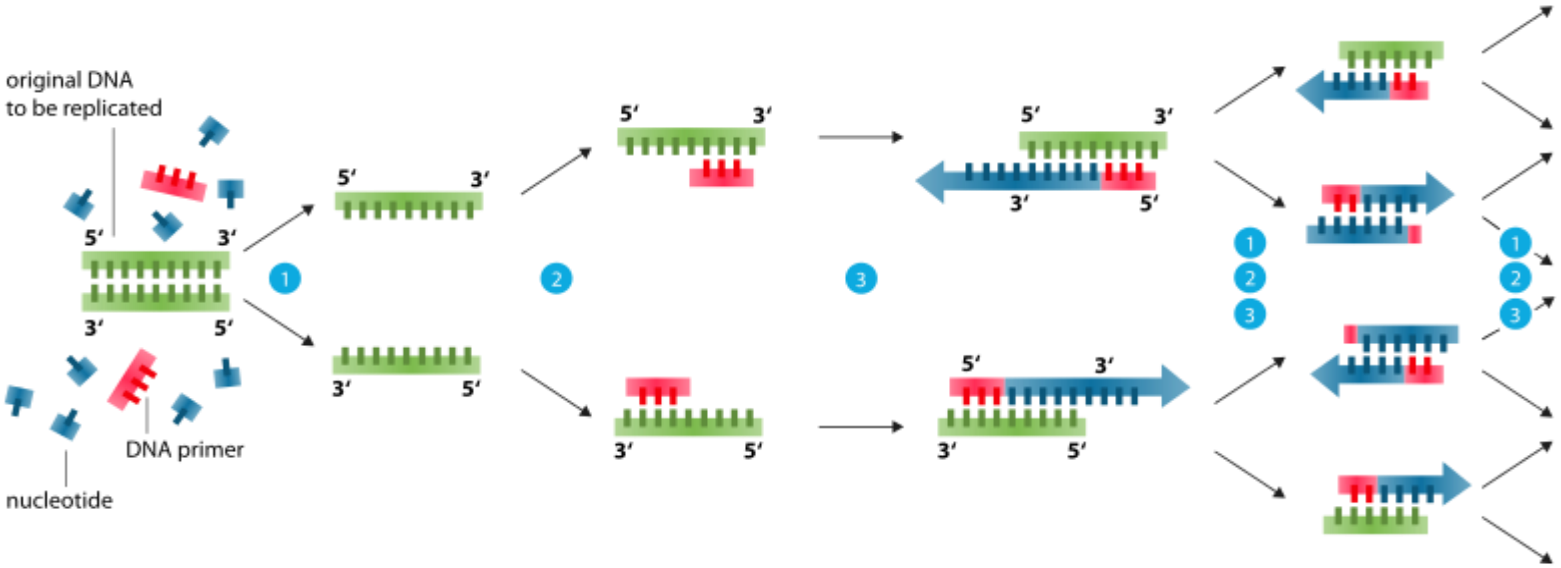
DNA cloning, mutation screening/validation, diagnosis of hereditary diseases, detection of infectious diseases, prenatal diagnosis, site-directed mutagenesis…

PCR

3 Steps: - denaturation

- annealing

- elongation



2/ Fluorescence *In Situ* Hybridization (FISH):

- Vizualisation of chromosomes or portions of chromosomes using fluorescent molecules
- Assessed on morphologically preserved chromosome preparations, fixed cells or tissue sections
- Identification of chromosomal alterations:

(ex: amplification/loss/translocation/ploidy determination)

- Validation of NGS and array CGH data

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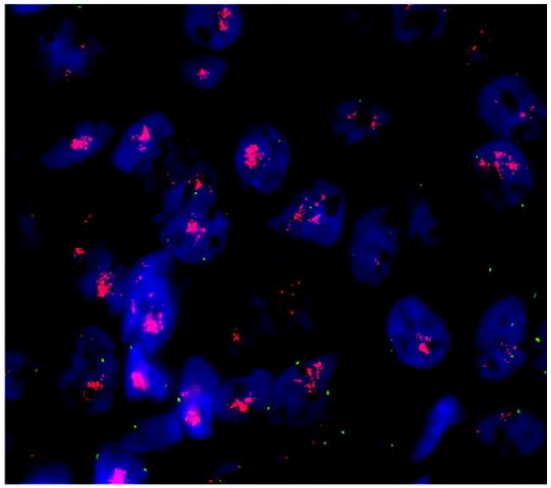
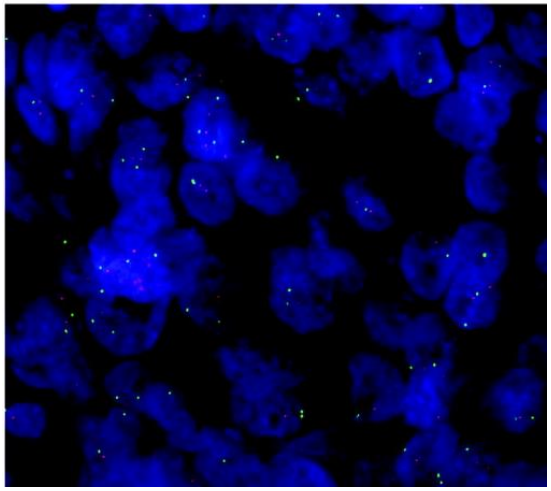
her2 FISH

HER2 amplified



HER2 non-amplified



her2* amplification: *her2*/CEP17 ≥2

Chromosome 17 centromere

***her2* gene**

* Wolff A. , Arch. Pathol. Lab. Med., 2014

3/ Other DNA techniques include:

Cloning, Southern blot, gel electrophoresis, DNA transfections,

Sanger sequencing, methylation analysis, array CGH…

NGS: Whole genome and exome sequencing

At the RNA level:

1/ Quantitative RT-PCR:

- Variant of PCR used to assess specific RNA expression (mRNAs, miRNAs,…)
- **2 steps:** - Reverse transcription (RNA → cDNA)
  - qPCR
- **2 main technologies:**

- SYBrGreen: non-specific fluorescent dyes that intercalate within dsDNA

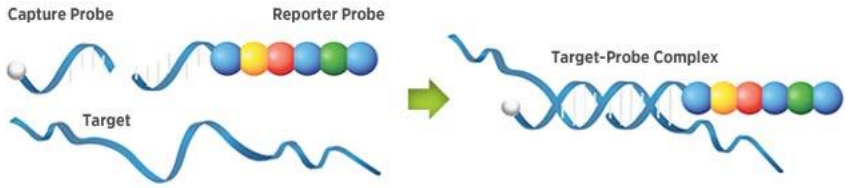
- Taqman: sequence-specific DNA probes labeled with a fluorescent reporter

- Validation of microarray data (frozen/FFPE)
- **21 genes Recurrence Score – FFPE samples**

(Prognosis and prediction of benefit from CT in ER+ BC pts)

2/ Nanostring technology

- Digital color-coded barcode technology
- Each color-coded barcode is attached to a single target-specific probe corresponding to a gene of interest



- Detection and counting of >100s different transcripts in one reaction
- High sensitivity (< 1 copy per cell)
- No amplification step
- **PAM50 classifier and prognosticator – FFPE samples**

3/ Other RNA techniques include:

- Gene expression profiling: Northern blot, microarray
- RNA-ISH

NGS: RNA-seq

At the protein level:

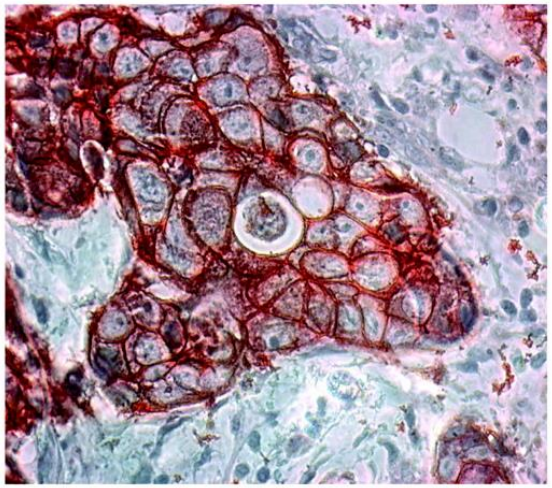
- Protein expression/modification/pathway analysis:

Western Blot, ELISA, IHC,…

- Protein localisation: IF, IHC
- Protein interaction: chromatin-IP, EMSA, co-IP, 2 yeast hybrid
- Protein identification: mass spectrometry

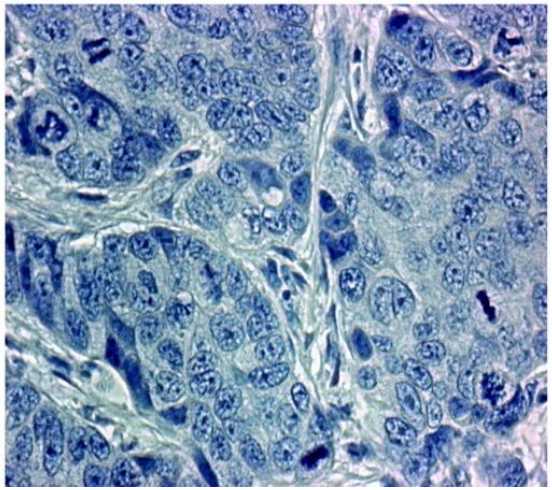
HER2 IHC

HER2 2+



→ FISH

HER2 negative

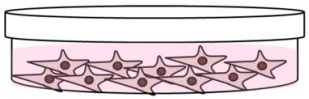


Cell lines in cancer study

Gene overexpression

Gene inhibition

Cell treatment



Proliferation

Migration/invasion

Drug sensitivity

Pathway analysis

DNA/RNA/proteins analysis

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From genes to proteins

Chromosomal aberrations

mRNAs

miRs

Deletions

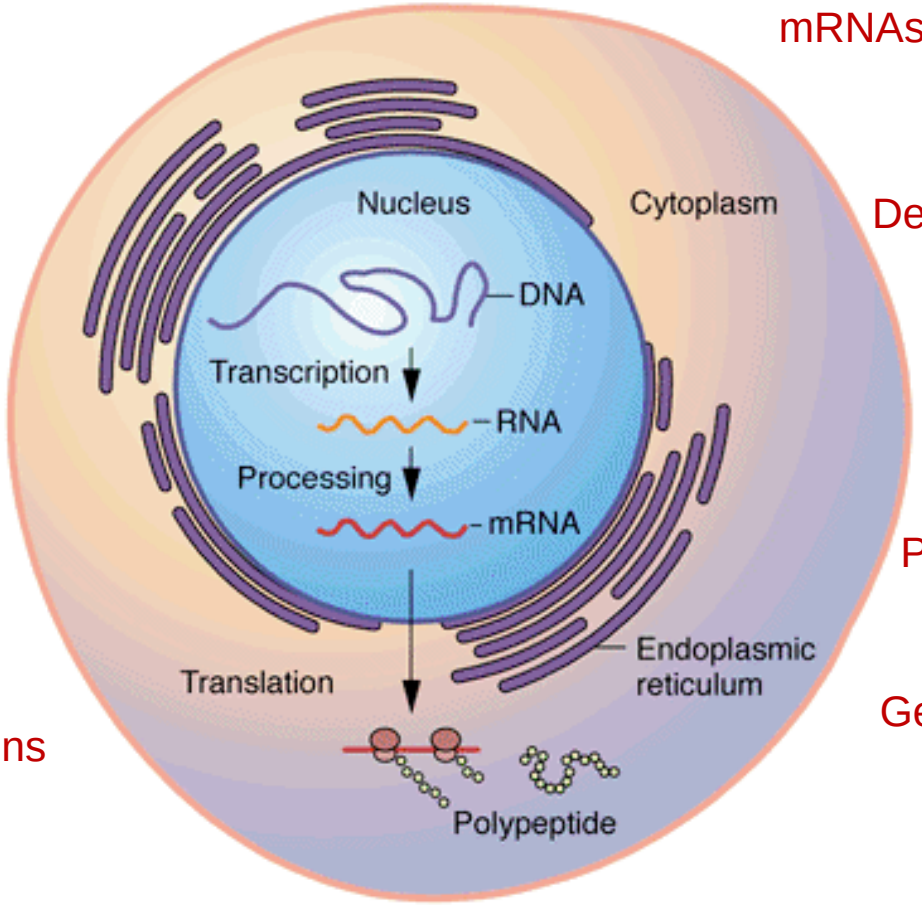
Protein localization

Mutations

Protein expression

Gene fusions

Amplifications



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Acknowledgements

BCTL – Jules Bordet Institute

Christos Sotiriou

Marion Maetens

Debora Fumagalli

Christine Desmedt

Michail Ignatiadis

Ghizlane Rouas

Delphine Vincent

Samira Majjaj

Naïma Kheddoumi

Norman Brown

Vinu Jose

Gabriele Zoppoli

Martine Piccart

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Thank you for your attention