MICROARRAY GENE EXPRESSION ANALYSIS:
CRITICAL ASPECTS OF TECHNOLOGY, WORKFLOW AND
METHODS

Dr. Jelena Kühn Georgijevic
FGCZ Tutorial day
Outline

• Overview on gene expression technology

• Principles of sample quality assessment

• Microarraying at FGCZ: Trends
## Commercial DNA microarrays

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Gene Expression Profiling:

**Known Exons**

**Unknown transcript**

**3'IVT arrays**

**Exon arrays**

**Tiling arrays**
Agilent Whole Genome Oligo Microarrays (4X44K format)

- Probe design: One oligo (60mer) per transcript
- 44K features per array, 4 arrays per slide
- Feature size ~ 65 microns
Agilent Gene Expression Microarray Formats

SurePrint HD Arrays

[Image of SurePrint HD Arrays]

65 μm features

Compatible with:
- Agilent B or C Scanner
Agilent Gene Expression Microarray Formats

Introducing

SurePrint HD Arrays

- 44K
- 65 µm features
- Compatible with:
  - Agilent B or C Scanner

SurePrint G3 Arrays

- 60K
- 30 µm features
- Compatible with:
  - Agilent C Scanner
Agilent and Affymetrix Gene Expression Arrays

Catalog arrays:

- Human
- Mouse
- Rat
- Dog (*Canis familiaris*)
- Arabidopsis thaliana
- Bovine
- Chicken
- Caenorhabditis elegans
- Fungus (*Magnaporthe grisea*)
- Rice (*Oryza sativa L. ssp.japonica*)
- Zebrafish (*Danio reio*)
- Frog (*Xenopus laevis*)
- Barley
- Bovine
- Citrus
- Drosophila
- Maize
- Medicago
- Poplar
- Plasmodium/Anopheles
- Pig
- Rhesus Macaque
- Soybean
- Sugar Cane
- Tomato
- Vitis vinifera (Grape)
- Wheat
- Xenopus laevis
- Custom design

...and many more...
mRNA → 5' AAAA 3' → AffinityScript
Oligo dT-Promoter Primer

1st strand cDNA

3' TTTT → Promoter 5'
2nd strand cDNA

5' AAAA → Anti-sense Promoter 3'

AffinityScript

T7 RNA Polymerase
Cy3-CTP, NTPs

cRNA

3' C C UUUU → 5'
2nd strand cDNA

5' AAAA → Anti-sense Promoter 3'
Purify cRNA

cRNA (antisense)

3' C C UUUU → 5'

B
RNA QUALITY

mRNA

5'           AAAA 3'

1st strand cDNA

2nd strand cDNA

AffinityScript
Oligo dT-Promoter Primer

3' TTTT Promoter 5'

5' AAAA Anti-sense Promoter

T7 RNA Polymerase
Cy3 CTP, NTPs

cRNA 5' C UUUU C 3'

2nd strand cDNA

Purify cRNA

cRNA (antisense) 3' C UUUU C 5'

functional genomics center zurich
Critical Aspect: RNA quality

Ideal situation:

• RNA integrity number $>9$

• High purity
  $A_{260/280} = 2$
  $A_{260/230} = 2-2.2$

• Plenty of RNA

80% of the samples submitted to our facility have this “profile”
RNA Isolation from cell culture and tissues
(plenty of material)

GE arrays (Affy and Agilent)
- Any column based purification + DNase treatment
- TRIzol + column purification

miRNA arrays (Agilent)
- TRIzol
- miRNA isolation kit

RNA seq
- TRIzol is the most advisable (all RNA population is kept)
FACS, LCM, FFPE
Low amount or degraded RNA

Quantification:
**Bioanalyzer** - RNA pico kit, use standards of known concentration

Quality check:
- RIN: define your own threshold
- RIN >4: the success rate is higher
- All the sample should have similar patterns

Amplification and labeling:
**Nugen Pico Kit**: OdT +randomers
cRNA quality

[Diagram showing the process of cRNA quality analysis]

1. cRNA synthesis
2. cRNA purification
3. Examination of cRNA quality

AffinityScript

Oligo dT-Promoter Primer

1st strand cDNA

2nd strand cDNA

T7 RNA Polymerase

Cy3-CTP, NTPs

Purify cRNA

cRNA (antisense)
HYB, Wash and Scan - Agilent

Scan asap!!
HYB, Wash and Scan - Affymetrix
Feature Extraction

The software automatically finds spots for Agilent microarrays

FE software quantifies spots and flags outliers

Generates raw expression data in text format

Out-put file
Microarrays @ FGCZ

Big and diverse user community

Many different GE projects

Many needs:

- various organisms on catalog arrays
- flexible experimental design
- low input material
- low cost
3′ IVT or Exon arrays? Agilent or Affymetrix?

**Affymetrix Exon** arrays

👍 Exon level (splicing variants) + gene level information
👎 Only 3 species: human, mouse, rat;
👎 More data to handle

**Affy 3′IVT arrays**

👍 many species, most popular, less input material
👎 high price (like an exon chip!)

**Agilent 3′IVT arrays**

👍 many species, one and two color, e-array
👎 no low input protocol
Agilent: One- or Two-color?

**Two** is recommended ONLY when the experiment has two conditions

- Cheaper !!
- Very sensitive
- Limited experimental design
- Possible dye bias/technical artifacts

**One color** is the most used approach: it can be used in any sort of design

- Flexible experimental design
- More expensive
Commercial microarrays @ FGCZ

NGS at FGCZ
Commercial microarrays service team

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