Sondas de ácidos nucleicos

Usos:

Para la detección de ácidos nucleicos: Southern-blot, Northern-blot, RNAse protection, Dot-blot, Colony-blot etc

Como reactivo: Ej. Para estudiar proteínas de unión ácidos nucleicos, para determinar estructura de RNAs etc.

Métodos para generar sondas:

Nick translation.
Random primers: DNA o RNA
Oligo dT
PCR: marcar primers o producto de PCR.
Primer extension
Transcripción in vitro.
Forforilación 5'.
Transferasa terminal.

Actividad específica: Cantidad de marca en relación a la masa total de sonda.

Southern-Blot

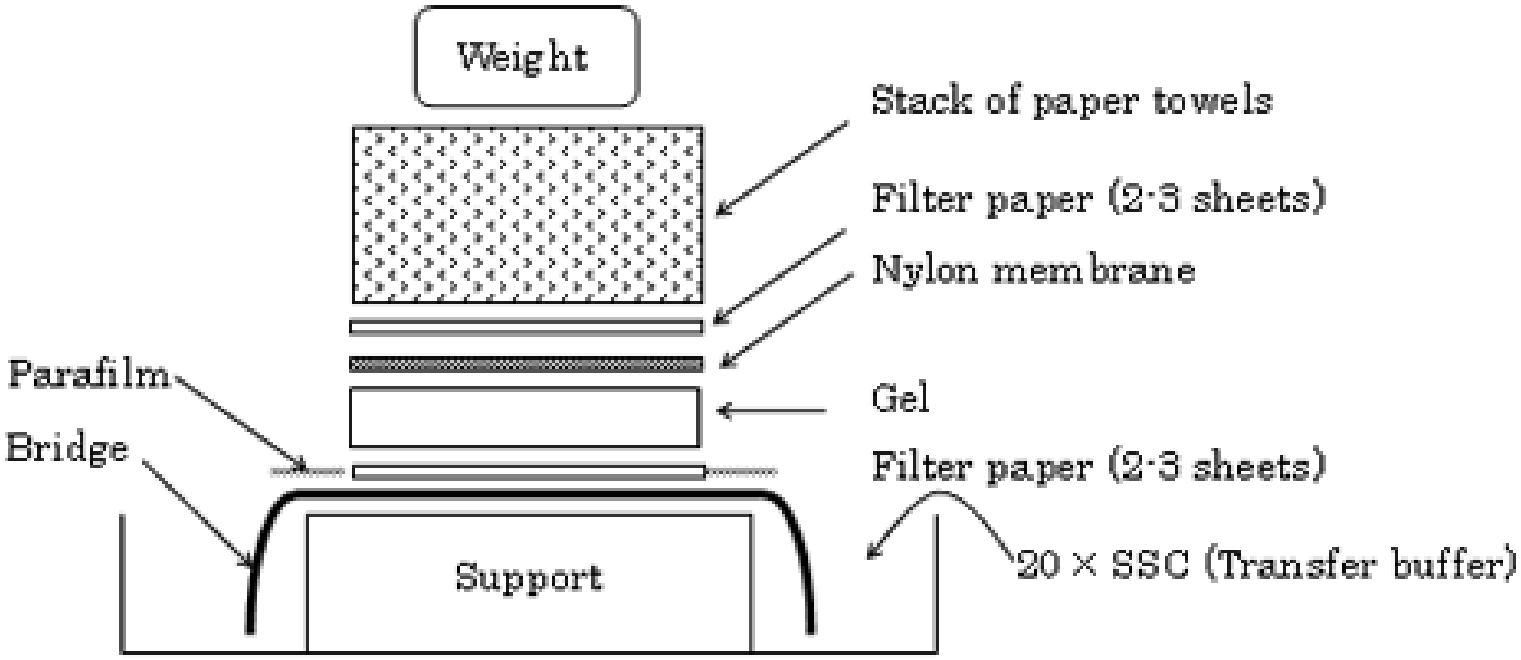
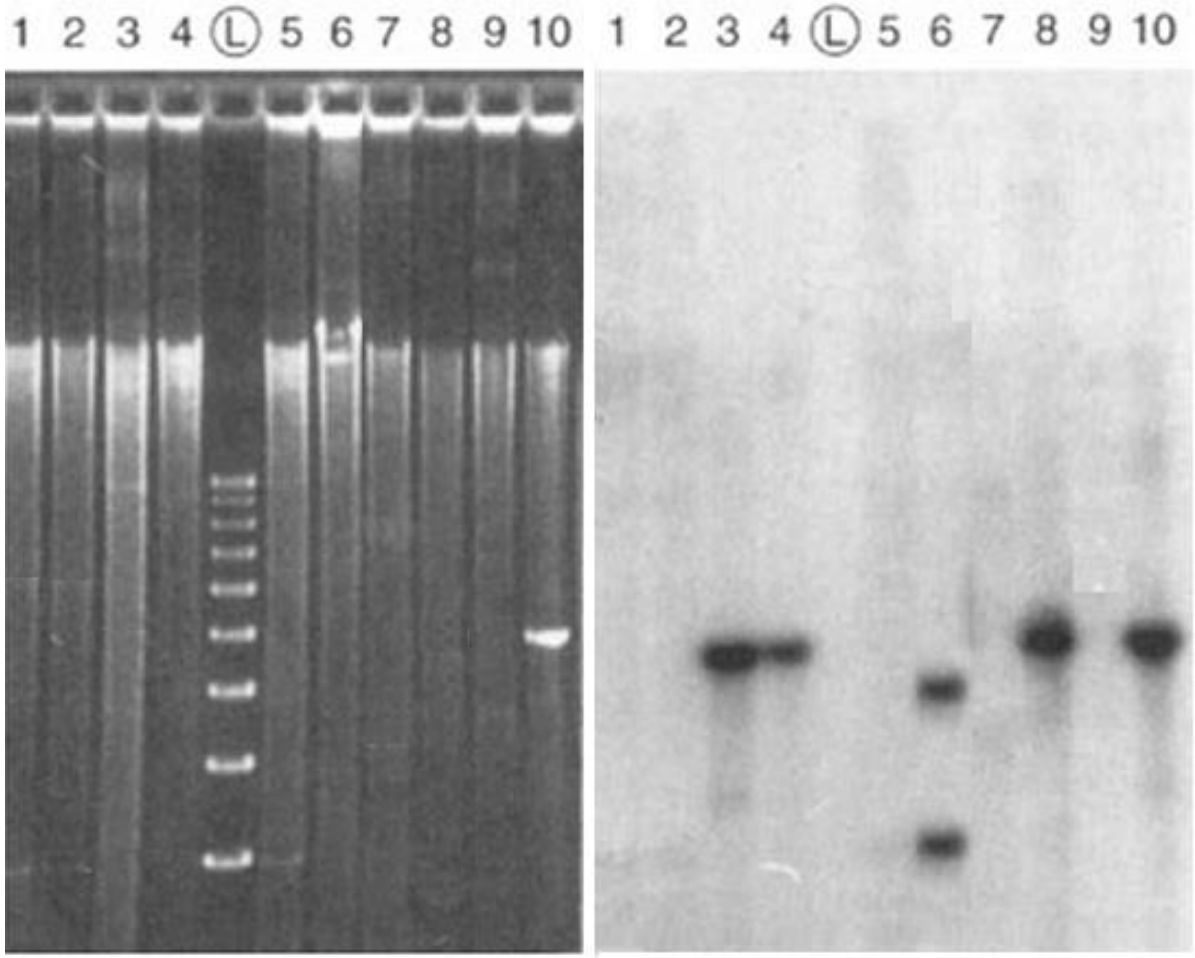
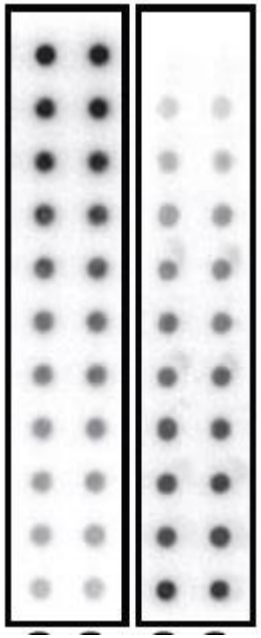


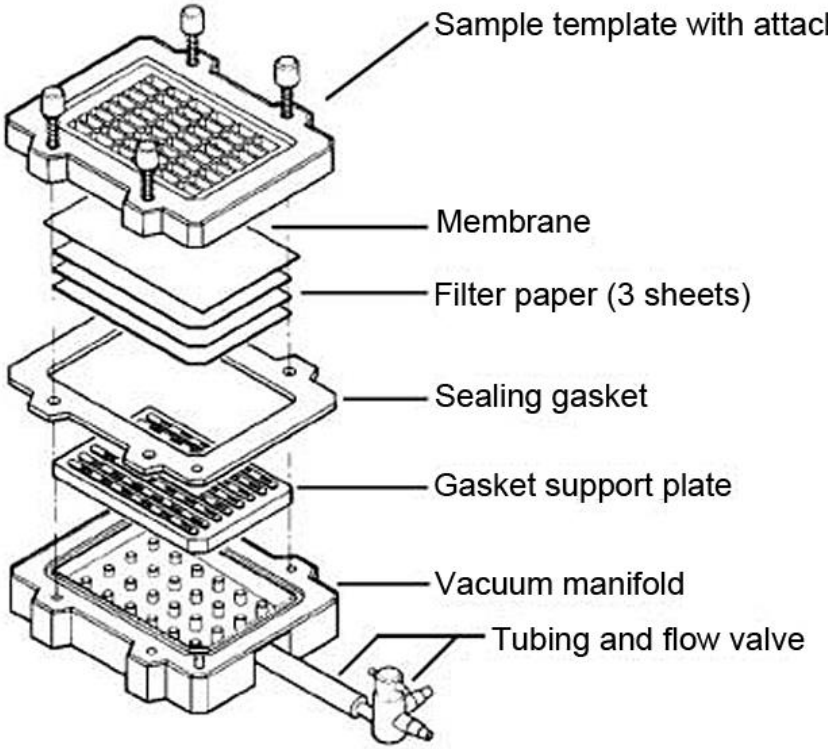
Fig. 1 Transfer Apparatus

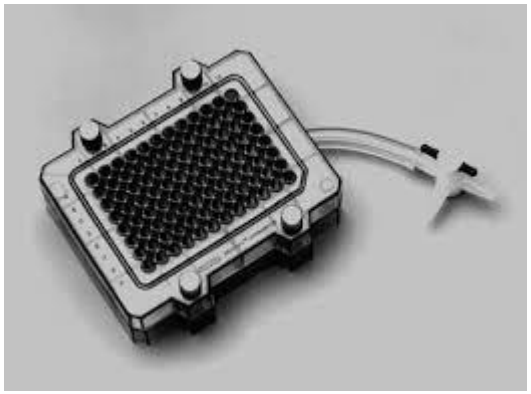

1 2 3 4 L 5 6 7 8 9 10
1 2 3 4 L 5 6 7 8 9 10

Dot-Blot

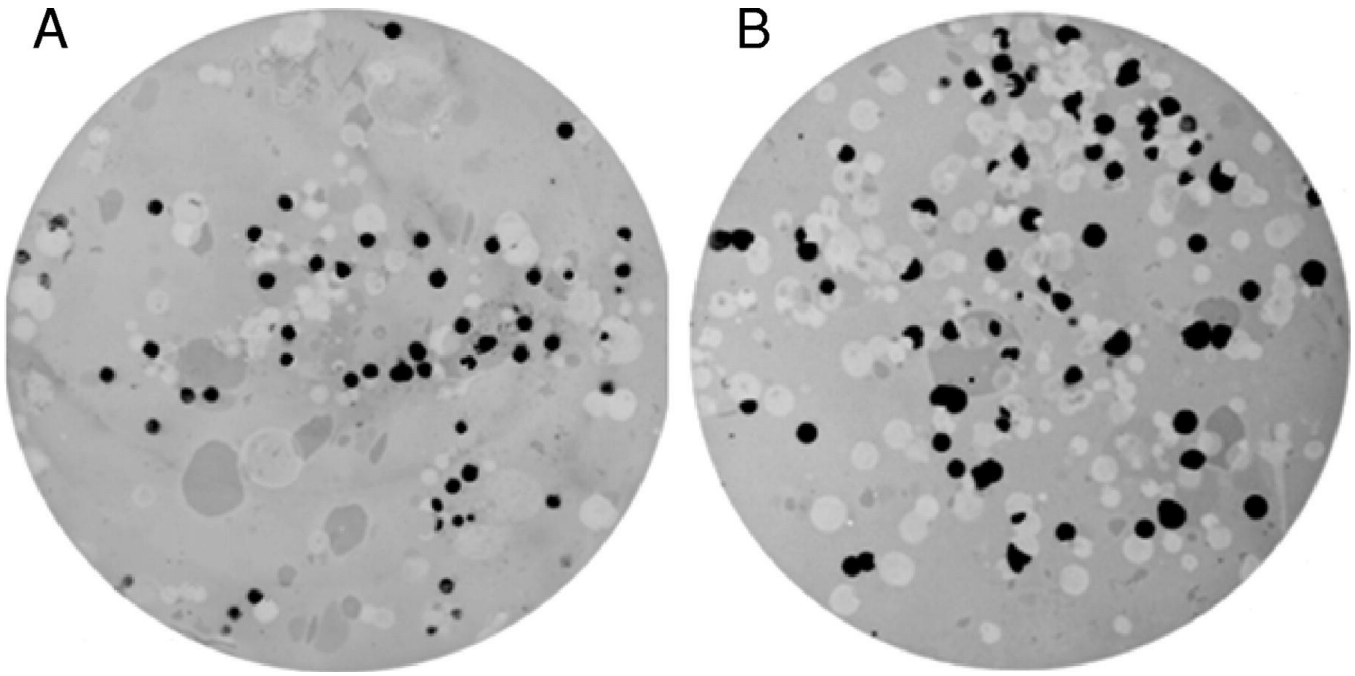



A
B
C
D
E
F
G
H
BIO-RAD
BIO DOT SF


Sample template with attached sealing screws
Membrane
Filter paper (3 sheets)
Sealing gasket
Gasket support plate
Vacuum manifold
Tubing and flow valve



Colony-Blot



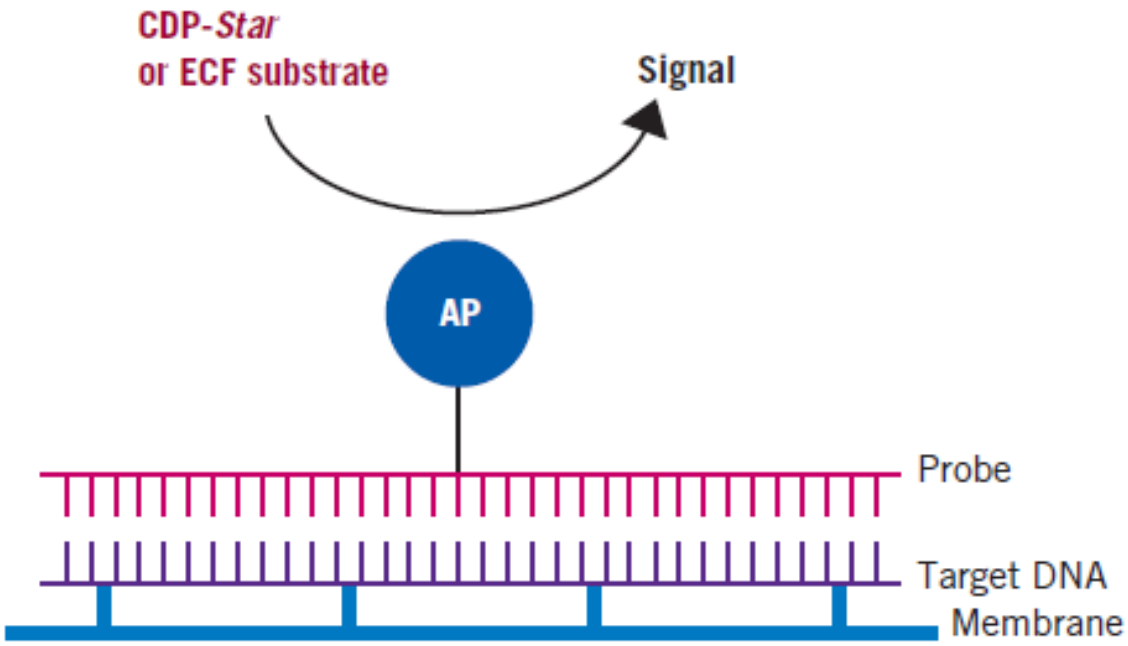


Fig 3. Outline of protocol for direct labelling method.

Amersham ECL™ Direct Nucleic Acid Labeling And Detection Systems

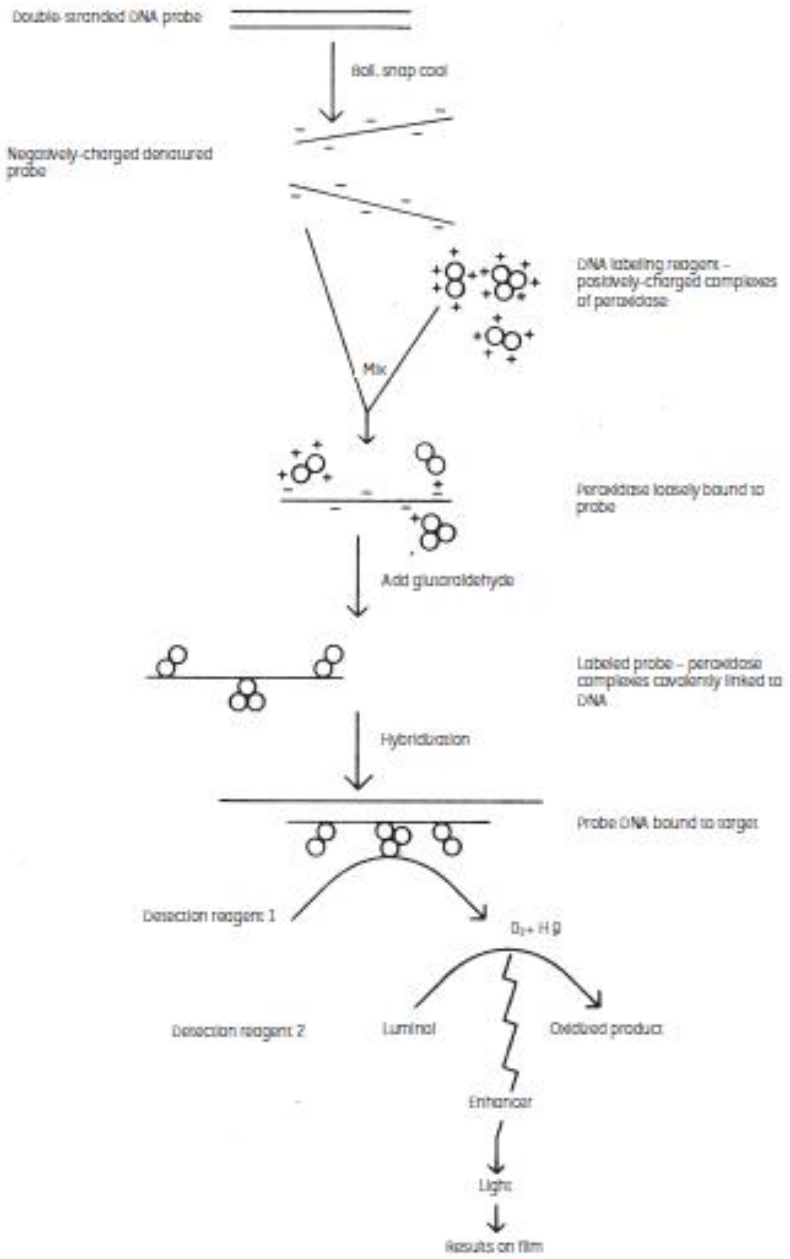
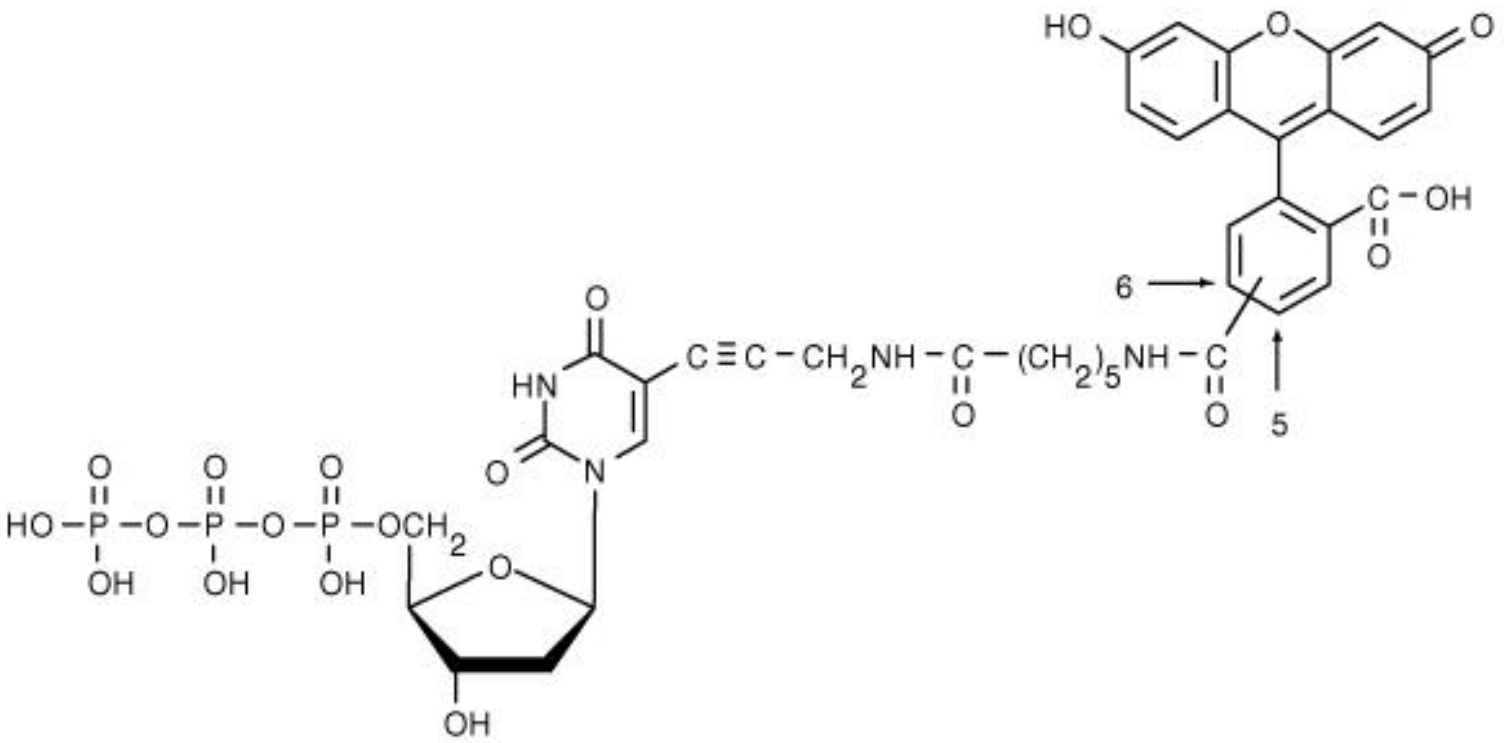
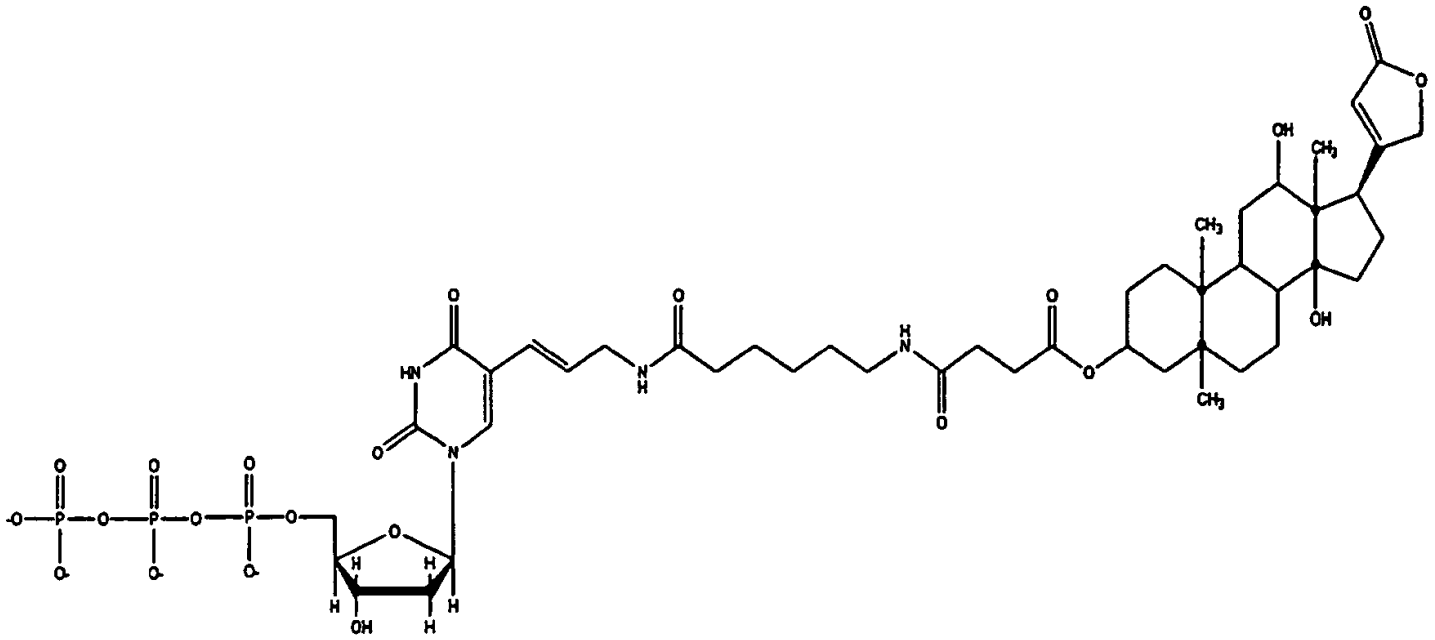


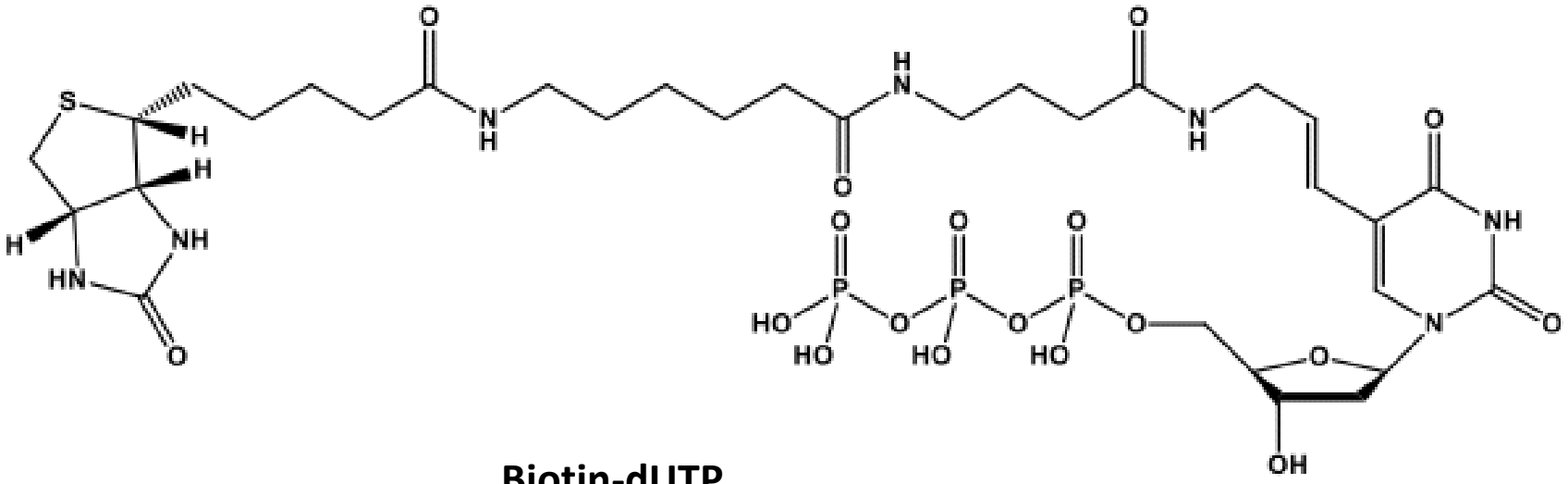
Figure 1. Principles of the ECL direct nucleic acid labeling and detection system.



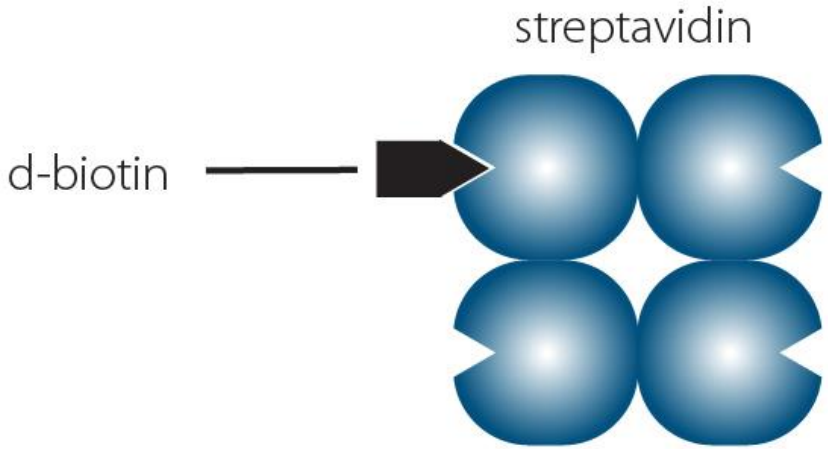
Fluoresceína dUTP



Digoxigenina dUTP



Biotin-dUTP



$K_d \approx 10^{-14}$ mol/L

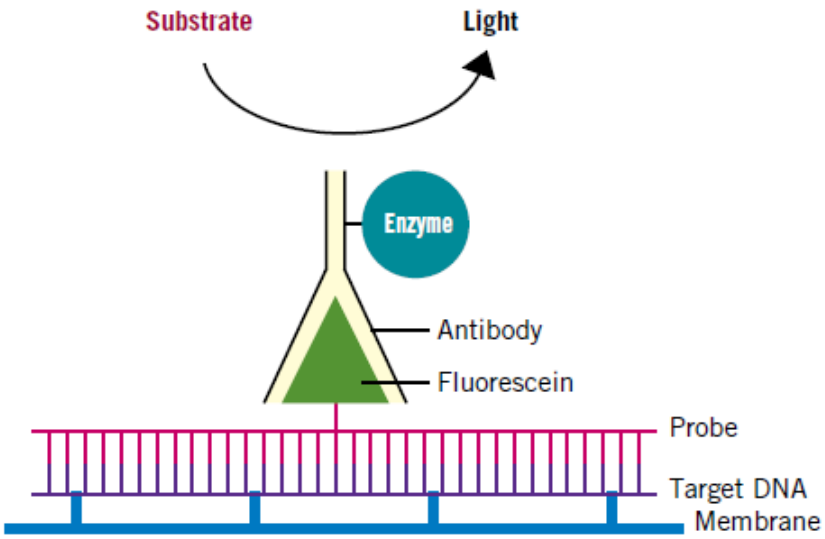


Fig 2. Outline of indirect labelling method.

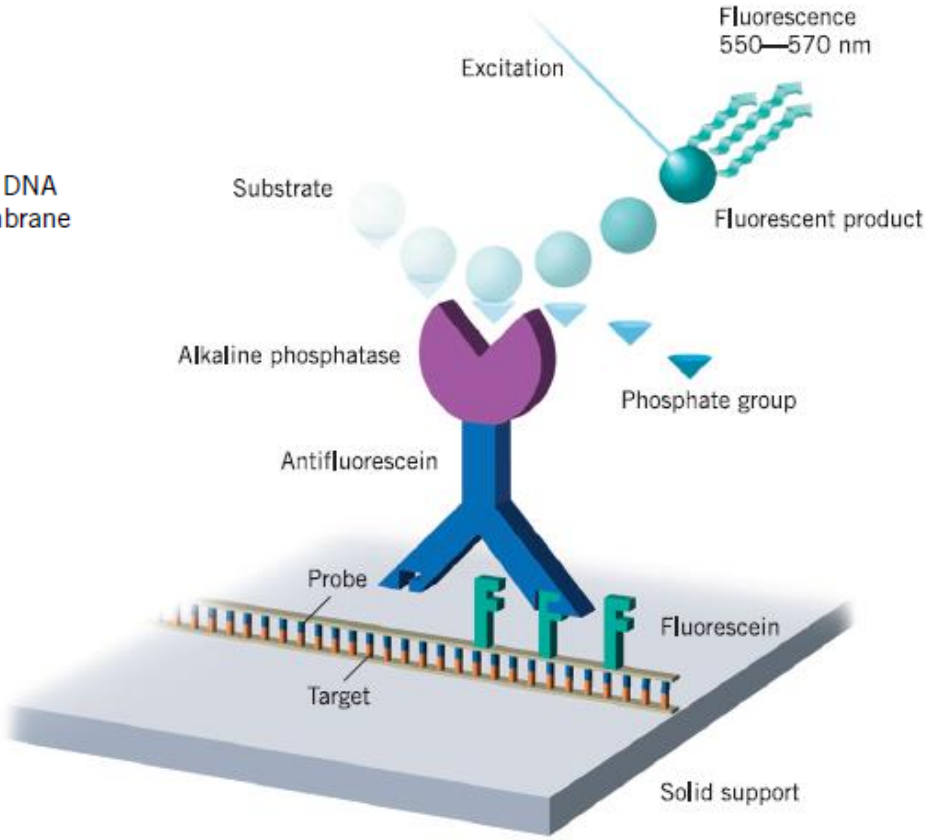
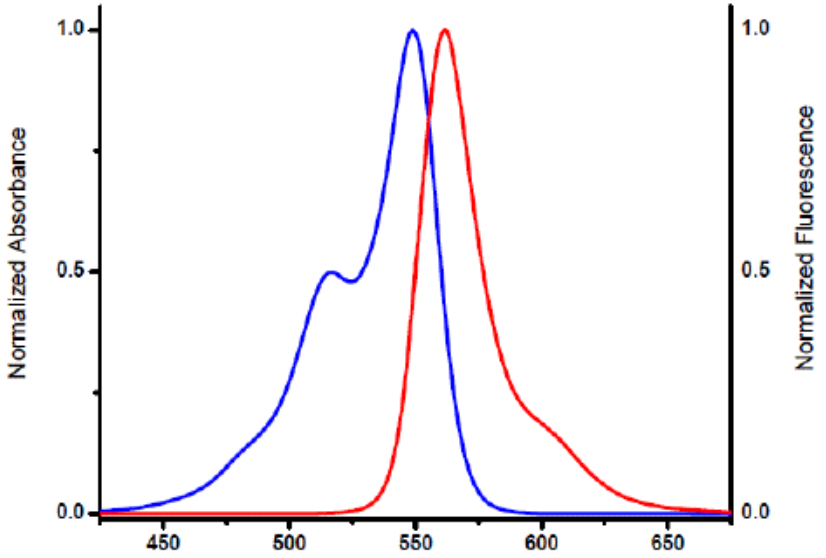


Table 1. Comparison of radioactive and non-radioactive nucleic acid labelling and detection systems.

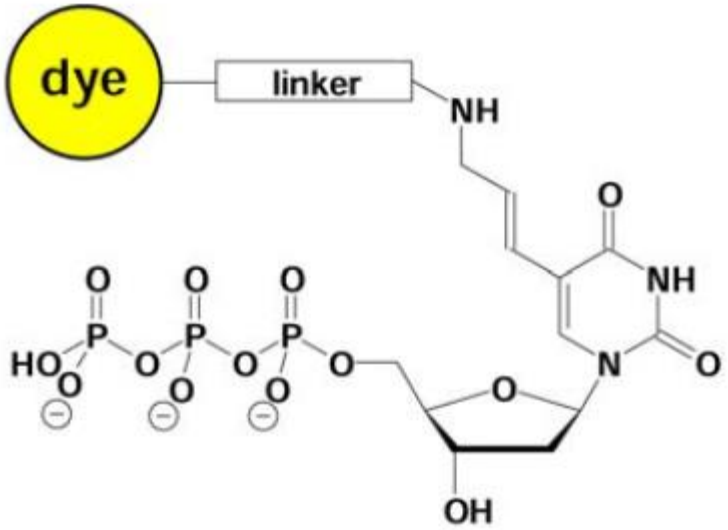
Labelling and detection system	Sensitivity	Application	Probe labelling time	Time from hybridization to detection
^{32}P	Down to 10 fg	All high sensitivity applications	5 min to 3 h	On film 1 h to 1 week
AlkPhos Direct	60 fg	All high sensitivity applications	30 min	1 h
ECL Direct	0.5 pg	High target applications	20 min	1–2 h
Gene Images Random-Prime with CDP-*Star* Detection	50 fg	High sensitivity Northern blots	30 min	3 h
Gene Images 3'-Oligolabelling with CDP-*Star*	0.1 pg	Oligonucleotide screening with stringency control	30 min	3 h
Gene Images Random-Prime with ECF	0.25 pg	Quantification	30 min	3 h
Gene Images 3'-Oligolabelling with ECF	120 pg	Quantification	30 min	3 h
ECL Random-Prime	0.5 pg	Medium target Southern blots with DNA probes	30 min	3h
ECL 3'-End Labelling	0.2 pg	Medium to high target Southern blots with oligonucleotide probes	30 min	3 h



Cy3 excitation and emission spectra.

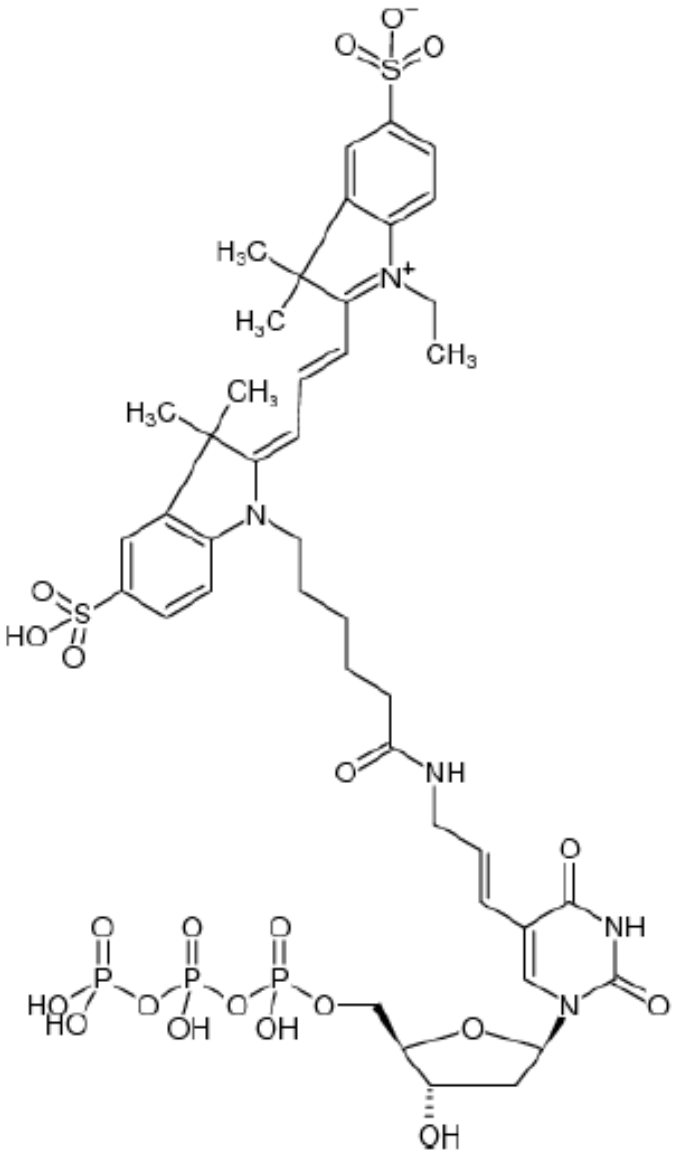
Spectroscopic data
Excitation maximum: λ_{Ex} = 550 nm
Emission maximum: λ_{Em} = 570 nm

Structure



Cy3-dUTP, the dye is attached via an optimized linker to aminoallyl-dUTP.

Molecular Structure:



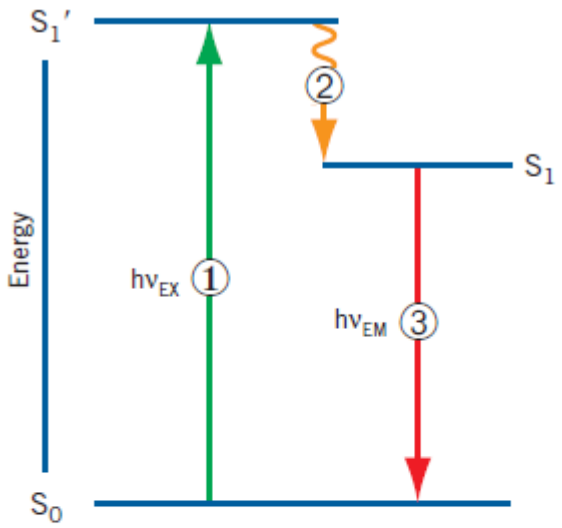
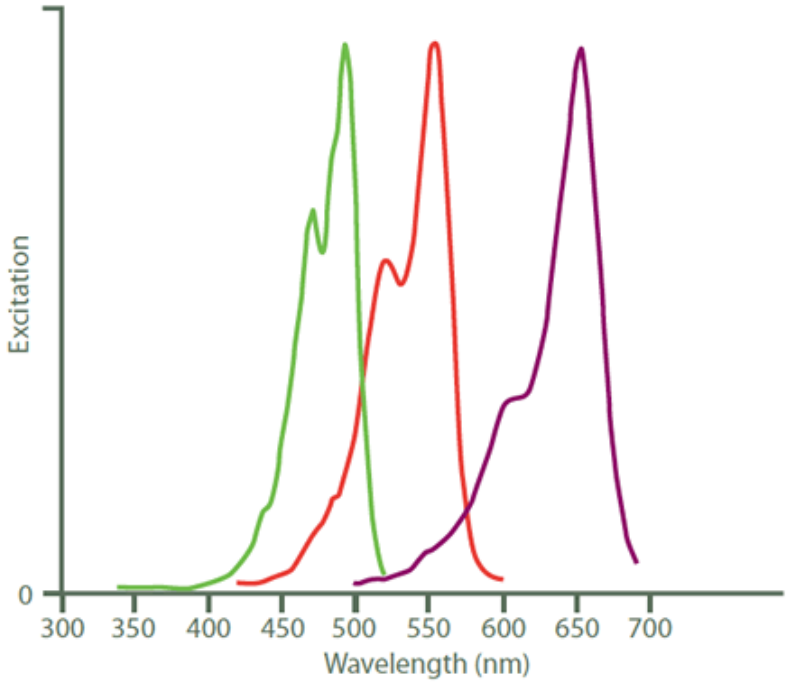
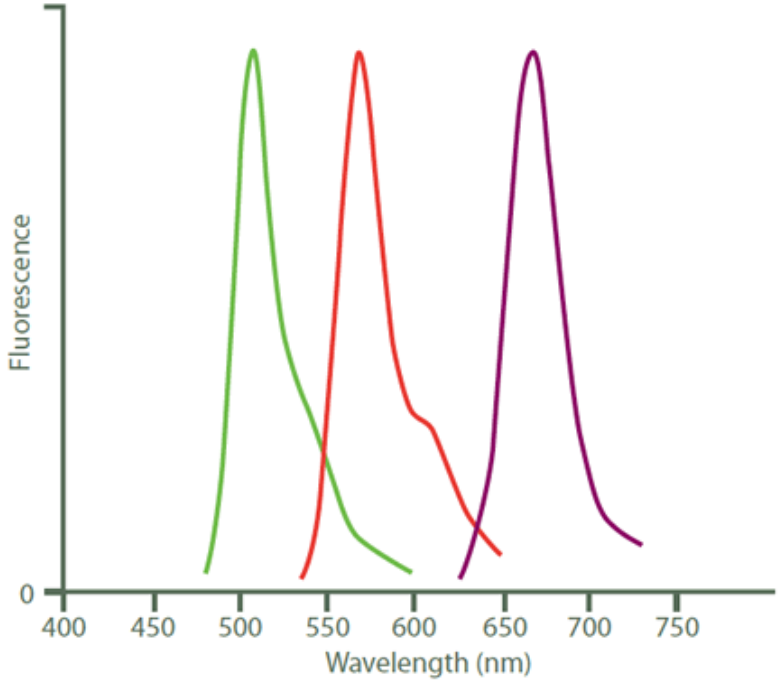


Fig 2. Jablonski diagram illustrating the processes involved in creating an excited electronic singlet state by optical absorption and subsequent emission of fluorescence. ① Excitation; ② Vibrational relaxation; ③ Emission.


Excitation
0
300 350 400 450 500 550 600 650 700
Wavelength (nm)


Fluorescence
0
400 450 500 550 600 650 700 750
Wavelength (nm)

Recommended PCR assay

20 µl PCR labeling assay

Component	Stock conc.	Amount	Final conc.
High yield buffer without $MgCl_2$	10x	2 µl	1x
$MgCl_2$ stock solution	25 mM	1.6 µl	2 mM
dATP	1 mM	2 µl	100 µM
dCTP	1 mM	2 µl	100 µM
dGTP	1 mM	2 µl	100 µM
dTTP	1 mM	1 µl	50 µM
Cy3-dUTP	1 mM	1 µl [1)]	50 µM [1)]
forward Primer	10 µM	1 µl	500 nM
reverse Primer	10 µM	1 µl	500 nM
Template DNA		0.1-10 ng	5-500 pg/µl
Taq Pol	5 units/µl	0.2 µl (1 unit)	0.05 units/µl
PCR grade H_2O		Fill up to 20 µl	

1) The optimal final concentration of the labeled nucleotide may vary depending on the application.

Typhoon™

Fig 1. Typhoon FLA 9500 is a high performance, versatile laser scanner for sensitive and quantitative measurements in a multiuser environment.

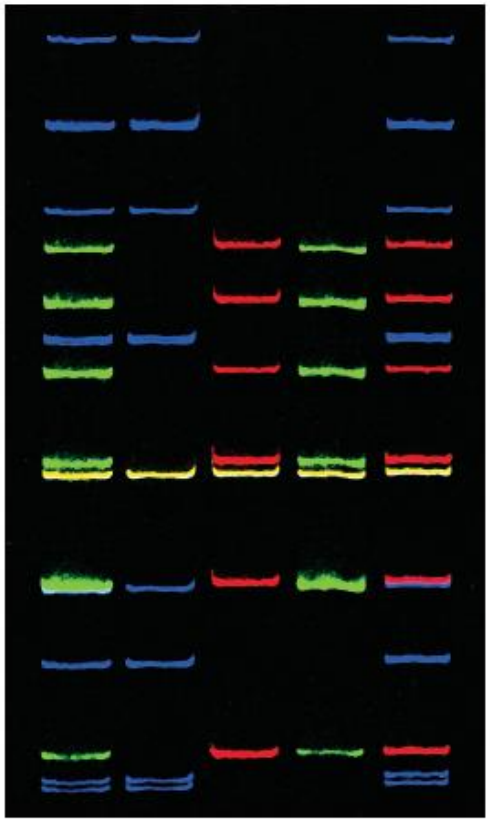


Fig 1. Fluorescently labelled DNA size ladders and PCR products loaded in the same lanes were electrophoretically separated in a polyacrylamide gel and imaged using Typhoon™ 8600 scanner. Fluorescein (green), Cy™3 (yellow), ROX™ (blue) and Cy5 (red) labels were used in amounts varying from 0.25 to 5 fmol per band.

Fig 19. Three-colour gel image of a DNA in-lane sizing experiment. The fluorochromes used were TAMRA™ (yellow), ROX (red), and fluorescein (green). The ROX and TAMRA bands are labelled DNA size ladders. The fluorescein fragments are PCR products of unknown size. Typhoon 8600 was used for image acquisition.

Microarrays

Se depositan decenas de miles de secuencias de DNA en una superficie pequeña, generalmente de vidrio (DNA chip). Las técnicas de *Northern blot* o qRT-PCR permiten estudiar unos pocos genes. Mediante *microarrays* se obtiene un perfil global de expresión.

Aplicaciones:

Cáncer: Clasificación de tumores, identificación genes supresores de tumores, biomarcadores, identificación de genes asociados con resistencia, *screening* de drogas etc

Asociación de genes con enfermedad.

Identificación de microorganismos patógenos, resistencia a antibióticos etc.

Genes expresados diferencialmente.

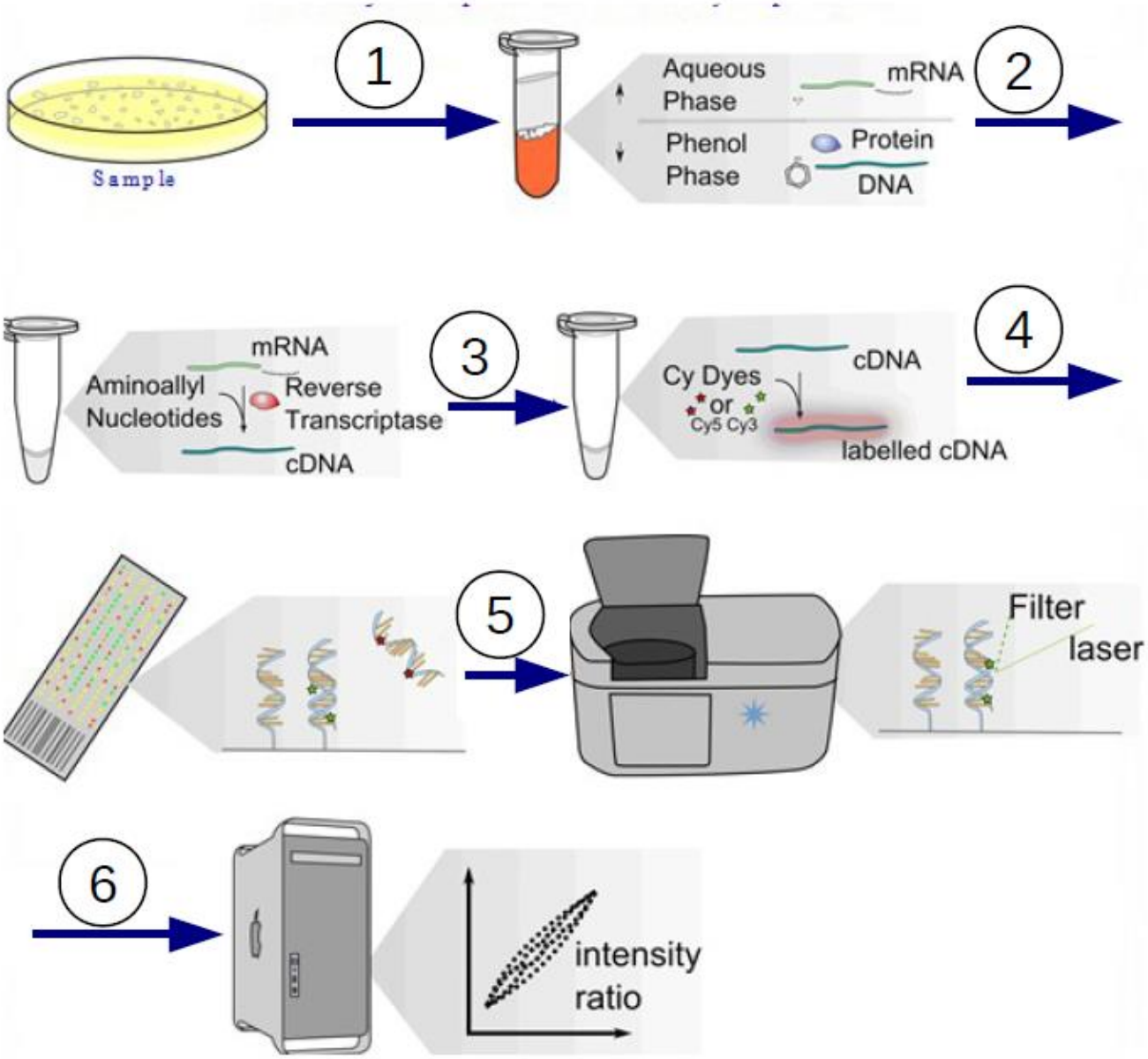
Limitaciones:

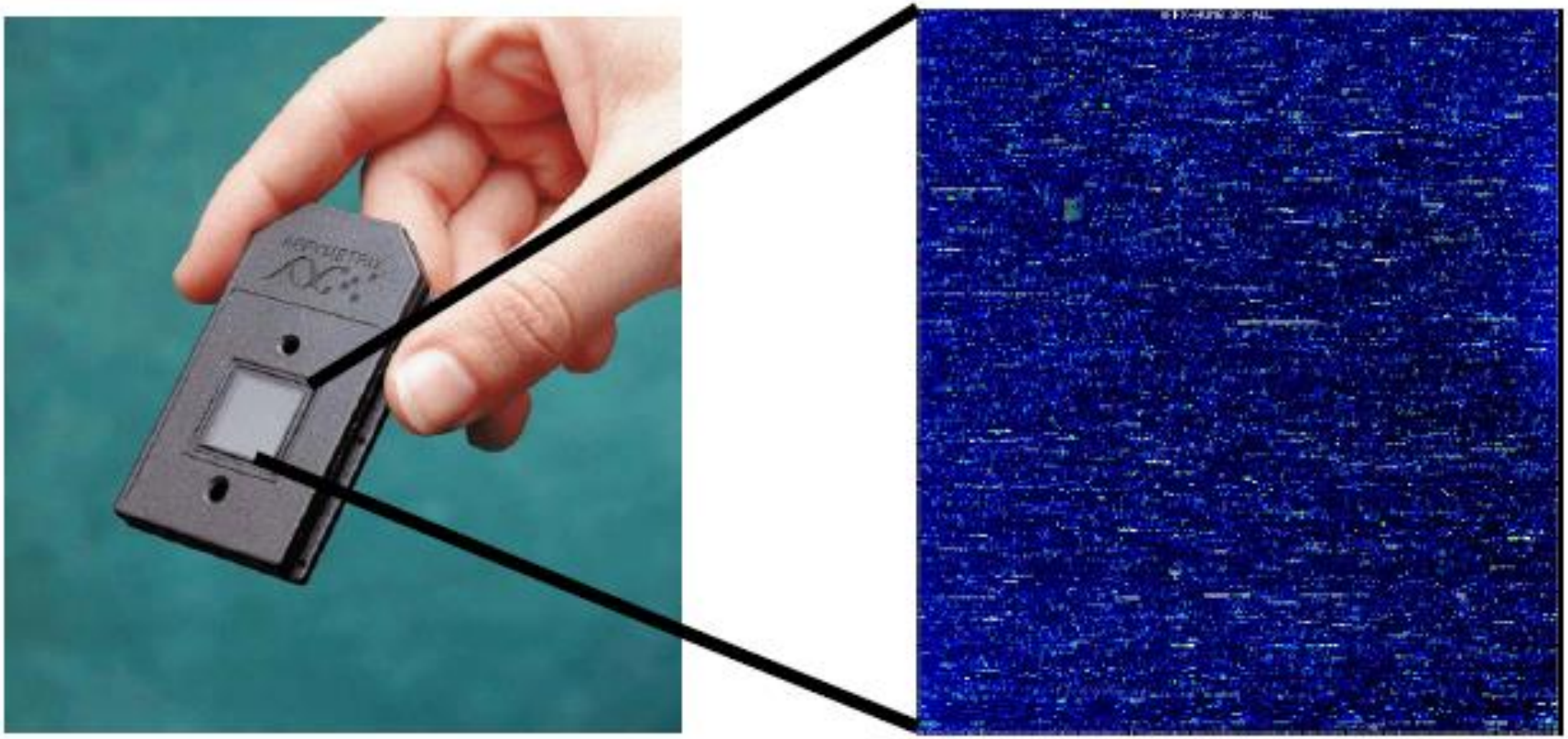
Solamente se detectarán secuencias para las cuales el *array* fue diseñado para detectar.

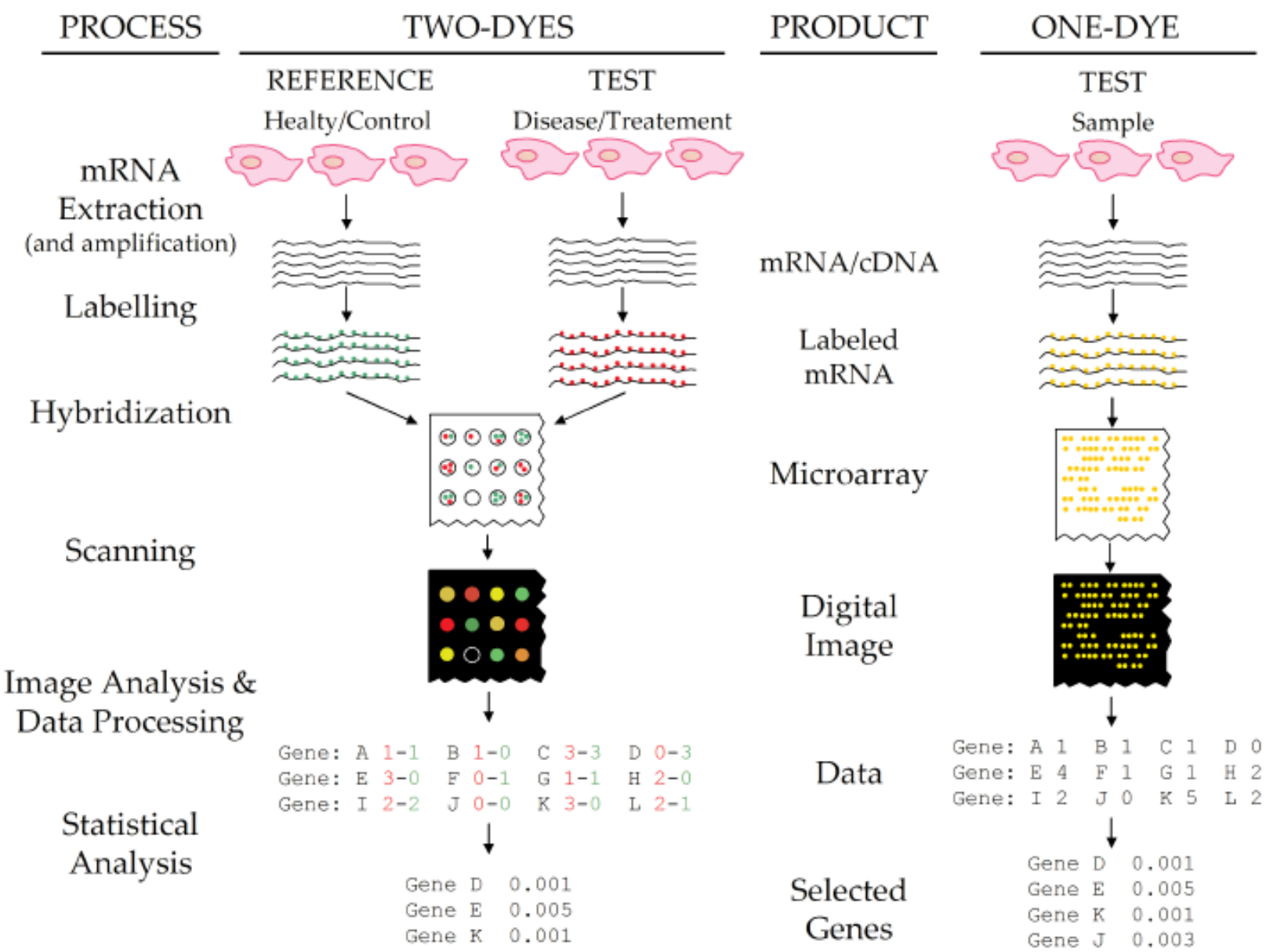
Muchas isoformas de cada gen.

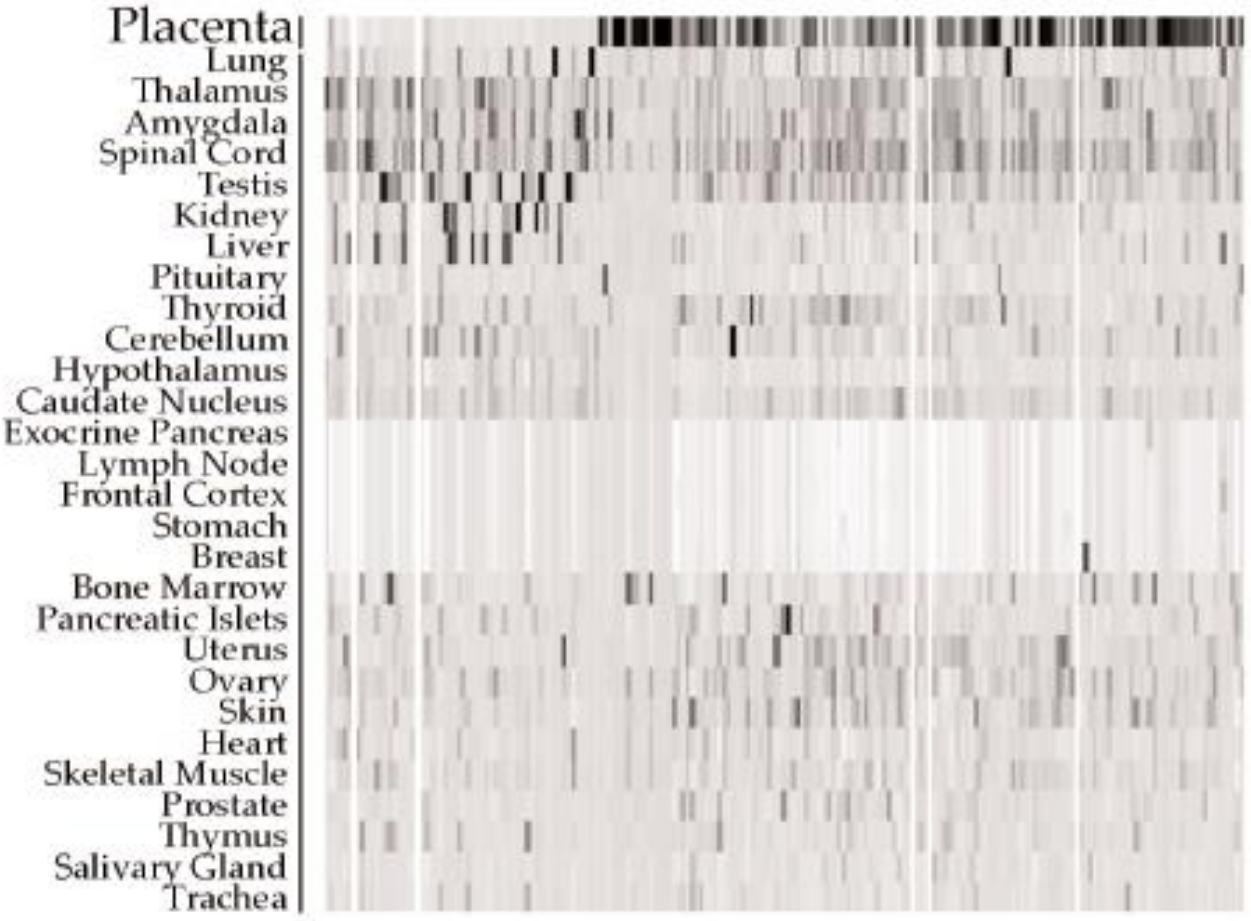
La cuantificación es indirecta.

Especificidad.


1
Sample
Aqueous Phase
mRNA
Phenol Phase
Protein
DNA
2
3
mRNA
Aminoallyl Nucleotides
Reverse Transcriptase
cDNA
4
cDNA
Cy Dyes
or
Cy5 Cy3
labelled cDNA
5
Filter
laser
6
intensity ratio




PROCESS
TWO-DYES
PRODUCT
ONE-DYE
REFERENCE
Healty/Control
TEST
Disease/Treatement
TEST
Sample
mRNA Extraction (and amplification)
Labelling
Hybridization
Scanning
Image Analysis & Data Processing
Statistical Analysis
mRNA/cDNA
Labeled mRNA
Microarray
Digital Image
Data
Selected Genes
Gene: A 1-1 B 1-0 C 3-3 D 0-3
Gene: E 3-0 F 0-1 G 1-1 H 2-0
Gene: I 2-2 J 0-0 K 3-0 L 2-1
Gene D 0.001
Gene E 0.005
Gene K 0.001
Gene: A 1 B 1 C 1 D 0
Gene: E 4 F 1 G 1 H 2
Gene: I 2 J 0 K 5 L 2
Gene D 0.001
Gene E 0.005
Gene K 0.001
Gene J 0.003


Placenta
Lung
Thalamus
Amygdala
Spinal Cord
Testis
Kidney
Liver
Pituitary
Thyroid
Cerebellum
Hypothalamus
Caudate Nucleus
Exocrine Pancreas
Lymph Node
Frontal Cortex
Stomach
Breast
Bone Marrow
Pancreatic Islets
Uterus
Ovary
Skin
Heart
Skeletal Muscle
Prostate
Thymus
Salivary Gland
Trachea

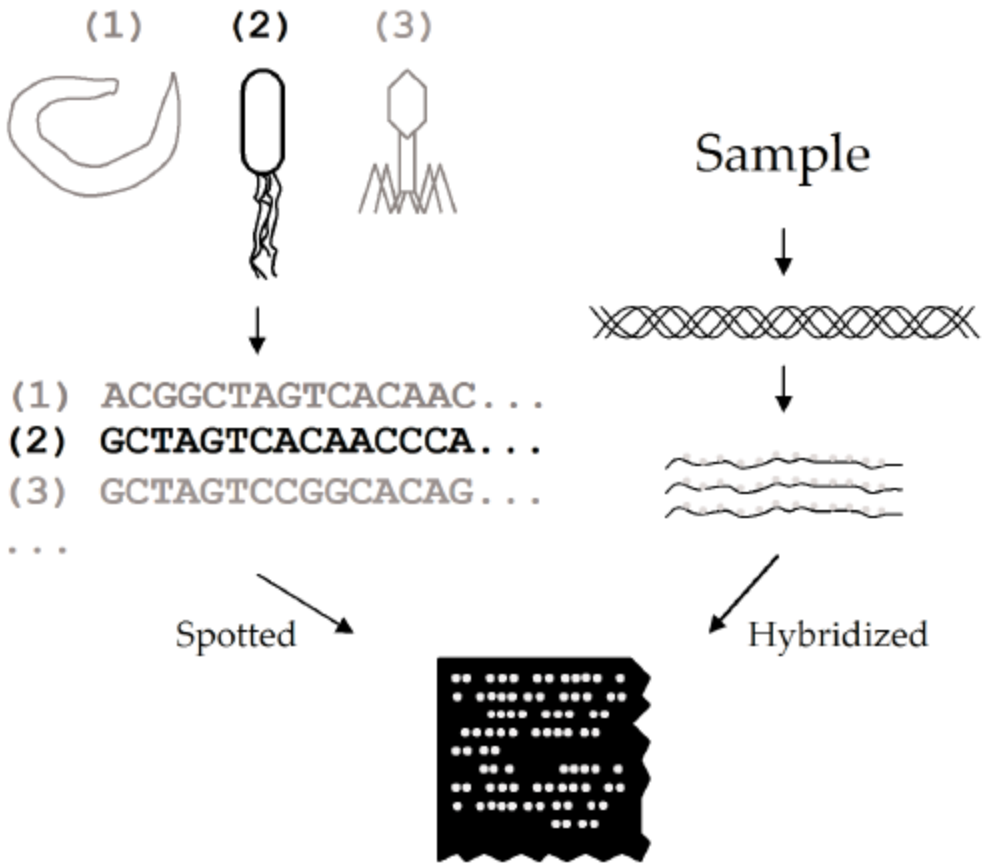


Figure 10. Multi-Pathogen Detection Using DNA Microarrays. Specific DNA sequences from disease-causing micro-organisms can be spotted on a microarray for pathogen detection.

PLOS ONE

RESEARCH ARTICLE

Identification of microRNAs with Dysregulated Expression in Status Epilepticus Induced Epileptogenesis

Mykaella Andrade de Araújo[1], Thalita Ewellyn Batista Sales Marques[1], Shirley Octacílio-Silva[2], Carmem Lúcia de Arroxelas-Silva[1], Marília Gabriella Alves Goulart Pereira[3], José Eduardo Peixoto-Santos[4], Ludmyla Kandratavicius[4], João Pereira Leite[4], Norberto Garcia-Cairasco[5], Olagide Wagner Castro[6], Marcelo Duzzioni[6], Geraldo Aleixo Passos[7], Maria Luisa Paçó-Larson[8], Daniel Leite Góes Gitaí[1]*

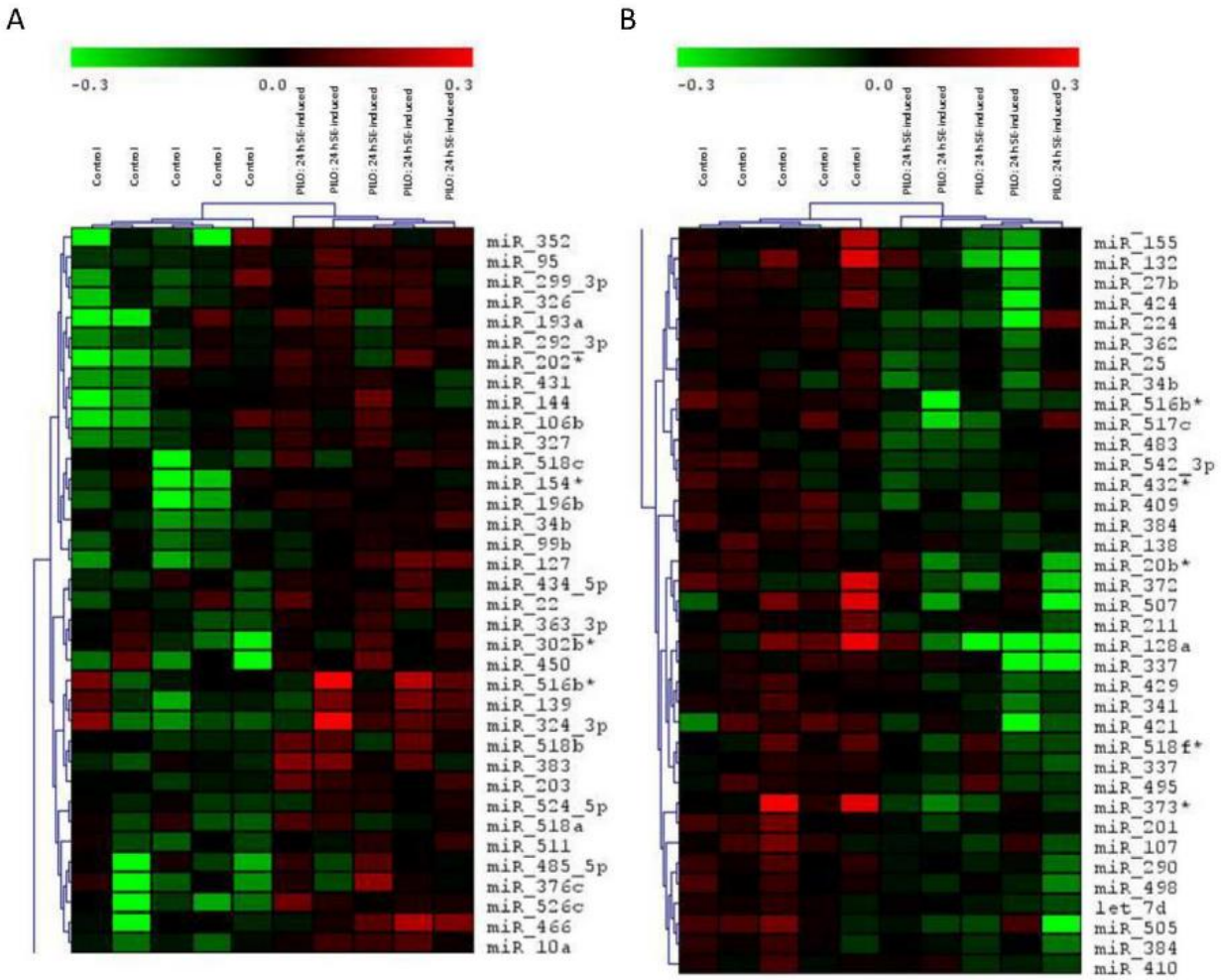


Fig 1. Hierarchical clustering of the 73 miRNA with significantly different expression in 24h post-SE hippocampus versus control experiment. Rows represent individual genes, and columns represent individual samples. The colorgram depicts high (red), average (black) and low (green) expression levels. A) Upregulated microRNAs. B) Downregulated microRNAs. (n = 5 for both H-PILO and control groups).