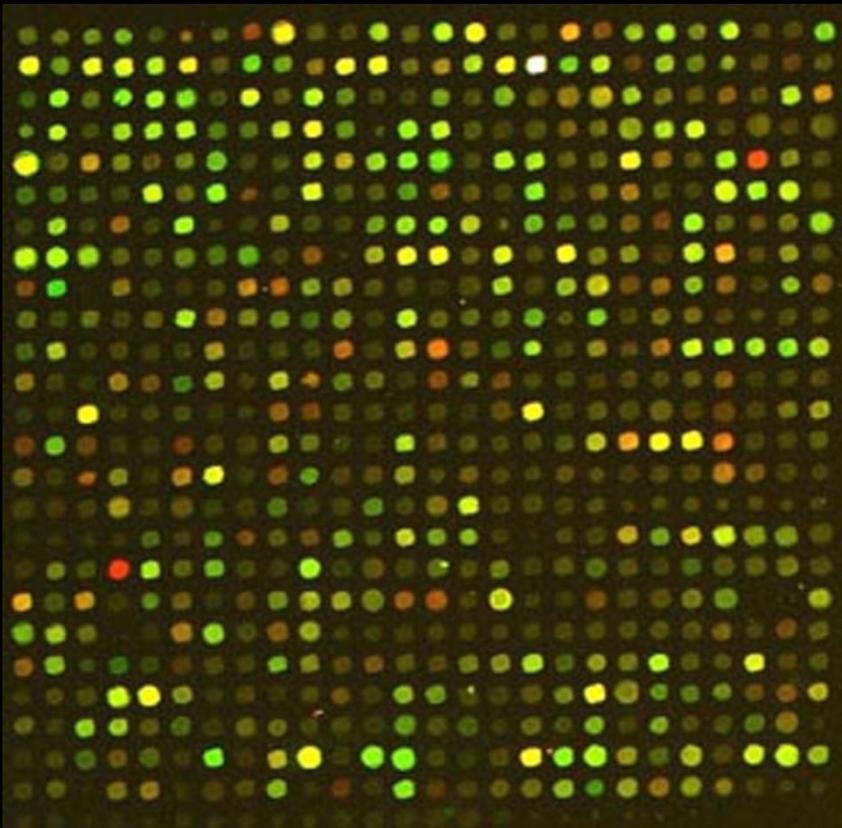
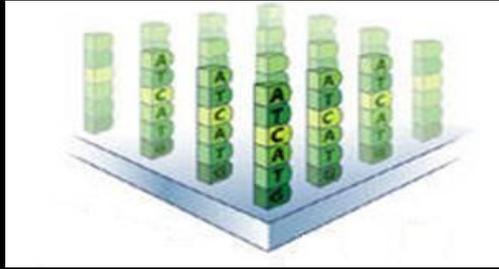


MICROARRAY AND SPLICING



Principio generale:

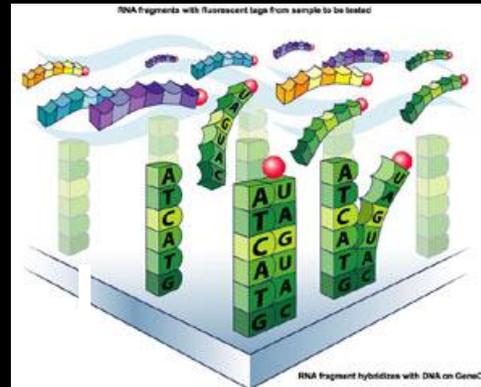


Probes su
vetrino

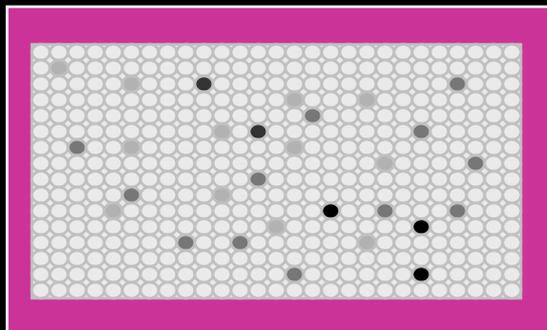
Targets marcati
radioattivamente o con
fluorofori



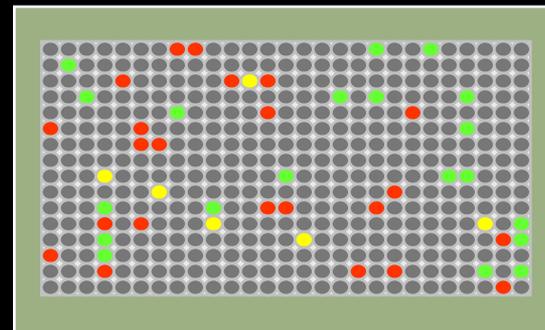
IBRIDAZIONE



Lettura dei Segnali, Quantificazione ed Interpretazione



radioattivo



fluorescenza

Possibilità di
confrontare due
campioni in un
unico passaggio



Oligonucleotide Array

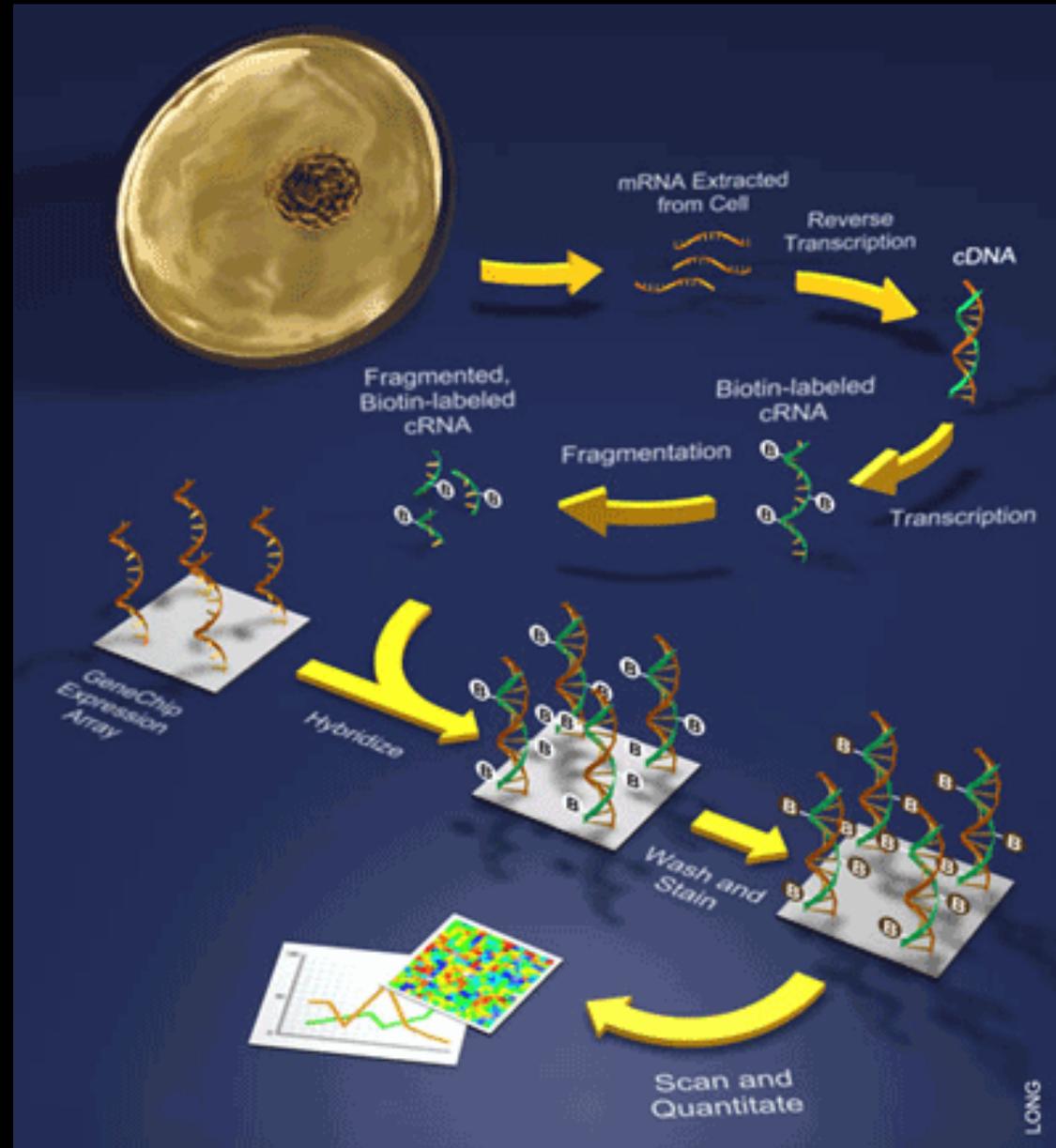
sonde 25bp long

11-16 sonde per ogni gene/esone

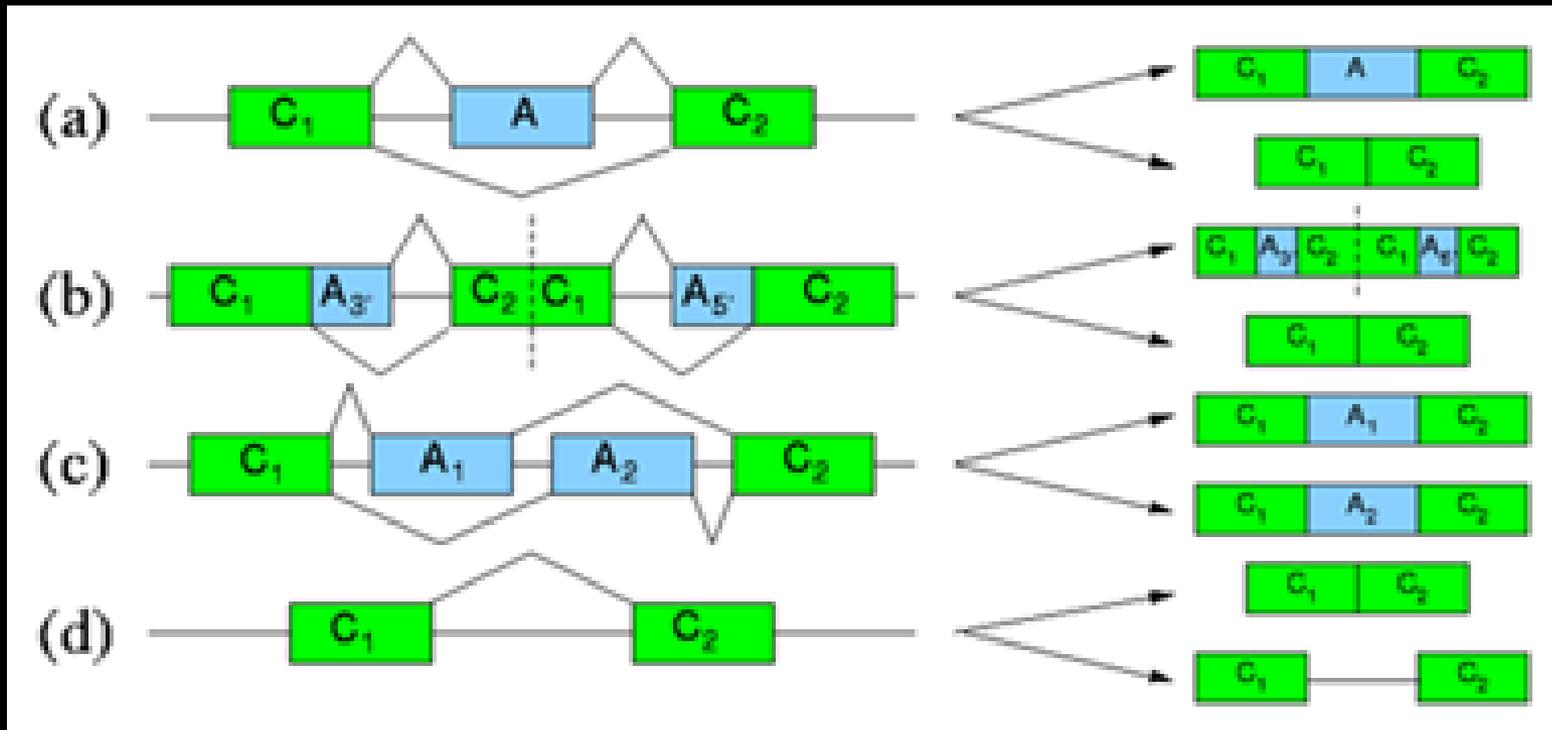
non si può sfruttare l'ibridazione per competizione

Usi:

1. Quantificazione della quantità di mRNA (espressione genica)
2. Comparazione di 2 campioni ibridati su due array separati

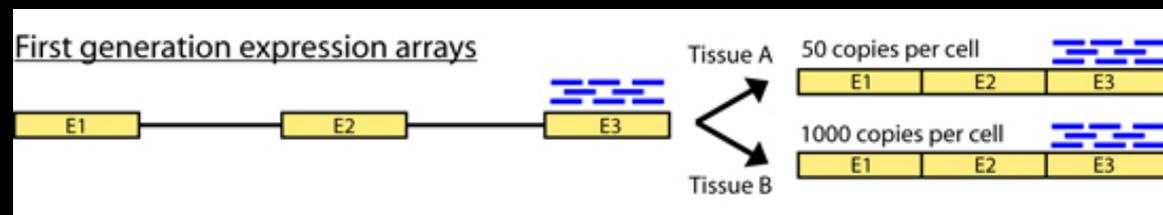


Eventi di Splicing

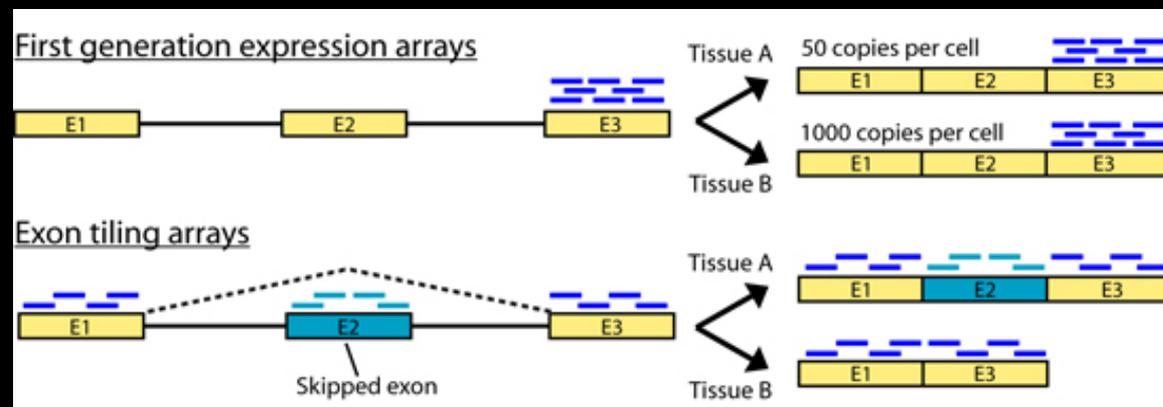


- a) exon inclusion/exclusion
- b) alternative 3'/5' splice site
- c) mutually excluded exons
- d) intron inclusion/exclusion

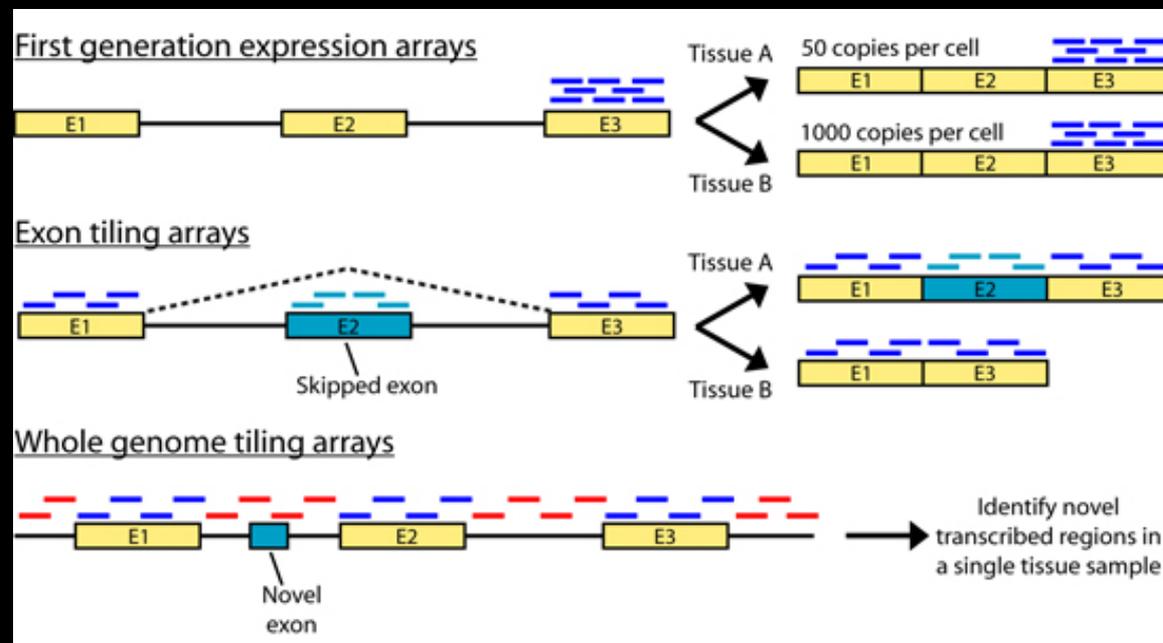
Disegno sonde per splicing array



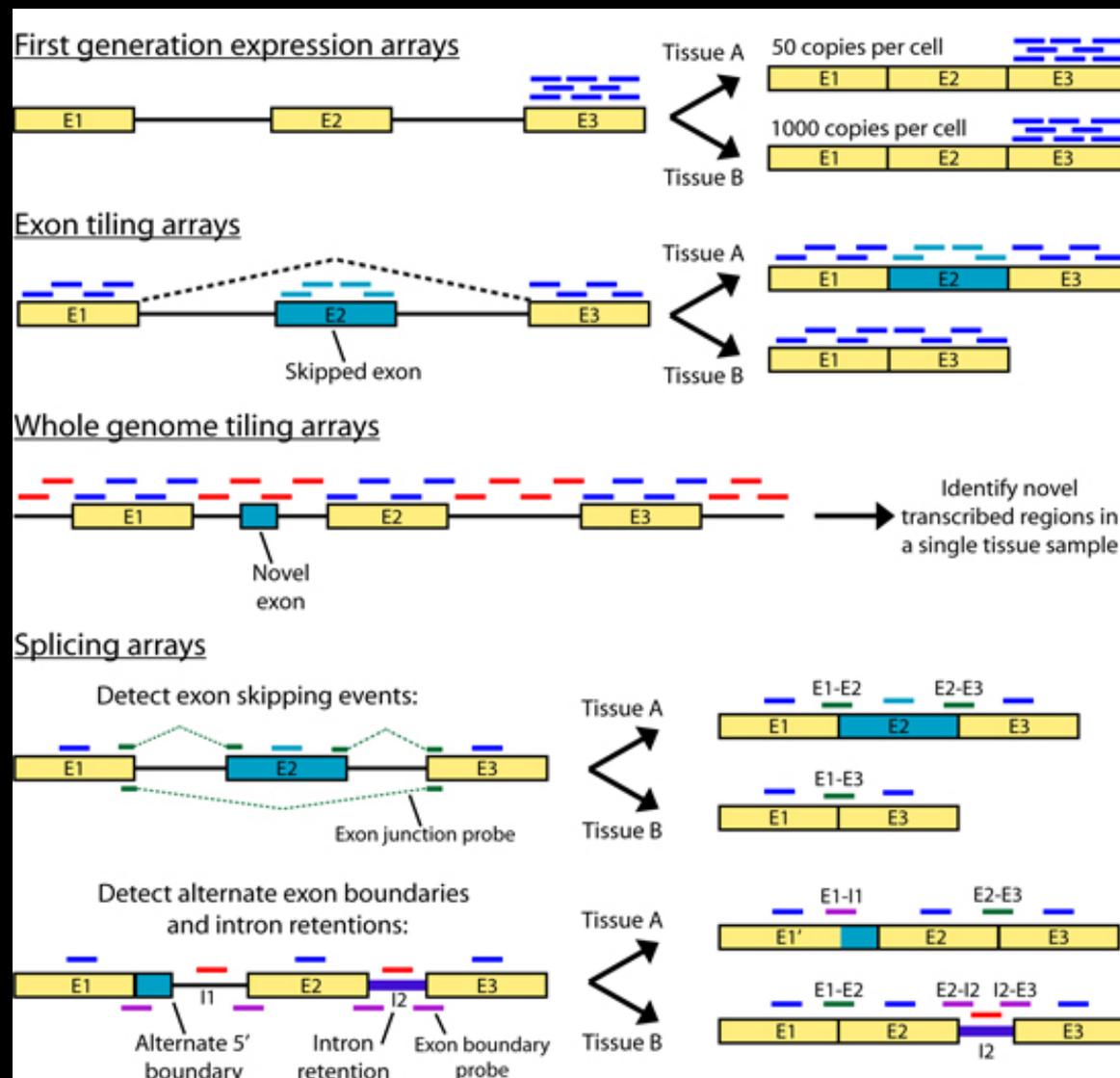
Disegno sonde per splicing array



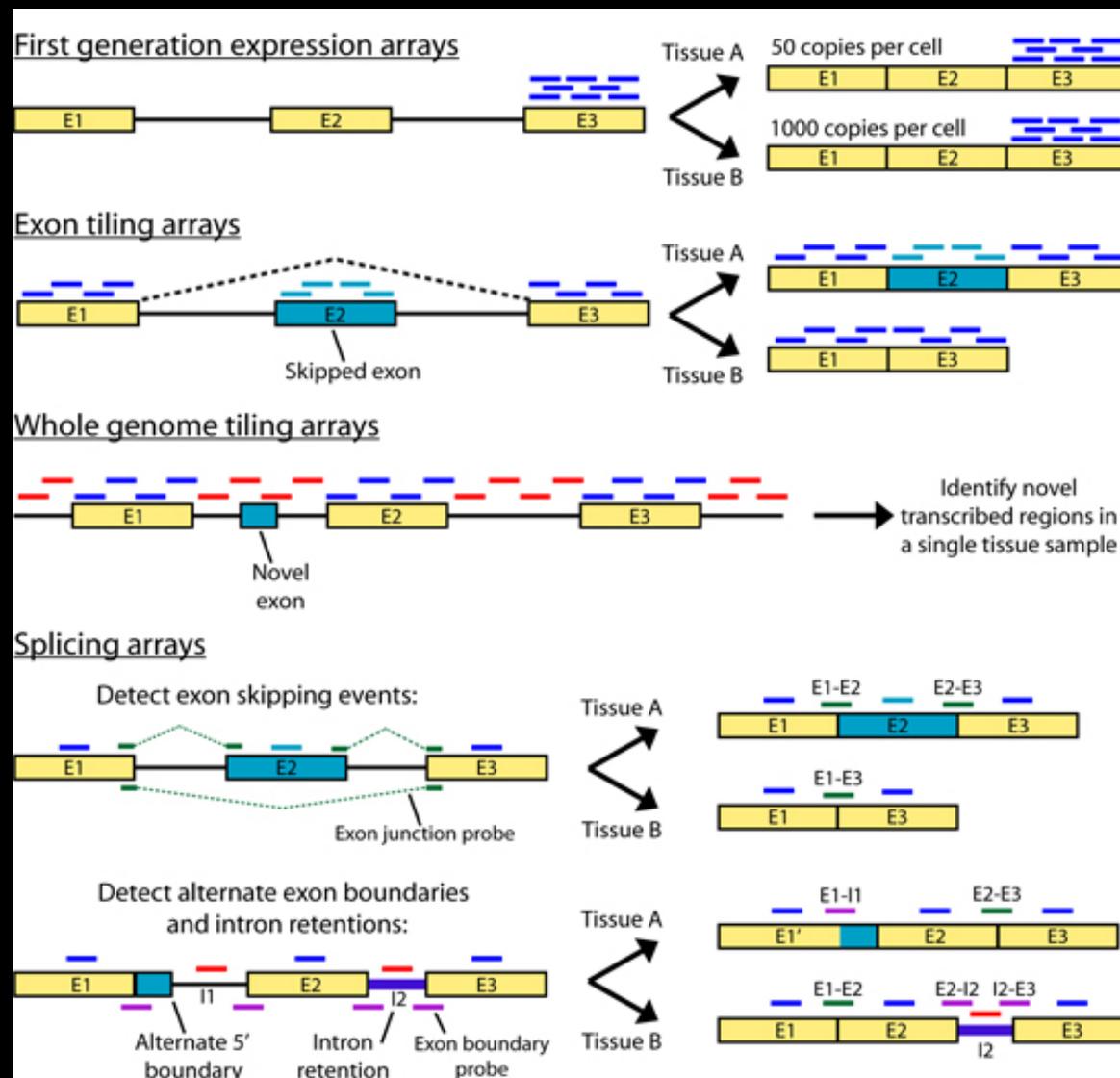
Disegno sonde per splicing array



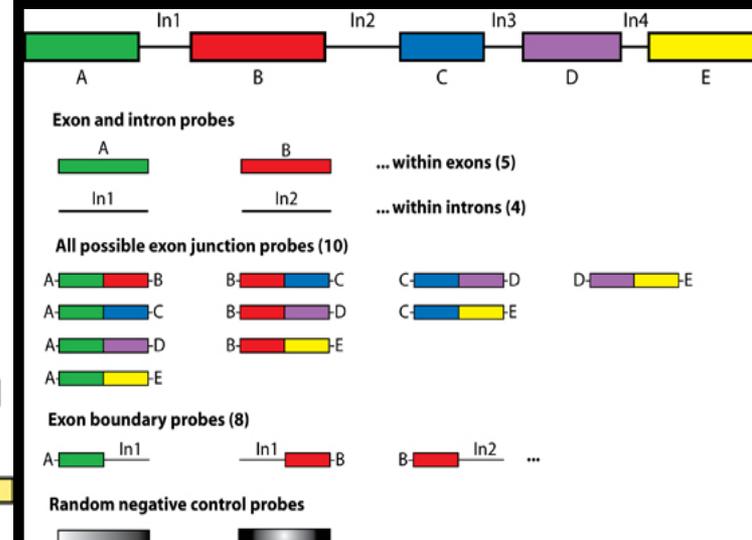
Disegno sonde per splicing array



Disegno sonde per splicing array

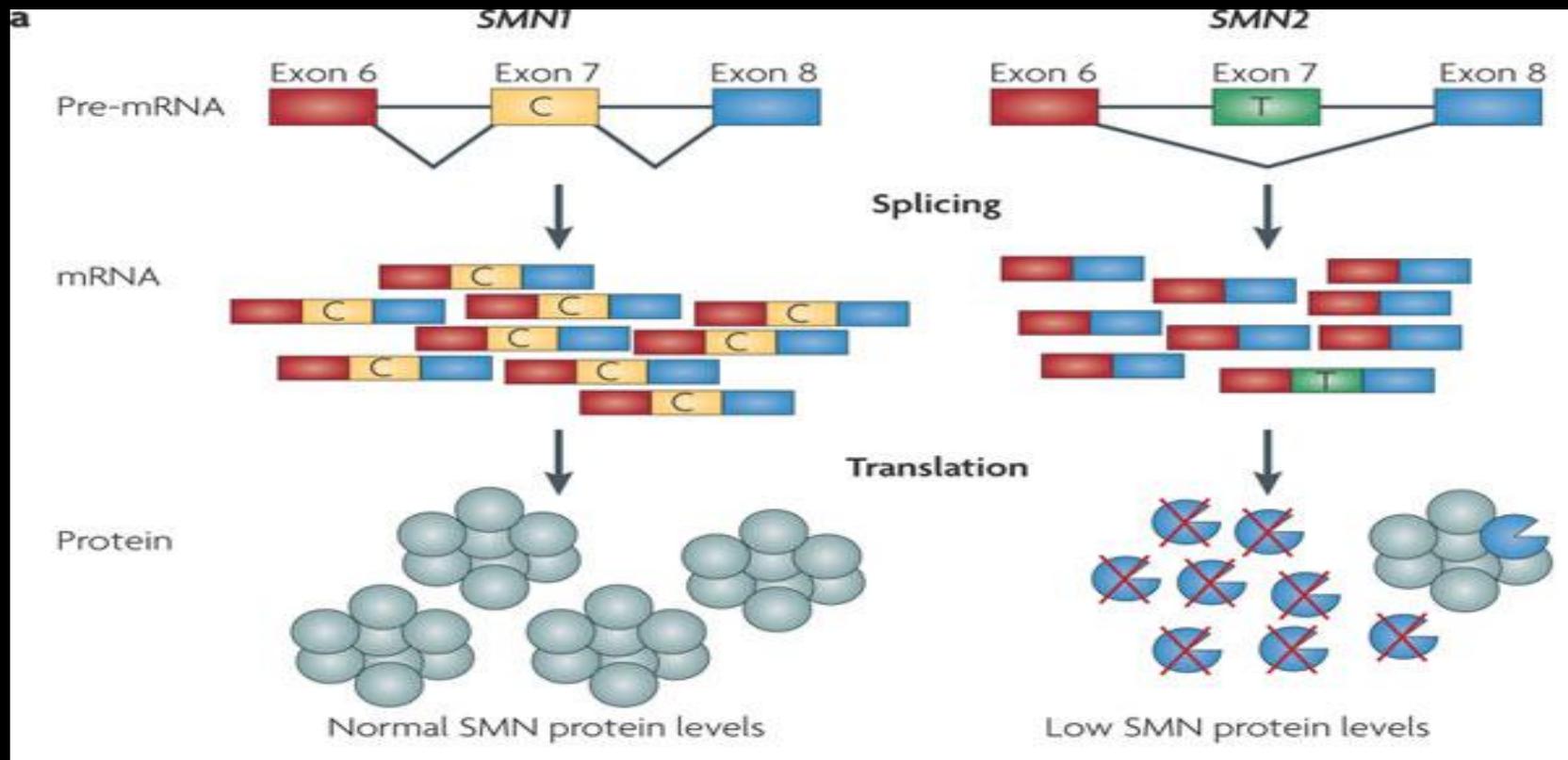


ALEXA array



Alternative Splicing Events Are a Late Feature of Pathology in a Mouse Model of Spinal Muscular Atrophy

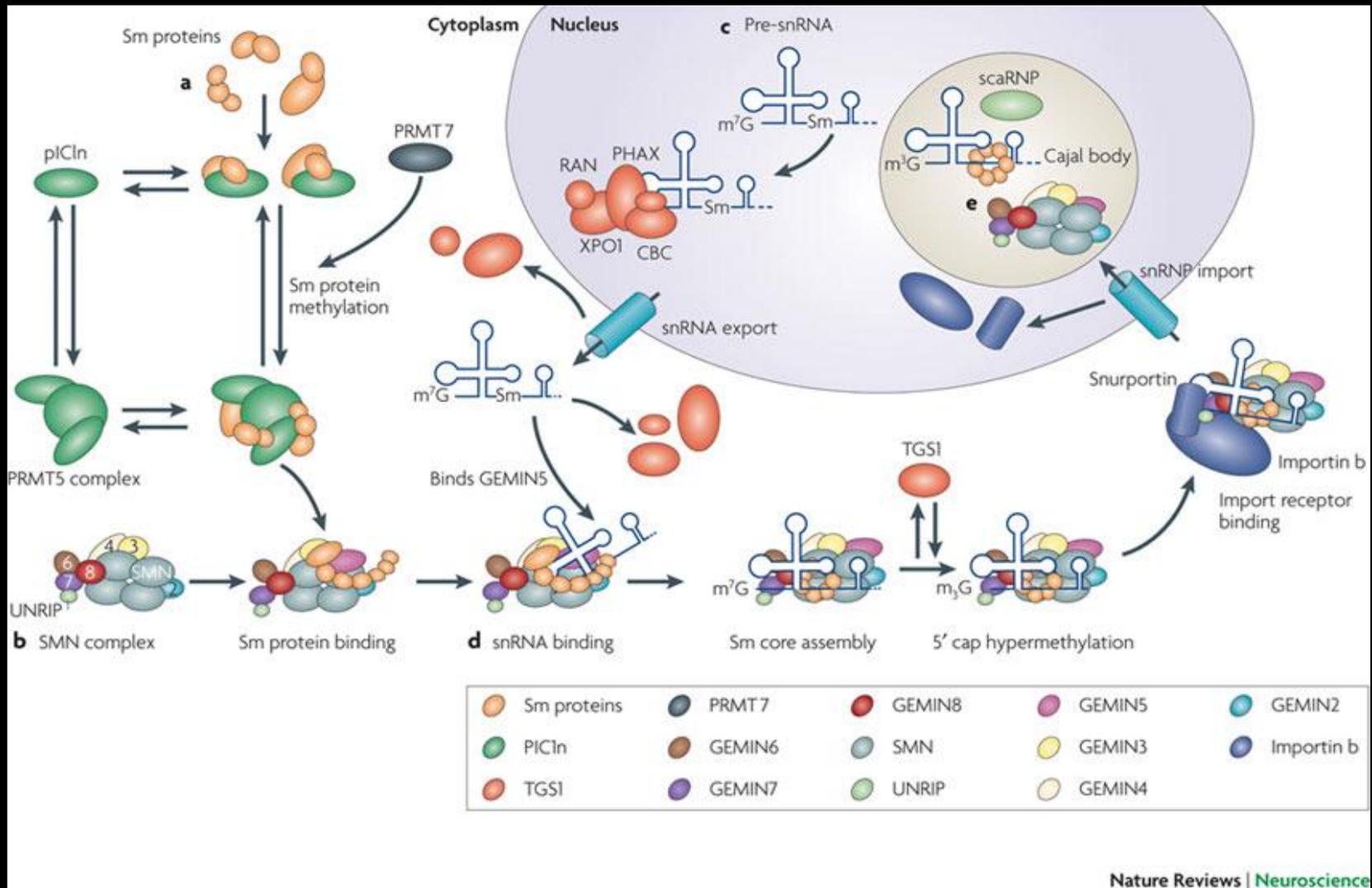
Dirk Bäumer¹, Sheena Lee¹, George Nicholson², Joanna L. Davies², Nicholas J. Parkinson¹, Lyndsay M. Murray³, Thomas H. Gillingwater³, Olaf Ansorge⁴, Kay E. Davies¹, Kevin Talbot^{1,5*}



Deletion of SMN1 gene → reduction of SMN protein level

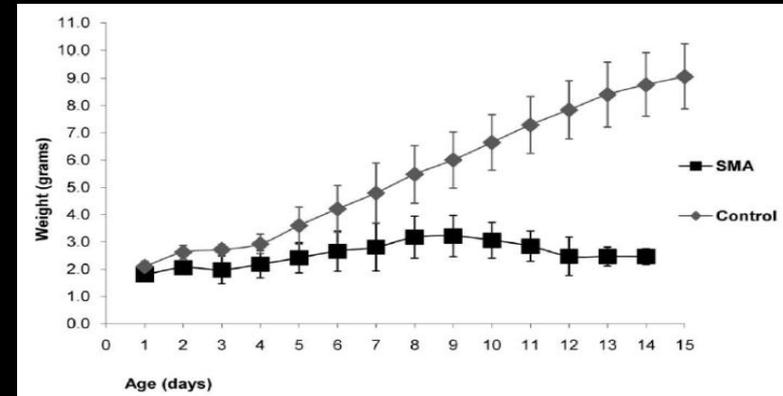
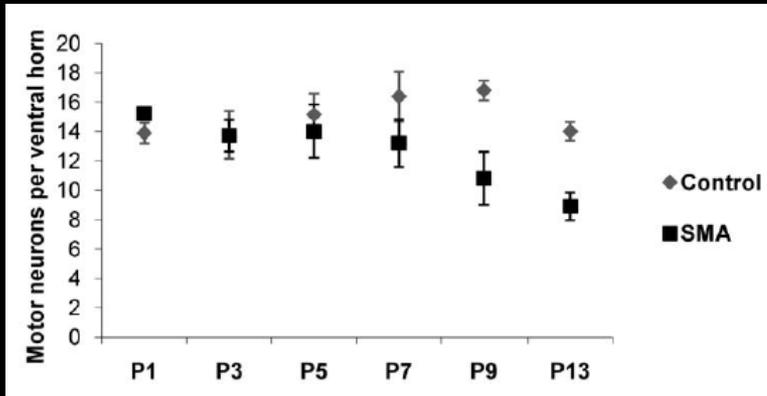
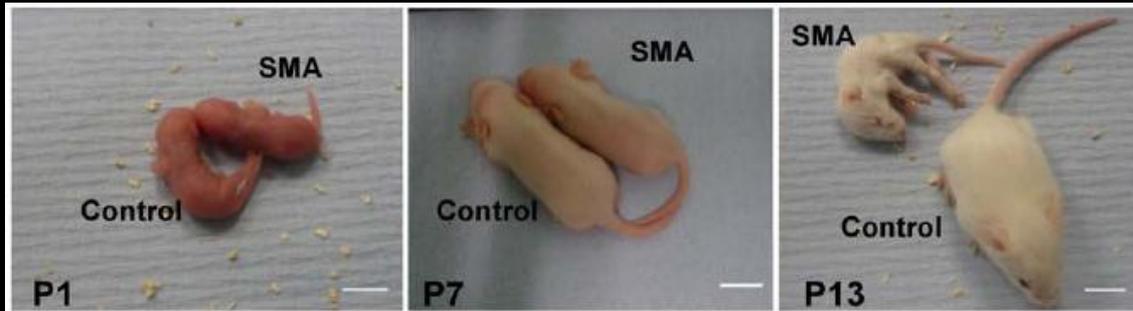
Paralogue SMN2 gene produce small amount of SMN protein

Correlation between SMN deficiency and motor neuron loss in SMA is poorly understood



SMN protein has important role in assembly of snRNP → splicing and gene expression

Dati preliminari e scopo

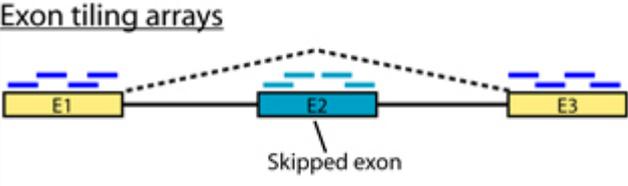


Le maggiori differenze si manifestano nelle fasi tardive della malattia

SMN deficiency ha effetto sull'espressione genica???

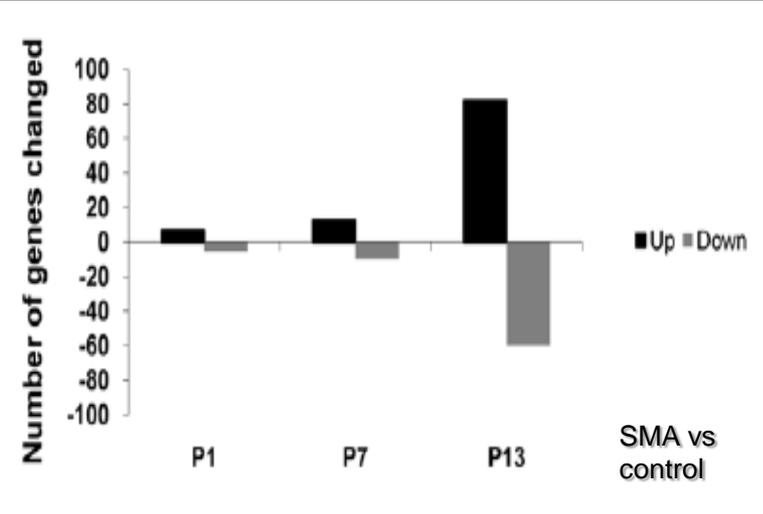
Eventi di splicing aberranti sono la causa della SMA???

Risultati

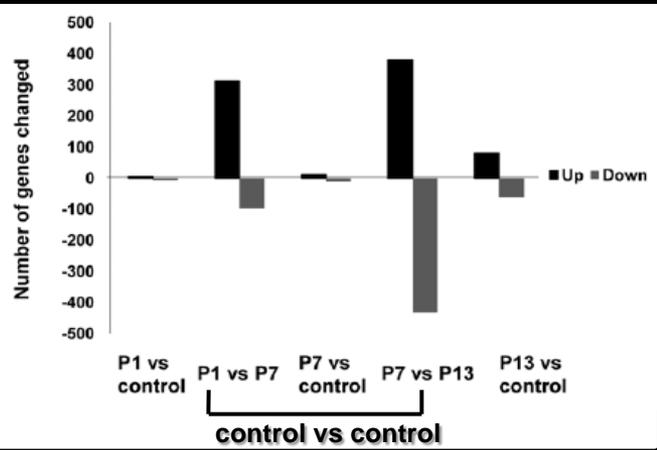


1 campione:1 chip:stadio di sviluppo
 espressione genica ed eventi di splicing

Global transcriptome changes:

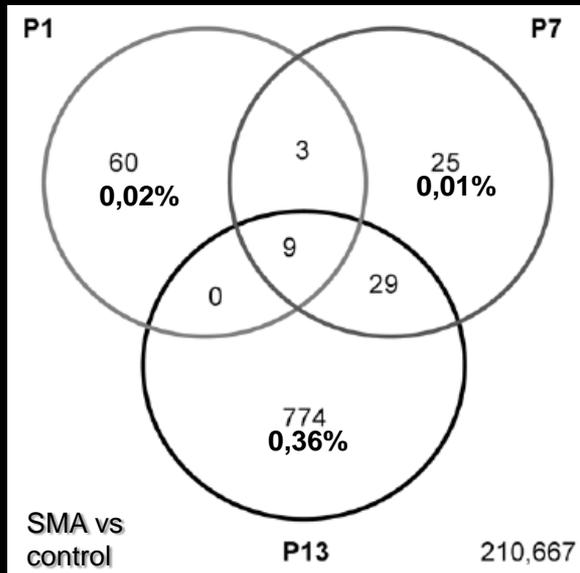


I cambiamenti avvengono nelle fasi tardive della malattia



Nel periodo post natale, nei topi wt, avvengono i maggiori cambiamenti a livello di espressione genica

Changes in splicing:



Gli eventi di splicing alternativo associato a SMA avvengono in prevalenza durante gli stadi avanzati della malattia

Discussione

Eventi di splicing alternativo associati a SMA avvengono tardivamente nella malattia, non contribuendo così nella patogenesi

I cambiamenti nello splicing “non sono dovuti dalla SMN deficiency, ma dalla progressione della malattia (stress cellulare)”

