Exploring Reverse Transcription for DNA MicroArrays

ATLANTIC MICROARRAY FACILITY

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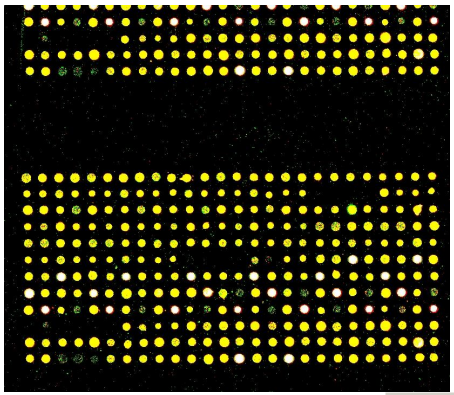
ABSTRACT

We designed 96 un-modified 50-mer DNA oligonucleotides, one for every 2 kb of the longest human genes known: Titin, Nebulin and Obscurin, all expressed in human muscle (Gene length ~100kb). DNA oligonucleotide targets for positive (α-Actin) and negative controls (β-Actin) for human muscle tissue were included and spotted on epoxide-coated glass slides at 1 - 50μM concentration to create a DNA microarray.
Oligo-dT, random and non-priming strategies were explored for reverse transcription (RT). Results were determined by microarray spot fluorescence analysis.
Our results suggest that random priming is the optimal method for expression analysis of long genes. This method of RT may be appropriate for alternatively spliced genes and for genes without unique probes in the 3'-region.

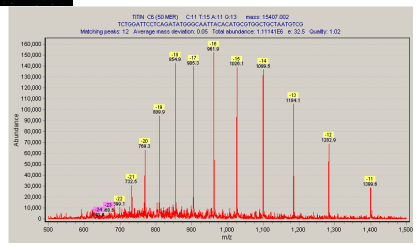
Methods: Un-modified 50-mer oligonucleotides (96 number count), synthesised at the Atlantic Microarray Facility, Beauséjour Medical Research Institute, Moncton, Canada, were used in this study. All synthesised oligonucleotides were analysed for quality by LC/ ES-MS on an Agilent 1100 LC/MSD system. Oligonucleotide solutions (30μM in Schott-Nexterion Spot buffer) were printed onto epoxysilane (Corning Epoxide) glass slides using a Genomic Solutions OmniGrid 100 arrayer equipped with PST SMT-S50 silicon pins.
mRNA was extracted from human muscle total RNA (Stratagene) was purified on RNA easy mini columns. Reverse transcription into cDNA was investigated with three procedures: (i) Oligo-dT (ii) Random and (iii) Non-priming.

Fluorescent labelling was achieved by direct incorporation of Cy3-dCTP and Cy5-dCTP with clean-up using a CyScribe GFx Purification Kit. Printed epoxysilane slides hybridized with cDNA were examined on an Axon Genepix 4000B fluorescence scanner.

Conclusions: Our results suggest that random priming is the optimal method for the expression analysis of long genes. This method of RT may be appropriate for alternatively spliced genes and for genes without unique probes in the 3'-region.



DNA microarray printed with 50 μm silicon pins on Corning Epoxide slides using an OmniGrid 100 microarrayer



Example of Electrospray mass spectrometric analysis of synthetic DNA 50-mer probes. Every DNA molecule was analysed for identity.

Nebulin NM_004543
Nebulin is a giant protein component of the cytoskeletal matrix that coexists with the thick and thin filaments within the sarcomeres of skeletal muscle. In most vertebrates, nebulin accounts for 3 to 4% of the total myofibrillar protein. It is suggested that alternative splicing may explain the developmental or tissue-specific size variants of nebulin. However, the nucleotide sequence information is not available for other spliced products. The mutations in the NEB gene are associated with recessive nemaline myopathy.
The complete sequence of human nebulin gene is 20670 bp long, contains 151 exon encodes a protein of size from 600 to 800 kD Oligonucleotide probes were designed every 1000bp.

Obscurin NM_052843
Obscurin is 800 kD protein expressed specifically in skeletal and cardiac muscle. The encoded protein contains 68 Ig domains, 2 fibronectin domains, 1 calcium/calmodulin-binding domain, 1 RhoGEF domain with an associated PH domain, and 2 serine-threonine kinase domains. This protein belongs to the family of giant sacromeric signaling proteins that includes titin and nebulin, and may have a role in the organization of myofibrils during assembly and may mediate interactions between the sarcoplasmic reticulum and myofibrils. While several transcript variants likely exist for this gene, the full-length nature of only one has been described to date. The complete sequence of human obscurin is 20409 bp long, contains 82 exons encodes a protein of approximately 720 kDa. Oligonucleotide probes were designed every 1000bp.

Titin
The giant muscle protein titin, also known as connectin, is a giant vertebrate striated muscle protein with critical importance for myofibril elasticity and structural integrity. The complete sequence of the human titin gene is 101461bp, contains 363 exons, which together code for 38 138 residues The product of this gene is divided into two regions, a N-terminal I-band and a C-terminal A-band. The I- band region of titin, largely composed of immunoglobulin-like (Ig) domains, is believed to be responsible for the molecule's extensibility and passive elasticity. Variability in the I-band region contributes to the differences in elasticity of different titin isoforms and, therefore, to the differences in elasticity of different muscle types. Up-to-date there are 5 identified isoforms.
Olignucleotide probes were designed every 2000bp.