# Center for Eukaryotic Structural Genomics

## Technology Dissemination Report

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| **CESG Tech Report No.** | 003 |
| **Title** | Use of a High-Resolution Genome Tiling Array to Identify the Presence of Target Genes in a cDNA Pool |
| **Research Unit** | Bioinformatics, Quality Assurance / Mass Spectrometry |
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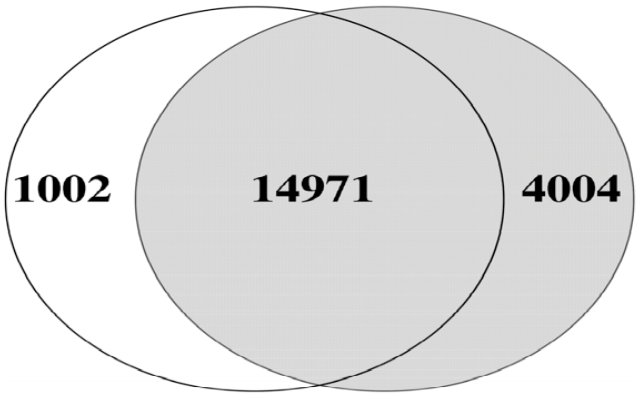


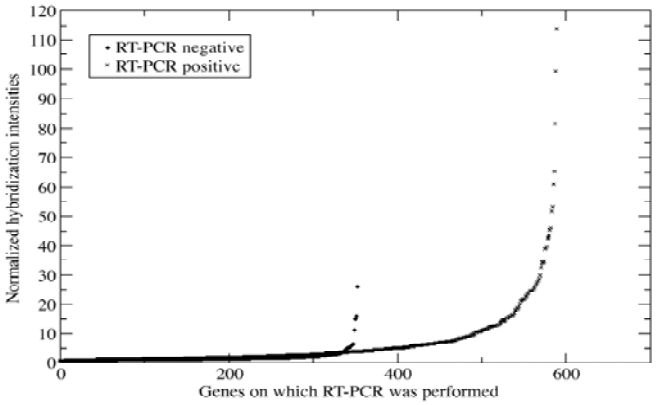


### Summary

Using a maskless photolithography method developed at the University of WIsconsin, we produced DNA oligonucleotide microarrays with probe sequences tiled throughout the genome of the plant *Arabidopsis thaliana*. RNA expression was determined for the complete nuclear, mitochondrial, and chloroplast genomes by tiling 5 million 36-mer probes [1]. These probes were hybridized to labeled mRNA isolated from liquid grown T87 cells, an undifferentiated Arabidopsis cell culture line. Transcripts were detected from at least 60% of the nearly 26,330 annotated genes, which included 151 predicted genes that were not identified previously by a similar genome-wide hybridization study on four different cell lines. In comparison with previously published results with 25-mer tiling arrays produced by chromium masking-based photolithography, 36-mer oligonucleotide probes made using maskless methods were found to be generally consistent (above right) but more useful in identifying intron-exon boundaries. Using two-dimensional HPLC tandem mass spectrometry, a small-scale proteomic analysis was performed with the same cells. A large amount of strongly hybridizing RNA was found in regions "antisense" to known genes. Similarity of antisense activities between the 25-mer and 36-mer data sets suggests that it is a reproducible and inherent property of the experiments. Transcription activities were also detected for many of the intergenic regions and the small RNAs, including tRNA, small nuclear RNA, small nucleolar RNA, and microRNA. Expression of tRNAs correlates with genome-wide amino acid usage. RT-PCR studies by CESG revealed good correlation between hybridization and the ability to clone the gene (above right).

Publication:

[1] Stolc, V., Samanta, M.P., Tongprasit, W., Sethi, H., Liang, S., Nelson, D.C., Hegeman, A., Nelson, C., Rancour, D., Bednarek, S., Ulrich, E.L., Zhao, Q., Wrobel, R.L., Newman, C.S., Fox, B.G., Phillips, G.N., Jr., Pak, J.W., Markley, J.L., and Sussman, M.R. (2005) Identification of transcribed sequences in *Arabidopsis thaliana* by using high-resolution genome tiling arrays. *Proc Natl Acad Sci* USA 102(12):4453-8.

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| **Acquiring the Technology** | For chips, contact www.nimblegen.com.<br>For T87 cells, contact Sebastian Bednarek bednarek@biochem.wisc.edu.<br>For other information, contact msussman@wisc.edu. |
| **Other Acknowledgements** | NASA |

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