

July
2006

Leica Laser Microdissection
Application Note

A vertical strip on the left side of the page features a green fluorescence microscopy image of rat brain cells. The top portion shows individual cells with bright green spots. The middle portion shows the same cells with red outlines drawn around them, representing laser microdissection. The bottom portion shows a more complex, interconnected network of green structures. The word 'resolution' is overlaid on the middle portion, with 're' in red and 'SOLUTION' in white.

reSOLUTION

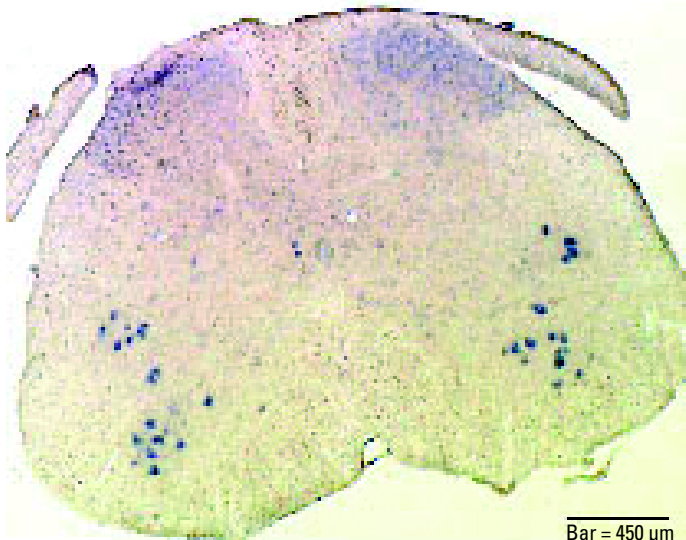
Quantitative RT-PCR
from Rat Brain

Quantitative RT-PCR from Rat Brain

Procedure:

Flash frozen mouse spinal cord was cut into 30 μm sections. 10 motor neurons in mouse spinal cord were collected using laser microdissection system. mRNA was isolated and subjected to qRT-PCR. This allowed confirmation of the increase of gene expression level in transgenic mouse.

C57BL/6J



GluR2 transgenic mouse

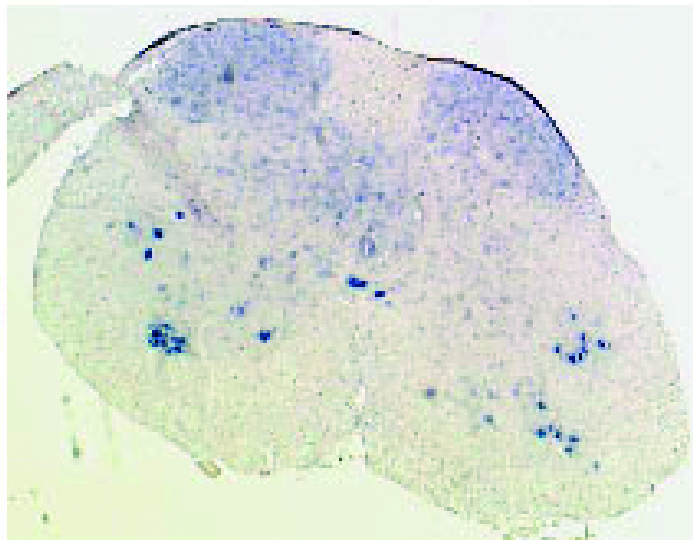


Fig. 1. *In situ* hybridization of ChAT (choline acetyltransferase). Motor neurons were visualized by expression of ChAT. Due to very small areas of motor neurons within the brain tissue, laser microdissection technique is very important to quantify the gene expressions with minimum of background coming from abundant mRNAs.

Bar = 450 μm

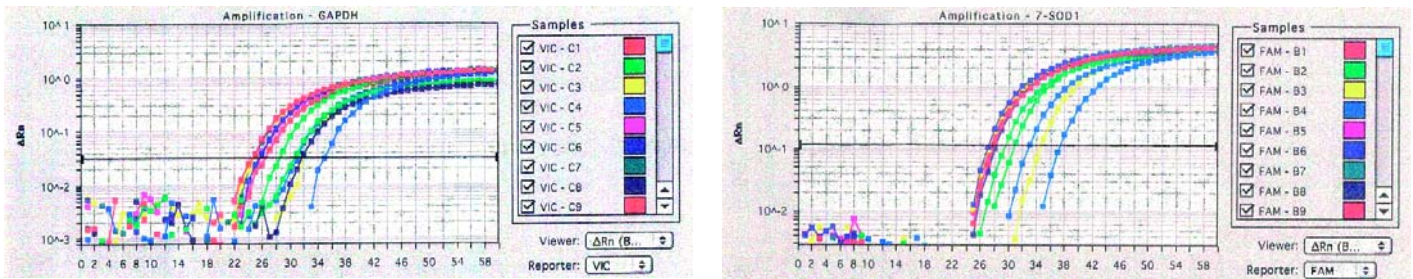


Fig 2. Quantitative real-time PCR of GAPDH and SOD1 (Sequence Detection System from Applied Biosystems). mRNA from 10 microdissected cells were purified with RNeasy Micro Kit (QIAGEN) and subjected to qRT-PCR.

GluR2 expression level in motor neurons of GluR2 transgenic mice

Mouse	GluR2	GluR3	GluR4	ChAT	SOD1
C57BL/6J (n=3)	1.00	1.00	1.00	1.00	1.00
GluR2-transgenic mice (n=3)	4.78 ± 0.85	1.02 ± 0.54	1.21 ± 0.26	1.17 ± 0.38	1.09 ± 0.31

Table 1. GluR2 expression in motor neurons of GluR2 transgenic mice. Data were normalized with GAPDH expression levels. Relative expression levels were compared with expression levels in C57BL/6J non-transgenic control mouse. Result: GluR2 expression level is particularly increased in GluR2 transgenic mice.

Acknowledgements: We would like to thank Dr. Hisako Sugimoto, Dr. Minako Kanno and Dr. Ryosuke Takahashi from Department of Neurology Tokyo Metropolitan Institute for Neurosciences, Japan for providing images and results.