

Taconic C57BL/6JBomTac, C57BL/6NTac and C57BL/10SgSnAiTac mice do not carry the *Nnt* mutation

Nicotinamide Nucleotide Transhydrogenase (NNT) is a transmembrane protein that catalyzes the interconversion of NADH and NADPH in the mitochondria. Under conditions of oxidative stress NADH will be converted to NADPH. This has very important implications in redox detoxification in the mitochondria since NADPH is used for the regeneration of reduced glutathione (GSH) and thioredoxin (TrxS₂). Studies suggest that the presence of *Nnt* is essential for mitochondrial defense against oxidative stress and for normal cellular metabolism (Huang et al. 2006). Furthermore, mitochondrial dysfunction has been implicated in a number of degenerative diseases, including Parkinson's disease (Cohen et al. 1997; Orth et al. 2001), Alzheimer's disease (Ko et al. 2001; Cottrell et al. 2001), Huntington's disease (Galas et al. 2004; Panov et al. 2002), diabetes (Toye et al. 2005; Freeman & Cox, 2006), cancer (Brandon et al. 2006), aging (Loeb et al. 2005), and cardiomyopathy (Marin-Garcia & Goldenthal, 2002).

Recently, several reports have documented that C57BL/6J mice carry a deletion in the *Nnt* gene, this includes exons 7 to 11 which is reflected as a 17.8 Kbp deletion (Huang et al. 2006, Toye et al. 2005, Freeman et al. 2006). For obvious reasons Taconic decided to screen the different C57BL/6 (C57BL/6JBom and C57BL/6NTac) and C57BL/10 (C57BL/10SgSnAiTac) strains that distributes to see if they carry the *Nnt* deletion.

Two sets of primers that have been published (Huang et al. 2006) were used. The first set of primers amplifies 312 bp (Fig. 1, upper gel) in intron 6. The following tested strains: C57BL/6JBomTac (B6JBom), C57BL/6NTac (B6/NTac), C57BL/10SgSnAiTac (B10/NTac), DBA/2NTac (DBA/2), and B6D2F1/Tac (B6D2F1) show the presence of the 312 bp band. The C57BL/6J strain from the Jackson Laboratories did not amplify the 312 bp band indicating the absence of the intron 6 DNA sequence. This corroborates data published by Huang et al. 2006, Toye et al. 2005 and Freeman et al. 2006.

The second set of primers was used to amplify across the breakpoint (Huang et al. 2006) giving a 547 bp product if the DNA carried the deletion. The only amplified band is the one found in the C57BL/6J mice (Fig. 1, bottom gel B6/J) from the Jackson Laboratories. Interestingly enough, the C57BL/6JBomTac does not present an amplified band (Fig. 1, bottom gel B6JBom). Taking together the amplification of intron 6 and the absence of the breakpoint amplified band indicate that the C57BL/6JBomTac does not present the deletion of exons 7-11 at the *Nnt* gene. The C57BL/6JBomTac was obtained through Taconic Europe in 1988. The original stock was received from The Jackson Laboratory in 1971. Therefore we can assume that the *Nnt* mutation arose at the Jackson Laboratory after 1971.

We can confidently say that none of Taconic's C57BL mice, C57BL/6JBomTac, C57BL/6NTac and C57BL/10SgSnAiTac, or our B6D2F1 mice carries the 17.8 Kbp deletion.

For more information please contact Dr. Ana Perez at (518) 697-3984 or by e-mail at ana.perez@taconic.com.

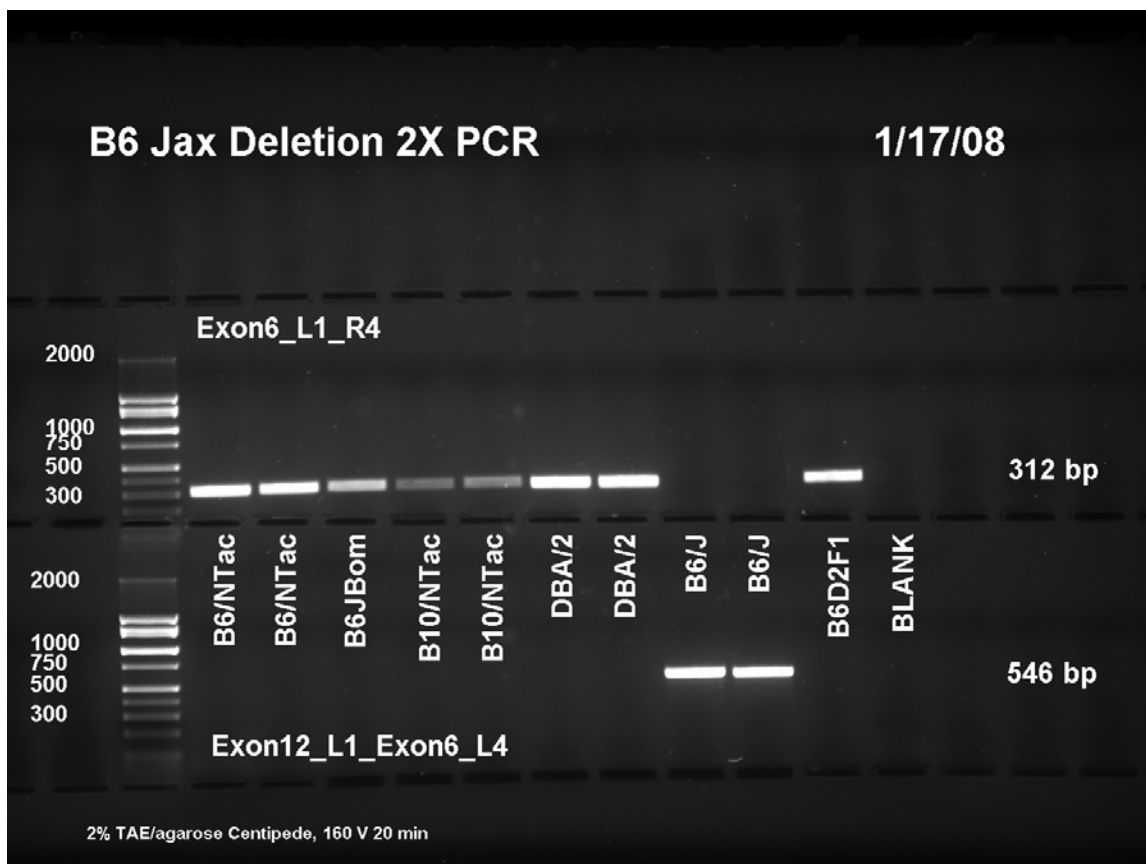


Figure 1. PCR reactions were done using primers Exon6_L1_R4 and Exon12_L1_Exon6_L4 (Supplementary data Table 3 and PCR conditions in Huang et al. 2006). Upper gel shows amplification of 312 bp band contained in intron 6 of the *Nnt* gene. Bottom gel shows amplification of 546 bp band across the breakpoint deletion between introns 6 and 12.

References:

- Brandon, M., Baldi, P. and Wallace, D.C. (2006) Mitochondrial mutations in cancer. *Oncogene* 25:4647-4662.
- Cohen, G., Farroqui, R., and Kesler, N. (1997) Parkinson disease: a new link between monoamine oxidase and mitochondrial electron flow. *Proc. Natl. Acad. Sci. USA* 94: 4890-4894.
- Cottrell, D.A., Blakely, E.L., Johnson, M.A., Ince, P.G. and Turnbull, D.M. (2001) Mitochondrial enzyme-deficient hippocampal neurons and choroidal cells in AD. *Neurology* 57:260-264.
- Freeman, H.C., Hugill, A., Dear N.T., Ashcroft, F.M. and Cox, R.D. (2006) Deletion of Nicotinamide Nucleotide Transhydrogenase A new quantitative trait locus accounting for glucose intolerance in C57BL/6J mice. *Diabetes* 55:2153-2156.
- Freeman, H. and Cox, R.D. (2006) Type-2 diabetes: a cocktail of genetic discovery. *Hum. Mol. Genet.* 15:R202-R209.
- Galas, M.C., Bizat, N., Cuvelier, L., Bantubungi, K., Brouillet, E., Schiffmann, S.N. and Blum, D. (2004) Death of cortical and striatal neurons induced by mitochondrial defect involves differential molecular mechanisms. *Neurobiol. Dis.* 15: 152-159.

Huang, T.T., Naeemuddin, M., Elchuri, S., Yamaguchi, M., Kozy, H.M., Carlson, E.J. and Epstein, C.J. (2006) Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxide dismutase. *Hum. Mol. Genet.* 15: 1187-1194.

Ko, L.W., Sheu, K.F., Thaler, H.T., Markesbery, W.R. and Blass, J. P. (2001) Selective loss of KGDHC-enriched neurons in Alzheimer temporal cortex: does mitochondrial variation contribute to selective vulnerability? *J. Mol. Neurosci.*, 17: 361-369.

Loeb, L.A., Wallace, D.C. and Martin, G.M. (2005) The mitochondrial theory of aging and its relationship to reactive oxygen species damage and somatic mtDNA mutations. *Proc. Natl. Acad. Sci. USA* 102:17993-17998.

Marin-Garcia, J. and Goldenthal, M.J. (2002) Understanding the impact of mitochondrial defects in cardiovascular disease: a review. *J. Card. Fail.* 8:347-361.

Orth, M. and Schapira, A.H. (2001) Mitochondria and degenerative disorders. *Am. J. Med. Genet.* 106: 27-36.

Panov, A.V., Gutekunst, C.A., Leavitt, B.R., Hayden, M.R., Burke, J.R., Strittmatter, W.J., and Greenamyre, J.T. (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat. Neurosci.* 5:731-736.

Toye, A.A., Lippiat, J.D., Proks, P., Shimomura, K., Bentley, L., Hugill, A., Mijat, V., Goldsworthy, M., Moir, L., Haynes, A., Quarterman, J., Freeman, H.C. Ashcroft, F.M. and Cox, R.D. (2005) A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. *Diabetologia* 48:675-686.