Provided by Tobias Suter

<table>
<tr><td colspan="3">Depts of Neurology and Neuropathology Neuroimmunology Unit
Group Leader: Asst. Prof. Dr. rer.nat. Burkhard Becher</td></tr>
<tr><td>DATE:
24.7.06 MP</td><td>Standard Operating Procedure
<u>Genotyping 2D2</u></td><td>Pages
1/1</td></tr>
</table>

DNA preparation: Qiagen **14-Oct-16**

Tested for GoTaq-Polymerase and –buffer (Promega)

Samples**: 2d2 x wsx #**

2d2 x CSF #

2d2 # 80

2d2 x TNFa ko #

Controls: 2d2 # 846,847

PCR:

Primers: yellow box

Va3.2: 5'-CCCG GGCA AGGC TCAG CCAG TCTC CTG-3'

Ja18: 5'-GCGG CCGC AATT CCCA GAGA CATC CCTC C-3'

Mix: x

Template DNA	1 µl
5X PCR buffer (GoTaq)	5 µl
25 mM MgCl	2.7 µl
10 mM dNTP mixture	0.5 µl
Primer 1(10µM)	0.25 µl
Primer 2	0.25µl
Taq DNA Polymerase (GoTaq)	0.125 µl
H2O	15,175 µl
	25 ul

PCR amplification (~Prog 42):

94°C 4'

94°C 30''

60°C 1' 35x

72°C 1'

72°C 5'

4°C ∞

Instead of this cycle, the cycle of Bcl-x can be used

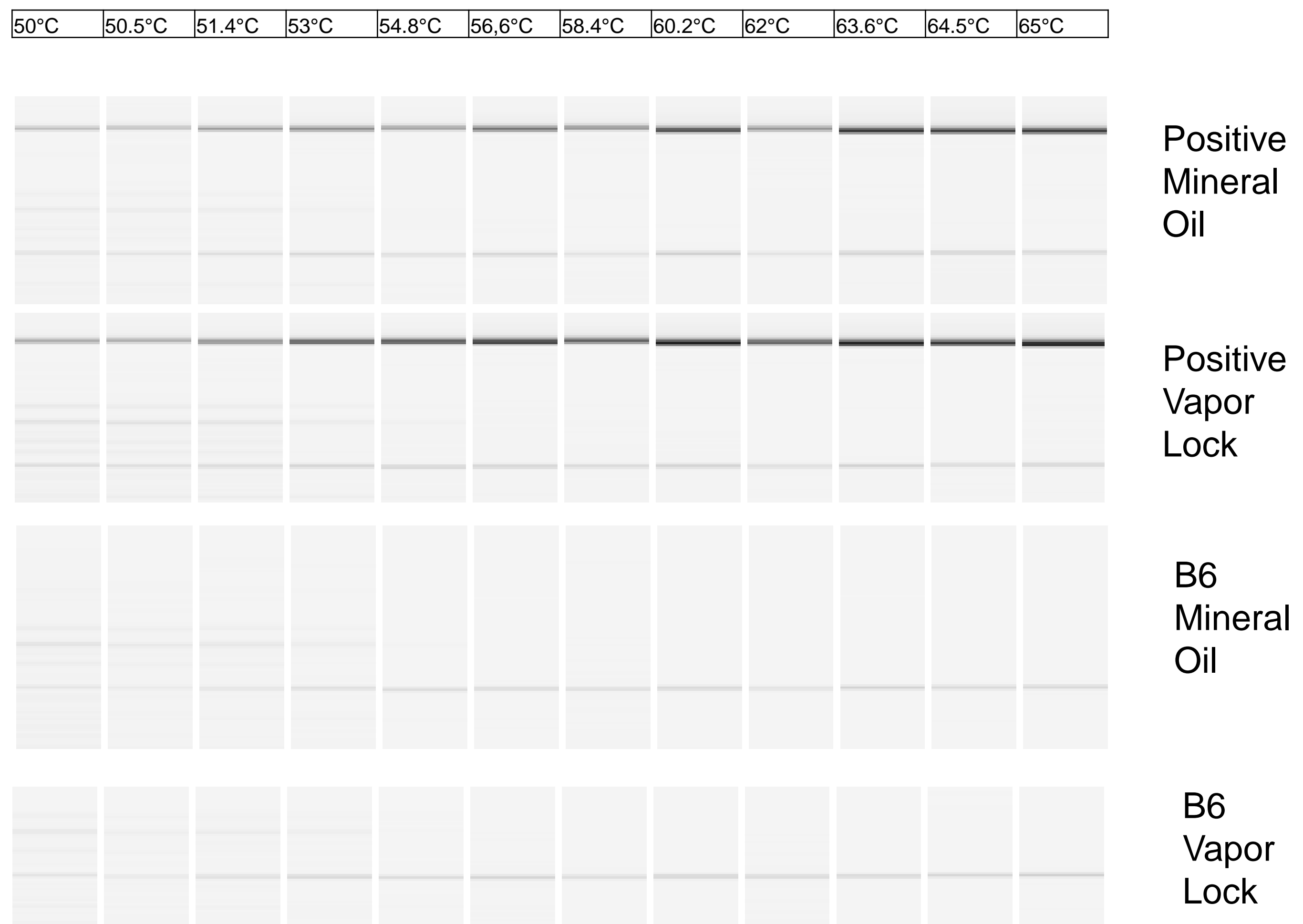
Analyze:

1% agarose gel 700bp

band at around 700 bp: 2D2

2D2 22.01.2015 (Qiaxcel SHPS1_2D2 gradient_22012015)

A gradient was run in a positive and B6 controls. Mineral Oil and Vapor Lock samples coverage was also compare. 20ul were use in both cases



17.02.2015

Annealing temperature change from 57°C to 60°C

2016

mTUBB247 was previously used as internal control. Actin has been tested, showing better results. The internal control has been exchanged