As received by J. Stanley from the Rupar lab, dated November 13, 2012:

The purpose of this document is to outline the rationale and evidence for the request to move mice injected with lentivector from level 2 plus to level 2.

The reason for such a move is that we have ongoing experiments that require longer timeframes than can be afforded by the level 3 recertification schedule.
We currently have a group of mice that were injected with vector in September of this year with the experiment set to end at the beginning of March of next year.

The lines of evidence that support moving these mice to level 2 are two-fold.

1) Our own data (provided) shows that 2 months following vector injection into the brain no vector DNA is found outside of the brain. Five tissues – lung, liver, kidney, spleen and ovaries/testes - were testing using a very sensitive method to detect vector DNA. There was no evidence seen indicating the presence of vector in these tissues, opposed to brain tissue in which vector was, as expected, observed.

2) From established data it is known that mice are not permissive hosts of HIV or lentivectors. Mice therefore cannot support replication of infectious HIV-1 and recombinant lentivectors. This means the risk of any shedding is extremely low. It is for this reason that the National Institutes of Health (in their document located at http://oba.od.nih.gov/oba/rac/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf) indicate that mice may be moved to BSL-1 7 days and 1 bedding change after injection with lentivectors. This is followed by many university animal centres such as the University of Ottawa.

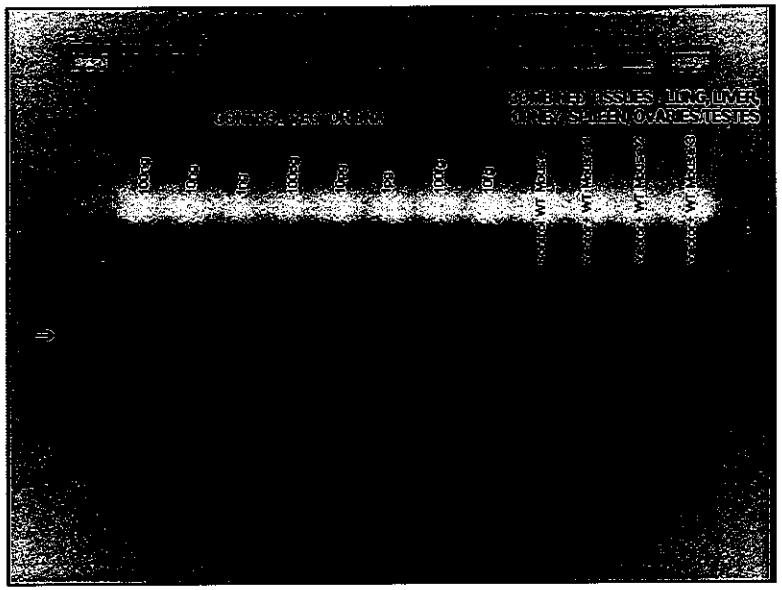

Figure 1

Figure 1 – The first 8 marked lanes show amplification of the vector DNA fragment from decreasing amounts of template control vector. This establishes that amounts of vector present as low as 1 picogram are readily detectable.

The last 4 lanes show amplification for the vector DNA fragment from DNA from the combined listed visceral organs. The first is a control mouse not injected with vector (so no amplification is expected) and the other 3 are from vector injected mice. No amplification is visible for any of these mice, suggesting that vector is not reaching these organs following injection.

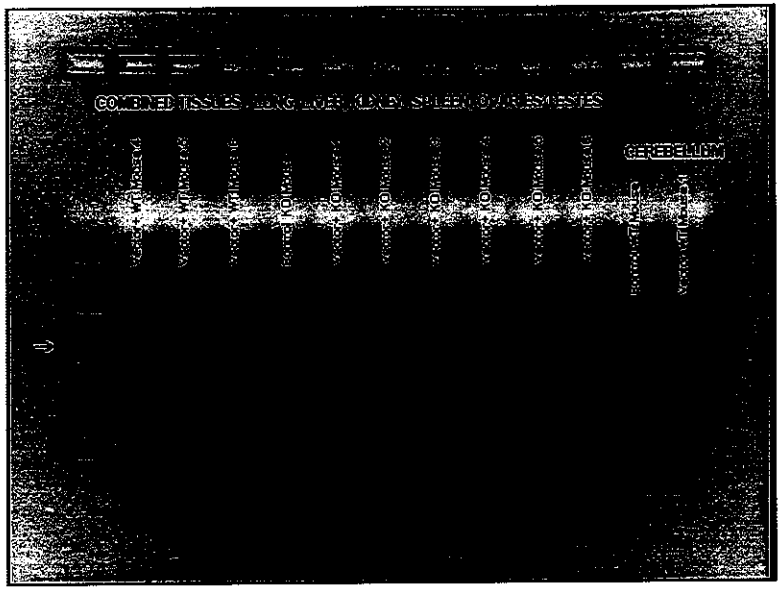

Figure 2

Figure 2 – The first 10 lanes show a continuation of amplification from combined visceral tissue DNA. No amplification is seen in any of these samples, again suggestive that vector is not "escaping" the CNS and entering other tissues.

The last 2 lanes are amplifications of the same vector fragment from cerebellar DNA of the same series of mice. The control mouse sample as expected shows no amplification as there was no exposure to vector. The vector injected mouse sample also shows no amplification indicating either that vector is not reaching the cerebellum or is present at less than 1 picogram in the reaction.

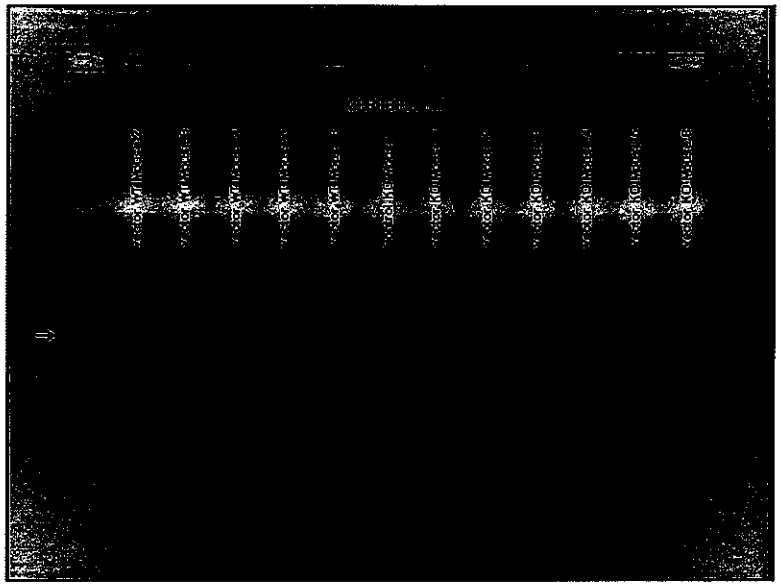

Figure 3

Figure 3 – This gel shows the amplifications from the cerebellar DNA of the remaining mice in the series. A faint band is clearly visible for mouse "Vector WT Mouse 4". This suggests that vector may be present in the cerebella of the vector injected mice in amounts lower than detectable by this method. In order to increase sensitivity, the products of the primary PCR reactions shown in these first 3 gels were used as template for secondary PCR reactions. These are referred to as "reamplifications".

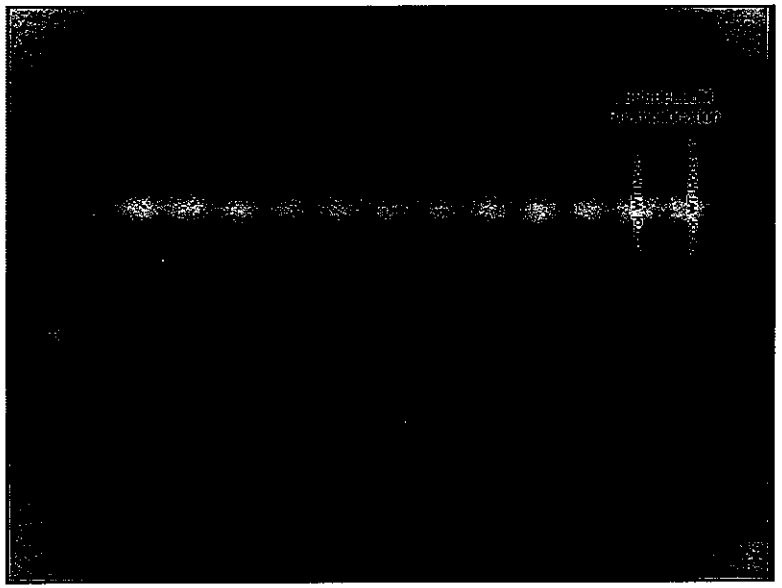

Figure 4

Figure 4 – All but the last 2 lanes should be ignored as they are from an unrelated series of amplifications.

The control mouse sample shows no reamplification of the vector DNA product as expected.

The vector injected mouse sample reamplification clearly shows the presence of the vector DNA product; now visible due to the increased sensitivity of PCR product reamplification.

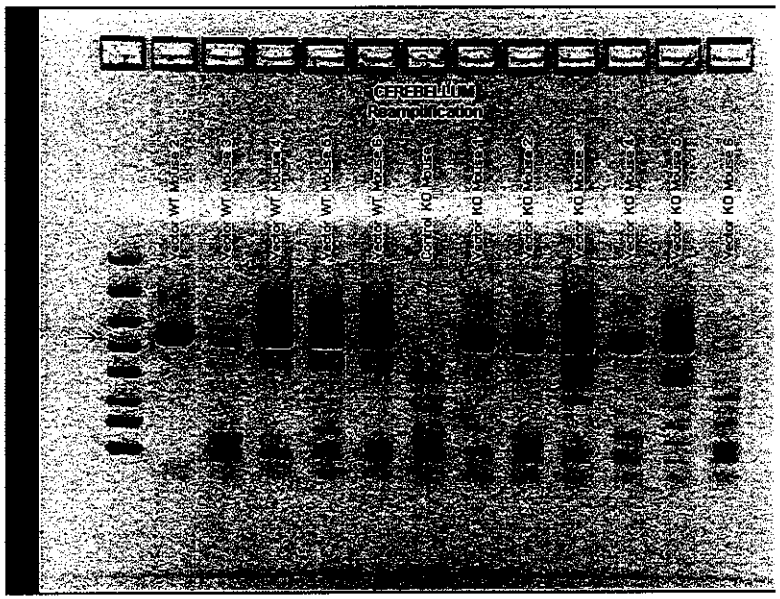

Figure 5

Figure 5 - This gel shows the reamplifications from the cerebellar DNA PCR products of the remaining mice in the series.

All but the control KO mouse reaction show a visible vector DNA product band (albeit 2 faintly).

This verifies that the vector leaves the area of injection (the lateral ventricle area) and reaches the cerebellum, but in minute amounts.

(The other fainter bands are characteristic of reamplifications)

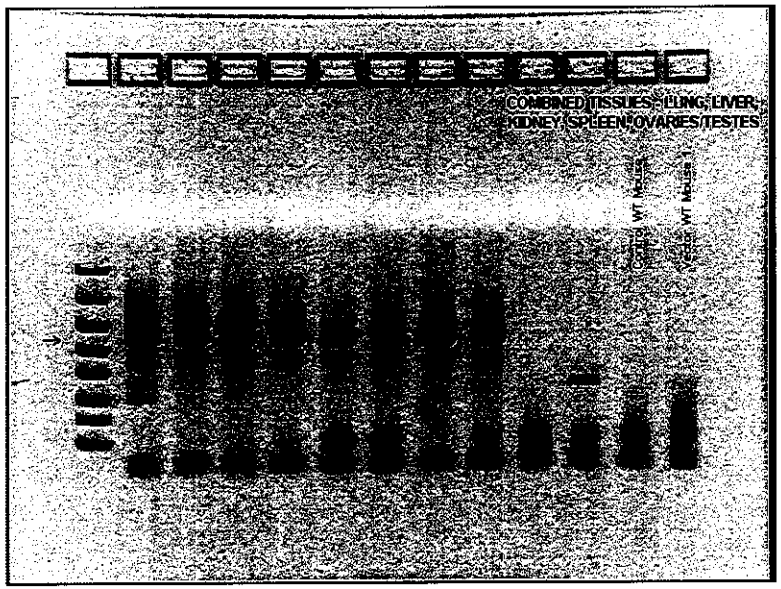

Figure 6

Figure 6 - All but the last 2 lanes should be ignored as they are from an unrelated series of amplifications.

This begins the series of reamplifications from the combined visceral tissue DNA samples.

As expected no band is seen in the control mouse sample. However, no band is seen in the vector exposed mouse sample as well.

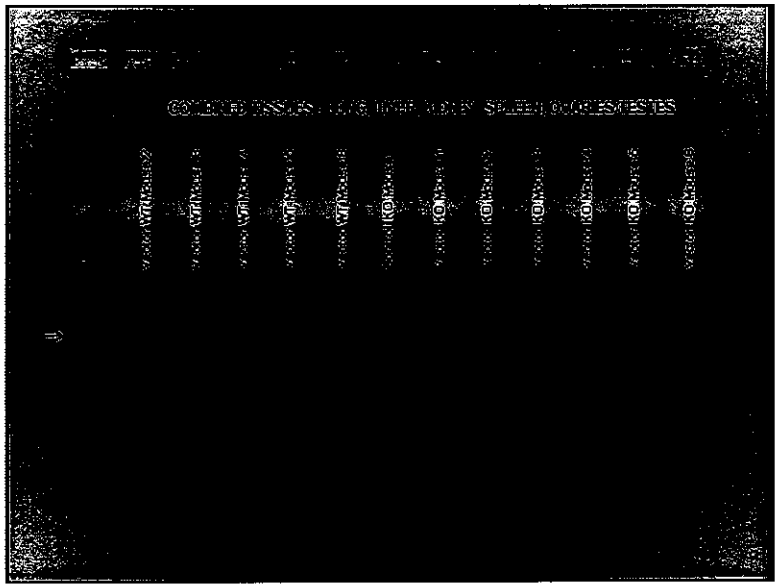

Figure 7

Figure 7 - This gel shows the reamplifications from the combined tissue DNA PCR products of the remaining mice in the series.

Only 1 mouse in this series shows a band in the correct location on the gel to be indicative of vector presence. This was investigated and is shown on the gel below (Figure 8).

All other samples indicate that vector does not "escape" the CNS and reach any of these visceral organs.

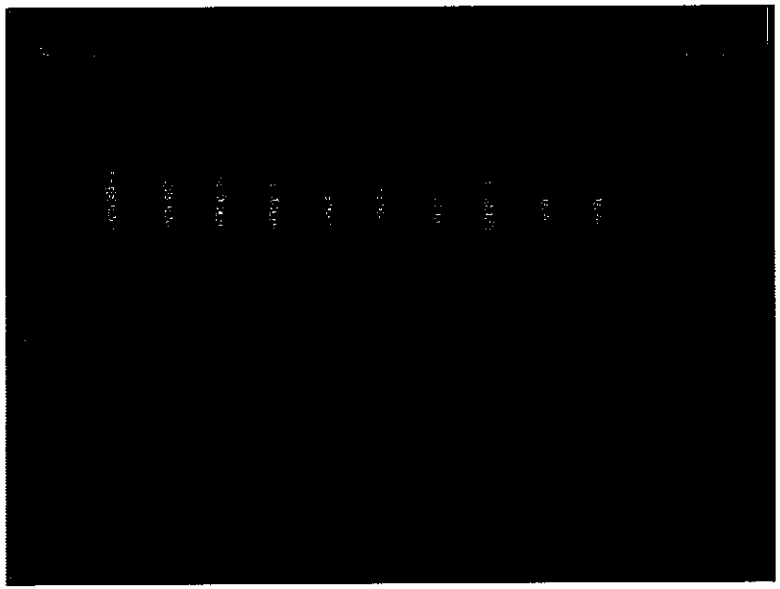

Figure 8

Figure 8 – As only one combined tissue sample reamplified and it was faint, DNA was isolated from each individual tissue and amplified. Amplification was done using 2 different amounts of DNA as template. For the 300ng samples, template DNA from these individual tissues is present at at least 6 times that in the previous combined samples.

Clearly there are no amplification products visible, which establishes that vector does not reach tissues outside the CNS.