

July 16, 2009

Dear Biosafety Committee Members,

We have recently added lentiviral plasmids encoding small RNA fragments (shRNA for gene knockdown) and mammalian pCMV6 expression plasmids encoding full length human cDNA (for gene expression) to our permit (UWO-BIO-0191). We would like to request you to allow our lab to be designated as BSL2. We understand that the risks with lentiviral vectors include 1) the potential to generate replication-competent virus particles, and 2) oncogenesis. However, we would like to request you to consider the nature of the vector system and the application/use when designating the containment level for our lab. In our lab, we will use the vector system in which 1) the vector and packaging functions are separated (3<sup>rd</sup> generation biosafety), and 2) the human gene has not been reported to be oncogenic (in the case of pCMV6). We will **not** use the plasmids for viral particle generation or transfection of producer/packaging cells. In fact, we have submitted the catalogue numbers for the plasmid we will be using (please see the modification form and attached appendices; catalogue numbers are also listed below). These items do not contain the packaging plasmids necessary for producing virus particles. We are also forwarding the email from Santa Cruz Biotechnology that states the nature of the vector system (3<sup>rd</sup> generation; multiple plasmids required to produce viral particles). Finally, our experiments are very similar to transient transfection of primary cells with siRNA which requires BSL2.

If you have any questions, please do contact me.

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<b>Catalogue Number</b>	<b>Vendor</b>	<b>Description</b>	<b>Use</b>
SC320234	Origene	IGF2 cDNA in pCMV6	Transfection only
sc-29358-sh	Santa Cruz	IGF1R shRNA plasmid	Transfection only
sc-37193-sh	Santa Cruz	IGF1 shRNA plasmid	Transfection only
sc-39576-sh	Santa Cruz	IGF2 shRNA plasmid	Transfection only
sc-37118-sh	Santa Cruz	IGFR2 shRNA plasmid	Transfection only
sc-108083	Santa Cruz	copGFP control plasmid	Transfection only