A note to investigators:

Thank you for expressing interest in our modified rabies virus system. This document provides basic information regarding the reagents most commonly requested from our lab. In general, we provide the basic materials necessary for producing high titer B19G- and EnvA-pseudotyped rabies virus from a provided starter stock.

For all cell lines, it is recommended that you thaw, expand, and refreeze aliquots so that you will have plenty of low-passage cells for future use. **Do this before you begin to expand the starter stock of rabies virus!**

For plasmids, it is recommended that you transform competent cells with the provided aliquots, grow up bacterial cultures, Maxiprep the plasmid DNA, and sequence the relevant sequences of DNA to verify plasmid integrity.

You will wish to initially amplify the starter stock of SAD-ΔG-GFP by growing the virus in BHK-B19G cells, and freeze aliquots for future use. If you wish to make EnvA-pseudotyped rabies virus, you will need to perform another round of amplification followed by pseudotyping in BHK-EnvARGCD cells (see associated protocols for detailed instructions).

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**Starting materials:**

SAD-ΔG-GFP: This is a supernatant containing the glycoprotein gene-deleted rabies virus SAD-ΔG-GFP. To amplify this virus, it must be grown in a complementing cell line that provides the rabies B19 glycoprotein.

BHK-B19G: This is the complementing cell line that expresses the rabies B19 glycoprotein, allowing for amplification of glycoprotein gene-deleted rabies virus.

BHK-EnvARGCD: This is a cell line that expresses the avian sarcoma and leukemia virus (ASLV) envelope protein EnvA, fused to the cytoplasmic domain of rabies glycoprotein. These cells will allow you to produce EnvA-pseudotyped rabies virus from a B19G-pseudotyped viral stock.

293T-TVA800: These cells express TVA800, a high-affinity binding partner for EnvA, and are necessary for the titration of EnvA-pseudotyped rabies virus, since this virus cannot normally infect mammalian cells.

pCAG-DsRed2: This is a plasmid carrying the DsRed2 construct (Addgene #15777).

pCMMP-TVA800: This is a plasmid carrying the coding sequence for TVA800 (Addgene #15778), a high-affinity binding partner for EnvA. Expression of this protein allows for selective infection of EnvA-pseudotyped rabies virus in mammalian nervous tissue.

pHCMV-RabiesG: This is a plasmid containing the coding sequence for the rabies B19 glycoprotein (Addgene #15785). Expression of this protein allows glycoprotein gene-deleted rabies viruses such as SAD-ΔG-GFP to form functional infectious particles that can retrogradely spread to connected cells.