**Table 1: Rescue from DNA and amplification of native-coat stock**

|  |  |  |  |
| --- | --- | --- | --- |
|  | B7GG1 | Neuro2a-N2cG2 | HEK-GT |
| Stably-expressed transgenes | T7 polymerase + SAD-B19G | CVS-N2cG | Optimized T7 polymerase (oT7) + optimized SAD-B19G (oG) |
| Selection markers | Fluorescence | Fluorescence | Antibiotic resistance genes |
| Transfected genes | Vector + N,P, G & L | Vector + T7, N,P,G & L | Vector + N,P & L |
| Transfection efficiency | Low | Low | High |
| Growth conditions | 3% CO2 at 35°C | 3% CO2 at 35°C | 5% CO2 at 37°C |
| Rescue timeline | 10-11 days | 10-11 days | 5-6 days |
| Initial amplification timeline | 9-11 days | 14-21 days | Not required |
| Compatibility | SAD-B19 (CVS-N2c possible, but not tested | CVS-N2c only | Both SAD-B19 and CVS-N2c |

**Table 2: Pseudotyping of rescued vectors**

|  |  |  |  |
| --- | --- | --- | --- |
|  | BHK-EnvA1 | Neuro2a-envA2 | BHK-eT |
| Stably-expressed transgenes | envA or envB | envA | envA + TVA |
| Selection markers | Fluorescence | Fluorescence | Antibiotic resistance genes |
| Growth conditions | 3% CO2 at 35°C | 3% CO2 at 35°C | 5% CO2 at 37°C |
| Pseudotyping timeline | 7-10 days | 28 days | 4-6 days |
| Requirements for pseudotyping | Large stock of native-coat particles | Large stock of native-coat particles | Trace amounts of either native-coat or evA pseudotyped stock |
| Titer | Low 10^8 typical | Low 10^7 typical | High 10^9 typical |
| Native-coat background | 10^2 typical | Not detectable | Not detectable |

1. Osakada, F. & Callaway, E. M. Design and generation of recombinant rabies virus vectors. *Nat Protoc* **8**, 1583–601 (2013).

2. Reardon, T. R. *et al.* Rabies virus CVS-N2cδG strain enhances retrograde synaptic transfer and neuronal viability. *Neuron* **89**, 711–724 (2016).