Production of lentiviral vectors pseudotyped with influenza neuraminidase (NA)

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Abstract

Lentiviral vectors pseudotyped with influenza surface glycoproteins represent an alternative to wild type virus for serological assays. The influenza neuraminidase (NA) has the ability to bud and release new virions with or without the contribution of Haemagglutinin (HA). Influenza pseudotypes expressing NA alone, or with HA, can be produced to evaluate the antibody response against NA using an enzyme-linked lectin assay (ELLA). The expression of an avian HA with human NAs has enabled the detection of specific antibody reponses against the human circulating subtypes of NA. This protocol describes the efficient production of NA pseudotypes for these ELLA assays. The pseudotypes have H11 HA on the surface as this will not react with human sera.

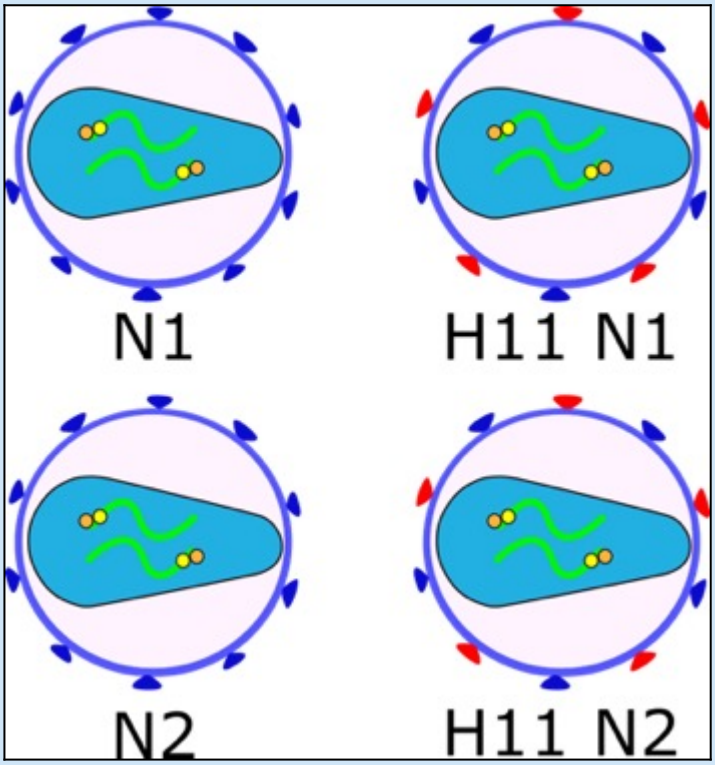

Figure 1. Cartoon schematic of lentiviral vectors pseudotyped with NA or HA+NA for use in ELLA assays.

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Guidelines

phCMV1-H11 (A/duck/Memphis/546/1974 (H11N9)) was kindly provided by Dr Davide Corti, Institute for Research in Biomedicine, Switzerland. N1/Cal09 and N2/Tex12 were synthesised

(WT sequence) and subcloned into plasmid expression vector pI.18 (Cox et al. 2002) by Genscript (U.S.A). HIV-1 derived packaging plasmid pCMVΔR8.91 (p8.91) was obtained from Dr Yasu Takeushi, University College London, originating from the laboratory of Dr Didier Trono (Naldini et al. 1996; Zufferey et al. 1997). Plasmid pCSGW originated from (Demaison et al. 2002), the GFP gene was replaced by that encoding firefly luciferase, forming the plasmid pCSFLW by Novartis Vaccines, Italy.

Protocol

Step 1.

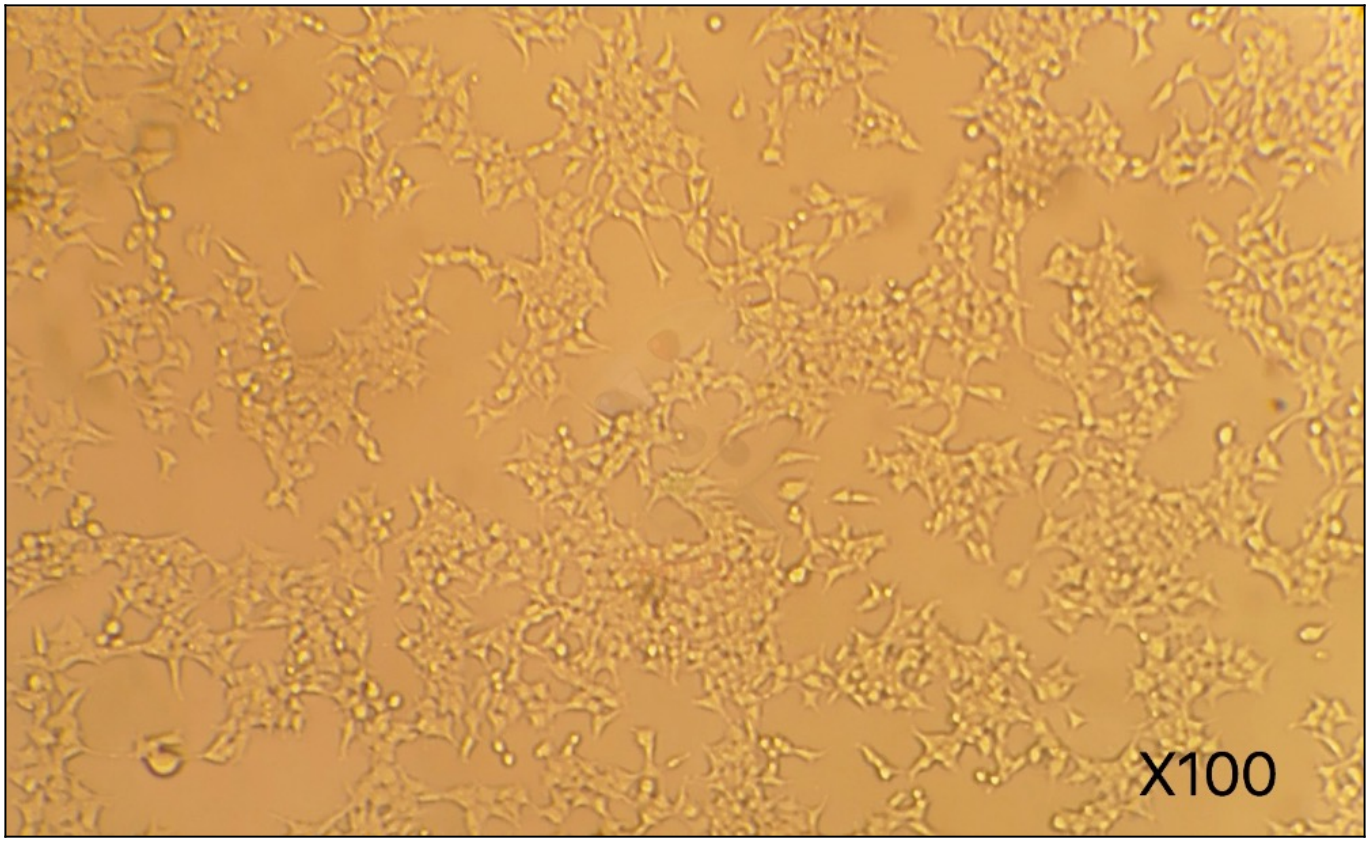
Prepare HEK293T/17 cells according to standard protocol. Production of lentiviral PV was carried out by co-transfection of HEK293T/17 cells using branched polyethylenimine (PEI) or Endofectin Lenti™ transfection reagents.

Step 2.

Briefly, a DNA mix of 0.5 µg of phCMV1-H11, 0.5 µg of p8.91, 0.75 µg of pCSFLW and either 1 µg of N1/Cal09 or N2/Tex12 was mixed in 200µl Opti-MEM™ and incubated for 20min with PEI (17.5µl of 1mg/ml PEI per well of a 6-well plate) or 25 min with Endofectin Lenti™ (3 µl/µg of DNA) per well of a 6-well plate.

Step 3.

This mix was transferred to 60-90% confluent HEK293T/17 cells.



Step 4.

Cell culture medium (DMEM+10% FBS +1% Penicillin/Streptomycin) was changed after overnight incubation at +37°C 5% CO2 in a humidified incubator.

TEMPERATURE

37 °C Additional info:

Step 5.

Cells were left for a further 48h and supernatants harvested 72h post transfection, and sterile filtered through a 0.45µm filter (Merck Millipore).