Production of Nanoblades

is classically performed upon transfection of HEK293T cells. Quality of producer cells is crucial (passage number, rate of division) : we noted important variations in VLP production depending on the producer cell clone. Best results were obtained in our lab using Gesicles-producer 293T cells from Clontech takara or Lenti-X 293T transfected with JetPrime (Polyplus). Transfection mix includes several plasmids: GAG-CAS9 (18%), GAGPOLmlv (40%), one or several (up to 4 was tested)gRNA coding constructs (40%), VSV-G (0,75-1%) and pBRL (0,75-1%). Absence of either VSVG or BRL in the mix result in very low VLP efficiency. Use of producer cells stably expressing GAGPOL mlv was poorly efficient in our hands. Endotoxin-free plasmids are not particularly required. 24-48h after transfection, massive syncitia appear due to the association of both fusogenic envelopes. Depending on the cell layer state, these detach from the dish or not, but efficient Nanoblades were harvested in both situations.