Lentivirus transduction of primary mammary epithelial cells (Peter Dijkgraaf)

MEC Growth Medium:
DMEM/F-12 (Gibco cat # 11330, 442 ml per 500 ml)
10% FBS 50 ml per 500 ml
100 U/ml Penicillin G 5 ml 100x Pen/Strep per 500 ml
100 µg/ml Streptomycin
5 µg/ml Insulin 2.5 ml of 1 mg/ml per 500 ml
50 µg/ml Gentamicin 500 µl of 50 mg/ml per 500 ml
1 µg/ml Hydrocortisone 50 µl of 10 mg/ml (in 100% EtOH) per 500 ml
10 ng/ml mouse EGF (Roche cat # 855-731, 50 µl of 100 µg/ml per 500 ml)
All media should be sterilized through a 0.22 µm filter and can be stored up to one month at 4 °C!

Transducing primary MECs
1) Transfer 2 million MECs to a well of a 24-well ultra low adhesion plate, add 10 million TUs (colony forming units) of concentrated virus and top of with MEC growth medium so that the final volume is 800 µl per well. Mix by pipeting up and-down.
2) The MECs will aggregate overnight at 37 °C in the virus-containing medium.
3) Wash the MEC aggregates twice with 10 ml of HANKS (with Ca2+ and Mg2+) to dilute out any unbound virus. Resuspend the pellet so that there are 20,000 MECs / µl of HANKS.
4) Inject 10 µl of aggregates (equivalent to 200,000 MECs) into each cleared fat pad.

Tips and Suggestions
- The MaSC frequency of 'fresh' primary MECs isolated as described above is approximately 1/13,000. The MaSC frequency of 'transduced' MECs after the overnight in suspension infection is approximately 1/29,000. These values may improve if the MECs are resuspended in Matrigel. We have not yet determined the MaSC frequency of the frozen MECs either before or after transduction, but we anticipate these to be significantly lower.
- For the resuspension of the aggregates, we assume no cell loss during the overnight in suspension infection and the washing of transduced MECs. However, cell counts have revealed that you loose about 25% of your starting material (ie, we routinely inject the aggregate equivalent of 150,000 MECs).