CULTURING HPDE CELLS

Medium:
There are two options for the medium and they both work equally well:

1. Keratinocyte SFM, + EGF + bovine pituitary extract (Invitrogen Cat#:17005042) supplemented with 1x antibiotic-antimycotic (Gibco Cat#15240-062)
2. Keratinocyte Basal Medium + supplied supplements (Lonza, Clonetics KBM, Cat#CC-3111)

Passaging:
1. Wash the cells with 5ml Hanks or PBS once (100mm plate)
2. Add 1ml of 0.5% TrypsinEDTA (Invitrogen Cat#15400-54) and leave in the incubator for 5-10 minutes. Note: if cells do not detach easily add a bit more trypsin.
3. Neutralize trypsin by adding the same volume of 0.1% trypsin inhibitor (Invitrogen, Cat#17075-029), then add 2 ml of medium and spin, discard the supernatant and plate the cells on new plate (8-10ml of growth media).

Notes:
Splitting ratio should not exceed 1:4 and the medium should be replaced every 2-3 days.
On average these cells are split 1-2 times a week depending on your experiments.
HPDE cells should not be split to thinly and are very difficult to subclone from single cells.
Using trypsin inhibitor and spinning subsequently is a necessary step and should never be skipped or you will lose your culture.

Freezing medium:
Growth medium with 10%DMSO or 10% glycerol.