Progeria culture conditions

Cell line: HGPS (HGADFN167, progeria research foundation)

Medium: MEM (cat#10370-021, Invitrogen) + 15% one-shot FBS(cat#16000-085, Invitrogen) + 1% pen-strep.

Procedure:

1. Frozen cells should be thawed into a 175 cm^2 flask containing 30 ml of medium and incubated @37C, 5% $C0_2$ and allowed to attach; change the media at the second day. Let the cells grow to 60-70% confluency, then split.
2. Trypsinize with 0.05% trypsin-EDTA. Split 1:5.
(a) Remove the media
(b) wash the cells with 1 X PBS once.
(c) suspend the cells with 5 ml 0.05% trypsin per T175 flask, or 30 ml 0.05% trypsin per 500 cm^2 square plate.
(d) add 7 ml (T175) or 50 ml (square plate) of media into trypsin-suspended cells; get 12 ml suspension per T175 flask or 80 ml per square plate.
(e) Centrifuge cell suspensions; aspirate supernatant; suspend cell pellets with 10 ml media (from a T175 flask), or 50 ml media (from a square plate)
(f) aliquot the cell suspension into 5 T175 flasks or 5 square plates, add fresh media to 30 ml(T175) or 100 ml (square plate).

3. Change the media every two days. Split cells when confluence reaches 70%.