Fibroblast culture conditions

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This protocol can be used for any primary fibroblast culture. Examples of cell strains (all from Coriell) grown using this protocol include:

Source Name	Refered to as	Tissue Source	Gender
AG20443	PF43	skin fibroblasts (71yo)	male
AG08395	PF95	skin fibroblasts (85yo)	female
AG08396	PF96	lung fibroblasts (85yo)	female

Fibroblast Culture Medium:
Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, cat. no. 11960) supplemented with 10% Fetal Bovine Serum (FBS; Invitrogen,), 2mM L-glutamine (Invitrogen, cat. no. 25030), and 0.1mM (0.7µl/100ml final media volume) 2-mercaptoethanol (Sigma, cat. no. M7522). Antibiotics can also be added at final concentrations of 50 units/ml penicillin and 50 g/ml streptomycin (Invitrogen, cat. no. 15070). Fibroblast Culture Medium is filter sterilized, stored at 4°C, and used for up to 2 weeks.

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%. These primary cells have a limited number of cell divisions. Fibroblasts typically start to display a flattened out morphology and longer doubling times towards later passages. Be sure to harvest primary cultures before they show either of these phenotypes.