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:

<table>
<thead>
<tr>
<th>Source Name</th>
<th>Refered to as</th>
<th>Tissue Source</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG20443</td>
<td>PF43</td>
<td>skin fibroblasts (71yo)</td>
<td>male</td>
</tr>
<tr>
<td>AG08395</td>
<td>PF95</td>
<td>skin fibroblasts (85yo)</td>
<td>female</td>
</tr>
<tr>
<td>AG08396</td>
<td>PF96</td>
<td>lung fibroblasts (85yo)</td>
<td>female</td>
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</tbody>
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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 cm² flask containing 30 ml of medium and incubated @37C, 5% CO₂ 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 cm² 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.