TABLE OF CONTENTS

1011

Cell type: human melanoma

Source: Suzanne Topalian

Medium: DMEM-HG, 20% FBS, 10% AIM-V, 1X NaPyruvate, 1X NEAA, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Low pigmentation. Grows quickly. These cells are very large (compared to HeLa or MNT-1). You will need to work out plating densities specific for this line. Plating 1.2 X 10^6 cells/ 10 cm dish should result in a 60% confluent dish the next day.

Splitting: Can split back 1:20 without slowing growth rate. Will reach confluency in ~3 days.

Transfection: use modified $CaPO_4$

Drug Selection: 1.6 mg/ml G418 for making stables. Use 0.8 mg/ml G418 when growing an already cloned stable line.

Frozen stocks: Found in Tower E racks 2 & 4

Notes: received 1011 cells two times from Topalian. To differentiate between the cells, the newest batch is called 1011-new.

293

Cell type: human embryonal kidney transformed with adenovirus type 5. Expresses adenovirus gene product E1.

Source: Paul Bates, UPENN

Medium: DMEM-10% FBS, L-glut, Pen/Strep

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows quickly, very small cells, like to pile up, very easy to dislodge from plate. When get too dense, whole monolayer lifts off plate by itself.

Splitting: Does fine with 1:20 splits.

Transfection: not determined

Drug Selection: not determined

Frozen stocks: Found in Tower E rack 2

Notes: Used as a packinging line for adenovirus production and titration (provides E1 gene product to E1 deficient viruses).

293T

Cell type: human embryonal kidney, expresses SV40 large T antigen

Source: in the lab already

Medium: DMEM-10% FBS, L-glut, Pen/Strep

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows quickly, very small cells, like to pile up, very easy to dislodge from plate. When get too dense, whole monolayer lifts off plate by itself.

Splitting: Does fine with 1:20 splits.

Transfection: Highly transfectable by modified $CaPO_4$. Treat plates with 0.1% gelatin in H_2O prior to transfection (15 min, RT, wash 1X with media).

Drug Selection: not determined

Frozen stocks: Found in Tower E rack 2

Notes: used for producing recombinant retrovirus by triple transfection with VSV env, gag/pol, and gene of interest.

1D4B

Cell type: hybridoma secreting rat anti-mouse lamp1 (IgG2a)

Source: DSHB

Medium: H-Y (Sigma #H9014), 10% FBS, 50 mg/ml Gentamycin

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics:

Splitting:

Transfection: N/A

Drug Selection: N/A

Frozen stocks: Found in Tower E racks 3 & 4

Notes:

BLM

Cell type: melanoma (human?)

Source: Carl Figdor?

Medium: probably DMEM-HG, 20% FBS, 10% AIM-V, 1X NaPyruvate, 1X NEAA, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics:

Splitting:

Transfection:

Drug Selection:

Frozen stocks: Found in Tower E rack 2

Notes: negative for Pmel17, Tyrosinase, and TRP1 by IFM. See Amer J. Path. (1993) v. 143, p. 1579 for refs.

CaCO2

Cell type: human colonic epithilum tumor, expresses α1-antitrypsin

Source: Gary Wu, UPENN

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows in clusters. Never reaches full confluency, instead grows up in little hills.

Splitting: If split back even 1:10, takes a long time to recover.

Transfection: not determined

Drug Selection: not determined

Frozen stocks: Found in Tower E rack 4 and Tower F rack 4

Notes: used as positive control for expression of α1-antitrypsin.

COS-1

Cell type: monkey kidney?

Source: in lab already

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics:

Splitting:

Transfection:

Drug Selection:

Frozen stocks: Found in Tower E rack 2

Notes:

D47

Cell type: hybridoma secreting Mab to HIV strain IIIB envelope glycoprotein V3 loop

Source: Bob Doms, UPENN

Medium: Iscoves, H/T, 10% FBS, Pen/Strep, L-glut

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics:

Splitting:

Transfection: N/A

Drug Selection: N/A

Frozen stocks: Found in Tower E rack 2

Notes:

HeLa

Cell type: human cervical carcinoma

Source: already in lab

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows rapidly. Plating 1.5 X 106 cells/ 10 cm dish or 2.6 X 105 cells/ well of 6-well dish should result in 60% confluency next day.

Splitting: Can split back 1:40 without slowing growth rate. Will reach confluency in ~3 days.

Transfection: use regular or modified CaPO4

Drug Selection:

Frozen stocks: Found in Tower E racks 2 & 4

Notes:

HepG2

Cell type: human hepatoma, expresses α1-antitrypsin

Source: Cell Center

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows in clusters. Never reaches full confluency, instead grows up in little hills.

Splitting:

Transfection: not determined

Drug Selection: not determined

Frozen stocks: Found in Tower E rack 2 and Tower F rack 4

Notes: used as positive control for expression of α1-antitrypsin

LOVO

Cell type: colon, adenocarcinoma, human. Lack furin activity.

Source: ATCC # CCL-229

Medium: DMEM-HG, 20% FBS, 10% AIM-V, 1X NaPyruvate, 1X NEAA, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: Grows fairly rapidly in this media only.

Splitting: Usually split back no more than 1:20. Split 1 confluent T75 1:20 into 6 well, should be 50% confluent next day.

Transfection: Lipofectamine

Drug Selection: not determined

Frozen stocks: Found in Tower E rack 2

Notes: widely used to determine if furin is the enzyme responsible for endoproteolytic cleavage of your favorite protein. Can rescue furin activity by transfection.

Melan-a

Cell type: mouse melanocyte

Source: Vince Hearing?

Medium: RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME, 200 nM TPA (medium must be treated with βME day before use it to inactivate something toxic in the FBS; TPA gets added directly to the cells, not to the bottle)

Freezing medium: 85% medium (above), 5% FBS, 10% DMSO

Growth characteristics: Low pigmentation. Grows quickly. These cells are very small compared to melan-si.

Splitting: Can split back 1:20 without slowing growth rate. Will reach confluency in ~3 days. To split wash 2X with PBS, then add 5 ml 200 μg/ml EDTA, 250 μg/ml trypsin in HBSS without Ca^{++} or Mg^{++}, rinse cells, and remove all but 1 ml.

Transfection: use FuGENE

Drug Selection: never determined, see melan-si for best guess

Frozen stocks: Found in Tower E racks 2 & 4

Notes: βME is not required for growth of melan-a but I always have grown them with it to save making up two bottles of medium (one for melan-a and one for melan-si)

Trypsin-EDTA mix:

20 ml Gibco Trypsin·EDTA (0.25% trypsin/ 1mM EDTA)
170μl 0.5M EDTA
180 ml 1X HBSS w/o Ca^{2+}, Mg^{2+} or phenol red
Filter

Melan-si

Cell type: mouse melanocyte carrying the silver mutation of pmel17

Source: Dorothy Bennett

Medium: RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME, 200 nM TPA (medium must be treated with βME day before use it to inactivate something toxic in the FBS; TPA gets added directly to the cells, not to the bottle)

Freezing medium: 85% medium (above), 5% FBS, 10% DMSO

Growth characteristics: High pigmentation. Grows very slowly. These cells are very large (compared to melan-a or MNT-1). Cells need keritinocytes to proliferate therefore cells are cocultured with mitotically inactivated XB2 keratinocyte feeder cells (see below)

Splitting: I never split back more than 1:5. Splitting back any further greatly reduces the growth rate. To split wash 2X with PBS, then add 5 ml 200 μg/ml EDTA, 250 μg/ml trypsin in HBSS without Ca^{++} or Mg^{++}, rinse cells, and remove all but 1 ml. Replace medium every 3-4 days.

Transfection: use electroporation

Drug Selection: 0.7 mg/ml G418 for making stables. Use 0.35 mg/ml G418 when growing an already cloned stable line.

Frozen stocks: Found in Tower E racks 2 & 4

Notes: At least 2 hours before plan to split melan-si cells, thaw XB2 feeder cells. Plate 1 vial of feeders/T75 in DMEM-10% FBS. When feeders are all adhering to the plate, split melani-si cells. Aspirate medium off feeders and add the melan-si cells plus RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME to the flask. Add 200 nM TPA. See appendix for XB2 Feeder preparation.

<u>Trypsin-EDTA mix:</u>

20 ml Gibco Trypsin·EDTA (0.25% trypsin/ 1mM EDTA)
170μl 0.5M EDTA
180 ml 1X HBSS w/o Ca^{2+}, Mg^{2+} or phenol red

Filter

LePoole melanomas (F002, F003, F010)

Cell type: human melanoma cells with reported deficiency in expression of Pmel17 (all) and MART-1 (F002 only)

Source: Obtained from I. Caroline LePoole, Loyola Univ., Chicago, IL

Medium: Iscove's modified Dulbecco's medium/ 10% heat-inactivated normal human AB serum (from Gemini)/ glutamine/ penicillin/streptomycin/ fungizone/ 10μg/ml ciprofloxacin

Freezing medium: 75% medium (above), 15% FBS, 10% DMSO

Growth characteristics: Grow very slowly, particularly F010. Each has its own characteristics:

F002: these cells are somewhat small and grow in nice tight clumps. They are the fastest of the bunch, but double in maybe a week.

F003: these cells are very large (compared to HeLa or MNT-1).

F010: these cells are huge, but grow very very slowly. (NOTE to Dawn - they have not been split yet!)

Splitting: These cells are very sensitive to trypsin, so you need to be careful. I split only 1:2 or 1:3 at most. To split wash with PBS, then add 2.5 ml 200 µg/ml EDTA, 250 µg/ml trypsin in HBSS without Ca^{++} or Mg^{++} (like for melan-a, melan-si cells), rinse cells, and remove all but 1 ml. Replace medium 3X per week.

Transfection: N/A

Drug Selection: N/A

Frozen stocks: Found in Tower E racks 7

Notes:

Melan-si clone sp1

Cell type: mouse melanocyte carrying the silver mutation of pmel17 stably transfected with wt human pmel17

Source: stable made by J. Berson

Medium: RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME, 200 nM TPA, 0.35 mg/ml G418 (medium must be treated with βME day before use it to inactivate something toxic in the FBS; TPA and G418 get added directly to the cells, not to the bottle)

Freezing medium: 85% medium (above), 5% FBS, 10% DMSO

Growth characteristics: High pigmentation. Grows very slowly. These cells are very large (compared to HeLa or MNT-1). Cells need keritinocytes to proliferate therefore cells are cocultured with mitotically inactivated XB2 keratinocyte feeder cells (see below)

Splitting: I never split back more than 1:5. Splitting back any further greatly reduces the growth rate. To split wash 2X with PBS, then add 5 ml 200 μg/ml EDTA, 250 μg/ml trypsin in HBSS without Ca^{++} or Mg^{++}, rinse cells, and remove all but 1 ml. Replace medium every 3-4 days.

Transfection: N/A

Drug Selection: 0.35 mg/ml G418

Frozen stocks: Found in Tower E racks 5 & 6

Notes: At least 2 hours before plan to split sp1 cells, thaw XB2 feeder cells. Plate 1 vial of feeders/T75 in DMEM-10% FBS. When feeders are all adhering to the plate, split sp1 cells. Aspirate medium off feeders and add the sp1 cells plus RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME to the flask. Add 200 nM TPA and 0.35 mg/ml G418.

The feeder cells will die off much more rapidly than normal because they are not G418 resistant [I made a G418 resistant XB2 line that can be used to make

G418 resistant feeders, but it grows very slowly, worth trying though]. To add more feeder cells without splitting the sp1 cells, thaw feeders, add to 10 ml medium in a tube, pellet, aspirate, and resuspend in RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME, 200 nM TPA, 0.35 mg/ml G418. Replace the medium on the sp1 cells with the resuspended XB2 feeders. I pellet to get rid of the DMS0.

See appendix for XB2 Feeder preparation.

MNT-1

Cell type: human melanoma

Source: Vince Hearing?

Medium: DMEM-HG, 20% FBS, 10% AIM-V, 1X NaPyruvate, 1X NEAA, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: High pigmentation; at confluency cells should be obviously black (by eye). Grows slowly. Plating 4.5 X 10^5 cells/ well of 6-well dish should result in a 50% confluent well the next day. 3 X 10^6 cells/ 10 cm-dish should result in a 60% confluent dish the next day. Give cells 3 days to sit down on coverslips.

Splitting: If split back more than 1:5, will slow growth rate. A 1:5 split will reach confluency in ~3 days.

Transfection: Nothing works well. Try FuGene or LT-1 to get a few percent transfected.

Drug Selection: 1.5 mg/ml G418 for making stables. Use 0.75 mg/ml G418 when growing an already cloned stable line. 0.4 mg/ml Hygromycin for making stables.

Frozen stocks: Found in Tower E racks 2 & 4

Notes: .

MOP8

Cell type: murine

Source: already in lab

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics:

Splitting:

Transfection:

Drug Selection:

Frozen stocks: Found in Tower E rack 2

Notes: used as a positive control for recombinant murine retrovirus infection (cell known to be susceptible to infection).

NIH-3T3

Cell type: murine fibroblast

Source: already in lab

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: grows fast, very small

Splitting: can split 1:40

Transfection:

Drug Selection:

Frozen stocks: Found in Tower E rack 2

Notes: used as a positive control for recombinant murine retrovirus infection (cell known to be susceptible to infection).

XB2/XB2 Feeders

Cell type: murine keratinocyte

Source: Dorothy Bennett

Medium: DMEM-HG, 10% FBS, L-glut, P/S

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: grows slowly

Splitting: see appendix

Transfection: electroporation used to make stable

Drug Selection: use 0.35 mg/ml G418 for maintaining stable because that is the concentration tolerated by melan-si.

Frozen stocks: XB2 cells found in Tower E racks 2 & 4.
"*XB2 Feeder*" cells found in Tower E rack 3

Notes: See appendix for preparation of mitotically inactivated XB2 cells, which are used to support the growth of Melan-si cells.

XB2 neo/ XB2 neo Feeders

Cell type: murine keratinocyte stably transfected with pSV2-neo (mixed clone; resistant to G418)

Source: J. Berson

Medium: DMEM-HG, 10% FBS, L-glut, P/S, 0.35 mg/ml G418

Freezing medium: 75% DMEM, 15% FBS, 10% DMSO

Growth characteristics: absolutely stagnant in presence of G418. Must remove drug to get any growth.

Splitting: See appendix.

Transfection:

Drug Selection: 0.35 mg/ml G418

Frozen stocks: XB2 neo found in Tower E rack 6
"*XB2 neo Feeders*" found in Tower E rack 3

Notes: See appendix for preparation of mitotically inactivated XB2 neo cells, which are used to support the growth of Melan-si clone sp1.

XB2 Feeder Preparation

I. Growing "XB2" cells

Reagents

DMEM-10% FBS, Pen/Strep, L-glut
Trypsin

Protocol

- Thaw 1 vial of "XB2" (*not* "*XB2 feeder*") cells at 37°C. Add cells to 5 ml DMEM-10 in a T25.
- Split cells next day into a T75. When splitting, use 1 ml trypsin/T25 or 2ml trypsin/T75 and after dilution in DMEM-10, *always spin out cells* to get rid of trypsin. [the original protocol calls for 2 washes in PBS- 200 μg/ml EDTA, followed by PBS-EDTA + 250 μg/ml trypsin, but my way has been working]
- Split cells next day into 2-T162s. If want to continue carrying the line to freeze cells or do another feeder prep, also plate some back into a T75. When the T162's are confluent, proceed to making feeders. [Alternatively, split the T162s into 4 flasks and make feeders when they are confluent. I don't recommend making feeders from more than 4 flasks at a time. Once I have XB2 cells growing, I usually do enough feeder preps to accumulate 30 or more vials of feeders. Don't forget to freeze more XB2 cells when needed.]

II. Making "XB2 Feeder" cells

Reagents

DMEM-10% FBS, Pen/Strep, L-glut
Trypsin
500 μg/ml Mitomycin C (100X).
Sigma M-0503 (2 mg)
dissolve 2 mg powder in 4 ml ddH_20
filter sterilize
protect from light
store at 4°C
stable at 4°C for 3 months

[stable indefinitely at -80°C, but to store at -80°C, must rapid freeze to avoid precipitation]

Protocol to mitotically inhibit cells

- Aspirate media from confluent T162s.
- Replace with fresh DMEM-10 containing 5 µg/ml mitomycin C (25 ml/T162).
- Incubate cells for 3 hrs at 37°C.
- Wash cells 1X with DMEM-10 (10 ml/T162).
- Add DMEM-10 (10 ml/T162) and incubate 10 min at 37°C.
- Trypsinize cells using 2 ml trypsin/T162. Add 18 ml DMEM-10/T162.
- Count cells (let cells sit in DMEM-10 for at least 5 minutes after trypsinization to give them time to round up - they are much easier to count that way) and resuspend at 2 X 10^6 cells/ml.
- Aliquot cells 0.5 ml/vial = 1 x 10^6 cells/vial (call them "XB2 Feeders"), slow freeze at -80°C, and transfer to liquid N_2.
- 2 T162s may yield anywhere from 8-20 vials, depending upon their initial density, which is very hard to judge.

Melan-si Cell Culture

Reagents

RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME (medium must be treated with βME day before use to inactivate something toxic in the FBS)

Freezing medium: 85% medium (above), 5% FBS, 10% DMSO

40 μM TPA (200X)
tetradecanoyl phorbol acetate a.k.a. PMA (phorbol 12-myristate 13-acetate)
Sigma P-8139, MW = 616.8 g/mol
First make 2 mM concentrated stock in ethanol: dissolve 5 mg in 4.053 ml ethanol, aliquot and store at -80°C (did not filter sterilize, prepared in TC. hood).
Then make 200X working stock in PBS + 0.1% BSA. Dilute 2 mM stock 1:50 in sterile PBS + 0.1% BSA, aliquot and store at -80°C. Stable at 4°C for 2 weeks protected from light.

200 μg/ml EDTA, 250 μg/ml trypsin, in HBSS w/o Ca^{++} or Mg^{++}.
100 ml 0.05% trypsin (= 500 μg/ml), 0.53 mM EDTA (=200 μg/ml) in HBSS w/o Ca^{++} or Mg^{++}. (Gibco-BRL #25300-054)
100 ml HBSS w/o Ca^{++} or Mg^{++} (Gibco-BRL# 14170-104)
106 μl 0.5M EDTA, pH 8.0.

Cell Culture

Thawing: At least 2 hours before plan to thaw melan-si cells, thaw XB2 feeder cells. Plate 1 vial of feeders/T75 in DMEM-10% FBS. When feeders are all adhering to the plate, thaw melan-si cells. Aspirate medium off feeders and add RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME plus the melan-si cells to the flask . Add 200 nM TPA. [The cells take close to 2 weeks to achieve confluency after thawing. I think it may help to plate the cells in a T25 after thawing. Just used 1/3 the amount of feeders].

Splitting: At least 2 hours before plan to split melan-si cells, thaw XB2 feeder cells. Plate 1 vial of feeders/T75 in DMEM-10% FBS. When feeders are all adhering to the plate, split the melan-si cells. Wash cells 2X with PBS, then add 5 ml 200 μg/ml EDTA, 250 μg/ml trypsin, rinse the monolayer, and remove all but 1 ml.

Once cells detached, dilute in RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME. Aspirate medium from feeders and add melan-si cells, more RPMI 1640, 10% FBS, L-glut, P/S, 0.1 mM βME if needed, and 200 nM TPA. Do not split more than 1:5. Splitting back any further greatly reduces the growth rate. Replace medium every 3-4 days.

Freezing: Freeze cells in 85% medium (above), 5% FBS, 10% DMSO. I do not treat with PTU prior to freezing as Dot Bennett suggested. I have been freezing 3 vials/T75.

Notes:

- Because the feeders are constantly dying off, the culture will always be full of cell debri. Gently knock the flask before viewing under the microscope to dislodge debri.
- When plating cells in different size flasks, adjust the amount of feeders you add. One vial of feeders is used for 1-T75.
- If plating for IFM, do not use feeder cells - the cell debri gives incredibly dirty results. Also, you can grow for 1 passage without feeders if you want to do biochemistry in their absence. By day 3 or 4 after plating the cells on feeders, the majority of feeders seem to be dead.
- Cells can not grow in the absence of TPA, even for one day.