PATU-8988T cell culture conditions

From: Duke/UNC/UTA/EBI ENCODE group
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1. Source of cells : Paula Herman and Matt Freedman (Harvard). Cells are commercially available from DSMZ - the German Resource Centre for Biological Material - http://www.dsmz.de/index.htm

2. Lineage of cells : established in 1985 from the liver metastasis of a primary pancreatic adenocarcinoma from a 64-year-old woman. Elsasser et al., Virchows Arch B Cell Pathol Incl Mol Pathol 61: 295-306 (1992), PubMed ID 1348891

3. Donor information : female (64 yo)

4. Karyotype : human near-triploid karyotype with 2% polyploidy - 68(63-70)<3n>XXX, -4, +6, +6, +8, +11, -15, -16, -17, -19, +20, -21, -22, +mar, der(1)t(1;?)(p11;?), i(6p), del(6)(q23)x2, der(8)t(8;?)(q24.3;?), der(8)t(8;?)(p11;?)x2, der(11)t(11;?)(p13;?)x2, del(12)(p12.3), dup(14(q11q12), del(17)(p12), der(17)t(17;?)(p13;?) - sideline with additional der(3)t(3;?)(q11;?) - 1p11 and 8q24 breakpoints previously reported in pancreatic cancer

5. Medium: DMEM (Invitrogen #10569-010) + 10% FBS + 1% pen-strep.

Procedure:

1. Frozen cells should be thawed into a 75 cm^2 flask containing 15 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 fill out the dish, then split.
2. Trypsinize with 0.05% trypsin. Split 1:6 – 1:10
(a) Remove the media
(b) rinse the cells with 1 X PBS.
(c) add 1 mL 0.05% trypsin to cells, incubate 5’ at 37°C
(d) add 9 mL of media into 1 mL trypsin-suspended cells; get 10 mL suspension.
(e) count cells (about 18 million/T75 flask when confluent)
(f) centrifuge 5’ at 1000rpm, RT
(g) discard media, suspend cells in 10mL DMEM, plate at desired density

3. Make sure media does not turn orange or yellow. For production, we grow these cells either in 15 cm dishes or T175 flasks.
(a) check the culture every day to make sure (i) media do not turn orange or yellow; (ii) confluence is less than 75%. Otherwise, change media and split cells.
4. Grow to 75% confluence before harvesting for experiments

Comments:
These cells grow extremely fast so check them every day.