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**We use this protocol and it's working**

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## Abstract

An amalgamation of cell line data in the lab. Its origin and standard culture conditions.

## Overview

- 1 All cells are maintained in humidified incubators at 37°C, 5% CO<sub>2</sub>, unless otherwise specified.

All cells are routinely tested for mycoplasma infection using a PCR-based test (Universal Mycoplasma Detection Kit, ATCC Cat. No. 30-1012K)

Dates of purchase are provided. Aliquots of purchased cells lines are frozen at P0, P1 and P2 from original stocks and P2 are defrosted and used for experimentation as standard.

Cell lines confirmed by short tandem repeat analysis are marked with \*.

- 2 Media for cell lines:

**R10:** RPMI (Gibco Cat. No. 2240-089) + 10% fetal bovine serum (Gibco Cat. No. 26140079) + 100 U/mL Penicillin and Streptomycin (Gibco Cat. No. 15140122)

**D10:** DMEM (Corning Cat. No. MT10013CV) + 10% fetal bovine serum (Gibco Cat. No. 26140079) + 100 U/mL Penicillin and Streptomycin (Gibco Cat. No. 15140122)

**D10-SP (without sodium pyruvate):** DMEM (Corning Cat. No. 10-017-CV) + 10% fetal bovine serum (Gibco Cat. No. 26140079) + 100 U/mL Penicillin and Streptomycin (Gibco Cat. No. 15140122).

Unless otherwise indicated, adherent cells are passaged using trypsin-EDTA (0.05%, Gibco Cat. No. 25300054). Some lines require 0.25% trypsin/1mM EDTA without Ca<sup>2+</sup> Mg<sup>2+</sup> (EMD Millipore, Cat. No. SM-2003-C), or this buffer diluted to 0.2% trypsin.

Cells transfected with plasmid are maintained with **blasticidin** (Thermo Fischer Scientific, Cat. No. A1113903) or **puromycin** (Sigma Aldrich, Cat. No. P9620-10ML).

## Lung

- 3 Non-small cell lung cancers:

Lung adenocarcinomas:

1. A549 (RRID:CVCL\_0023) was purchased from the American Type Culture Collection (ATCC) in March 2019; grown in R10 and split 1/20 twice per week.

1A. \*A549-GFP/luciferase (Gift of Daniel Vallera)

1B. A549-NucLightRed (generated by lentiviral transduction from ATCC stocks; Michael Kaminski 2019) grown with puromycin selection for maintenance (0.5 µg/mL)



2. NCI-H322 (RRID:CVCL\_1556; purchased May 2019, Sigma Aldrich) grown in R10 and split 1/3 to 1/6 twice per week.
3. NCI-H522 (RRID: CVCL\_1567) grown in R10 and split 1/3 to 1/6 twice per week.

Large cell lung carcinoma:

1. NCI-H460 (RRID:CVCL\_0459; purchased June 2019, ATCC) grown in R10 and split 1/8 three times per week.
- 1A. \*NCI-H460-GFP/luciferase (Gift of Daniel Vallera) as above but selected with blasticidin (100 µg/mL)

## Melanoma

- 4 SK-Mel-3 (purchased from ATCC, 2022)  
CHL-2 (purchased from ATCC, 2022)

## Prostate

- 5 Castration resistant prostate cancer (CRPC):
  1. LnCAP (RRID: CVCL\_0395) grown in R10, harvest with 0.2% trypsin.
  - 1A. LnCAP acclimated to grow in hypoxia (Gift of Xcell Biosciences)
  2. \*C4-2
  3. \*C4-2B (Gift of Xcell Biosciences)
  - 3A. C4-2B acclimated to grow in hypoxia (Gift of Xcell Biosciences)
  4. \*PC3 (Gift of Xcell Biosciences)
  - 4A. PC3 acclimated to grow in hypoxia (Gift of Xcell Biosciences)
  - 4B. \*PC3-NuclightRed (Gift of Scott Dehm)

## Mesothelioma

- 6 Pleural epithelioid mesothelioma:
  1. NCI-H2452 (RRID:CVCL\_1553)
  2. NCI-H226 (RRID:CVCL\_1544 ) obtained from Daniel Vallera in 2009.
  3. \*NCI-H2461-GFP/luciferase (Gift of Manish Patel) grown in R10 and split 1/20 twice per week.
  4. YOU (RRID:CVCL\_N819; Gift of Raffit Hassan, 2021, see Li et al. <http://www.ncbi.nlm.nih.gov/pubmed/15274292>) grown in R10 and split 1/4 twice a week.
  5. HAY (RRID:CVCL\_N813; Gift of Raffit Hassan, 2021, see Li et al. <http://www.ncbi.nlm.nih.gov/pubmed/15274292>) grown in R10 and split 1/4 twice per week.
  - 5A. HAY-luciferase/GFP created by Blake Jacobson, Manish Patel's lab, 2021) grown in R10 with 10µg/mL blasticidin for selection of the GFP/luciferase.

6. ORT (RRID:CVCL\_N815; Gift of Raffit Hassan, 2021, see Li et al. <http://www.ncbi.nlm.nih.gov/pubmed/15274292>) grown in R10 and split 1/2 twice per week.
7. ROB (RRID:CVCL\_N818; Gift of Raffit Hassan, 2021, see Li et al. <http://www.ncbi.nlm.nih.gov/pubmed/15274292>) grown in R10 and split 1/4 twice per week.
8. PET (RRID:CVCL\_N816; Gift of Raffit Hassan, 2021, see Li et al. <http://www.ncbi.nlm.nih.gov/pubmed/15274292>) grown in R10 and split 1/2 twice per week.

Sarcomatoid mesothelioma:

1. NCI-H2691 (RRID:CVCL\_A551)
2. \*NCI-H2373 (RRID:CVCL\_A533) grown in R10 and split 1/5 twice per week.
- 2A. \*NCI-H2373-GFP/luciferase (Gift of Manish Patel) grown in R10 and split 1/5 twice per week. Selected with blasticidin (2 µg/mL).

## Leukemia

- 7 Chronic myelogenous leukemia
  1. \*K562 (RRID:CVCL\_0004; purchased March 2015, Cat. No. CCL-243, ATCC) cultured in R10, split down to  $1 \times 10^5$  cells/mL three times per week.
  - 1A. \*K562-Nuclight Red (Gift of Fate Therapeutics, created using Incucyte Nuclight Red Lentivirus Reagent, EF1a, Puro; Sartorius, Cat. No. 4625) expression maintained with 1 µg/mL puromycin.

Acute myeloid leukemia

1. HL-60 (RRID:CVCL\_0002; purchased from April 2017, Cat. No. CCL-240, ATCC) grown in R10, split down to  $2 \times 10^5$  cells/mL two to three times per week.
- 1A. \*HL-60-luciferase (no GFP)

Childhood acute monocytic leukemia

1. THP-1 (RRID:CVCL\_0006; purchased December 2015, Cat. No. TIB-202, ATCC) cultured in R10 with 50 µM β-mercaptoethanol. Split down to  $2-4 \times 10^5$  cells/mL. Subculture when cell concentration reaches  $8 \times 10^5$  cells/mL. Do not allow the cell concentration to exceed  $1 \times 10^6$  cells/mL.
2. MV-4-11 (RRID:CVCL\_0064; purchased February 2015, Cat. No. CRL-9591, ATCC)

Plasma cell myeloma

1. MM1.S (RRID:CVCL\_8792; purchased December 2021, Cat. No. CRL-2974, ATCC) cultured in R10. Scrape to split 1/2 twice or three times per week. Spin at 125g 5min.
- 1A. \*MM1.S-NuclightRed (Gift of Fate Therapeutics)

## Breast

- 8 Triple negative breast adenocarcinoma:
1. MDA-MB-231 (RRID: CVCL\_0062; purchased from ATCC, August 2021) cultured in D10. Treat with 0.25% trypsin for 1-2 min only and split 1/10 three times a week.

## Lymphoma

- 9 Epstein-Barr virus-related Burkitt lymphoma
1. Raji (RRID:CVCL\_0511; purchased March 2015, Cat. No. CCL-86, ATCC) cultured in R10, split down to  $4 \times 10^5$  cells/mL three times per week.
    - 1A. \*Raji-Nuclight Red (Gift of Fate Therapeutics, created using Incucyte NucLight Red Lentivirus Reagent, EF1a, Puro; Sartorius, Cat. No. 4625) expression maintained with  $1\mu\text{g/mL}$  puromycin.
  2. Daudi (RRID:CVCL\_0008; purchased April 2018, Cat. No. CCL-213, ATCC) cultured in R10, split down to  $3\text{--}5 \times 10^5$  cells/mL; should not exceed  $3 \times 10^6$  cells/mL.

## Gut

- 10 Colon adenocarcinoma
1. CaCO-2 (RRID: CVCL\_0025) purchased from ATCC before 2016 (Schohl et al. Targeted Oncology, 2016, doi:10.1007/s11523-015-0391-8) Cultured in Eagle's Minimum Essential Medium (ATCC Cat. No. 30-2003) + 20% fetal bovine serum (Gibco Cat. No. 26140079) + 100 U/mL Penicillin and Streptomycin (Gibco Cat. No. 15140122); split 1/2 once per week.
  2. \*HT29 (RRID:CVCL\_0320; purchase May 2022, Cat. HTB-38, ATCC) P0 to P2 were cultured in McCoy's 5A medium modified (30-2007, ATCC); P3 onwards grown in R10. All split 1/8 3 times per week, down to  $40,000 \text{ cells/cm}^2$ .
    - 2A. \*HT29-Nuclight Red (Gift of Daniel Vallera, 2022) expression maintained in  $0.5\mu\text{g/mL}$  puromycin.
    - 2B. \*HT29-GFP/luciferase (Gift of Daniel Vallera, 2022) expression maintained in  $10\mu\text{g/mL}$  blasticidin.
  3. \*T24 (Gift of Manish Patel's lab) grown in R10 and split 1/20 twice per week.

## Endometrium

- 11 Endometrial adenocarcinoma
1. Ishikawa 3-H-12 (same as ECC-1) sensitive
  2. Ishikawa 3-H-12 (same as ECC-1) resistant

## Head and Neck



- 12 Human papilloma virus-negative Head and Neck Squamous Cell Carcinomas (HNSCCs):
1. \*Cal27 (RRID:CVCL\_1107; Gift of Ramon Garcia-Escudero, Spain, received in 2021) cultured in 15 mL D10/T75, split 3 times a week at 0.1 mil. cells/ mL, harvested using 0.25% trypsin (3 mL for T75, 5 mL for T150) for 3.5 minutes.  
1A. \*Cal27-Nuclight Red (generated by Melissa Khaw, using Incucyte NucLight Red Lentivirus Reagent, EF1a, Puro; Sartorius, Cat. No. 4625, August 2021), puromycin selection at 1.5 µg/ mL.
  2. \*Cal33 (RRID:CVCL\_1108; Gift of Ramon Garcia-Escudero, Spain, received in 2021) cultured in 15mL D10/T75, split 3 times a week at 0.1 mil. cells/ mL, harvested using 0.25% trypsin (3 mL for T75, 5 mL for T150) for 3.5 minutes.  
2A. \*Cal33-Nuclight Red (generated by Melissa Khaw, using Incucyte NucLight Red Lentivirus Reagent, EF1a, Puro; Sartorius, Cat. No. 4625, August 2021), puromycin selection at 1.5 µg/ mL.

## Ovarian

- 13
1. OVCAR3 (NCI, received March 2017)
  2. OVCAR4 (NCI, received March 2017)
  3. OVCAR5 (NCI, received March 2017)
  4. \*OVCAR8 (NCI, received March 2017) Culture in R10. Seed at 11,000 cells/cm<sup>2</sup> when subculturing.  
4A. OVCAR8-Nuclight Red (made in 2018 by Laura Bendzick and Caitlin Ryan)
  5. \*SK-OV-3 (NCI, received March 2017)

## Brain

- 14
1. \*BT12 (Gift of Crystal Mackall) grown in D10 with Glutamax.  
1A. \*BT12 B7H3-knockout (Gift of Crystal Mackall; <https://doi.org/10.1038/s41591-020-0821-8>)
  2. BT16
  3. LN229 (ATCC, received December 2023) Grown in D10. See complete subculture protocol here: <https://www.atcc.org/products/crl-2611>

## NK cell lines

- 15 Malignant non-Hodgkin's lymphoma
1. \*NK-92 (RRID:CVCL\_2142; Gift of Kerry Campbell, Philadelphia, 2018)  
(see also *Assessing IL-15 bioavailability - 'the bioassay'*) NK-92 are maintained in Alpha Minimum Essential Medium plus ribonucleosides and deoxyribonucleosides (12571, Gibco), β-mercaptoethanol (0.1mM, M7522, Sigma Aldrich), recombinant human IL-2 (100U/mL, NDC 65483-116-07, Prometheus), horse serum (12.5%, 26050088, Fisher



Scientific), fetal bovine serum (12.5%, 26140079, Gibco), Penicillin and Streptomycin (100U/ml, 15140122, Gibco). Split down to  $2 \times 10^5$  -  $3 \times 10^5$  cells/mL three times per week. NK-92 freezing medium: 50% FBS; 40% NK92 media; 10% DMSO. Freeze no more than  $5 \times 10^6$  cells/mL

## T cell lines

- 16 Childhood T lymphoblastic leukemia  
1. Jurkat clone E6.1 (RRID:CVCL\_0367; purchased June 2015, Cat No. TIB-152, ATCC)

## Miscellaneous

- 17 Mouse mast cell neoplasm  
1. P815 (ATCC Cat. No. TIB-64, purchased August 2017, RRID:CVCL\_2154) cultured in D10; split down to  $1 \times 10^5$  cells/mL three times per week.
- 18 Human Dermal Microvascular Endothelial Cells  
1. HDMEC (Sigma Aldrich Cat. No. S100-05A)  
Human Microvascular Endothelial Cells - Lung  
1. HMVEC-L (Lonza Cat. No. CC-2527)
- 19 Renal clear cell carcinoma  
1. Caki-1 (RRID:CVCL\_0234; purchased July 2018, Cat. No. HTB-46, ATCC)  
2. Caki-2 (RRID:CVCL\_0235; purchased March 2020, Cat. No. HTB-47, ATCC)
- 20 Mouse fibroblast  
1. L cells (RRID:CVCL\_4536; purchased February 2016, Cat. No. CRL-2648, ATCC)
- 21 Osteosarcoma  
1. U2OS (RRID:CVCL\_0042; purchased March 2020, Cat. No. HTB-96, ATCC)  
Note - you cannot treat this cell line effectively with mycoplasma removal agent (Cat. No. BUF035, BioRad)