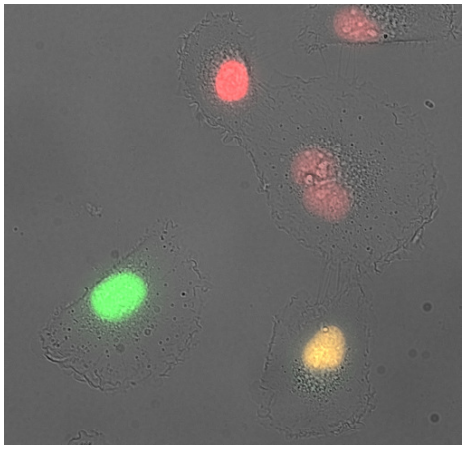

DIC and fluorescence image of Fucci-G_1 Orange and Fucci-S/G_2/M Green in HT1080 cells.

Fucci-HT1080 stable cell line

The day before transfection, 1×10^6 HT1080 cells per 10 cm dish were seeded in 10 ml MEM supplemented with 10% fetal bovine serum, penicillin and streptomycin. The cells were incubated at 37°C in 5% CO_2. The cells were co-transfected with 5 µg pFucci-G_1 Orange (expression vector Code No. AM-V9003) and 5 µg pFucci-S/G_2/M Green-Hyg (expression vector Code No. AM-V9010) with PolyFect® Transfection Reagent (QIAGEN) according to the manufacture's protocol. Twenty-four hours after transfection, the medium was replaced with 10 ml fresh medium containing 300 µg/ml G418 and 50 µg/ml Hygromycin B.

After 3 days, the transfected cells were passaged from a 1:4 split onto a 10-cm dish with fresh medium, and the remaining cells stocked (for backup). When confluent, the cells were cloned by limited dilution seeding of 1 cell/well onto a 96-well plate or stocked (for backup). Isolated clones of the cells were established in the wells.

Cloned cells were imaged by fluorescence microscopy.

Fluorescence microscopy

Fucci-HT1080 cells were grown on a 35-mm glass-bottom dish in 2 ml HBSS containing 20 mM HEPES/NaOH (pH 7.0). Images were acquired using a fluorescence microscope equipped with an objective lens (Olympus, ×40), differential interference contrast (DIC) optical components, and interference filters. Excitation and an emission filter were used at BP 470/20 and D510/40M for pFucci-S/G_2/M Green and BP520-540HQ and BA555-600HQ for pFucci-G_1 Orange, respectively. The dichroic mirror used was series DM 420DCLP.

Image acquisition and analysis were performed using MetaMorph analysis tools.

NOTE: The optical filter and mirror used were optimized for our experiment. We recommend selecting the filter set most appropriate for your experiment.