

Selection and screening of stable mammalian cell lines

Stable cell lines are essential tools for genetic and phenotypic studies. To establish such lines, the transgenic cells which carry a defined genotype or exhibit a desired phenotype must be enriched or isolated from the parental population using a selectable marker. These markers typically encode either survival advantages such as an antibiotic resistance or detectable traits such as a fluorescence marker.

Selection can occur in a single step by applying selective pressure through antibiotics, which kill non-transgenic cells (*Alternative A*). Alternatively, fluorescence or surface markers allow rapid enrichment through screening and physical isolation (*Alternative B*).

Refer to 0036 for banking and tracking of cell lines.

Risk assessment

<ul style="list-style-type: none"> – Work with human-derived material or transgenic cell lines (BSL-2) ▷ Wear gloves, safety glasses, lab coat □ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE 	 <p>Reviewed: Apr 28, 2023</p>
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Procedures

A > Antibiotic selection of mammalian cell lines

□ Culture medium with antibiotics

- (1.) Determine the minimum effective antibiotic concentration for selection using a matrix titration format.
 - Prepare a 96-well plate with 100 µL of culture medium in all wells.
 - Perform a row-wise dilution of the antibiotic: Add 100 µL of the highest concentration to row A, and perform 1:2 serial dilutions down to row G. Leave row H as a no-antibiotic control.
 - In each row, seed column 1 with 100 µL of a 4.0×10^4 mL⁻¹ cell suspension. Mix gently and transfer 100 µL to column 2. Continue serial dilution across columns to column 12. Discard excess.
 - Incubate the plate under standard conditions.
 - Assess cell survival under the microscope after 3–7 d.

Hint: This layout allows assessment of both cytotoxicity and clonogenic potential. Antibiotic potency depends on cell line, passage number, seeding density, and batch variability of the substance at hand.

This is why: If wells in row H with very few cells (columns 10–12) still show growth, the cell line is likely capable of clonal outgrowth from single cells.

- (2.) Select cells using the lowest antibiotic concentration that resulted in complete killing of non-transgenic cells. Include a negative control (non-transfected) to verify selection stringency. Useful ranges:

Cell line	Blasticidin S	G418 (Geneticin)	Hygromycin B	Puromycin	Zeocin
Typical duration	10 days	14 days	4 days	4 days	10 days
293T	2–10 µg/mL	Not determined	Not determined	1.0–5.0 µg/mL	Not determined
HeLa	5–10 µg/mL	Not determined	Not determined	1.0–2.0 µg/mL	Not determined
MCF 10A	2–10 µg/mL	150–400 µg/mL	100 µg/mL	0.5–2.5 µg/mL	750 µg/mL
iPS	10–20 µg/mL	Not determined	Not determined	Not determined	Not determined

Hint: Concentrations marked ‘not determined’ have not been validated in this lab. Consult the literature or perform a kill curve before use.

- (3.) Maintain selection by feeding cells regularly. Expand once resistant colonies are visible.
- (4.) *Optional:* Perform a second round of limiting dilution under selection to ensure the selected cell population is monoclonal.

B > Screening of mammalian cell lines

- (1.) Physically separate the population into clones using limiting dilution, pipette-based cell picking, or flow cytometry (FACS). ☒

Hint: For manual picking under a microscope, seed 1.0×10^4 cells into a 6-well plate and abduct single cells with a pipette.

Hint: Certain cell types need a critical number of neighboring cells to support growth, making it more difficult to obtain pure clones. Use pre-conditioned medium or culture the cells on soft agar, methylcellulose, or in 96-well plates with communicating channels.

- (2.) Feed cells regularly and monitor for clonal outgrowth. Split as needed.

Critical: In 96-well plates, border wells lose volume faster than center wells. Top up edge wells regularly to prevent desiccation. ←

Note: Depending on growth rate, single-cell clones may require 2 to reach usable density for expansion and analysis.

- (3.) Analyze each clone for expression of the transgene or phenotypic marker.

Hint: Use immunofluorescence, flow cytometry, reporter activity, or PCR depending on the marker type.

Troubleshooting

Antibiotic selection of mammalian cell lines

In Step 3:

- No surviving colonies after selection
 - o Verify the antibiotic is active by testing on untransfected cells at the working concentration. Some antibiotics degrade in medium after prolonged storage.
 - o Increase transfection efficiency before selection. Even well-optimized chemical transfections rarely exceed 50% for most cell lines.
 - o Extend the recovery period between transfection and antibiotic addition to allow marker gene expression.
- Resistant clones do not express the gene of interest
 - o Verify transgene expression in a transient assay to rule out vector loss.
 - o Assess whether transgene toxicity, silencing, or recombination may have occurred during integration.
 - o Try linearizing the vector before transfection.

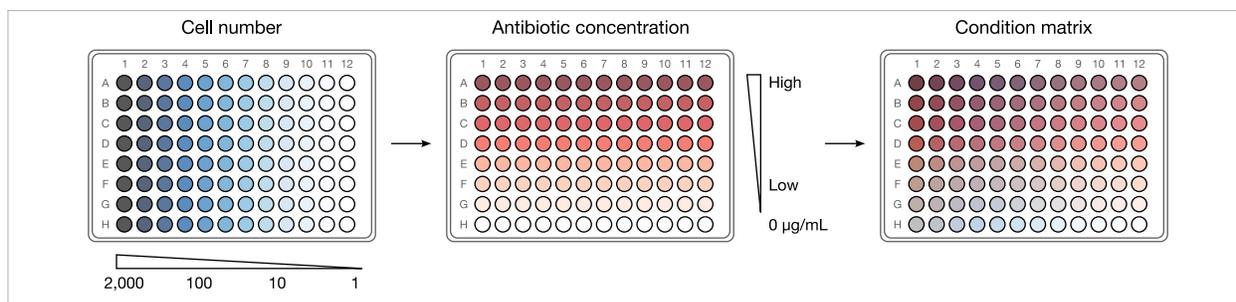
Screening of mammalian cell lines

In Step 1:

- Clones do not grow after separation
 - o Allow four to five weeks for outgrowth before clone picking. Some cells require extended recovery before expansion.

Resources

Antibiotic selection of mammalian cell lines



In Step 1: Matrix titration plate setup. Antibiotic concentrations are diluted across rows (A–G); cell density decreases across columns (1–12). Row H serves as a no-antibiotic control.

Change log

2023-04-28 Benjamin C. Buchmuller Adaptation as SOP.

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