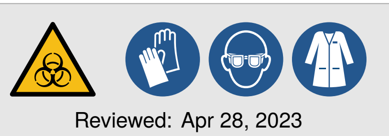
# Selection and screening of stable mammalian cell lines

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 SOP 0036 for banking and tracking of cell lines.



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*This is a bench card. Full protocol available online.*

## *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 $4.0 \times 10^4$ $mL^{-1}$ 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.

(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 |

(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).

(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.

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

Recipe (available online) Resources (available online) Troubleshooting (available online) Notes (available online)

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