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Blasticidin titration of cancer cell lines

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1 Works for me dx.doi.org/10.17504/protocols.io.bgz6jx9e

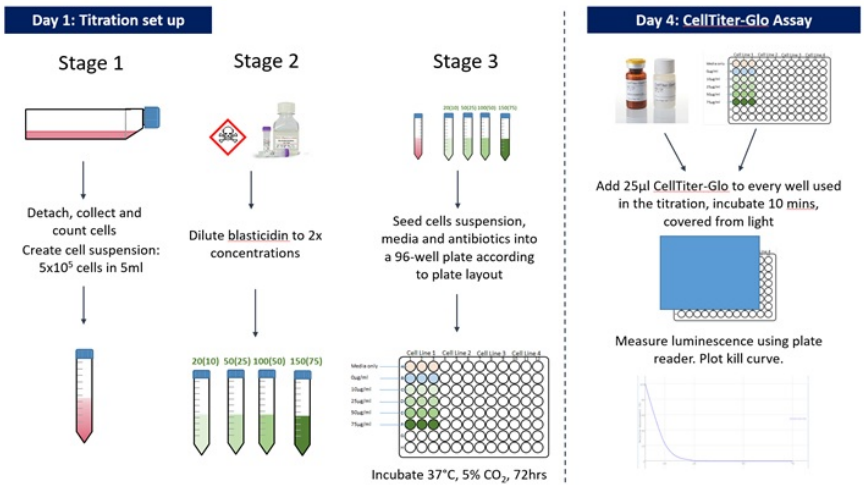
Cellular Generation and Phenotyping

Emily Souster

ABSTRACT

This protocol is used to identify the optimum blasticidin concentration for the selection of Cas9 positive cancer cell lines.

Process diagram:



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COLLECTIONS

Whole genome CRISPR screening in cancer cell lines

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PARENT PROTOCOLS

Part of collection

Whole genome CRISPR screening in cancer cell lines

GUIDELINES

- Ensure the cell suspension is mixed thoroughly to create an even single cell suspension before plating.
- All steps involved in the plate set up, including seeding cells, media, antibiotics and CellTiter-Glo should be carried out using reservoirs and multi-channel pipettes where possible to avoid ergonomic strain and to maintain homogenous solutions throughout.
- It is essential to use black 96-well plates in this protocol, as luminescence can carry over into neighbouring wells in clear plates.

MATERIALS

NAME	CATALOG #	VENDOR
CellTiter-Glo(R) 2.0 Assay	G9241	Promega
Falcon™ 15mL Conical Centrifuge Tubes	14-959-53A	Fisher Scientific
TrypLE™ Express Enzyme (1X), no phenol red	12604021	Thermo Fisher
Reagent Reservoir	9510047	Thermo Fisher
DPBS	14190	Invitrogen - Thermo Fisher
10mg/ml Blasticidin	ant-bl-1	InvivoGen
Black walled 96 well plate	10419822	Fisher Scientific

MATERIALS TEXT

Select an appropriate culture media for your cell line. Common culture medias used for cancer cell lines are serum supplemented Advanced DMEM F-12 or RPMI, in the presence of pen-strep.

Equipment

Light Microscope
Microbiology safety cabinet (MSC)
Pipette Boy
Stripettes
Pipettes and tips
Centrifuge
Multichannel Pipette and tips
37 °C , 5%CO_2 incubator
Plate reader

SAFETY WARNINGS

- Blasticidin is toxic if swallowed and harmful in contact with skin.
- CellTiter-Glo is harmful to aquatic life with long lasting effects.

BEFORE STARTING

- Pre-warm culture media to room-temperature.
- Thaw a vial of 10mg/ml Blasticidin.

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Day 1: Titration plate set up

1 Detach, collect and count cells by following Steps 1-8 of the protocol: Passaging adherent cancer cell lines.

2 Resuspend 5x10^5 cells in **5 mL** of culture media, at a concentration of 1x10^5 cells/ml.

3 Using a 10mg/ml stock of blasticidin, prepare four dilutions, at 2x final concentration by diluting the stock in media as show in Table 1, column C & D. (When the 2x antibiotic concentration is diluted with an equal volume of cell suspension it will result in the final concentration show in Table 1, column B).

- Prepare a minimum of 5ml of each 2x antibiotic to that the volume is adequate for loading a multi-channel pipette without bubbles.
- Antibiotic dilutions should be prepared fresh on the day that they are required.

2 x concentration (µg/ml)	Final concentration (µg/ml)	10mg/ml stock blasticidin (µl)	Media (ml)	Total (ml)
20	10	10	4.99	5
50	25	25	4.975	5
100	50	50	4.95	5
150	75	75	4.825	5

Table 1. Preparation of blasticidin concentrations using 10mg/ml stock to achieve a 2x concentration.

A wider range of blasticidin concentrations can be used if necessary, for example 1µg/ml- 200µg/ml, depending on the cell type and the sensitivity of the cell line.

Blasticidin is toxic if swallowed and harmful in contact with skin.

4 Using a multi-channel pipette, add **75 µl** cell suspension to the first 3 wells of rows B-F in a 96-well plate (row A is used as a control with no cells, to subtract background luminescence).

Always seed 3 wells per row as the titration is carried out in triplicate. Therefore, a 96-well plate can be used to titrate up to 4 cell lines at a time (see Fig. 1).

5 Using a multi-channel pipette, add **150 µl** media to the first 3 wells of row A, and **75 µl** media to the first 3 wells

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of row B in a 96-well plate.

6 Pipette **75 µl** of the blasticidin 2x concetrations into the first 3 wells of rows C-F, to achieve the final concentrations as per the plate layout shown in Fig. 1.

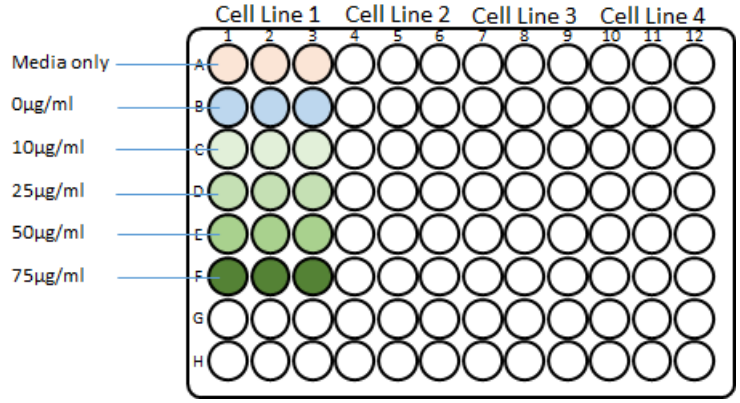

Figure 1. Plate layout for blasticidin titration of one cell line.

7 Incubate at **37 °C** , 5% CO_2 for approximately 72 hours.

Day 4: Assessing cell viability using CellTiter-Glo at 72 hours

8 Thaw CellTiter-Glo 2.0 reagent and equilibrate to room-temperature prior to use. Mix by gently swirling to obtain a homogeneous solution.

- The CellTiter-Glo reagent can be stored at **-20 °C** and is stable for up to 4 freeze-thaws; thawed reagent can be kept at **4 °C** for up to 5 months.
- CellTiter-Glo is light sensitivie so should be stored in tin foil, and used in a cell culture hood with the light off where possible.

9 Remove the 96-well plate from the incubator and allow to equilibrate to room-temperature for 15 minutes.

10 Using a multi-channel pipette, add **25 µl** CellTiter-Glo reagent to each well (1:6 dilution) and mix by gently rocking the plate back and forth. Incubate at room-temperature for 10 minutes (wrap plate in blue roll/foil or keep away from light where possible).

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11 Use an appropriate plate reader to record the luminescence of each well.

The plate reader should be set to an integration time of 1 second per well, and optimised for a peak emission wavelength of 560nm.

12 Create a kill curve as follows:
- Average the triplicate luminescence values to get a single value for each condition.
- Subtract the average background luminescence (row A, media only) from the other averaged vaues.
- Divide the average luminescence for 10, 25, 50 and 75µg/ml by the 0µg/ml average to get a relative percentage viability.
- Plot these values on a graph to create a kill curve.

The 'kill concentration' is the lowest concentration of blasticidin which results in death of approximately 100% of cells after 72 hours.

For example, the 'kill concentration' in Fig. 2 is 25µg/ml.

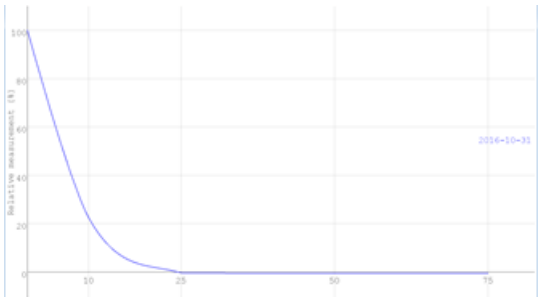

Figure 2. Kill curve for a blasticidin titrated cancer cell line.

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