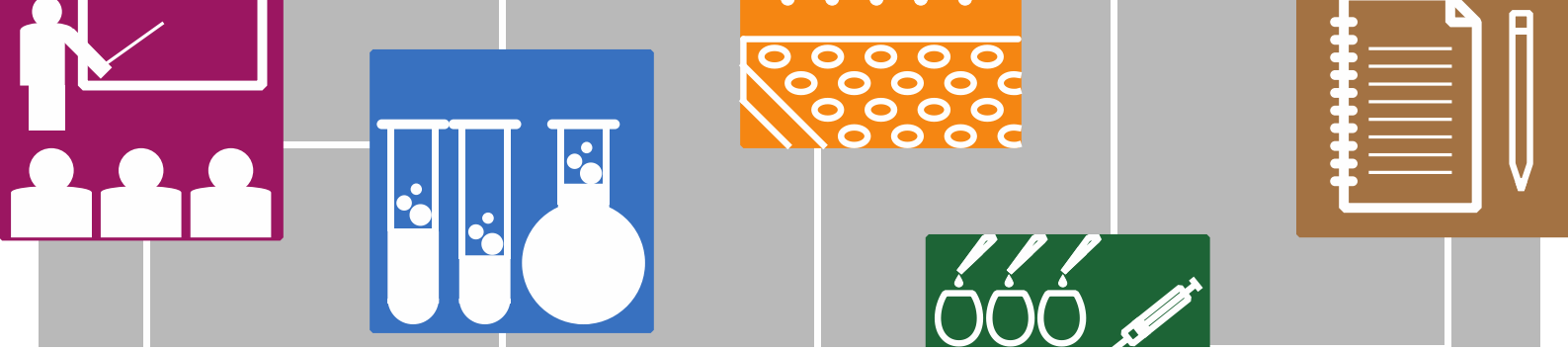

Clonogenic assay

Evaluation of the survival of clonogenic cells following treatment is an important aspect of experimental cancer therapy. The clonogenic assay is a common *in vitro* assay to determine the intrinsic radiosensitivity of many types of cells. The assay can be used to establish reproductive cell survival after treatment with radiation, cytotoxic agents alone or in combination with irradiation. It is based on the principle that after treatment, damaged cells can occasionally still divide a few times or survive as sterile (non-reproductive) cells. Only cells that are still reproductive and able to form a colony are considered to ultimately survive. Hence, clonogenic cells are cells that have the capacity to produce an expanding family of descendants (defined as > 50 cells, i.e., a number reached after at least 6 cell doublings since $2^6 = 64$).

The clonogenic capacity of cells is presented as plating efficiency (PE), which is defined as the number of colonies formed per number of seeded cells:

PE = number of colonies formed / number of seeded cells.

It is important to note that untreated cells are not 100% clonogenic and the plating efficiency varies between different cell lines or experimental conditions. To get insight in the effect of treatment, the P.E. of treated and untreated cells is used to calculate the Surviving fraction (SF) which is defined as the ratio of the PE of treated and untreated cells:

SF = PE of treated cells / PE untreated cells.

An outline and example of the clonogenic assay is provided in the figure below.

Tip: By determining the PE and SF at increasing treatment doses, a clonogenic survival curve can be generated. Such a curve can be used to compare the effect of single treatment vs. combination treatment. In case of irradiation in combination with drugs, a shift of the curve to the left indicates radiosensitization and a shift to the right radioprotection.

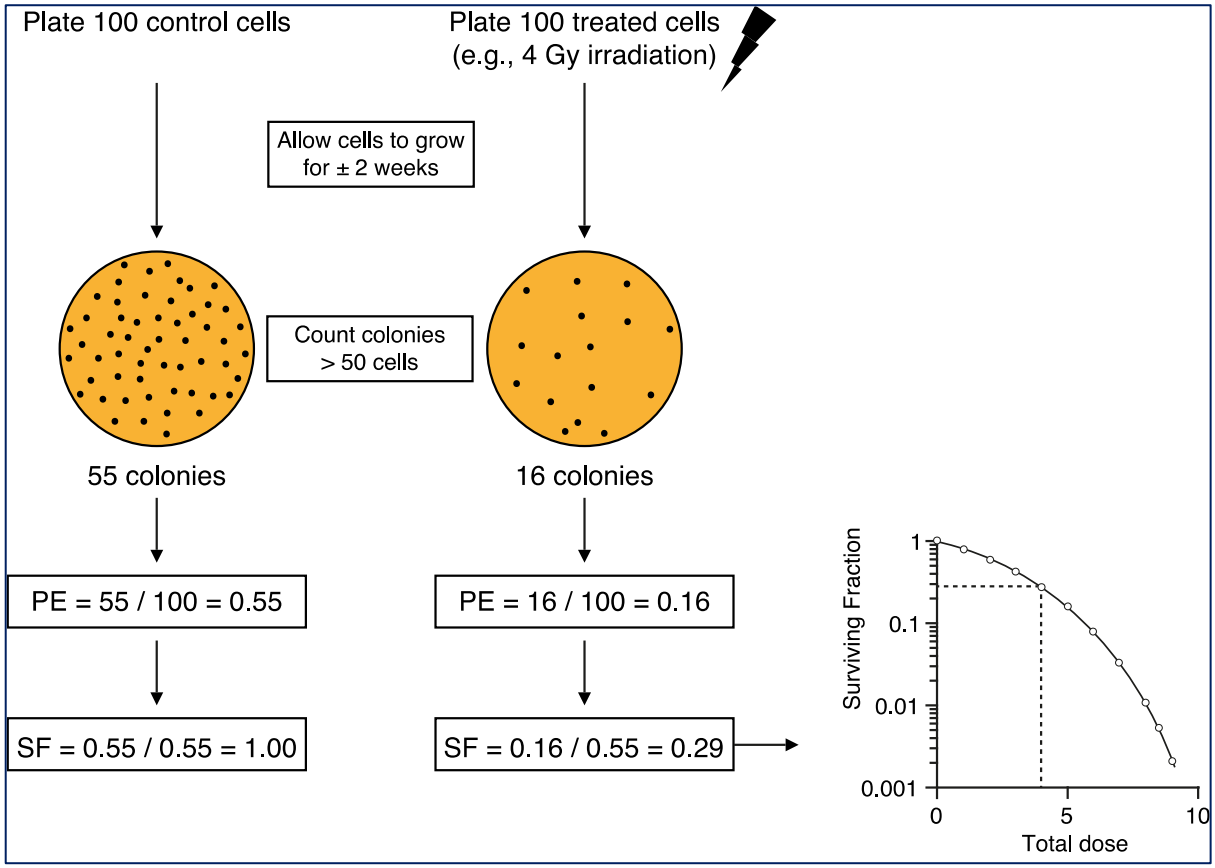
Plate 100 control cells
Plate 100 treated cells
(e.g., 4 Gy irradiation)
Allow cells to grow
for ± 2 weeks
Count colonies
> 50 cells
55 colonies
16 colonies
PE = 55 / 100 = 0.55
PE = 16 / 100 = 0.16
SF = 0.55 / 0.55 = 1.00
SF = 0.16 / 0.55 = 0.29
Surviving Fraction
1
0.1
0.01
0.001
0
5
10
Total dose