COLONY ASSAY

1. Drug treatment after plating cells

- Seed MEFs on 24-wells plates.

- Trypsinize 6-well (10 cm^2) with 0.5 ml TVP (5 min 37 °C).
  Add 0.5 ml complete + β+ LIF and resuspend cells.
  Count cells → 4 - 8x10^6 cells per ml.

- Seed 500 cells per 2 cm^2 well:
  1 µl cells in 4 ml complete + β + LIF for 8 wells of a 24-wells plate.
  Use Eppendorf multipet to pipet 0.5 ml medium with cells in each well.

- Next day:

  . (incubate cells with 40 µM O^6-benzylG for 1 h at 37 °C if methylating or chloroethylating drugs are tested).
  . incubate with drug for 1 h at 37 °C.
  . wash cells with PBS.
  . add per well 0.5 ml complete + β + LIF.

- Count colonies after 3 days (200-250 colonies in well without drug).

2. Drug treatment before plating cells

- Seed MEFs on 24-wells plates.

- Trypsinize 6-well (10 cm^2) with 0.5 ml TVP (5 min 37 °C).
  Add 0.5 ml complete + β + LIF and resuspend cells.
  Count cells → 4-8 x 10^6 cells per ml.

- Pipet 100-150 µl cells in 8 ml complete + β + LIF.

- Use Eppendorf multipet to pipet 0.5 ml medium with cells in Nunc vials.

- Add 10 µl drug of appropiate concentation to each vial.

- Incubate at 37 °C for 1 h.

- Transfer 10 µl of treated cells to 24 wells containing 0.5 ml complete + β + LIF.

- Count colonies after 3 days (200-250 colonies in well without drug).

O^6-benzylG (20 mM)

O^6-benzyl-Guanine	4.82 mg
DMSO	1 ml
