ES CELL CULTURE

1. On MEFs

- Coat tissue culture flasks or wells with 0.1% gelatin for 30 min at RT or ON at 4°C.
- Thaw irradiated MEFs at 37 °C; spin cells down through 10 ml complete medium (5 min at 1200 rpm) and seed cells onto gelatin-coated surfaces. Incubate ON at 37 °C/5% CO_2.
- Thaw frozen ES cells at 37 °C; spin cells down through 10 ml complete medium + β + LIF (5 min at 1200 rpm).
- Resuspend ES cells and seed onto approximately twice the original surface. Flasks are gazzed with 5% CO_2 / 95% air, close lid not too tightly. Incubate at 37 °C / 5% CO_2.
- Refresh medium next day.
- Refresh medium when buffer is exhausted (orange-yellow); generally, after one to three days.
- Passage cells at confluency or when medium is yellow within one day after refeeding: generally this occurs after 3-5 days of culturing.
    - Wash cells with small volume of PBS.
    - Trypsinize with 1 ml of TVP(10x) per T25 for 10 min at 37 °C.
    - Detach cells by careful shaking and rapping.
    - Add 1 ml of medium and resuspend to single cells with a 10ml pipette or blue tip.
    - Reseed cells 1 : 5.
- ES cells can be frozen normally in 10% DMSO in complete medium - β - LIF.

2. On BRL-conditioned medium

- Culture ES cells on 0.1% gelatin-coated flasks or wells.
- Use <u>60% BRL + β + LIF</u>.
- Use <u>TVP(2x)</u>.

<u>NOTES</u>:

- Take care that the trypsin is always at least diluted 10-fold!!
- ES cells grow in tightly packed patches, slightly rounded, with sharp, well defined edges.
- No single cells should be visible in the patches.
- No differentiated cells should be present between the patches

3. Surfaces and volumes

	cm^2	ml medium	ml TVP	ml gelatin 0.1%
96-well	0.3	200 µl	25 µl	50 µl
4-well/24-well	1.8	0.5 - 0.75	0.1	0.3
12-well	4	1.5	0.25	0.5
6-well	10	3	0.5	1
grid plate	20	7	1	2
T25 flask	25	5 - 8	1	2.5
large plate r=8.5	57	10	2.5	6
T75 flask	75	25	3	7.5
T175 flask	175	60	7	15
Roller bottle	850	≥150		