Growth *D. radiodurans* **(ATCC BAA-816) in DRM without Mn supplementation (no-Mn DRM)**

I. DRM ingredients (for 1000ml DRM):

		Final concentration:
H_2O	854.7 ml	
10x Potassium Phosphate buffer 0.2M (pH 8.0)[1]	100 ml	20mM
100x $(NH_4)_2SO_4$, 1.5M (Sigma)[2] (**optional**)	10 ml	15mM
Autoclave and add sterilized before adding the following:		
$MgCl_2$ 0.8M (Sigma)	1 ml	0.8mM
$CaCl_2$ 0.18M (Sigma)	1 ml	0.18mM
Fructose 20% (Sigma)	20 ml	0.4%
Vitamins Mix (Sigma)	10 ml	10 mg/ml
12 Amino acids[3]	800 µl	200µg/ml
L-Leucine[4]	2.5 ml	25µg/ml

[1]10x Potassium Phosphate buffer 0.2M (pH 8.0):
K_2HPO_4, 1.0M (174.0 g/L) (Sigma) - 188 ml/L
KH_2PO_4, 1.0M (136.0 g/L) (Sigma) - 12 ml/L

[2]100x $(NH_4)_2SO_4$, 1.5M (Sigma) - 198 g/L

[3]Amino acids: His, Phe, Ala, Arg, Gly, Lys, Met, Pro, Ser, Thr, Val (Sigma), Glu (Gibco BRL) - stock 250 mg/ml total:
Ala – 1 g
Gly – 2 g
Ser – 2 g
His – 1 g
Glu – 1 g
Met – 2.5 g
Pro – 1 g
Arg – 1 g
Thr – 1 g
Val – 1 g
Lys – 2 g
Phe – 0.5 g
Total 16 g
Add water to 64 ml

[4]Leu – stock 10 mg/ml in 0.01M HCl

DRM plates:

1. Mix on warm stirrer 2x MM Mixture for 500 ml:

   354.7 ml H_2O
   100 ml 5x Phosphate buffer, 0.2M (pH 8.0)
   10 ml 100x $(NH_4)_2SO_4$, 1.5M
   1 ml 1000x $MgCl_2$, 0.8M
   1 ml 1000x $CaCl_2$, 0.18M
   20 ml 20% fructose

2. Melt in microwave oven 500 ml 3% Nobel agar[5] (1 min +1 min or less) and pour in 2xMM mixture. Mix well #1 + #2 (with stirrer) and put in 50°C water bath for 20-30 min.
3. Then add (on stirrer):

   10 ml 100x Vit mix
   800 µl amino acids[3]
   2.5 ml L-Leucine[4]

[5]Washed Noble agar preparation:

36 g Noble agar (DIFCO) + 4g (~10%) is required to prepare 1200 ml 3% agar

1. Mark 1200 ml-level on flask.
2. Add 1L 1mM EDTA (8.0) (in deionized water) to 40g Nobel agar, mix by hand and leave for 20 min, remove liquid and add 1mM EDTA again (final volume about 1-1.2L; three times total; for all 3 washes use 2 L 1mM EDTA).
3. Remove EDTA as much as possible using 50ml pipette (the rest volume of agar must be about 600 ml).
4. Add deionized water (ddw), up to 2 L, mix well, wait 20 min, and replace with fresh ddw four more times (5 water washing finally). Remove water carefully, with pipette at the border water/agar, try to remove as much water as possible.
5. Last time fill flask with water completely.
6. Remove as much water as possible and add ddw up to 1200 ml.
7. Autoclave.
8. After autoclaving, mix very well and pour in sterile bottles or flasks suitable for microwave, and keep them at room temperature.

D. radiodurans **(R1) cells grown without Mn supplementation:**

R1 cells were pre-grown on a DRM plate (2.5 nM Mn) for 4-5 days at 32^0C.
R1 cells from a plate were inoculated into 15 ml (50 ml tubes) of DRM (all components agreed the DRM protocol) supplemented with 2.5 nM Mn (OD_{600} ~ 0.2) and incubated at 32^0C overnight (OD_{600} ~ 1.0). Next day R1 cells were spun down and resuspended in freshly prepared 10 ml of DRM (no Mn supplementation) to OD_{600} ~ 0.1 and incubated over 2 nights to OD_{600} ~ 1.0. Then R1 cells were transferred to 15 ml tubes and irradiated on ice followed by plating with standard dilutions.