

# The cells grow at 33C

## Cell Culture Media and Reagents

### DMEM + 10 % FBS

- Remove 50 ml of medium from 500ml medium bottle
- Add 50 ml of Fetal Bovine Serum (FBS)
- Add 5 ml of Pen/Strep/Glu solution of a 100X solution (Gibco #10378-016)
- Add 5 ml of [G418] 40 mg/ml (400 microg/ml final)
- Store at + 4°C

### [G418] 40 mg/ml

- 600 mg in 15 ml PBS
- filter with 0.2 µm filters
- store at -20°C

### Freezing medium

- DMEM
- 20% FBS
- 10% DMSO
- 1:100 PGS solution

### Thawing process

- warm the vial up at 37C
- when thawed, put in a tube with medium
- spin at 1200 rpm for 4 minutes
- resuspend pellet in the desired volume of medium WITHOUT G418
- incubate at 33C

The presence of G418 makes the recovery from the thawing process more difficult. Start to add G418 when the cells look fine (usually at the first passage after the thawing)

### Growth conditions

The cells **grow at 33°C** in DMEM + 10% FBS + Pen Strep Glutamine + G418  
I usually grow them until they are about 75% confluent, and then I split them by trypsinization.