## Before you start

See 'Medium & Reagents for JM8.N4 cells' protocol for all reagents
Thaw and pre-warm trypsin
Pre-warm PBS
Pre-warm required media
Pre-warm gelatin

| Dish/Flask/Plate | Volume of Gelatin/PBS | Volume of Trypsin | Volume of Media |
|---|---|---|---|
| 6 well plate | 3ml | 500µl | 4.5ml |
| 96 well plate | 100µl | 25µl | 175µl |
| 10cm Petri dish | 8ml | 1.5ml | 8.5ml |

## Things you'll need

An aspirator & sterile filter-free tips/pipettes
Pipette aid & plastic pipettes
3 x liquid reservoirs
Multichannel pipette and 200µl Rainin filtered tips.
*We have tried several brands of tips, and find that the bore of the Rainin 200µl tip is optimal for dissociating cells.*

## For passaging cells in 96-well plates (Falcon #353916) or flasks (Corning #430167):

***JM8.N4 cells should be passaged every 48 hours at a 1:6 dilution***

1. Remove the parent *flask* from the incubator and observe under the microscope. Check cells are healthy and confluent
2. Aspirate old media
3. Wash with pre-warmed PBS
   *Add PBS to the side of the flask, and slowly tilt to gently wash the cells.*
4. Add pre-warmed trypsin.
   *Gently swirl the flask to cover all cells with trypsin*
5. Incubate at 37$^{o}$ for 10 minutes
6. While cells are incubating, coat fresh flasks with 0.1% gelatine (1 minute), aspirate and add pre-warmed media
7. After incubation, gently swirl the flask again and add 5 volume of medium to inactivate the trypsin.
   *Pipette up and down gently 3 to 4 times to disperse cells*
8. Transfer 1/6$^{th}$ of cell suspension to each of the fresh flasks.
9. Incubate the plates in an incubator at 37ºC with 5% $CO_2$.
10. Check cells the following day and media change to remove all traces of trypsin and dead cells.

*Example: Passing a confluent 96 well plate into 4 daughter plates.*

1. Coat four fresh, sterile 96-well tissue culture plates with 100 µl gelatin.
   *Incubate at room temperature for >10 minutes.*
2. Remove the parent 96-well plate from the incubator and observe under the microscope. Check cells are healthy and confluent
3. Aspirate old media
4. Wash each well with 100 µl pre-warmed PBS
   *Add PBS to the side of the wells*
5. Add 25 µl pre-warmed trypsin.
   *Gently tap the plate to ensure all cells are covered with trypsin*
6. Incubate at 37$^{o}$ incubator for 10 minutes

7. While cells are incubating, remove gelatin from the daughter 96-well plates and add 150 µl fresh, pre-warmed medium to each well
8. After incubation, gently tap the plate again and add 175 µl medium to inactivate the trypsin. *Gently pipette up and down 3 to 4 times to disperse cells.*
9. Gently transfer 50 µl of the cell suspension to each of the four daughter 96-well plates.
10. Incubate the plates in an incubator at 37ºC with 5% $CO_2$.
11. Check cells the following day and media change to remove all traces of trypsin and dead cells

12. **<u>For splitting 96-well plates:</u>**

*Example: Splitting a 96-well plate 1:4.*

1) Gelatinise 4 X 96-well plates with 100µl 0.1% gelatin.
2) Remove the 96-well plate from the incubator and observe under the microscope. Check cells are healthy and confluent.
3) Using multichannel aspirator, remove old media from wells.
4) Using electronic multichannel pipette, add 100µl of PBS to each well.
5) Aspirate off PBS.
6) Add 25µl 2 x Trypsin + glucose.
7) Gently tap plate and incubate for 8-10 minutes.
8) While cells are incubating, remove gelatin using aspirator and add 150µl of pre-warmed media to each well.
9) After incubation, gently tap the plate again, and check that cells have lifted off the plate.
10) Add 175µl of media to the cells.
11) Use the Eppendorf multichannel pipette and the 200µl Rainin tips for the split:
    i. Use a fresh tip for each well i.e. a whole box of 96 tips will be required
    ii. Gently aspirate the first column of the master plate, up and down about 5 times, then transfer 50µl of cell suspension to the first column of each of the new plates, gently mixing the cell suspension in the new media.
    iii. Discard tips, and repeat for the next 11 columns.
12) Label plates and incubate.
13) Check cells the following day and media change to remove all traces of trypsin and dead cells.