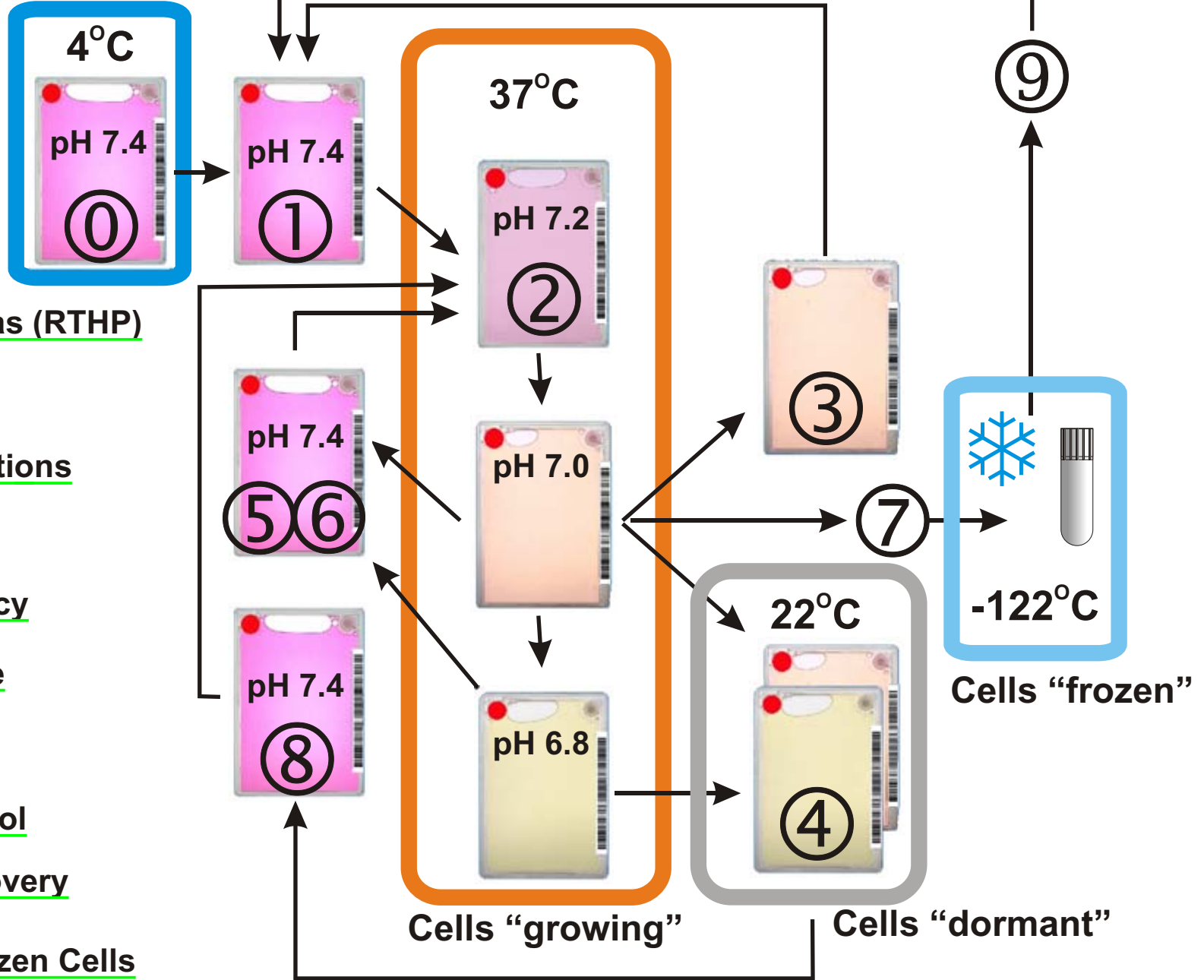


MAP OF PROCEDURES (pH GUIDANCE)

“Pre-filled Petakas”

Copyright 2004 Celartia Ltd.



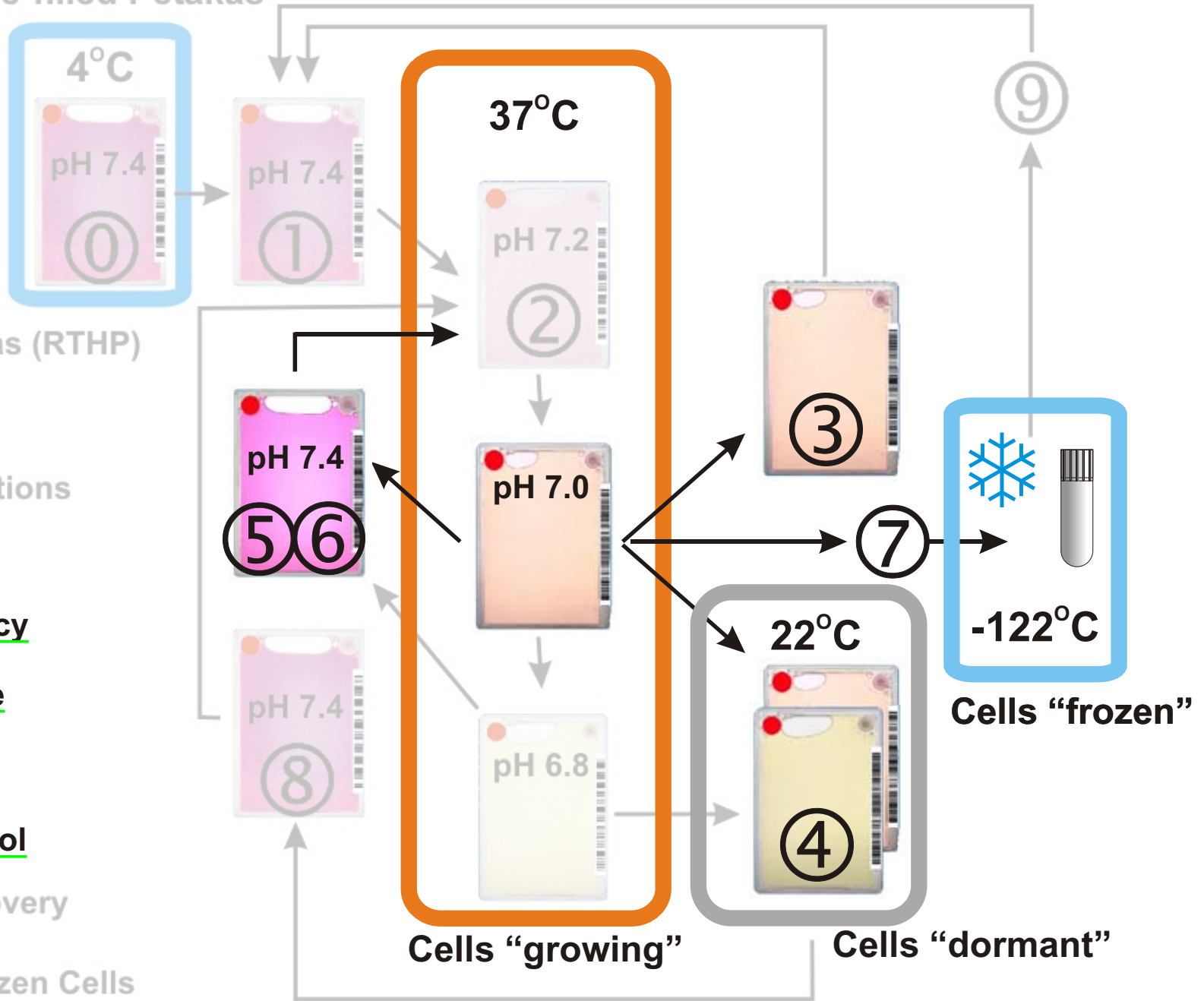
- ① Pre-filled Petakas (RTHP)
- ② Seeding Cells
- ③ Incubation Positions
- ④ Cell Harvesting
- ⑤ *In vitro* Dormancy
- ⑥ Media Exchange
- ⑦ pH Adjustment
- ⑧ Freezing Protocol
- ⑨ Dormancy Recovery
- ⑩ Recovering Frozen Cells

IF THE MEDIA REACH pH 7.0 THESE ARE YOUR BEST OPTIONS!

"Pre-filled Petakas"

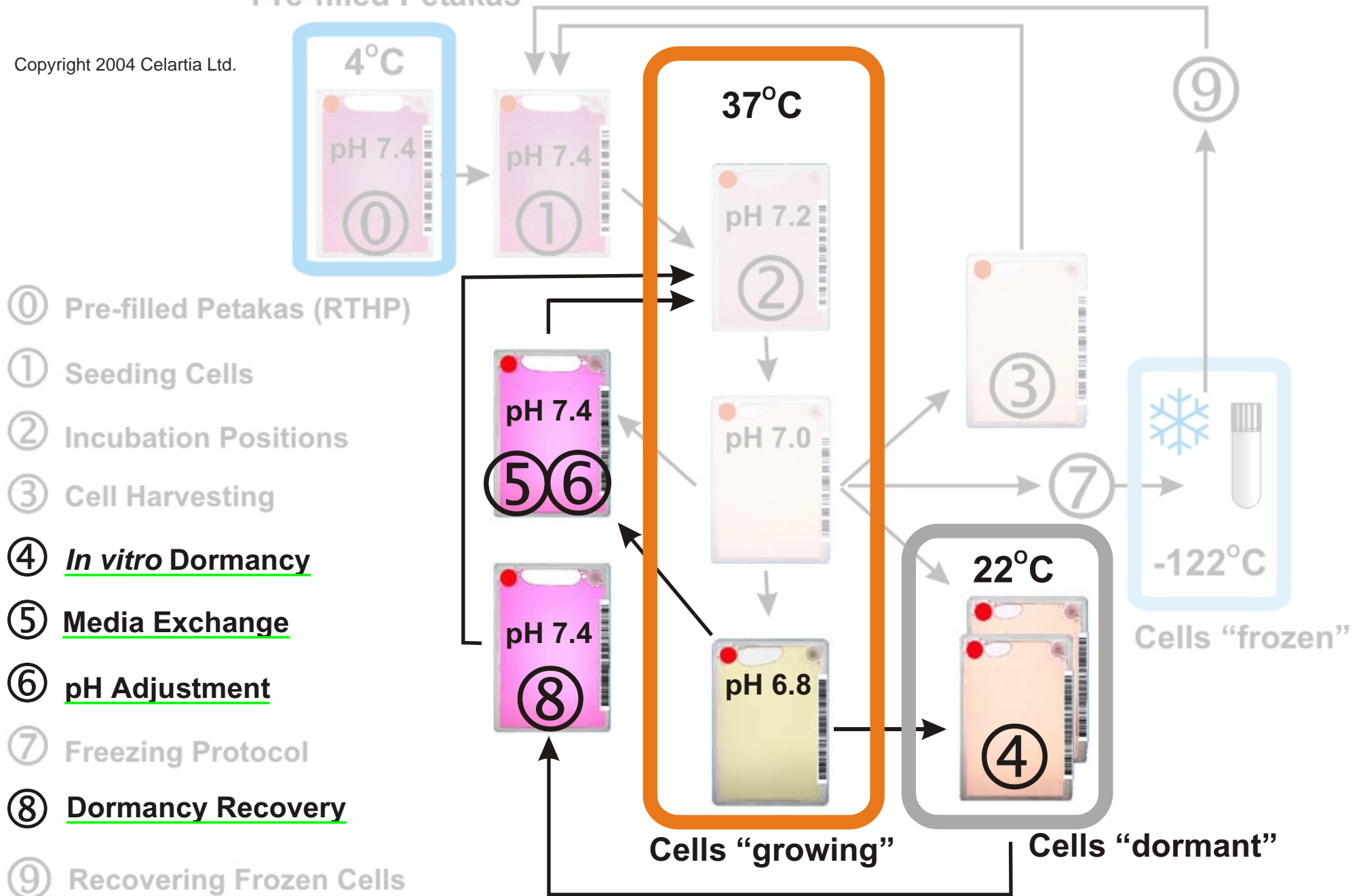
Copyright 2004 Celartia Ltd.

- ① Pre-filled Petakas (RTHP)
- ② Seeding Cells
- ③ Incubation Positions
- ④ Cell Harvesting
- ⑤ In vitro Dormancy
- ⑥ Media Exchange
- ⑦ pH Adjustment
- ⑧ Freezing Protocol
- ⑨ Dormancy Recovery
- ⑩ Recovering Frozen Cells



IF THE MEDIA GO BELOW pH 6.9 THESE ARE YOUR BEST OPTIONS!

Copyright 2004 Celartia Ltd.



CULTURING CELLS IN SERUM SUPPLEMENTED MEDIA

1 SEEDING THE CELLS

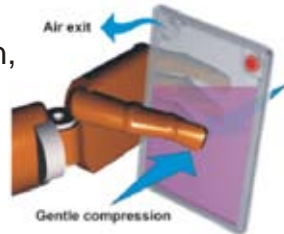


Holding Petaka in vertical position!

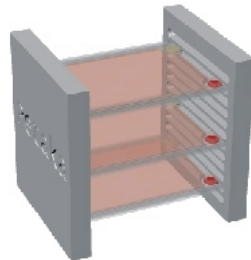
1. Inject 1.2 mL of Serum for 5%
2. Inject 0.5 mL of Antibiotic solution
3. Inject X mL of Cell suspension
4. Inject 21-X mL of Medium

TOTAL: 23 mL

5. Holding Petaka in vertical position, PRESS it to expel residual air



6. Holding the pressure, Protect the filter with a Filter Protector

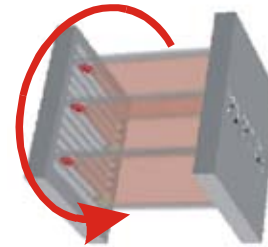
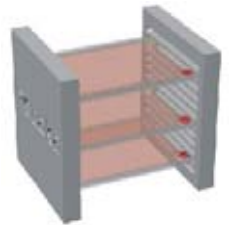


7. Incubate Petaka according with the Cell type (see INCUBATION POSITIONS)

2 INCUBATION POSITIONS

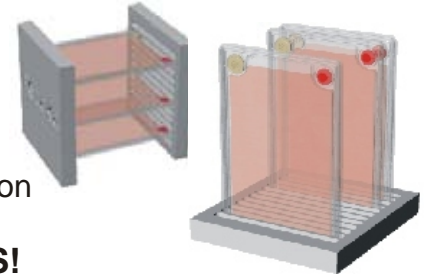
A) ADHERENT CELLS!

1. After seeding ALWAYS incubate at least 1 h in HORIZONTAL



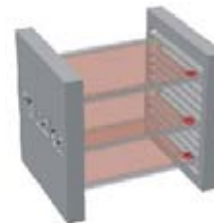
2. For cell growth in both sides of Petaka, after 20-30 minutes in Horizontal position one side, flip rack 180° and incubate at least 12 h in horizontal position on the second side

3. After complete cell attachment, incubate as long as needed in either horizontal or vertical position



B) NON-ADHERENT CELLS!

1. ALWAYS incubate in HORIZONTAL position



HARVESTING CELLS

ADHERENT CELLS WITH TRYPSIN-EDTA



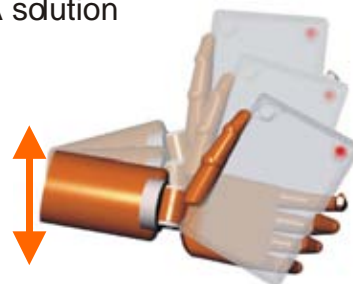
1. Remove Filter protector



2. Disinfect port with 90% Ethanol



3. Inject 4 mL of 0.25% Tyrp sine-EDTA sdution

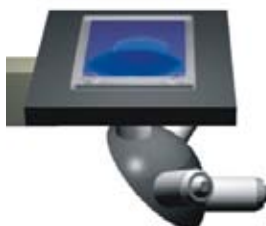


4. Shake softly for 10-15 seconds



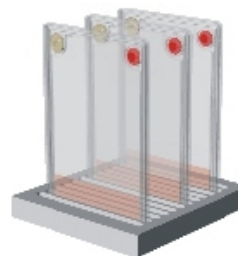
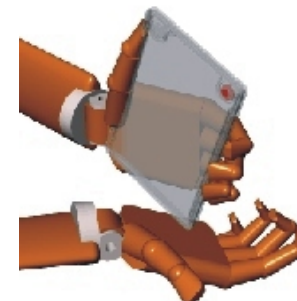
5. Incubate at 37°C for 3 min in horizontal position

6. Control Cell detachment under the microscope



WHEN CELL ARE DETACHED!

7. Tap Petaka 2-3 times



8. Leave Petaka 1-2 minutes in VERTICAL position



9. Withdraw the cell concentrate

For NON Adherent Cells Click here

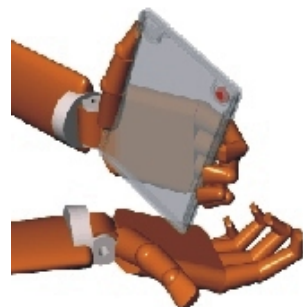
HARVESTING CELLS

NON ADHERENT CELLS

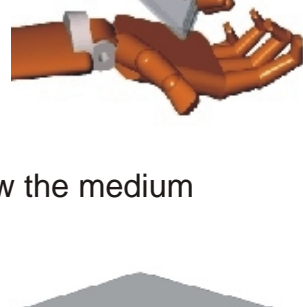
1. Remove Filter protector



2. Disinfect port with 90% Ethanol



3. Tap Petaka 2-3 times



4. Slowly withdraw the medium



5. Transfer the medium containing the cells into a centrifuge tube and concentrate by centrifugation



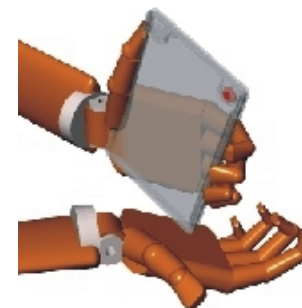
ALTERNATIVELY!

1. Remove Filter protector

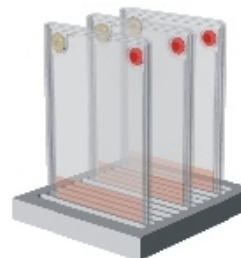


2. Disinfect port with 90% Ethanol

3. Tap Petaka 2-3 times



8. Leave Petaka 1-2 HOURS in VERTICAL position



4. Slowly withdraw the lowermost medium with concentrated cells



For ADHERENT Cells Click here

“READY TO HOST” PETAKAS (RTHP)

PREPARING “READY TO HOST” PETAKAS

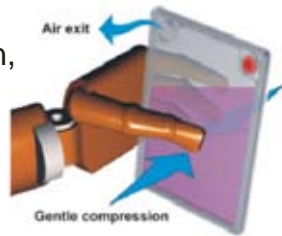


Holding Petaka in vertical position!

1. Inject 0.5 mL of Antibiotic solution
2. Inject 19 mL of Medium

TOTAL: 19.5 mL

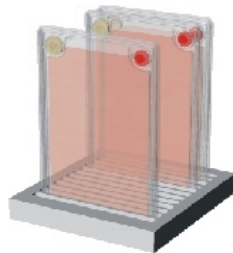
5. Holding Petaka in vertical position, PRESS it to expel residual air



6. Holding the pressure, Protect the filter with a Filter Protector



7. Keep these Petakas refrigerated at 4°C In vertical position in stands or in plastic bags (zip bags)



Dehydration and pH should be controlled if RTHP are kept In refrigerator for months!

SEEDING CELLS IN “RTHP”

1. Remove Filter protector from RTHP



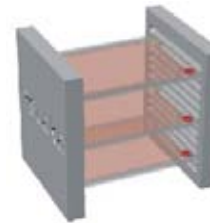
2. Disinfect port with 90% Ethanol

3. Warm RTHP in incubator, at 37°C for 20-30 min in VERTICAL position



4. Inject 1.5 mL of Serum (*)
5. Inject up to 2mL of cell suspension

6. Shake softly for 10-15 seconds



7. Incubate in HORIZONTAL position

For INCUBATION POSITIONS Click here

1

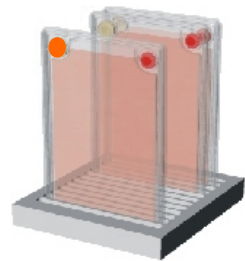
KEEPING CELLS IN IN VITRO "DORMANCY" (ONLY ADHERENT CELLS)

4 PUTING THE CELLS IN "DORMANCY"

8 RECOVERING CELLS FROM "DORMANCY"

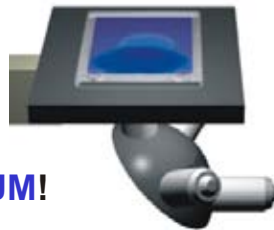
1. WITHDRAW Petakas from the incubator

2. Protect filters with Filter protector



3. Keep Petakas at 20-25°C in vertical position, away from direct sun light, or Infr-Red sources.

6. Periodically Control Cell shape under the microscope



DO NOT CHANGE THE MEDIUM!

In-dormancy survival time depends on:

- A) Cell type
- B) Culture Media
- C) Type of Buffer (in the media)
- D) Stability of the environmental variables

1. Remove Filter protector from dormant Petakas



2. Disinfect port with 90% Ethanol

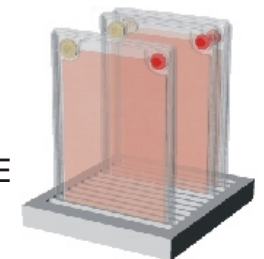
3. Slowly withdraw the medium



4. Inject same volume of NEW medium



5. Incubate at 37°C, in vertical position, at least for 24 hours before cell SUBCULTURE



FREEZING CELLS



1. Remove Filter protector

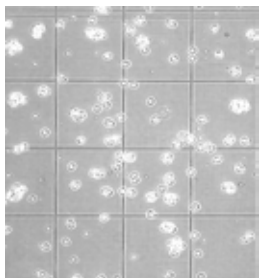


2. Disinfect port with 90% Ethanol



3. FOLLOW HARVESTING CELLS
PROTOCOL **3**

4. Transfer the medium containing the
CELLS to conical tubes.
Wash the cells twice with PBS.



5. Count the cells

5. Concentrate the CELLS
by centrifugation in conical tubes.

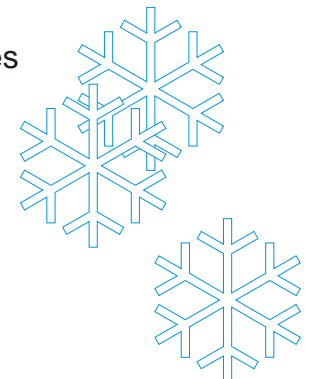


6. Resuspend the cell pellet in
FREEZING MEDIUM at a final concentration:
1,000,000 cells per mL of medium

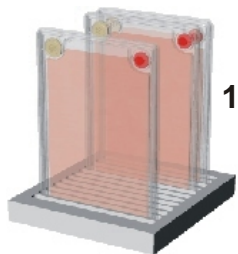


7. Transfer 1.5 mL of cell suspension
into each Cryotube

8. Proceed freezing the Cryotubes
(stepwise) up to -120°C and
store in LIQUID NITROGEN



RECOVERING FROZEN CELLS



1. PREPARE "READY TO HOST" PETAKAS

2. Remove Filter protector



3. Disinfect port with 90% Ethanol

4. Warm RTHP in incubator, at 37°C for 10-20 minutes in VERTICAL position



5. Inject 3.0 mL of Serum (*)

6. Thaw the cryotube in a disinfected water bath at 38°C for 1-3 minutes. Be sure the ice is melted

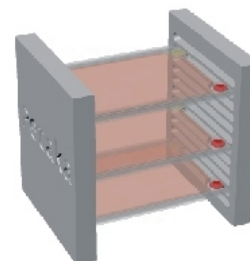
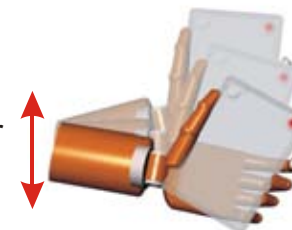


7. Extract the cell suspension from the cryotube with a 1 mL tip



8. Transfer the cell suspension into a Petaka (RTHP)

9. Shake softly for 10-15 seconds



10. Incubate Petaka according with the Cell type (see INCUBATION POSITIONS)

For INCUBATION POSITIONS Click here

pH ADJUSTMENT OF GROWING CULTURES

5 pH ADJUSTMENT WITH SODIUM BICARBONATE

1. Remove Filter protector from RTHP



2. Disinfect port with 90% Ethanol

3. Withdraw 1mL of Medium



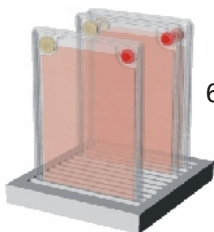
4. Inject 1 mL of 7.5% Sodium Bicarbonate solution sterile



5. Shake softly for 10-15 seconds



6. Return Petakas to the incubator



6 MEDIA EXCHANGE

1. Remove Filter protector from dormant Petakas



2. Disinfect port with 90% Ethanol

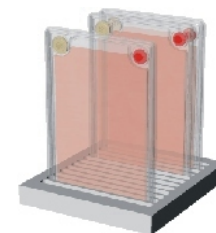
3. Slowly withdraw the medium



4. Inject same volume of NEW medium



5. Incubate at 37°C, in vertical position, at least for 24 hours before cell SUBCULTURE



For INCUBATION POSITIONS [Click here](#)

1

In Situ MONITORING ADHERENT CELLS VIABILITY (TRYPAN BLUE METHOD)

1. Remove Filter protector from RTHP



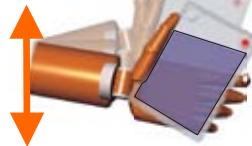
2. Disinfect port with 90% Ethanol

3. Withdraw 1mL of Medium

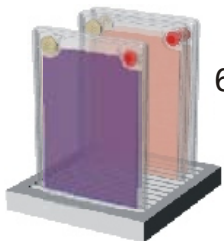


4. Inject 1mL of Trypan Blue 0.4%
"sterile" solution

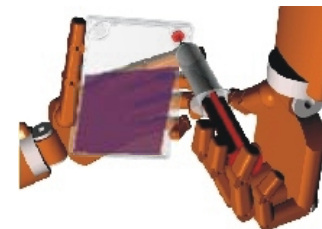
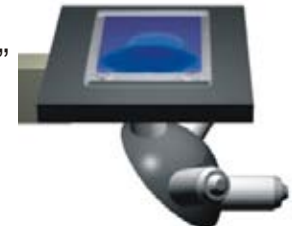
5. Shake softly for
10-15 seconds



6. Leave Petaka in vertical position
for 10 minutes at room temperature



7. Count proportion of "blue-stained"
cells versus unstained per field

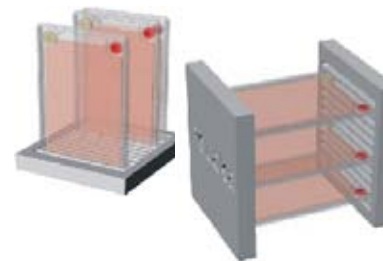


8. Slowly withdraw the medium

9. Inject same volume of NEW medium



10. Incubate in the adequate position



For INCUBATION POSITIONS Click here