Sub-process A - Dissociation of the cells

1. One flask containing adherent cells stored at 37°C, 5% CO_2
2. Get the flask from incubator
3. Empty the medium from the flask and throw it away
4. Add PBS (10ml)
5. Rotate the flask to put PBS in contact with the cells
6. Empty the PBS from the flask and throw it away
7. Add trypsin (2ml) in the flask
8. Rotate (45° width/length) the flask to put trypsine in contact with the whole area (cells).

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9. Store the flask at 37°C, 5% CO_2
10. Wait between 3 and 5 minutes
11. Get the flask from the incubator
12. Agitate (violently) the flask to have the cells detached
13. Add [between4-8ml] of medium on the cells
14. Rotate the flask to put medium in contact with the trypsinated cells
15. Pipet up & down the liquid to dissociate the cells. (The end of the pipet must be in contact with the bottom of the flask)
16. At this step the cells can wait up to 15 minutes before being starting the sub-process B

*

* **At room temperature/MSC Class II environment**

Sub-process B - Pooling

1. n_A flasks from A - step 16 ($n_A \leq 8$)
2. If $n_A > 1$:
    1. aspirate the cell suspension from flask # i with $1 < i < n_A$
    2. Dispense the cell suspension into flask#1
    3. Repeat until all the flasks are pooled together in the flask#1 ($i = n_A$)
3. If $n_A = 1$, go to process C

*

Process C - Plating

1. 1 flask from A - step 16
2. Add new medium to the flask (Volume V_F = 20 mL max.)
3. Transfer volume V_T into n_C flasks as $V_T = V_F / n_C$, knowing that $1 < n_C < 20$
4. Rotate each flask to put medium/cells in contact with the flask's bottom area.
5. Incubate the flasks at at 37°C, 5% CO_2 for 24 hours

*

*** At room temperature/MSC Class II environment**

Sub-process D - Transfection I

- A 50ml Falcon vial containing a 3 plasmids mix is manualy put on the workplan (vial 1)
- A 50ml Falcon vial containing the transfectant is manualy put on the workplan (vial 2)

1. Add medium to the vial 1
2. Transfer 60 to 100 µL of transfectant from vial 2 to vial 1
3. Vortex 5-10 sec
4. Incubate at room temperature for 5-10 minutes

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Sub-process E - Transfection II

1. Distribute the transfection medium in 5 platted flasks from the incubator (end of process C)
2. Put the flask at 37°C, 5% CO_2
3. Add 5 ml of new medium in each flask from process D
4. Put the flask back in the incubator (72H)

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*** At room temperature/MSC Class II environment**

Sub-process F - Harvesting

1. From Process E – step 4
2. Pooling of 5 flasks (process B)
3. Transfer in a Corning® 250mL PP Centrifuge Tube
4. Add 30-50 ml of 1-PEG to the tube
5. Gently agitate the tube

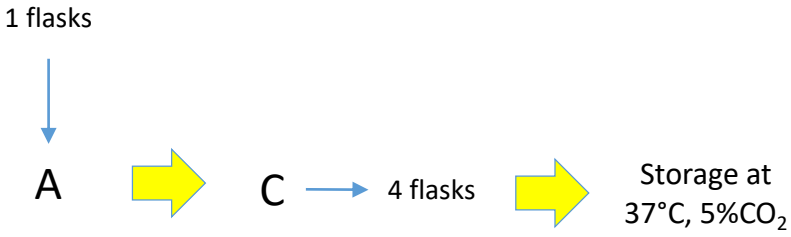
*

*** At room temperature/MSC Class II environment**

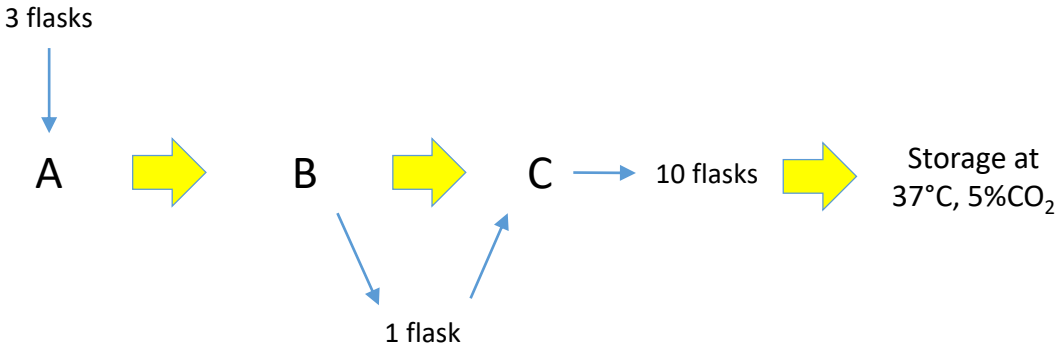
Consummables references

consumable	Provider	Ref.
Flask	ThermoFisher scientific	Nunc™ Cell Culture Treated EasYFlasks™, T175, filter
Triple layer flask	ThermoFisher scientific	Nunc™ Cell Culture Treated TipleFlasks™, T175, filter
Centrifuge tube	Corning	Corning® 250mL PP Centrifuge Tubes with Plug Seal Cap, Sterile, (Product #430776)
50 mL tube	Fisher Scientific	Product #14-432-22

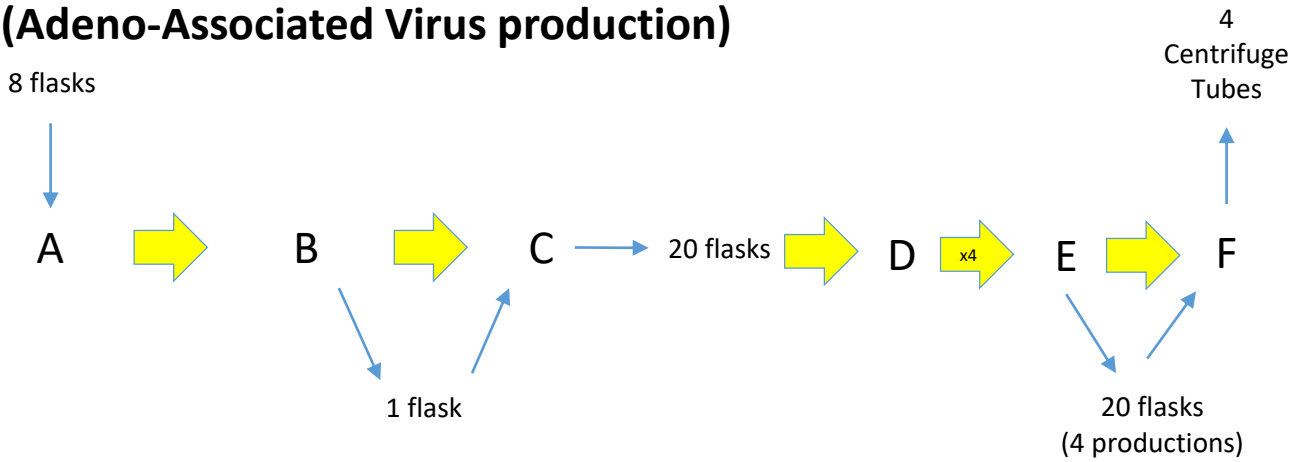
PROCESS I : « Small » maintenance (culture of HEK cells in flasks – low yield)



PROCESS II : « Big» maintenance (culture of HEK cells in flasks – high yield)



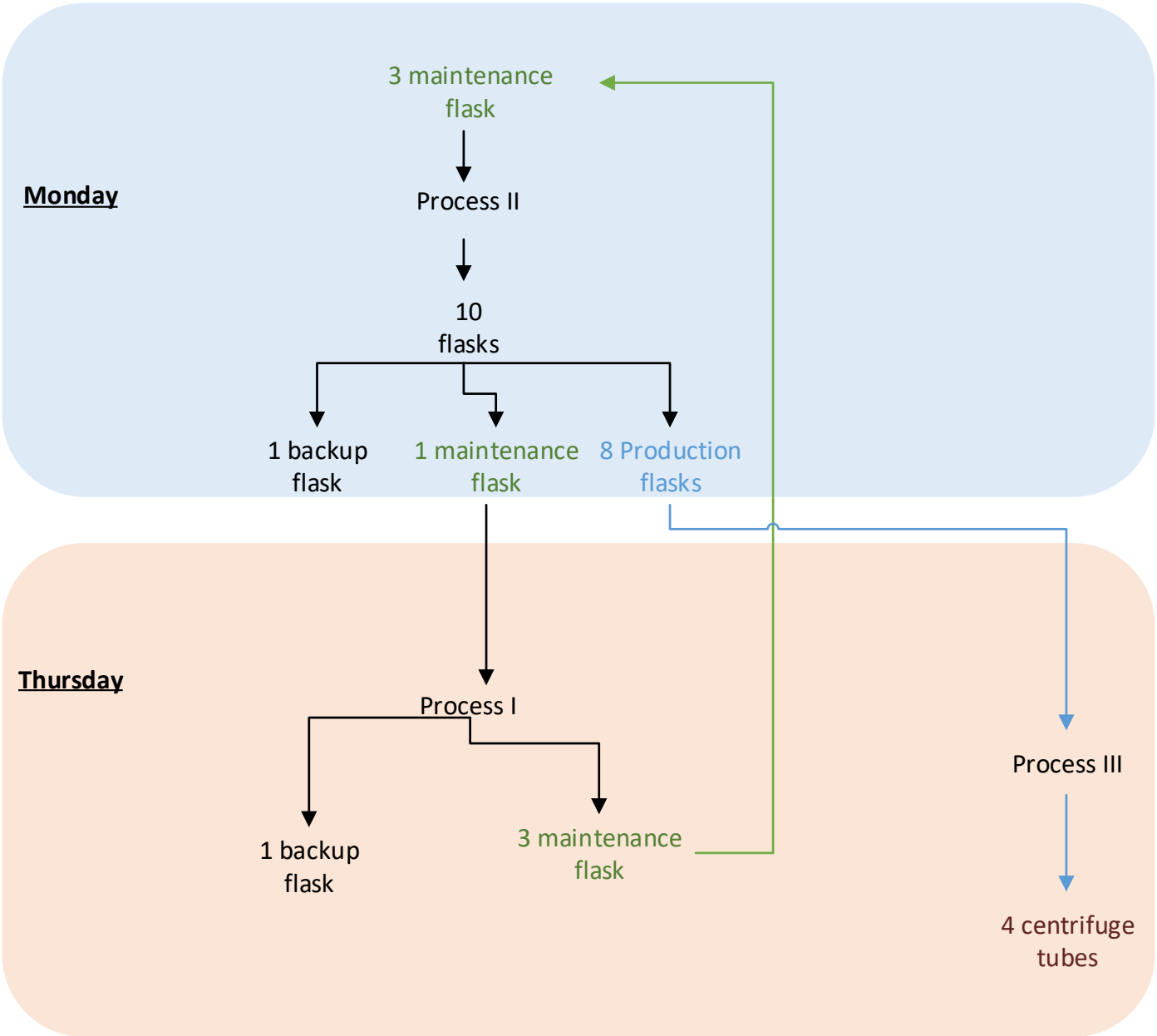
PROCESS III : Production (Adeno-Associated Virus production)



NB#1: This processes should be run using normal T175 flasks or triple layer T175 flasks.

NB#2: Two diffrent types of flasks will never be present at the same time in the system.

Current weekly organization



NB#3: The current manual rate is **12 productions/week**,