April 2014 Revised DM Based on AR & VL	**Standard Operating Procedure** Mouse reconstitution	Pages 1/2

Reconstitution:

Irradiate **pups on d1** (only if you see the milk spot and blood in the cage looks dry) – **d4** at the irradiation facility with **1Gy (1:25 min).** Bring pups in inner irradiation boxes.

The person that irradiates is responsible for entering them into the system:

On the day of irradiation/reconstitution, mice need to be moved from the Z-MUENZ license to 148/2011.

1. In iRATS: add the pups to the system for weaning, but don't move them: On the main page of the cage, select them, execute an advanced transfer and there, change license/ mark them as 'in experiment' and put severity level to '1').
2. On the score sheets, add the pups on the breeding sheet. Check the mice and document it for three days in a row.
3. Add the 148/2011 to the cage card.

Reconstitute them 5-7 hours after irradiation.

Factors to take into account when choosing HFL (if there are no vials left from the HFL used in last reconstitution)

- How many cells you need? – **$2x10^5$ cells/mouse** but leave 2x for injection error (e.g. 10 pups → 12 x 0.2 x 10^6=2.4 x 10^6 needed + recovery is not optimal → start with ~3 x 10^6 frozen cells)
- Are more pups coming? – e.g. 8 pups are usually not enough for an experiment → if the gap is ~2 weeks, use HFL that will be enough to reconstitute both batches
- If HFL has less than 90% CD34+, adjust # of cells to be injected
- Ideally use earlier HFL

Thawing CD34+ cells

Get changed 1st and prepare everything else before thawing the cells

To take down:

- ice bucket (spray it, get ice and close with a lid)
- yellow tips
- p200 pipette
- needles (if there are less than 2 boxes left → re-order)
- syringe (wash syringe and needles first in 70% Ethanol, NON-ketonized and then in PBS)
- cells

1) prepare 2ml of PBS in V-bottom falcon
2) thaw vial in water bath and remove as soon as there is a small piece of ice left (to keep at 4°)
3) use pasteur pipette to transfer cells to the falcon
4) remove 10 µl for counting and another 10 µl as backup
5) top up with 15ml PBS and spin at 500xg for 5min
6) count cells at the microscope in the P2 room
7) resuspend cells in the correct volume of PBS (20 µl/mouse + 2x for injection error, e.g. 10 pups → 240 µl)
8) transfer to small eppendorf tube

After injection:

1. Clean the syringes with PBS and then 70 % Ethanol
2. Remove the cells you thawed from the N2 list
3. Correct the number of vials left in the "HFL overview" list.
4. Fill in the "reconstitution overview spread sheet"
5. Put HFL and R# in
    a. mouse book and on cage card
    b. score sheet

    *pups reconstituted on the same day with the same HFL have the same reconstitution number