

## Cutaneous Wound Healing Assay (from Monika Krampert)

1. anesthetize the mice, shave the back and wipe it with ethanol
2. cut wounds with 5–6 mm diameter using a biopsy punch. Grab skin from the dorsal midline and place cardboard backing on one side of the skin fold. Using biopsy punch, press into skin with twisting motion until you punch through both layers of skin stopping when you reach the cardboard backing. Do this twice to make a total of 4 wounds per mouse, two on each side of the dorsal midline.

It is quite important to have the wounds allways at equal distance from the midline, because the tension of the skin is not equal all over the mouse back, at this influences the contraction of the wounds.

3. at the desired time point (we usually start with 5–day old wounds for a preliminary experiment) sacrifice the mice and cut out the wounds including a few millimeters of the surrounding skin (if you want to prepare RNA or protein lysates, it is important to take allways the same amount of intact tissue with you, for histology it does not matter)

4. place the wounds for histology on a millipore filter for stabilisation (any membrane that is resistant to organic solvents such as xylene) and cut exactly in two halves. Embedd the 1/2–wounds either directly in tissue frezing medium (for cryo–sections) or fix overnight with 4% PFA or 95% ethanol/1% acetic acid (we prefer the latter for immunostainings, because most antibodies work better on this fixation) and embedd in paraffin, so that the sectioning can start in the middle of the wound.