Transfection

1. 100 µl of the cell culture (**10^8** cells per ml) is fixed with 200 µl fixing solution
2. 10 µl of the cell culture are placed in a counting chamber
3. The cells are counted under a microscope
4. The cells are centrifuged at room temperature (RT) by **3310 rpm for 5 min**
5. Take the supernatant off and wash the pellet with **1 ml of transfection buffer**
6. The cells are centrifuged again at RT by **3310 rpm for 5 min** and take the supernatant off
7. Add 150 µl transfection buffer per transfection to the pellet
8. Before the DNA constructs are added to the cell culture, they are sterilized for **5 min by 95°C** in the PCR cycler
9. Put **150 µl cell culture** in electroporation cuvettes and add **1–20 µg of the DNA (~50 µl)** construct.
10. For a **negative control,** add 50 µl of distilled water to the 150 µl cell culture in the electroporation cuvettes
11. The cuvette with the cell / DNA suspension is placed in the Nucleofector and the transfection is carried out with the **program U-033**
12. The suspension is pipetted from the cuvette into **1 ml BHI medium** (→ Basic Protocol: BHI medium for the cellcultures aftee transfection) into an Eppi and **incubated at 27 ° C** overnight