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| Step 3/6  Optional: Chromatin pre-clearing  Time  Operation 20min  1h | 3 Optional: Chromatin pre-clearing    Pre-clearing in our hand can reduce both the background and IP signals. Pre-clear when both of them are too high.  1) Add 10µl of pre-washed magnetic beads (prepared from step one) per 100µl sheared chromatin in 1.5 ml. Rotate at 4°C for 1 h.  Attention:  Count the volume of the beads solution used, as the ChIP buffer in the beads solution will remain in the ChIP tube, which can increase the total volume. Also consider the material loss during the process.  Deduct the volume of the beads solution from the ChIP buffer added to the sheared chromatin in step2, will give you approximate 100µl per IP.  2) Place tubes on magnetic rack, open the lid and let sit for 1 min, transfer the supernatant into a new siliconized tube and discard the beads. These are the pre-cleared chromatin ready to be ChIPed. |