|  |  |
| --- | --- |
| Step 5/6  Washes  Time  1h operation +1.5h waiting | 5 Washes  Add 40µl PIC per 1mL wash buffers to make the Complete wash Buffer.  Wash the Chromatin two times with Low Salt Wash Buffer and two times with High Salt Wash Buffer:  8) Brief spin the tubes/8-strip to bring down the liquid caught on the lid.  9) Place the tubes into the Magnetic rack, wait 1 minute and discard the Buffer.  10) Add 100µl of ice-code Low Salt Wash Buffer and incubate for 5~10min at at 4°C on a rotating wheel (40 rpm).  11) Repeat step 8-10 once.  12) Repeat wash (step 8-10) with 100µl ice-code High Salt Wash Buffer twice.  !! Attention: Do not disturb the captured beads attached to the tube wall. Washes are to isolate the chromatin complexes that are specifically attached to the beads by antibody binding (chromatin-antibody-beads complex). Do not vortex between washes, instead invert tubes to resuspend the chromatin-antibody-beads complex and keep them intact.  13) Repeat step 8-9 to collect the beads. Add 100µl Hot Elution Buffer to the beads and 90µl Elution Buffer to the Input and transfer them to a new tube/strips. This is to remove the non-specific binding on the tube wall and lids.  12) Put the strips in a PCR block at 65°C for 1.5~2h.  13) Place the strips on a magnetic rack, open the lid and let sit for 1 min, transfer the liquid to a PhaseLock tube (brief spin down the agarose in the PhaseLock tube first).  Add an extra 100µl Hot Elution Buffer to the beads, let sit for 1min then transfer the liquid to the same PhaseLock tube to collect the residual molecules left.  Now you have 200µl DNA ready for isolation. |