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| Step 1/6  Binding antibodies to magnetic beads  Time:  30m +2h waiting  Operation=30min  Step1+Step2 =1h;  Pre-clear=1h  Vol.  11 µl ChIP Buffer/11µl Beads/IP  Beads:  Use protein A coated beads for H3K9me3 antibody we have.  Antibody:  Fill in Antibody table | 1 Binding antibodies to magnetic beads    1) Wash twice the Protein A/G-coated paramagnetic beads with ice-cold ChIP Buffer: Add ChIP Buffer, suspend the beads in ChIP Buffer, then centrifuge for 5 minutes at 1,300 rpm, discard the supernatant and keep the beads pellet.  For each IP, use 11µl stock solution of beads, adds 22µl ChIP Buffer to 11µl stock solution of beads. Scale accordingly.  !! Attention:  Prepare same amount of beads for each IP if pre-clearing is desired.  Use 1.5 ml siliconized tubes to avoid non-specific bindings.  Keep the beads homogenously in suspension at all times. Variation in the amount of beads can lead to lower reproducibility.  2) After washing, resuspend the beads in ChIP Buffer. For each IP, resuspend the beads to the same concentration as the stock (11 µl ChIP Buffer/11 µl beads/ IP). For 2 IPs, simply add 22 µl of ChIP Buffer. 4 IP, 44 µl ChIP Buffer. Scale accordingly.  !! Attention: Use siliconized tubes/8-strips to reduce non-specific binding.  3) Aliquot 90 µl of ChIP Buffer per IP to a newly prepared tube.  4) Add 10 µl of pre-washed Protein A/G-beads per IP tube made up 100µl total per IP. (Optional: Add IgG antibody to reduce non-specific bindings to IgG)  5) Add specific antibody and control antibodies. The amount of antibody varies depends on the antibody used, the binding capacity of 10µl magnetic beads is ~3µg.  6) Incubate the tubes/8-strips at 40 rpm on a rotating wheel for at least 2 hours at 4°C. |