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| Step 6/6  DNA isolation  Time  1h operation  Vol.  250 ->24µl | 6 DNA isolation  For DNA sequencing, we use the phenol-chloroform to get high concentration double stranded DNA.  Phenol-chloroform method:  1) Brief spin the phase lock tube to bring down the agrose.  2) Add DNA and equal volume phenol:chloroform:isoamyl alcohol into the PhaseLock tube. Mix well by vertex.  3) Spin at 13,000g for 5 min at room temperature.  4) Transfer the supernatant to a new 1.5 tube.  5) Add 1/10 of the vol. (10µl if use 100µl) 3M NaOAc and 1µl of LPA. Vortex to mix well\*.  6) Add 2.5× Vol. (250µl if use 100µl) ice-cold 100% EtOH. Mix well by vertex.  7) Put the tubes at -20°C to precipate for 30min up to overnight.  8) Centrifuge DNA at 13,000g for 15~30min at 4°C.  9) Remove the supernatant and add 1ml 70% EtOH, let the tube sit for 5 min to allow the salt to dissolve. Then centrifuge at 13,000g for 5 min at 4°C to attach the pellet to the bottom.  Repeat this wash step once more.  10) Remove the EtOH and let the pellet to dry for 5min at room temperature.  !! Attention:  Phenol:chloroform:isoamyl alcohol are toxic and can burn the skin! Wear gloves and operate in fume hood.  LPA pellet are very loosely attached at the bottle of the tip. Use 200µl PAGE gel loading tips to remove the last 100µl supernatant.  11) Add 24µl DNA elution Buffer. Add 3µl into a new tube for qPCR validation and the rest 20µl for library construction.  Add 17µl DNA EB to 3µl, made up 20µl. Use 2µl per PCR, it can be used for 3 amplicons with triplicates. If use 8µl for qPCR validation, add 32 µl DNA EB to 8µl, can be used for 6 amplicon with triplicates. Scale accordingly.  Pause point: The purified DNA from ChIP and input samples could be stored at -20°C for months.  \* LPA doesn’t dissolve in 70% EtOH, make sure it is evenly suspended before add in 100% EtOH. |