Isopropanol precipitation procedure with KAPA Express Extract

Alcohol precipitation is commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation is mediated by the addition of isopropanol to the supernatant removed from the KAPA Express Extract reaction.

1. **Add an equal volume of room-temperature isopropanol to the DNA solution and mix.**
   Use all solutions at room temperature to minimize co-precipitation of any salt. This is then left at room temperature for >5 min.

2. **Centrifuge the sample at 16,000 x *g* for 30 min** *(at 4 °C if possible, but not essential)*.
   Centrifugation at 4 °C prevents overheating of the sample. With the small volumes usually used with the Express Extract, centrifugation may be carried out at room temperature.

3. **Carefully discard the supernatant without disturbing the pellet**.
   A small white-ish or glassy pellet may be visible at the bottom of the tube (ethanol precipitation pellets are most often white). This is the nucleic acids and should not be disturbed. If there is no visible pellet, there is still likely to be nucleic acid present but at a lower concentration.
   Tip *- Marking the outside of the tube or uniformly orienting micro-centrifuge tubes before centrifugation allows the pellet to be more easily located. Care should be taken when removing the supernatant as pellets from isopropanol precipitation are usually loosely attached to the side of the tube. Carefully tip the tube with the pellet on the upper side to avoid dislodging the pellet. If the samples are valuable, the supernatant should be retained until recovery of the precipitated DNA has been verified.*

4. **Add 500 µL of room-temperature 70% ethanol to the DNA pellet**.
   Do not pipette the pellet since this can shear the DNA. Gently vortex for 1 min, or leave for 5 min at room-temperature and invert several times until the pellet is no longer attached to the bottom of the tube. This step is to remove any co-precipitated salt and replaces the isopropanol with the ethanol, making the DNA easier to re-dissolve.

5. **Centrifuge at 16,000 x *g* for 5 –15 min** *(at 4 °C if possible, but not essential).*
   Tip *- Centrifuge the tube in the same orientation as previously to recover the DNA in a compact pellet.*

6. **Carefully decant the supernatant without disturbing the pellet**.

7. **Air-dry the pellet for 5 –20 min (depending on the size of the pellet) until residual ethanol has evaporated.**
   Tip *– It is easier to centrifuge the pellet again for 2 min to pellet and remove any residual ethanol with a P10 tip. This will reduce the drying time (reducing the chance of contamination and DNA damage). Do not over-dry the pellet (e.g., by using a vacuum evaporator) as this will make DNA difficult to re-dissolve.*

8. **Re-dissolve the DNA in a suitable buffer.**
   Choose an appropriate volume of buffer according to the downstream application and the desired final DNA concentration. Recommended buffers are 10 mM Tris-Cl, pH 8.0 or TE. The volume added should typically be around 50 µl to keep the DNA in a concentrated form. This can then be diluted afterwards once the concentration has been determined. Do not re-dissolve in water for long term storage since the DNA will degrade in a few days. To avoid shearing DNA, do not pipette or vortex at high speed. It may take a short time before the DNA has re-dissolved, leaving at room temperature or at 37 °C for 30 min can help with high MW DNA.