

### **Additional chloroform extraction with alcohol precipitation**

1. Remove 500  $\mu$ l of Chromosomal DNA and place into an eppendorf tube
2. Add 1200  $\mu$ l of Chloroform
3. Mix gently 1-2 min
4. Spin at max speed for 5 minutes
5. Remove top layer and place in a new tube (be careful, the interface is hard to see)
6. Add 40  $\mu$ l 3M Sodium Acetate
7. Add 400  $\mu$ l Isopropanol
8. Mix and incubate for 15 minutes
9. Spin for 15 minutes at maximum RPM
10. Pour off supernatant and remove the last of the liquid making sure not to disturb the pellet.
11. Add 500  $\mu$ l of 70% Ethanol
12. Mix and spin at Max RPM for 5 minutes
13. Repeat steps 10-11
14. Remove liquid, air dry ~10 minutes
15. Resuspend DNA in 100  $\mu$ l TE