21. septenber 2020

Special Protocol: Extarction of fungus DNA by use of FATGK kit

Additional equipment:
- RNase A (optional),
- 96~100% ethanol,
- Acid-washed glass beads, 500 ~ 700 µm
- Lyticase, 200 U for one preparation
- Sorbitol buffer (1M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol)

1. Transfer 1~ 5 x10^6 of cultures (fungal/ yeast cells) to a 1.5 ml microcentrifuge tube. (not provided)
2. Add 1 ml of **PBS (not provided)** to the cells and resuspend the cells by pipetting.
3. Descend the cells by centrifuging at 5,000 x g for 2 min and discard the supernatant completely.
4. Resuspend the cells in **550 µl of Sorbitol buffer (1M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol) and add 200 U of lyticase. mix well by vortexing**. Incubate the sample at 37 °C for 30 min.
   Caution: Sorbitol buffe 14 mM of ß-mercaptoethanol is hazardous to human health. perform the procedures in a chemical fume hood.
5. **(Optional)** If RNA-free genomic DNA is required, add 8 µl of 50 mg/ml **RNase A** (not provided) and incubate for 2 min at room temperature.
6. Descend the cells by centrifuging at 5,000 x g for 10 min. Remove the supernatant completely.
7. Add 350 µl **FATG1 Buffer** and mix well by pipetting.
8. **Mix well by Plus-vortexing for 10 minutes.**
9. Add 20 µl of **Proteinase K** (10 mg/ml) **and mix well by vortexing.** Incubate at 55 °C for 15 min, vortex 30 seconds for every 5 minutes incubation.
10. Centrifuge the sample mixture at 5,000 x g for 1 min and transfer 200 µl of the supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
11. Add 200 µl of **FATG2 Buffer** and mix well by pipetting.
12. Add 200 µl of **ethanol** (96-100%) and mix well by pulse-vortexing for 10 seconds.
13. Place a **FATG Mini Column** in **Collection Tube**. Transfer the sample mixture (including any precipitate) carefully to **FATG Mini Column**. Centrifuge at 11,000 x g for 30 second **then place the FATG Mini Column to a new Collection Tube.**
14. Add 400 µl of **W1 Buffe**r to the **FATG Mini Column.** Centrifuge at 11,000 x g for 30 sec. Discard the flow-through and then place the **FATG Mini Column** back to the Collection Tube.
    --Make sure ethanol has been added into W1 Buffer when first use.
15. Add 750 µl of **Wash Buffe**r to the **FATG Mini Column.** Centrifuge at 11,000 x g for 30 sec. Discard the flow-through and then place the **FATG Mini Column** back to the Collection Tube.
    --Make sure ethanol has been added into Wash Buffer when first use.
16. Centrifuge at full speed (~ 18,000 x g) for an additional 3 min to dry the column.
    Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
17. Place the **FATG Mini Column** to **Elution Tube**.
18. Add 50 ~100 µl of **Elution Buffer or ddH_2O** to the membrane center of the **FATG Mini Column**. Stand **FATG Mini Column** for 3 min.
    Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
19. Centrifuge at full speed (~ 18,000 x g) for 1 min to elute total DNA.
20. Store total DNA at 4°C or -20°C.