# Protocol of DNA barcode duckweeds by *atpF-atpH* marker

1. Order primer pairs for marker atpF-atpH and prepare them into 5pmol work solution.

   Forward: ACTCGCACACACTCCCTTTCC

   Reverse: GCTTTTATGGAAGCTTTAACAAT

2. Extract total genomic DNA using CTAB method (see our protocol).
3. Quantify DNA concentration by Nanodrop.
4. PCR mix:

| | |
|---|---|
| DNA | 1ul (50-100ng) |
| Primer | (1+1)ul (5pmol each) |
| H2O | 9.5ul |
| Red taq mixture | 12.5ul |
| Total | 25ul |

5. PCR program

| | | |
|---|---|---|
| 94°C | 2min | |
| 94°C | 15s | |
| 50°C | 15s | 35 cycles |
| 72°C | 40s | |
| 72°C | 5min | |

6. Purify PCR products with ExoSap-IT™.
7. Directly sequence purified PCR products on an ABI3730 automated sequencer using the same primers as in the PCR reactions.

## Chemical

JumpStart™Redtop® Ready-Mix™Reaction Mix (P1107, Sigma)

ExoSap-IT™(USB Corp.)

## Reference


1. Hollingsworth PM FL, Spouge JL, Hajibabaei M, Ratnasingham S, et al (2009) A DNA barcode for land plants. Proc Natl Acad Sci U S A 106: 12794-12797.
2. Wang W, Wu Y, Yan Y, Ermakova M, Kerstetter R, et al. (2010) DNA barcoding of the Lemnaceae, a family of aquatic monocots. BMC Plant Biology 10: 205.