Extraction of high quality DNA for Genome Sequencing

Modified from protocols of Prof. Pietro Spanu (Imperial College, London) and T. M. Fulton, J. Chunwongse, S. D. Tanksley, Pl Mol Biol Rep 13, 207 (1995)

Reagents required

BUFFER A: 0.35 M **sorbitol**
0.1 M **Tris**-HCl, pH 9
5 mM **EDTA**, pH 8

BUFFER B: 0.2 M **Tris**-HCl, pH 9
50 mM **EDTA**, pH 8
2 M **NaCl**
2% **CTAB**

BUFFER C: 5% Sarkosyl (N-lauroylsarcosine sodium salt SIGMA L5125)

Potassium Acetate 5M (KAc precipitate polysaccharides) **pH 7.5**
RNAse A (10 mg/ml)
Proteinase K (20 mg/ml)
PVP 1 %
Chloroforme:Isoamylalcool (24:1)
Sodium Acetate (NaAc) 3M
Isopropanol 100%
Ethanol 70%

Qiagen Genome-tip 100/G or 500/G and Qiagen QC, QBT and QF Buffers

Lysis Buffer	**For 17.5 ml**
2.5 volume of Buffer A	6.5 ml
2.5 volume of Buffer B	6.5 ml
1.0 volume of Buffer C	2.6 ml
PVP 0.1 %	1.75 ml
Proteinase K 0.1 mg	125 µl

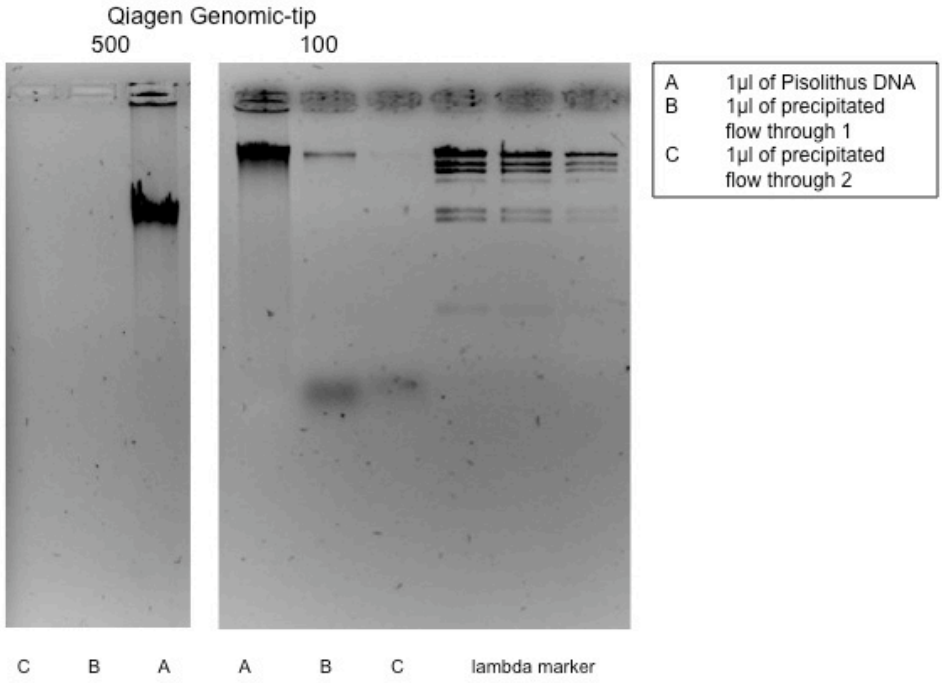
- Pre-warm Buffer B at 65°C,
- Prepare the Lysis Buffer: use 17.5 ml per 500mg of starting material
- Grind tissue with liquid nitrogen in a mortar, and transfer 500 mg of finely ground powder in a 50 ml Falcon tube
- Add 17.5 ml of lysis Buffer to the tube and mix by vortexing
- Incubate 30 min at 65°C, mix frequently by inverting the tube
- Add 5.75 ml (0.33 vol) of KAc (5M), mix by inverting the tube and incubate 30 min on ice
- Centrifuge for 20 min at 5,000 g at 4°C
- Transfer the supernatant in a new 50 ml Falcon tube and add 1 vol of Chloroforme:Isoamylalcool (24:1)
- Centrifuge for 10 min at 4,000g at 4°C
- Transfer the aqueous phase in a 50 ml centrifuge tube (e.g. Nalgene)
- Add 100 µl of RNAse A (10mg/ml) and incubate 90-120 min at 37°C
- Add 1/10 vol of NaAc and 1 vol of Isopropanol (RT) and incubate 5 min at room temperature
- Centrifuge for 30 min at 10,000 g at 4°C
- Discard the supernatant
- Wash the pellet with 2ml of ethanol (70%) and centrifuge for 10 min at 10,000 g at 4°C
- Discard the supernatant and dry the pellet for 5 min at RT
- Resuspend the pellet in 500 µl of TE at 65°C and store at -80°C

Use four extractions of 500 mg starting material for one Qiagen genomic-tip 500/G column

- Combine 4x 500µl DNA in TE from four extractions and add 8ml QBT buffer
- Equilibrate the Qiagen Genomic-tip 500/G with 10 ml of QBT buffer
- Place the Genomic-tip column on a new 50 ml Falcon tube and load the DNA extract. Keep the flow through (Flowthrough 1).
- Wash the Genomic-tip columns twice with 15 ml of QC buffer. Keep the flow through (Flowthrough 2).
- Place the Genomic-tip column onto a 50 ml centrifuge tube and elute DNA with 15 ml of prewarmed (50 °C) QF buffer.
- Add 1/10 vol of NaAc and 1 vol of Isopropanol (RT) and incubate 5 min at room temperature
- Centrifuge for 30 min at 10,000 g at 4°C
- Discard the supernatant,
- Wash the pellet with 2ml of ethanol (70%) and centrifuge for 5 min at 10,000 g at 4°C
- Discard the supernatant and dry the pellet for 5 min at RT
- Resuspend the pellets in 250 µl of TE at 65°C and store at -80°C

- Prepare a 1% agarose gel and load 1-5µl of the genomic DNA sample, the precipitated flow trough 1 and 2.

Example of gel :



Additional remarks

For Pisolithus species and many other ECM fungi, the younger the mycelium, the better. We have noticed that mycelial age should not exceed 30 days or else pigments will be a problem.

Use gloves all the time.

Use of chemical hood when using the chloroform:isoamylalcool mix.

The mycelium must be ground in liquid nitrogen to a very fine powder, otherwise the DNA yield is poor !

During grinding and weighing, never allow the samples to thaw.

Once weighed, the samples were kept on ice until the extraction buffers were added.

Unless otherwise explicitly stated in the protocol, the tubes were always kept on ice.

Using Qiagen Genomic-tip 500/G columns is often better than using Genomic-tip 100/G.
By starting with 4 X 500 mg mycelium, you risk to pass the 100 ug capacity of the column and you will end up with DNA in the flow-through.
This makes it almost always mandatory to recover DNA that has not been retained in the column, adding additional work. In addition, residual contaminants and pigments are more efficiently eliminated by using the 500/G columns (see gel photo).