Extraction of the genomic DNA from *M. larici-populina* urediniospores (developed by Francis Martin's group at INRA, adapted from classic CTAB protocol):

1. Genomic DNA was extracted from a total of 1 g of *M. larici-populina* urediniospores. Aliquots of 25 mg urediniospores were placed in 2 ml Eppendorf tube, with two 3-mm-diameter tungsten carbide beads (Qiagen) and 1 mg Polyvynilpyrrolidone (PVP).
2. The spores were homogenized for 1 minute at 30 Hz using a MM 200 Mixer Mill (Retsch - Qiagen) and then suspended in 600 µl of 65°C CTAB buffer (CTAB 2%, Tris pH9 0.1 M, NaCl 1.4 M, EDTA 0.02 M, ß-mercaptoethanol 0.2%). The content of 40 Eppendorf tubes was pooled in two 50 ml centrifuge tubes. After carefully mixing by inverting, the tubes were incubated for 30 min at 65°C.

Note: Extraction of DNA relies on breaking urediniospores at this point. Mechanical damage with tungsten carbide beads and then lysis in CTAB buffer (CTAB is a strong cationic detergent) kills most urediniospores here.

3. One volume of phenol/chloroform/isoamyl alcohol (50:48:2) (Euromedex) was added to each tube. The content of the tube was carefully mixed and then centrifuged at 8000 *g* for 10 min. The aqueous phase was transferred to a new 50 ml tube and the tube containing the spore debris was eliminated.

Note: Centrifugation effectively pellets debris from lysed spores as well as still-viable spores. Very few viable spores (if any) should be carried over as long as the pellet remains intact and the researcher does not attempt to remove all of the liquid from the old tube to put into the new tube.

4. One volume of chloroform was added to each tube. The content of the tube was carefully mixed and then centrifuged at 8000 *g* for 10 min. The aqueous phase was transferred to a new 50 ml tube. This step was repeated twice.
5. Two volumes of ice-cold (- 20°C) isopropanol was added to each tube. The tubes were incubated at -20°C for 1 hr in order to pellet the DNA. The tubes were centrifuged at 10.000 *g* for 30 min. The supernatant was eliminated and the DNA pellet was washed twice with 1 ml of ethanol 70%, air-dried, and then resuspended in 2 x 250 µl of Ultrapure water.

Note: if any viable urediniospores were still present before this step, the action of isopropanol and ethanol kills all the remaining viable spores.

6. The DNA extracted was pooled in a new Eppendorf tube and RNA was digested with DNase-free RNase A at 37°C for 1 hr.

7. One volume of phenol/chloroform/isoamyl alcohol (50:48:2) (Euromedex) was added to the tube. The content of the tube was carefully mixed and then centrifuged at 13.000 *g* for 10 min. The aqueous phase was transferred to a new Eppendorf tube.

8. One volume of chloroform was added to the tube. The content of the tube was carefully mixed and then centrifuged at 13.000 *g* for 10 min. The aqueous phase was transferred to a new Eppendorf tube. This step was repeated twice.

9. In order to pellet the DNA, 2.5 volume of ethanol 100% and NaCl (to a final concentration of 0.2 M) were added. The content of the tube was carefully mixed and

then centrifuged at 13.000 *g* for 20 min at 4°C. The supernatant was eliminated and the DNA pellet was washed twice with 1 ml of ethanol 70%, air-dried, and then resuspended in about 500µl Tris buffer (Tris 10 mM, pH 8).