Complete chromatin immunoprecipitation (ChIP) protocol

Two-week old seedlings grown on MS plates were spray inoculated with *B. cinerea* spores ($5x10^5$ spores mL^{-1}) or half-strength grape juice at ZT0 and harvested at ZT18. Two g of tissue were cross-linked in fixation buffer (0.4 M sucrose, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1 mM PMSF and 1 % (v/v) formaldehyde) at RT under vacuum for 30 min. The cross-linking reaction was quenched with 2 M glycine, added to a final concentration of 0.125 M and vacuum infiltrated for 10 min. The seedlings were ground to a fine powder in liquid nitrogen and cross-linked chromatin complexes were isolated in 25 mL of cold nuclei isolation buffer (0.25 M sucrose, 15 mM piperazine-N,N'-bis 2-ethanesulfonic acid (PIPES) pH 6.8, 5 mM $MgCl_2$, 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, 0.9 % (v/v) Triton X-100, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail). The solution was filtered through two layers of miracloth, incubated on ice for 30 min and then centrifuged at 11 000 x *g* for 15 min at 4°C. The pellets were resuspended in 600 µL of cold nuclei lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 % (w/v) SDS, 0.1 % (w/v) sodium deoxycholate, 1 % (v/v) Triton X-100, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail) and sonicated for 18 min on a cycle of 20 s at maximum power followed by a 40 s rest using a QSonica Q700 sonicator. The sonicated chromatin was then clarified by centrifugation at 4°C for 10 min at 13 800 x *g.*

Pre-blocking was performed by incubating 500 µL of protein A/G PLUS beads (Santa Cruz Biotechnology, USA), with 100 µg salmon sperm DNA (Sigma-Aldrich), 1 mg mL^{-1} BSA in 2 mL ChIP dilution buffer (16.7 mM Tris-HCl pH 8, 167 mM NaCl, 1.2 mM EDTA, 1.1% (v/v) Triton X-100, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail) for 1 h at 4°C with rotation. Thereafter the beads were collected by centrifugation and resuspended in 500 µL of ChIP dilution buffer. For input samples, 30 µg of chromatin was made up to 100 µL with Nuclear lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 % (w/v) SDS, 0.1 % (w/v) sodium deoxycholate, 1 % (v/v) Triton X-100, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail) and diluted with 400 µL of Elution buffer (0.5 % (w/v) SDS, 0.1 M $NaHCO_3$). For immunoprecipitation, 80 µg of chromatin was made up to a final volume of 100 µL with nuclear lysis buffer and diluted with 900 µL of ChIP dilution buffer. The diluted chromatin was then pre-cleared by adding 30 µL pre-blocked protein A/G PLUS beads, followed by rotation for 1 h at 4°C to reduce non-specific binding. The pre-cleared chromatin solutions

were collected by centrifugation at 15 000 x g for 10 min and immunoprecipitated with 5 µg anti-GFP N-terminal antibody (Sigma-Aldrich) overnight at 4°C with rotation. Negative control immunoprecipitations were performed using samples treated in the same manner but without the addition of an antibody, or with chromatin from Col-0 plants immunoprecipitated with anti-GFP N-terminal antibody.

Following overnight immunoprecipitation, an additional 40 µL of pre-blocked protein A/G PLUS beads was added to each sample, and incubated for a further 2 h at 4°C. Immunoprecipitated samples were sequentially washed for 8 min with gentle rotation at 4°C with 1 mL of the following buffers: once with low salt wash buffer (150 mM NaCl, 0.2 % (w/v) SDS, 0.5% (w/v) Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH8, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail) once with high salt wash buffer (as above but with 500 mM NaCl), once with LiCl wash buffer (0.25 M LiCl, 1 % (v/v) NP-40, 1 % (w/v) sodium deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH8, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail) and then three times with TE wash buffer (10 mM Tris-HCl pH8, 1 mM EDTA, 1 mM PMSF and 1x Halt Protease Inhibitor Cocktail). The DNA-protein complexes were released with 250 µL of elution buffer (0.5 % (w/v) SDS, 0.1 M $NaHCO_3$) by incubation at 65°C for 15 min with gentle agitation followed by incubation at room temperature for an additional 15 min with rotation. Samples were collected by centrifugation at 4 500 x g for 5 min at room temperature. The supernatant was removed and transferred into a new microfuge tube, and the elution step repeated. The two eluates were combined and 20 µL 5 M NaCl was added to the ChIP samples and the 30 µg input samples, followed by incubation at 65°C overnight to reverse the cross-links. The following day, protein digestion was performed by adding 10 µL of 0.5 M EDTA, 20 µL of 1 M Tris-HCl pH 6.5 and 1 µL of 10 mg mL^{-1} Proteinase K (New England BioLabs) to each sample followed by incubation at 45°C for 2 h. DNA from immunoprecipitated and input chromatin was purified by phenol/chloroform extraction.