arr3,4,5,6,7,8,9 mutant

For construction of the *arr3,4,5,6,7,8,9* septuple mutant the T-DNA insertions mapped to the following positions relative to the ATG:

Mutant	Locus	Insertion site	Mutant	Locus	Insertion site
arr3	At1g59940	801	*arr7*	At1g19050	642-660
arr4	At1g10470	817	*arr8*	At2g41310	35
arr5	At3g48100	689	*arr9*	At3g57040	782
arr6	At5g62920	1021			

Chromatin Immuno-Precipitation (ChIP)

ChIP was conducted with modifications after Wang, H., Tang, W., Zhu, C. & Perry, S. E. A chromatin immunoprecipitation (ChIP) approach to isolate genes regulated by AGL15, a MADS domain protein that preferentially accumulates in embryos. *Plant J* **32**, 831-43 (2002).

1. fix 500 mg of tissue with 0,3% formaldehyde in MC buffer for 1h under vacuum (turn on and off vacuum 4 times and give sufficient time to adapt)
2. wash 3 times with MC buffer
3. wet Miracloth filter with M1 buffer (without protease inhibitor)
4. grind tissue under liquid N2
5. transfer ground tissue to Miracloth filter
6. add 15 ml M1 buffer and filtrate slurry through Miracloth into a falcon tube
7. reapply flow-through 2 times
8. spin flow-through (1,000g; 10 min; 4°C)
9. wash pellet carefully 5 times with 1 ml M2-Buffer (2,000g; 5 min; 4°C)
10. wash pellet with 1ml of M3 Buffer (2,000g; 5 min; 4°C)
11. resuspend crude nuclear pellet in 1 ml Sonication buffer
12. solubilize chromatin on ice using sonicator with microtip (Branson 450; output 3; Cont., 8 * 5'')
13. centrifuge (top speed; 5 min; 4°C)
14. remove 250 µl and freeze (for input DNA controls and size check)
15. mix supernatant with equal amount of IP buffer (750 µl)
16. preabsorb 1h-ON at 4°C with 7.5 µl preimmune serum

17. spin (top speed; 5 min; 4°C)
18. mix supernatant with with 40 µl protein G-Sepharose (Sigma, 50% slurry in 10 mM Tris, pH 7.5 and 150 mM NaCl)
19. incubate on a rotating wheel for 1h at 4°C
20. spin (top speed; 5 min; 4°C)
21. divide supernatant in 2 equal fractions and transfer to fresh tubes
22. add 2.5µl of specific antiserum to one tube and preimmune serum to other tube
23. incubate on rotating wheel for 1h at 4°C
24. spin (top speed; 2 min; 4°C)
25. mix supernatant with 20µl protein G-Sepharose (Sigma)
26. incubate on a rotating wheel for 1h at 4°C
27. pellet beads (top speed; 2 min; 4°C; save supernatant as ‚post bind fraction')
28. wash 5 times with 1ml IP-Buffer on rotating wheel for 10 min at RT
29. transfer beads and wash to a new tube
30. spin (top speed; 2 min; RT) and remove supernatant fully
31. add 100 µl ice cold glycine elution buffer
32. vortex 30'', then spin (top speed; 1 min; RT)
33. transfer supernatant to fresh tube with 50 µl of TRIS buffer (1M; pH9)
34. repeat 31 to 33 twice
35. spin (top speed; 2 min; RT)
36. transfer supernatant to fresh tube and add 1 µl RNase A (10 mg/ml)
37. incubate for 15min at 37°C
38. optional: save an aliquot to check for protein
39. add 1.5 µl proteinase K (18,2 mg/ml, Roche Diagnostics)
40. incubate ON at 37°C
41. add 1.5 µl proteinase K (18,2 mg/ml, Roche Diagnostics)
42. incubate at 65 °C for 6h
43. remove proteins by phenol/chloroform extraction
44. precipitate DNA with 2.5 vol ethanol, 1/10 vol 3M NaAc pH 5.4, and 1 µl glycogen
45. resuspend DNA in 10-30 µl 10 mM Tris, pH 8

ChIP buffers

MC-Buffer:

10 mM sodium phosphate, pH 7
50 mM NaCl
0.1 M sucrose

M1 buffer

M3 Buffer with 1M 2-methyl 2,4-pentanediol

M2 buffer

M1 buffer with 10 mM MgCl2
0.5% Triton X-100

M3 buffer

10 mM sodium phosphate, pH 7
0.1 M NaCl
10 mM beta- mercaptoethanol
Complete Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannheim, Germany)

Sonication buffer

10 mM sodium phosphate, pH 7
0.1 M NaCl
0.5% Sarkosyl
10 mM EDTA
Complete Protease Inhibitor Cocktail (Roche Diagnostics)
GmbH)
1 mM PEFABLOCK (Roche Diagnostics)

IP buffer

50 mM Hepes, pH 7.5
150 mM KCl
5mM MgCl2
10 µM ZnSO4
1% Triton X-100
0.05% SDS

Glycine elution buffer

0.1 M glycine
0.5 M NaCl
0.05% Tween-20
pH 2.8