**Supplementary material**

**Table S1.** Binding parameters for the interaction between MIR167A or its mutant alleles and DBD of MoCdc5, TvMyb2, or AtCdc5 by BLI.

**Table S2.** Binding parameters of the interaction between MoCdc5-DBD or its mutant alleles and U6 RNA by BLI.

**Table S3.** PCR primers used in this study.

**Figure S1.** DBDs of Myb and Cdc5 contact with distinct DNA sequences. (a) In Myb-DNA complex structures, several positively charged residues on the second and third helixes, including R84, R87, R89, K138, and T143 in TvMyb2, and K128, E132, R133, N179, and T188 in c-Myb, contact with DNA directly. (b) DBDs of Cdc5 and Myb specifically interact with a CTCAGCG motif and a AAC(G/T)G motif, respectively, suggesting that Cdc5 is not a canonical Myb domain protein.

**Figure S2.** Circular dichroism (CD) spectra of MoCdc5-DBD and its mutant alleles show standard α-helix characteristics. CD spectra of MoCdc5-DBD and its mutated alleles were all nearly superimposable from 195 nm to 260 nm. The experiment was recorded at a concentration of 0.2 mg/mL proteins in 20 mM MES (pH 6.0) and 150 mM NaCl. CD wavelength scans were collected between 195 nm to 260 nm using a 1-mm quartz cuvette.

**Figure S3.** Six conserved aromatic residues of MoCdc5-DBD play important roles in maintaining its structural stability. (a) Schematic representation of the structure of the first HTH motif (containing α1-α3) and second HTH motif (containing α4-α6) showing the spatial locations of six conserved aromatic residues, W9, W29, W48, W61, W80, and Y98 in MoCdc5-DBD. These six aromatic amino acids are arranged regularly, forming the hydrophobic core and are responsible for the structure and stability of the two HTH motifs. (b) CD spectra of MoCdc5-DBD and its mutant alleles at six conserved aromatic residues, W9, W29, W48, W61, W80, and Y98.

**Figure S4.** Point mutations of the five amino acid residues involved in DNA binding do not abolish expression level and stability of AtCdc5. Protein expression levels of Myc-Cdc5 and heat shock protein Hsc70 (loading control) from different transgenic *cdc5-2* lines were detected by western blot analysis. The primary antibodies, anti-Myc and anti-Hsc70, were used for western blot analysis.

**Figure S5.** Sequence alignment of Cdc5 and Myb proteins at the 100th amino acid. The 100th amino acids in Cdc5 proteins are mainly those with long side chains, that is, lysine, phenylalanine and tyrosine, which is different from that of Myb proteins. Red dotted lines show the boundary of Cdc5 and Myb. The 100th amino acids in Cdc5 and Myb proteins are highlighted in yellow and green, respectively.

**Figure S6.** Model of MoCdc5-DBD with two distinct nucleic acid-binding surfaces. Cdc5-DBD has two distinct nucleic acid-binding surfaces, one binding DNA similar to Myb-DBD and the other one participating in RNA binding.

**Table S1.** Binding parameters for the interaction between MIR167A or its mutated alleles and DBD of MoCdc5, TvMyb2, or AtCdc5 by BLI.

| Name | DNA sequences |
|---|---|
| MIR167A | TGTCTCAGCGGTGGAG |
| MSE (167A) | TGTACAACGGGTGGAG |
| MIR167A-1 | TGTACAAGCGGTGGAG |
| MIR167A-2 | TGTCTTCACGGTGGAG |
| MIR167A-3 | TGTCTCATATGTGGAG |

| | MIR167A | | | MSE(167A) | | |
|---|---|---|---|---|---|---|
| | KD* (M, e-08) | $K_{on}$ (1/Ms, e+04) | $K_{off}$ (1/s, e-03) | KD (M, e-08) | $K_{on}$ (1/Ms, e+04) | $K_{off}$ (1/s, e-03) |
| MoCdc5-DBD | 5.17±2.13 | 7.23±1.96 | 3.12±0.75 | 63.82±17.39 | 1.27±0.67 | 7.14±3.38 |
| Cdc5-DBD | 8.98±0.9 | 25.93±0.82 | 23.27±2.08 | 27.73±0.24 | 26.28±0.32 | 72.88±1.5 |
| TvMyb-DBD | 66.76±3.44 | 1.6±0.039 | 10.74±0.41 | 70.81±3.32 | 1.62±0.042 | 11.51±0.45 |

| | MIR167A-1 | | | MIR167A-2 | | | MIR167A-3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | KD (M, e-08) | $K_{on}$ (1/Ms, e+04) | $K_{off}$ (1/s, e-03) | KD (M, e-08) | $K_{on}$ (1/Ms, e+04) | $K_{off}$ (1/s, e-03) | KD (M, e-08) | $K_{on}$ (1/Ms, e+04) | $K_{off}$ (1/s, e-03) |
| MoCdc5-DBD | 17.01±4.71 | 11.43±2.73 | 17.86±2.11 | 19.05±5.08 | 9.58±0.39 | 18.19±4.76 | 20.89±5.15 | 8.83±0.75 | 17.99±3.37 |
| AtCdc5-DBD | 13.75±2.04 | 4.93±3.09 | 7.66±4.54 | 15.14±1.69 | 6.84±0.34 | 10.38±1.47 | 12.93±1.24 | 7.01±0.35 | 9.09±1.19 |
| TvMyb-DBD | 71.26±4.22 | 1.56±0.04 | 11.15±0.4 | 73.09±2.83 | 1.65±0.05 | 12.04±0.48 | 67.48±4.38 | 1.63±0.04 | 11.01±0.43 |

*KD, equilibrium dissociation constant. The experiments were repeated three times. Shown values are means ± s.d. of three experiments.

**Table S2.** Binding parameters of the interaction between MoCdc5-DBD or its mutated alleles and U6 RNA by BLI.

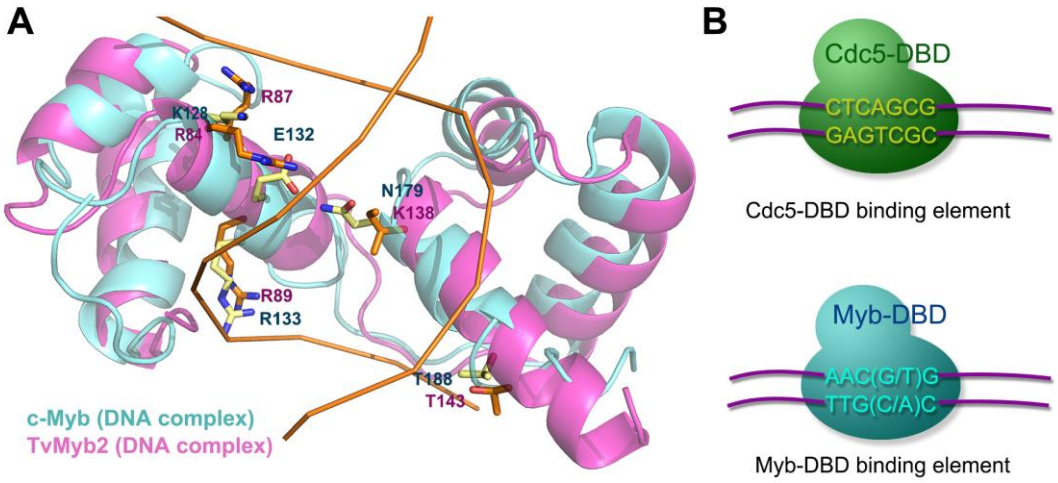
| | **KD* (M, e-08)** | **$K_{on}$ (1/Ms, e+04)** | **$K_{off}$ (1/s, e-03)** |
|---|---|---|---|
| MoCdc5-DBD | 1.38 ±0.05 | 3.85 ±0.03 | 0.53 ±0.02 |
| MoCdc5-DBD$^{R31M}$ | $^{\#}$ND | $^{\#}$ND | $^{\#}$ND |
| MoCdc5-DBD$^{R38N}$ | 4.10 ±0.06 | 6.92 ±0.08 | 2.84 ±0.02 |
| MoCdc5-DBD$^{K42A}$ | 2.80 ±0.07 | 5.51 ±0.08 | 1.54 ±0.03 |
| MoCdc5-DBD$^{K100A}$ | 1.63 ±0.05 | 5.20 ±0.07 | 0.84 ±0.02 |

*KD, equilibrium dissociation constant. The experiments were repeated three times. Shown values are means ± s.d. of three experiments. $^{\#}$ND, not determined due to the small binding affinity. Shown values are means ± s.d. of three experiments.

**Table S3.** PCR primers used in this study.

| Primer name | Description | Sequence 5' to 3' |
|---|---|---|
| CDC5-TOPO-F | CDC5 CDS cloning | CACCATGAGGATTATGATTAAGGG |
| CDC5-TOPO-R | CDC5 CDS cloning | TGCAGAAGCTTCCATGGCTATGG |
| MoCDC5 -DBD -F1 | MoCDC5 DBD cloning | CATGCCTGTCGTCAAAGGAGG |
| MoCDC5-DBD -F2 | MoCDC5 DBD cloning | CCTGTCGTCAAAGGAGGAGTTT |
| MoCDC5-DBD -R1 | MoCDC5 DBD cloning | GTCAGTGGTACCCATTAGTAAC |
| MoCDC5 -DBD -R2 | MoCDC5 DBD cloning | TCGAGTCAGTGGTACCCATTAGT |
| MoCdc5_R31M_F | R31M mutation | TTGAATCAATGGGCAATGGTCTCGTCACTGCTG |
| MoCdc5_R31M_R | R31M mutation | CAGCAGTGACGAGACCATTGCCCATTGATTCAA |
| MoCdc5_R38N_F | R38N mutation | TCGTCACTGCTGGCGAACAAGACGCCAAAGCAG |
| MoCdc5_R38N_R | R38N mutation | CTGCTTTGGCGTCTTGTTCGCCAGCAGTGACGA |
| MoCdc5_K42A_F | K42A mutation | GCGCGCAAGACGCCAGCGCAGTGCAAGGCGCGA |
| MoCdc5_K42A_R | K42A mutation | TCGCGCCTTGCACTGCGCTGGCGTCTTGCGCGC |
| MoCdc5_K45A_F | K45A mutation | ACGCCAAAGCAGTGCGCGGCGCGATGGAACGAG |
| MoCdc5_K45A_R | K45A mutation | CTCGTTCCATCGCGCCGCGCACTGCTTTGGCGT |
| MoCdc5_R47A_F | R47A mutation | AAGCAGTGCAAGGCGGCGTGGAACGAGTGGCTG |
| MoCdc5_R47A_R | R47A mutation | CAGCCACTCGTTCCACGCCGCCTTGCACTGCTT |
| MoCdc5_N92A_F | N92A mutation | GTGGGCCGCACGGCGGCCCAGTGCCTTGAGCGC |
| MoCdc5_N92A_R | N92A mutation | GCGCTCAAGGCACTGGGCCGCCGTGCGGCCCAC |
| MoCdc5_K100A_F | K100A mutation | CTTGAGCGCTACCAGGCGCTTCTCGACGAGGCC |
| MoCdc5_K100A_R | K100A mutation | GGCCTCGTCGAGAAGCGCCTGGTAGCGCTCAAG |

| | | |
|---|---|---|
| CDC5_R32M_F | R32M mutation | AACGAGAAGAGACGAGATCATAGCCCATTGGTTCTTACCA |
| CDC5_R32M_R | R32M mutation | TGGTAAGAACCAATGGGCTATGATCTCGTCTCTTCTCGTT |
| CDC5_R39N_F | R39N mutation | TTACACTGTTTAGCAGACTTATTAACGAGAAGAGACGAGATCCG |
| CDC5_R39N_R | R39N mutation | CGGATCTCGTCTCTTCTCGTTAATAAGTCTGCTAAACAGTGTAA |
| CDC5_K43A_F | K43A mutation | CAGCGAGCTTTACACTGTGCAGCAGACTTACGAACGAGAA |
| CDC5_K43A_R | K43A mutation | TTCTCGTTCGTAAGTCTGCTGCACAGTGTAAAGCTCGCTG |
| CDC5_K101A_F | K101A mutation | GCATGCTGCATCAAGGAGCGCCTCATACCTCTCAAGACAT |
| CDC5_K101A_R | K101A mutation | ATGTCTTGAGAGGTATGAGGCGCTCCTTGATGCAGCATGC |
| CDC5_MoDBD_F | Mosaic CDC5 | CACCATGCCTGTCGTCAAAGGAGGGGTTTG |
| CDC5_MoDBD_R | Mosaic CDC5 | ATAATTTTCATCCTTAGTGCAGGCCTCGTCGAGAAGCT |
| AtCDC5_infusion_F | Mosaic CDC5 | AGCTTCTCGACGAGGCCTGCACTAAGGATGAAAATTATGATGC |
| AtCDC5_infusion_R | Mosaic CDC5 | AGCTGGGTCGGCGCGTGCAGAAGCTTCCATGGC |
| MIR158a qF | Pri-MIR158a | GTGATGACGCCATTGCTCTTT |
| MIR158a qR | Pri-MIR158a | TGTGACTTTAGATGCCCTTGTTCA |
| MIR167a qF | Pri-MIR167a | TGTTGTGTTTCATGACGATGG |
| MIR167a qR | Pri-MIR167a | AGCTCACAAAATCAGACTGAAGA |
| MIR171a qF | Pri-MIR171a | CCGCGCCAATATCTCAGTA |
| MIR171a qR | Pri-MIR171a | TGTCTCCATTTCAACACACACA |
| MIR172b-F | Pri-MIR172b | TATTAAGGACTTGTAGGACTCA |
| MIR172b-R | Pri-MIR172b | TAATAGTACGTACACATAAATGG |
| miR156a stem loop RT | miR156a | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACGTGC TC |
| miR156a forward | miR156a | GCGGCGGTGACAGAAGAGAGT |
| miR167a stem-loop RT | miR167a | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCA ACTAGATC |
| miR167a Forward | miR167a | GGCGTC TGAAGCTGCCAGCAT |
| miR172a stem-loop RT | miR172a | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCAACATGCA G |
| miR172a forward | miR172a | GGCGTCAGAATCTTGATGATG |
| miR173 stem-loop RT | miR173 | GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACGTGA TT |
| miR173 forward | miR173 | GTTGGC TTCGCTTGCAGAGAG |
| U6 stem loop RT | U6 | GTGCAGGGTCCGAGGTTTTGGACCATTTCTCGAT |
| U6 forward | U6 | GGAACGATACAGAGAAGATTAGCA |
| Universal | | GTGCAGGGTCCGAGGT |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TvMyb2 (DNA complex)
B
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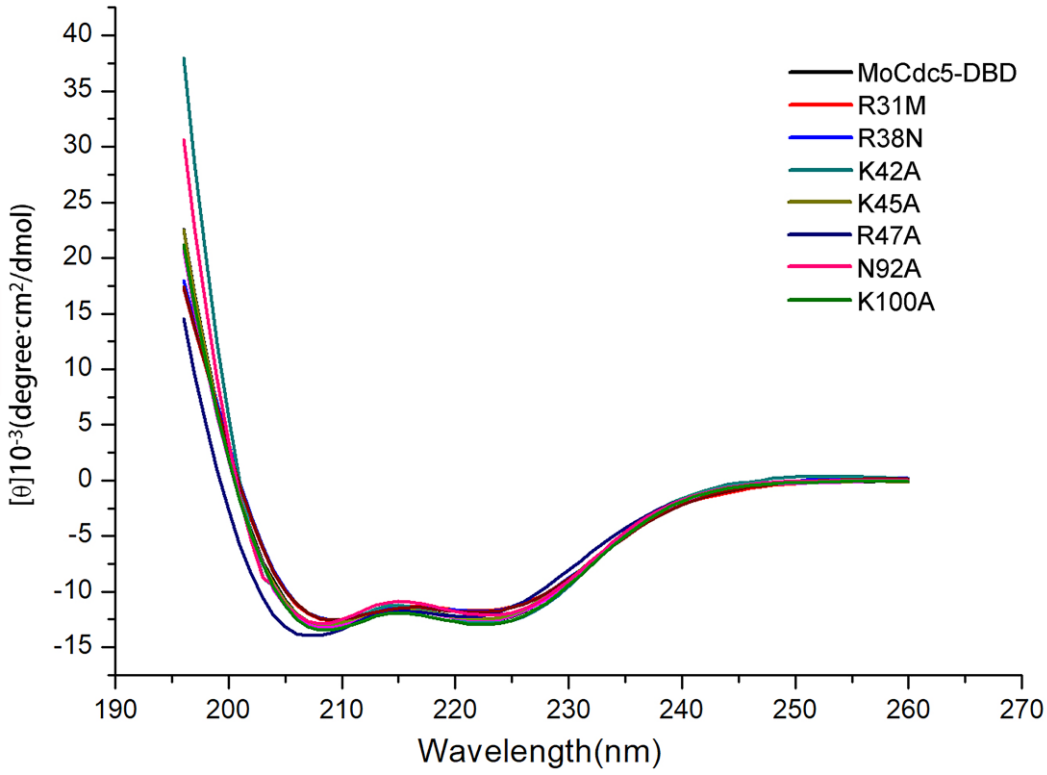
Myb-DBD binding element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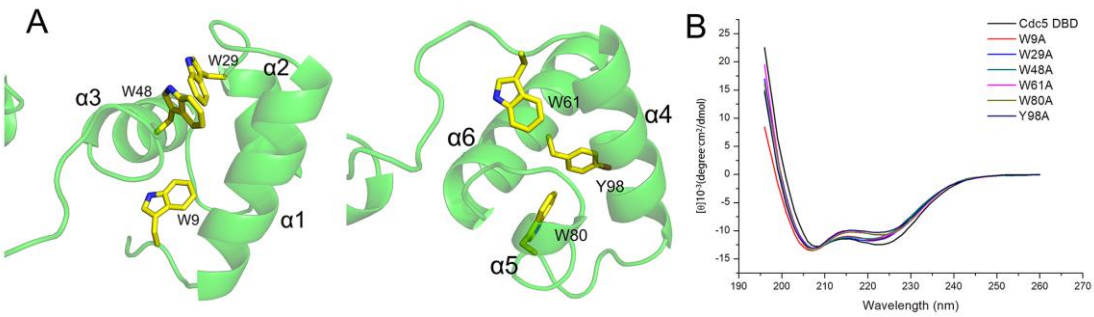
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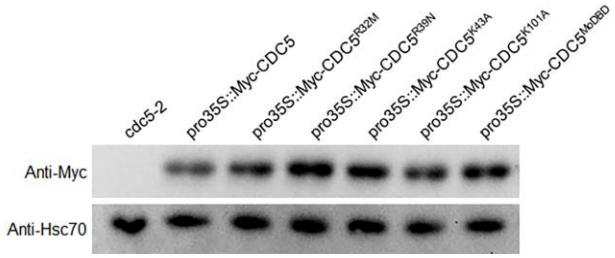
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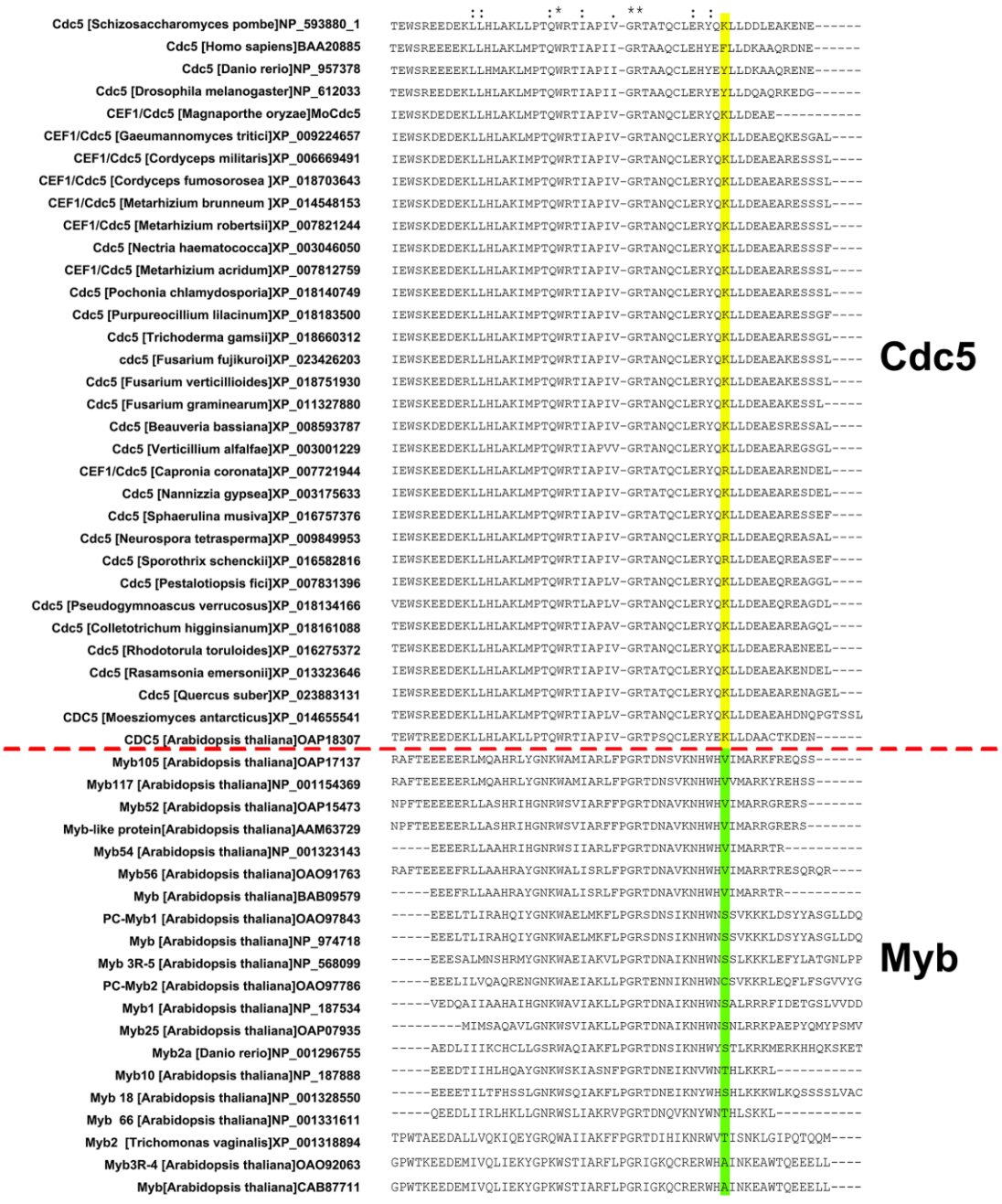
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Myb 3R-5 [Arabidopsis thaliana]NP_568099 -----EEESALMNSHRMYGNKWAEIAKVLPGRTDNAIKNHWNSSLKKKLEFYLATGNLPP
PC-Myb2 [Arabidopsis thaliana]OAO97786 -----EEELILVQAQRENGNKWAEIAKLLPGRTENNIKNHWNCSVKKRLEQFLFSGVVYG
Myb1 [Arabidopsis thaliana]NP_187534 -----VEDQAIIAAHAIHGNKWAVIAKLLPGRTDNAIKNHWNSALRRRFIDETGSLVVDD
Myb25 [Arabidopsis thaliana]OAP07935 ---------MIMSAQAVLGNKWSVIAKLLPGRTDNAIKNHWNSNLRRKPAEPYQMYPSMV
Myb2a [Danio rerio]NP_001296755 -----AEDLIIIKCHCLLGSRWAQIAKFLPGRTDNSIKNHWYSTLKRKMERKHHQKSKET
Myb10 [Arabidopsis thaliana]NP_187888 -----EEEDTIIHLHQAYGNKWSKIASNFPGRTDNEIKNVWNTHLKKRL----------
Myb 18 [Arabidopsis thaliana]NP_001328550 -----EEEETILTFHSSLGNKWSQIAKFLPGRTDNEIKNYWHSHLKKKWLKQSSSSLVAC
Myb 66 [Arabidopsis thaliana]NP_001331611 -----QEEDLIIRLHKLLGNRWSLIAKRVPGRTDNQVKNYWNTHLSKKL----------
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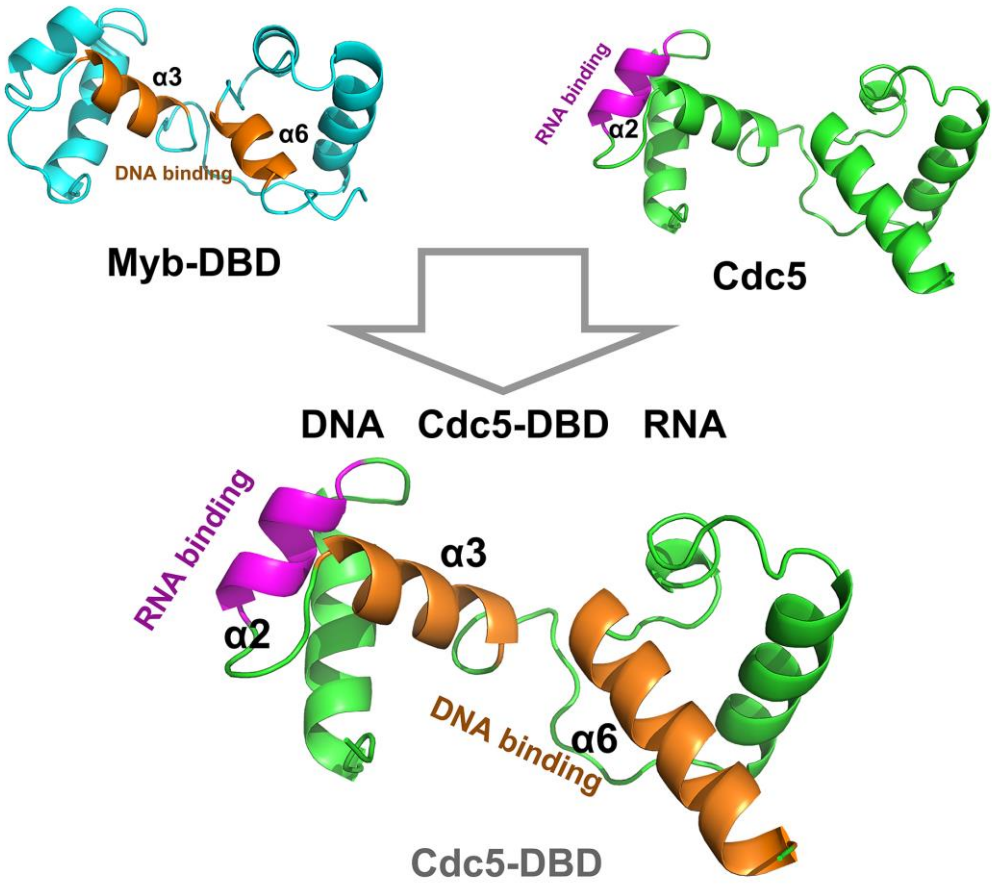
Myb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α6
DNA binding
Myb-DBD
RNA binding
α2
Cdc5
DNA Cdc5-DBD RNA
RNA binding
α3
α2
DNA binding
α6
Cdc5-DBD