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Enhanced Y1H assays for Arabidopsis

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Supplementary Figure 1 Description of clones and their sources

Supplementary Figure 2 AD-2μ with mating is the most sensitive assay.

Supplementary Figure 3 The number of confirmed regulatory interactions obtained.

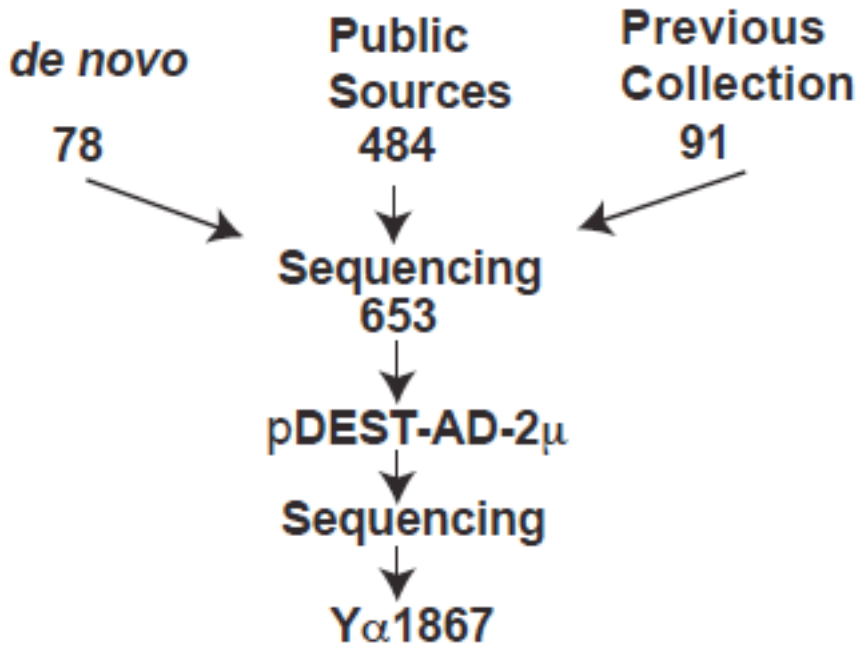
Supplementary Table 1 The 653 transcription factors in the stele collection

Supplementary Table 2 Identified transcription factor-promoter interactions.

Supplementary Table 3 Interacting transcription factors.

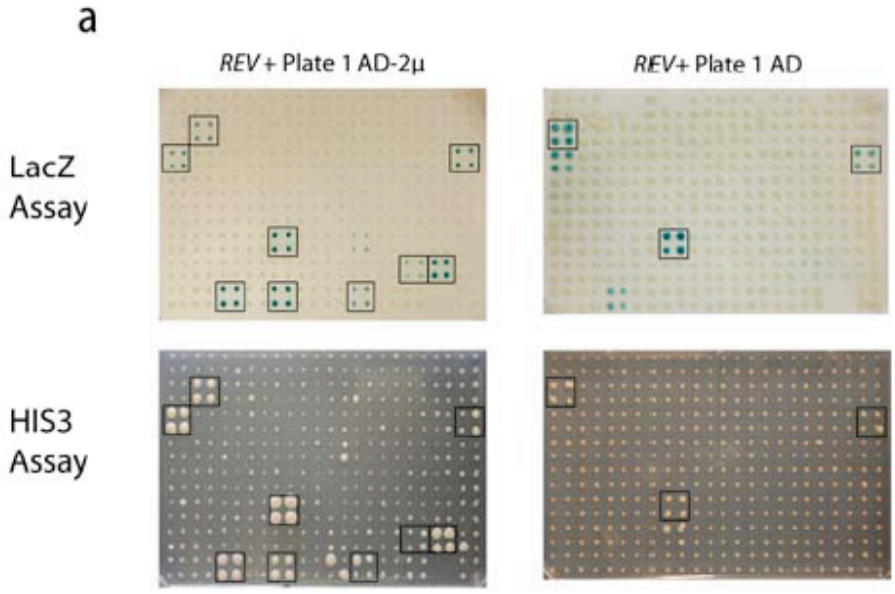
Supplementary Notes Identification of TFs Expressed in the Root Stele; Comparison of Direct Transformation vs. Mating and the High Copy Number and Low Copy Number AD Vector; and Comparison of Efficiency in Labor, Cost and Time

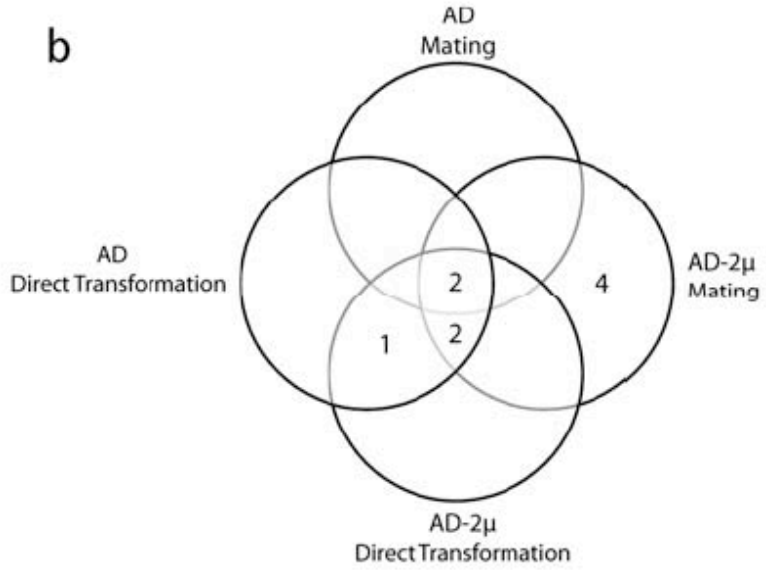
Supplementary Figure 1



Transcription factors in the resource were cloned de novo, obtained from our previous collection or from public sources, were fully sequenced, and then recombined with the activation domain vector. Clones were sequenced and then subsequently transformed in the Yα1867 yeast strain.

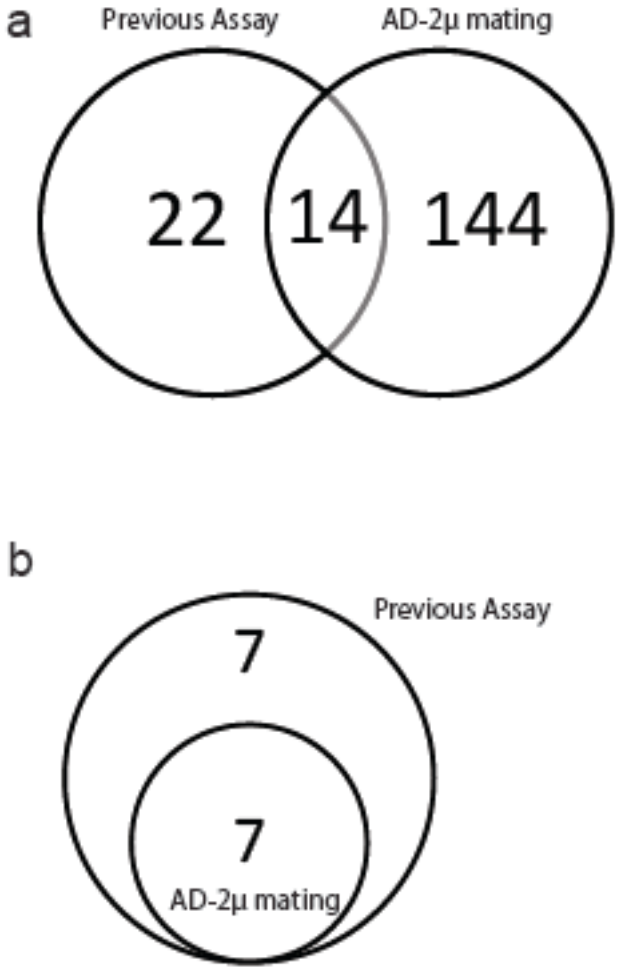
Supplementary Figure 2





Using the *REV* promoter, mating with the AD-2μ vector was the most sensitive assay. (**a**) Pictures of the reporter plates of screens resulting from mating of the *REV* promoter with transcription factor Plate 1 of the AD (low copy) and AD-2μ vectors. The black boxes indicate a positive interaction observed with both reporters (*HIS3* and *LacZ*). (**b**) Venn diagram of interaction overlap between the direct transformation and mating assays using the AD and AD-2μ vectors. There was 100% overlap between the interactions obtained for the direct transformation assays of the AD and AD-2μ vectors. There was a 25% overlap between the AD and AD-2μ vectors in the mating assay. There was 50% overlap of interactions obtained from mating with the AD-2μ vector and the direct transformation assays. Moreover, two of the positives detected with mating the AD-2μ vector were obtained as weak positives with only one reporter, *LacZ*, in the direct transformation assays with both vectors.

Supplementary Figure 3



A comparison of direct transformation versus mating with the different activation domain vectors and the number of confirmed regulatory interactions identified. (**a**) Venn diagram showing the number of interactions obtained with the previous screen using direct transformation with the activation domain vector (left circle), mating with the AD-2μ vector (right circle) and their overlap. 14 out of 36 (39%) of the previously identified interactions were obtained. (**b**) Venn diagram showing the number of interactions from the previous screen which have been confirmed to be regulatory *in planta* (14), the interactions obtained from mating with the AD-2μ vector (7) and their overlap

Supplementary Table 1

AGI	pENTR Source	Gene Model	Family
At1g22985	Brady	AT1G22985.1	AP2/EREBP
At1g78080	Brady	AT1G78080.1	AP2/EREBP
At2g23340	Brady	AT2G23340.1	AP2/EREBP
At2g44940	Brady	AT2G44940.1	AP2/EREBP
At3g16280	Brady	AT3G16280.1	AP2/EREBP
At3g60490	Brady	AT3G60490.1	AP2/EREBP
At4g36920	Brady	AT4G36920.1	AP2/EREBP
At5g51190	Brady	AT5G51190.1	AP2/EREBP
At1g04550	Brady	AT1G04550.1	AUX/IAA
At3g16500	Brady	AT3G16500.1	AUX/IAA
At4g28640	Brady	AT4G28640.1	AUX/IAA
At5g25890	Brady	AT5G25890.1	AUX/IAA
At2g43060	Brady	AT2G43060.1	BHLH
At3g06590	Brady	AT3G06590.1	BHLH
At3g23210	Brady	AT3G23210.1	BHLH
At3g25710	Brady	AT3G25710.1	BHLH
At3g56980	Brady	AT3G56980.1	BHLH
At3g62090	Brady	AT3G62090.1	BHLH
At4g02590	Brady	AT4G02590.1	BHLH
At4g36540	Brady	AT4G36540.1	BHLH
At2g22850	Brady	AT2G22850.1	BZIP
At3g19290	Brady	AT3G19290.1	BZIP
At4g37730	Brady	AT4G37730.1	BZIP
At5g06960	Brady	AT5G06960.1	BZIP
At3g21890	Brady	AT3G21890.1	C2C2-CO-LIKE
At1g07640	Brady	AT1G07640.1	C2C2-DOF
At1g29160	Brady	AT1G29160.1	C2C2-DOF
At1g64620	Brady	AT1G64620.1	C2C2-DOF
At2g28810	Brady	AT2G28810.1	C2C2-DOF
At2g37590	Brady	AT2G37590.1	C2C2-DOF
At3g45610	Brady	AT3G45610.1	C2C2-DOF
At3g55370	Brady	AT3G55370.1	C2C2-DOF
At3g61850	Brady	AT3G61850.1	C2C2-DOF
At2g18380	Brady	AT2G18380.1	C2C2-GATA
At4g36620	Brady	AT4G36620.1	C2C2-GATA
At5g25830	Brady	AT5G25830.1	C2C2-GATA
At2g01940	Brady	AT2G01940.1	C2H2
At2g28200	Brady	AT2G28200.1	C2H2
At3g05760	Brady	AT3G05760.1	C2H2

At5g03510	Brady	AT5G03510.1	C2H2
At1g54160	Brady	AT1G54160.1	CCAAT-HAP2
At5g47640	Brady	AT5G47640.1	CCAAT-HAP3
At4g14770	Brady	AT4G14770.1	CPP
At1g21450	Brady	AT1G21450.1	GRAS
At2g04890	Brady	AT2G04890.1	GRAS
At4g37650	Brady	AT4G37650.1	GRAS
At2g22840	Brady	AT2G22840.1	GRF
At1g23380	Brady	AT1G23380.1	HB
At2g16400	Brady	AT2G16400.1	HB
At2g22800	Brady	AT2G22800.1	HB
At2g34710	Brady	AT2G34710.1	HB
At2g46680	Brady	AT2G46680.1	HB
At4g37790	Brady	AT4G37790.1	HB
At5g06710	Brady	AT5G06710.1	HB
At5g53980	Brady	AT5G53980.1	HB
At5g60690	Brady	AT5G60690.1	HB
At1g77570	Brady	AT1G77570.1	HSF
At2g42830	Brady	AT2G42830.1	MADS
At3g57230	Brady	AT3G57230.1	MADS
At5g13790	Brady	AT5G13790.1	MADS
At1g16490	Brady	AT1G16490.1	MYB
At1g17950	Brady	AT1G17950.1	MYB
At1g18570	Brady	AT1G18570.1	MYB
At1g66230	Brady	AT1G66230.1	MYB
At3g06490	Brady	AT3G06490.1	MYB
At3g08500	Brady	AT3G08500.1	MYB
At3g11280	Brady	AT3G11280.1	MYB
At3g24310	Brady	AT3G24310.1	MYB
At3g46130	Brady	AT3G46130.1	MYB
At4g12350	Brady	AT4G12350.1	MYB
At4g22680	Brady	AT4G22680.1	MYB
At5g12870	Brady	AT5G12870.1	MYB
At5g37260	Brady	AT5G37260.1	MYB
At5g59780	Brady	AT5G59780.1	MYB
At1g02220	Brady	AT1G02220.1	NAC
At1g54330	Brady	AT1G54330.1	NAC
At1g71930	Brady	AT1G71930.1	NAC
At2g18060	Brady	AT2G18060.1	NAC
At2g27300	Brady	AT2G27300.1	NAC
At2g33480	Brady	AT2G33480.1	NAC
At3g15510	Brady	AT3G15510.1	NAC

At3g17730	Brady	AT3G17730.1	NAC
At4g29230	Brady	AT4G29230.1	NAC
At5g50820	Brady	AT5G50820.1	NAC
At5g64530	Brady	AT5G64530.1	NAC
At2g45680	Brady	AT2G45680.1	TCP
At1g13450	Brady	AT1G13450.1	TRIHELIX
At2g04880	Brady	AT2G04880.1	WRKY
At5g13080	Brady	AT5G13080.1	WRKY
At5g49520	Brady	AT5G49520.1	WRKY
At5g56270	Brady	AT5G56270.1	WRKY
At1g74660	Brady	AT1G74660.1	ZF-HD
At3g42790	de novo	AT3G42790.1	ALFIN-LIKE
At3g14230	de novo	AT3G14230.2	AP2/EREBP
At3g25730	de novo	AT3G25730.1	AP2/EREBP
At4g17490	de novo	AT4G17490.1	AP2/EREBP
At4g23750	de novo	AT4G23750.1	AP2/EREBP
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At5g07580	de novo	AT5G07580.1	AP2/EREBP
At5g10510	de novo	AT5G10510.2	AP2/EREBP
At5g18560	de novo	AT5G18560.1	AP2/EREBP
At5g52020	de novo	AT5G52020.1	AP2/EREBP
At5g61590	de novo	AT5G61590.1	AP2/EREBP
At5g61600	de novo	AT5G61600.1	AP2/EREBP
At1g19220	de novo	AT1G19220.1	ARF
At5g20730	de novo	AT5G20730.2	ARF
At1g09250	de novo	AT1G09250.1	BHLH
At1g51140	de novo	AT1G51140.1	BHLH
At2g22770	de novo	AT2G22770.1	BHLH
At2g31730	de novo	AT2G31730.1	BHLH
At3g17100	de novo	AT3G17100.1	BHLH
At3g23690	de novo	AT3G23690.1	BHLH
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At5g46760	de novo	AT5G46760.1	BHLH
At5g58010	de novo	AT5G58010.1	BHLH
At1g32150	de novo	AT1G32150.1	BZIP
At2g40950	de novo	AT2G40950.1	BZIP
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At4g36730	de novo	AT4G36730.1	BZIP
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At2g19380	de novo	AT2G19380.1	C2H2

At3g02860	de novo	AT3G02860.2	C2H2
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At3g19580	de novo	AT3G19580.1	C2H2
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At5g66730	de novo	AT5G66730.1	C2H2
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At3g20770	de novo	AT3G20770.1	EIL
At1g44810	de novo	AT1G44810.1	GEBP
At1g61730	de novo	AT1G61730.1	GEBP
At1g07520	de novo	AT1G07520.1	GRAS
At1g63100	de novo	AT1G63100.1	GRAS
At2g45160	de novo	AT2G45160.1	GRAS
At1g27050	de novo	AT1G27050.1	HB
At1g30810	de novo	AT1G30810.1	JUMONJI
At1g63490	de novo	AT1G63490.1	JUMONJI
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At1g63910	de novo	AT1G63910.1	MYB
At1g70000	de novo	AT1G70000.1	MYB
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At4g01680	de novo	AT4G01680.1	MYB
At4g34990	de novo	AT4G34990.1	MYB
At4g37260	de novo	AT4G37260.1	MYB
At4g38620	de novo	AT4G38620.1	MYB
At3g04070	de novo	AT3G04070.1	NAC
At5g09330	de novo	AT5G09330.1	NAC
At1g76350	de novo	AT1G76350.1	NIN-LIKE
At1g47270	de novo	AT1G47270.2	TUB
At1g13960	de novo	AT1G13960.1	WRKY
At1g29280	de novo	AT1G29280.1	WRKY
At1g69310	de novo	AT1G69310.1	WRKY
At3g01970	de novo	AT3G01970.1	WRKY
At1g69600	de novo	AT1G69600.1	ZF-HD
At4g32010	de novo	AT4G32010.1	ABI3/VP1
At5g17430	de novo	AT5G17430.1	AP2/EREBP
At2g46510	de novo	AT2G46510.1	BHLH
At4g37740	de novo	AT4G37740.1	GRF
At4g36740	de novo	AT4G36740.1	HB
At1g28470	de novo	AT1G28470.1	NAC

At1g62700	de novo	AT1G62700.1	NAC
At4g36160	de novo	AT4G36160.1	NAC
At3g10040	de novo	AT3G10040.1	TRIHELIX
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At3g11580	REGIA	AT3G11580.1	ABI3/VP1
At1g71450	REGIA	AT1G71450.1	AP2/EREBP
At3g20840	REGIA	AT3G20840.1	AP2/EREBP
At3g61630	REGIA	AT3G61630.1	AP2/EREBP
At4g18450	REGIA	AT4G18450.1	AP2/EREBP
At4g36900	REGIA	AT4G36900.1	AP2/EREBP
At1g59750	REGIA	AT1G59750.1	ARF
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At4g17880	REGIA	AT4G17880.1	BHLH
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At4g39070	REGIA	AT4G39070.1	C2C2-CO-LIKE
At5g02460	REGIA	AT5G02460.1	C2C2-DOF
At3g53340	REGIA	AT3G53340.1	CCAAT-HAP3
At2g29060	REGIA	AT2G29060.1	GRAS
At3g46600	REGIA	AT3G46600.1	GRAS
At4g36710	REGIA	AT4G36710.1	GRAS
At1g52150	REGIA	AT1G52150.1	HB
At4g00730	REGIA	AT4G00730.1	HB
At4g34610	REGIA	AT4G34610.1	HB
At5g59340	REGIA	AT5G59340.1	HB
At5g65310	REGIA	AT5G65310.1	HB
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At2g26150	REGIA	AT2G26150.1	HSF
At1g17310	REGIA	AT1G17310.1	MADS
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At1g48000	REGIA	AT1G48000.1	MYB
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At5g01200	REGIA	AT5G01200.1	MYB
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At4g25470	REGIA/SALK	AT4G25470.1	AP2/EREBP
At1g04250	REGIA/SALK	AT1G04250.1	AUX/IAA
At2g46990	REGIA/SALK	AT2G46990.1	AUX/IAA
At4g14550	REGIA/SALK	AT4G14550.1	AUX/IAA
At1g03970	REGIA/SALK	AT1G03970.1	BZIP
At3g21175	REGIA/SALK	AT3G21175.1	C2C2-GATA
At3g24050	REGIA/SALK	AT3G24050.1	C2C2-GATA
At4g24470	REGIA/SALK	AT4G24470.1	C2C2-GATA
At2g37430	REGIA/SALK	AT2G37430.1	C2H2
At2g48100	REGIA/SALK	AT2G48100.1	C2H2
At5g44180	REGIA/SALK	AT5G44180.1	HB
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At1g62300	REGIA/SALK	AT1G62300.1	WRKY
At1g68150	REGIA/SALK	AT1G68150.1	WRKY
At2g38470	REGIA/SALK	AT2G38470.1	WRKY
At1g25560	SALK	AT1G25560.1	ABI3/VP1
At3g18990	SALK	AT3G18990.1	ABI3/VP1
At1g14510	SALK	AT1G14510.1	ALFIN-LIKE
At2g02470	SALK	AT2G02470.1	ALFIN-LIKE
At3g11200	SALK	AT3G11200.1	ALFIN-LIKE
At5g05610	SALK	AT5G05610.1	ALFIN-LIKE
At5g26210	SALK	AT5G26210.1	ALFIN-LIKE
At1g12610	SALK	AT1G12610.1	AP2/EREBP
At1g12630	SALK	AT1G12630.1	AP2/EREBP
At1g13260	SALK	AT1G13260.1	AP2/EREBP
At1g19210	SALK	AT1G19210.1	AP2/EREBP

At1g21910	SALK	AT1G21910.1	AP2/EREBP
At1g22190	SALK	AT1G22190.1	AP2/EREBP
At1g22810	SALK	AT1G22810.1	AP2/EREBP
At1g28360	SALK	AT1G28360.1	AP2/EREBP
At1g28370	SALK	AT1G28370.1	AP2/EREBP
At1g36060	SALK	AT1G36060.1	AP2/EREBP
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At1g74930	SALK	AT1G74930.1	AP2/EREBP
At1g77640	SALK	AT1G77640.1	AP2/EREBP
At1g79700	SALK	AT1G79700.1	AP2/EREBP
At2g20880	SALK	AT2G20880.1	AP2/EREBP
At2g28550	SALK	AT2G28550.1	AP2/EREBP
At2g38340	SALK	AT2G38340.1	AP2/EREBP
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At2g44840	SALK	AT2G44840.1	AP2/EREBP
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At4g11140	SALK	AT4G11140.1	AP2/EREBP
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At4g34410	SALK	AT4G34410.1	AP2/EREBP
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At4g39780	SALK	AT4G39780.1	AP2/EREBP
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At5g61890	SALK	AT5G61890.1	AP2/EREBP
At5g67190	SALK	AT5G67190.1	AP2/EREBP
At1g19850	SALK	AT1G19850.1	ARF
At1g30330	SALK	AT1G30330.1	ARF
At1g34310	SALK	AT1G34310.1	ARF
At2g28350	SALK	AT2G28350.1	ARF
At2g33860	SALK	AT2G33860.1	ARF
At3g61830	SALK	AT3G61830.1	ARF
At5g37020	SALK	AT5G37020.1	ARF
At5g62000	SALK	AT5G62000.1	ARF
At1g04880	SALK	AT1G04880.1	ARID
At1g20910	SALK	AT1G20910.1	ARID
At1g76510	SALK	AT1G76510.1	ARID
At3g43240	SALK	AT3G43240.1	ARID
At1g04100	SALK	AT1G04100.1	AUX/IAA
At1g04240	SALK	AT1G04240.1	AUX/IAA
At1g51950	SALK	AT1G51950.1	AUX/IAA
At1g52830	SALK	AT1G52830.1	AUX/IAA
At2g22670	SALK	AT2G22670.1	AUX/IAA
At2g33310	SALK	AT2G33310.2	AUX/IAA
At3g04730	SALK	AT3G04730.1	AUX/IAA
At3g15540	SALK	AT3G15540.1	AUX/IAA
At3g17600	SALK	AT3G17600.1	AUX/IAA
At3g23030	SALK	AT3G23030.1	AUX/IAA
At3g23050	SALK	AT3G23050.1	AUX/IAA
At4g14560	SALK	AT4G14560.1	AUX/IAA
At4g29080	SALK	AT4G29080.1	AUX/IAA
At5g43700	SALK	AT5G43700.1	AUX/IAA
At5g65670	SALK	AT5G65670.1	AUX/IAA
At1g01260	SALK	AT1G01260.1	BHLH
At1g03040	SALK	AT1G03040.1	BHLH
At1g05710	SALK	AT1G05710.1	BHLH
At1g05805	SALK	AT1G05805.1	BHLH
At1g10120	SALK	AT1G10120.1	BHLH
At1g31050	SALK	AT1G31050.1	BHLH
At1g32640	SALK	AT1G32640.1	BHLH
At1g59640	SALK	AT1G59640.1	BHLH
At1g61660	SALK	AT3G56400.1	BHLH
At1g68810	SALK	AT1G68810.1	BHLH
At1g68920	SALK	AT1G68920.1	BHLH
At1g69010	SALK	AT1G69010.1	BHLH

At1g74500	SALK	AT1G74500.1	BHLH
At2g22760	SALK	AT2G22760.1	BHLH
At2g24260	SALK	AT2G24260.1	BHLH
At2g47270	SALK	AT2G47270.1	BHLH
At3g05800	SALK	AT3G05800.1	BHLH
At3g07340	SALK	AT3G07340.1	BHLH
At3g19860	SALK	AT3G19860.1	BHLH
At3g26744	SALK	AT3G26744.1	BHLH
At3g47640	SALK	AT3G47640.1	BHLH
At3g57800	SALK	AT3G57800.2	BHLH
At4g14410	SALK	AT4G14410.1	BHLH
At4g16430	SALK	AT4G16430.1	BHLH
At4g30410	SALK	AT4G30410.1	BHLH
At4g36930	SALK	AT4G36930.1	BHLH
At5g08130	SALK	AT5G08130.1	BHLH
At5g48560	SALK	AT5G48560.1	BHLH
At5g54680	SALK	AT5G54680.1	BHLH
At5g62610	SALK	AT5G62610.1	BHLH
At5g65640	SALK	AT5G65640.1	BHLH
At1g06070	SALK	AT1G06070.1	BZIP
At1g22070	SALK	AT1G22070.1	BZIP
At1g42990	SALK	AT1G42990.1	BZIP
At1g43700	SALK	AT1G43700.1	BZIP
At1g49720	SALK	AT1G49720.1	BZIP
At1g77920	SALK	AT1G77920.1	BZIP
At2g21230	SALK	AT2G21230.1	BZIP
At2g31370	SALK	AT2G31370.1	BZIP
At2g35530	SALK	AT2G35530.1	BZIP
At2g40620	SALK	AT2G40620.1	BZIP
At2g41070	SALK	AT2G41070.1	BZIP
At2g46270	SALK	AT2G46270.1	BZIP
At3g49760	SALK	AT3G49760.1	BZIP
At3g54620	SALK	AT3G54620.1	BZIP
At3g56850	SALK	AT3G56850.1	BZIP
At3g58120	SALK	AT3G58120.1	BZIP
At4g01120	SALK	AT4G01120.1	BZIP
At4g02640	SALK	AT4G02640.2	BZIP
At4g35040	SALK	AT4G35040.1	BZIP
At4g38900	SALK	AT4G38900.2	BZIP
At5g06950	SALK	AT5G06950.1	BZIP
At5g11260	SALK	AT5G11260.1	BZIP
At5g28770	SALK	AT5G28770,1	BZIP
At5g44080	SALK	AT5G44080.1	BZIP
At5g65210	SALK	AT5G65210.1	BZIP

At1g75540	SALK	AT1G75540.1	C2C2-CO-LIKE
At1g78600	SALK	AT1G78600.1	C2C2-CO-LIKE
At2g21320	SALK	AT2G21320.1	C2C2-CO-LIKE
At2g24790	SALK	AT2G24790.1	C2C2-CO-LIKE
At3g07650	SALK	AT3G07650.1	C2C2-CO-LIKE
At4g38960	SALK	AT4G38960.2	C2C2-CO-LIKE
At5g24930	SALK	AT5G24930.1	C2C2-CO-LIKE
At5g48250	SALK	AT5G48250.1	C2C2-CO-LIKE
At5g57660	SALK	AT5G57660.1	C2C2-CO-LIKE
At1g51700	SALK	AT1G51700.1	C2C2-DOF
At2g28510	SALK	AT2G28510.1	C2C2-DOF
At2g34140	SALK	AT2G34140.1	C2C2-DOF
At2g46590	SALK	AT2G46590.1	C2C2-DOF
At3g21270	SALK	AT3G21270.1	C2C2-DOF
At3g50410	SALK	AT3G50410.1	C2C2-DOF
At4g24060	SALK	AT4G24060.1	C2C2-DOF
At5g39660	SALK	AT5G39660.1	C2C2-DOF
At5g60200	SALK	AT5G60200.1	C2C2-DOF
At5g60850	SALK	AT5G60850.1	C2C2-DOF
At5g62430	SALK	AT5G62430.1	C2C2-DOF
At5g62940	SALK	AT5G62940.1	C2C2-DOF
At1g51600	SALK	AT1G51600.1	C2C2-GATA
At3g06740	SALK	AT3G06740.1	C2C2-GATA
At3g16870	SALK	AT3G16870.1	C2C2-GATA
At3g54810	SALK	AT3G54810.1	C2C2-GATA
At3g60530	SALK	AT3G60530.1	C2C2-GATA
At4g17570	SALK	AT4G17570.1	C2C2-GATA
At4g32890	SALK	AT4G32890.1	C2C2-GATA
At4g34680	SALK	AT4G34680.1	C2C2-GATA

At5g26930	SALK	AT5G26930.1	C2C2-GATA
At1g03840	SALK	AT1G03840.1	C2H2
At1g04850	SALK	AT1G04850.1	C2H2
At1g10480	SALK	AT1G10480.1	C2H2
At1g24625	SALK	AT1G24625.1	C2H2
At1g26610	SALK	AT1G26610.1	C2H2
At1g27730	SALK	AT1G27730.1	C2H2
At1g30970	SALK	AT1G30970.1	C2H2
At1g34370	SALK	AT1G34370.1	C2H2
At1g43860	SALK	AT1G43860.1	C2H2
At1g50670	SALK	AT1G50670.1	C2H2
At1g55110	SALK	AT1G55110.1	C2H2
At1g55460	SALK	AT1G55460.1	C2H2
At1g66140	SALK	AT1G66140.1	C2H2
At1g67030	SALK	AT1G67030.1	C2H2
At1g68130	SALK	AT1G68130.1	C2H2
At1g68360	SALK	AT1G68360.1	C2H2
At1g72050	SALK	AT1G72050.1	C2H2
At1g75710	SALK	AT1G75710.1	C2H2
At2g01650	SALK	AT2G01650.1	C2H2
At2g24500	SALK	AT2G24500.1	C2H2
At2g26940	SALK	AT2G26940.1	C2H2
At2g27100	SALK	AT2G27100.1	C2H2
At2g29660	SALK	AT2G29660.1	C2H2
At2g36930	SALK	AT2G36930.1	C2H2
At2g41940	SALK	AT2G41940.1	C2H2
At2g45120	SALK	AT2G45120.1	C2H2
At3g02790	SALK	AT3G02790.1	C2H2
At3g10470	SALK	AT3G10470.1	C2H2
At3g13810	SALK	AT3G13810.1	C2H2
At3g49930	SALK	AT3G49930.1	C2H2
At3g53600	SALK	AT3G53600.1	C2H2
At3g57480	SALK	AT3G57480.1	C2H2
At3g58070	SALK	AT3G58070.1	C2H2
At3g60580	SALK	AT3G60580.1	C2H2
At3g62240	SALK	AT3G62240.1	C2H2
At4g16610	SALK	AT4G16610.1	C2H2
At4g16845	SALK	AT4G16845.1	C2H2
At4g27240	SALK	AT4G27240.1	C2H2
At4g31420	SALK	AT4G31420.1	C2H2
At5g03740	SALK	AT5G03740.1	C2H2
At5g04390	SALK	AT5G04390.1	C2H2
At5g16470	SALK	AT5G16470.1	C2H2

At5g25160	SALK	AT5G25160.1	C2H2
At5g40710	SALK	AT5G40710.1	C2H2
At5g43170	SALK	AT5G43170.1	C2H2
At5g44160	SALK	AT5G44160.1	C2H2
At5g51230	SALK	AT5G51230.1	C2H2
At5g52010	SALK	AT5G52010.1	C2H2
At5g59820	SALK	AT5G59820.1	C2H2
At5g63280	SALK	AT5G63280.1	C2H2
At1g67910	SALK	AT1G67910.1	CAMTA
At5g64220	SALK	AT5G64220.1	CAMTA
At5g08190	SALK	AT5G08190.1	CCAAT-DR1
At5g23090	SALK	AT5G23090.1	CCAAT-DR1
At1g72830	SALK	AT1G72830.2	CCAAT-HAP2
At5g12840	SALK	AT5G12840.1	CCAAT-HAP2
At2g38880	SALK	AT2G38880.1	CCAAT-HAP3
At1g07980	SALK	AT1G07980.1	CCAAT-HAP5
At1g08970	SALK	AT1G08970.1	CCAAT-HAP5
At1g54830	SALK	AT1G54830.1	CCAAT-HAP5
At1g56170	SALK	AT1G56170.1	CCAAT-HAP5
At3g12480	SALK	AT3G12480.1	CCAAT-HAP5
At1g47870	SALK	AT1G47870.1	E2F/DP
At2g36010	SALK	AT2G36010.1	E2F/DP
At3g01330	SALK	AT3G01330.1	E2F/DP
At5g02470	SALK	AT5G02470.1	E2F/DP
At1g73730	SALK	AT1G73730.1	EIL
At2g27050	SALK	AT2G27050.1	EIL
At4g25210	SALK	AT4G25210.1	GEBP
At5g28040	SALK	AT5G28040.1	GEBP
At1g07530	SALK	AT1G07530.1	GRAS
At1g14920	SALK	AT1G14920.1	GRAS
At1g50420	SALK	AT1G50420.1	GRAS
At1g50600	SALK	AT1G50600.1	GRAS
At2g01570	SALK	AT2G01570.1	GRAS
At3g03450	SALK	AT3G03450.1	GRAS
At3g54220	SALK		GRAS

		AT3G54220.1	
At3g60630	SALK	AT3G60630.1	GRAS
At4g00150	SALK	AT4G00150.1	GRAS
At4g17230	SALK	AT4G17230.1	GRAS
At5g48150	SALK	AT5G48150.1	GRAS
At5g52510	SALK	AT5G52510.1	GRAS
At5g59450	SALK	AT5G59450.1	GRAS
At2g36400	SALK	AT2G36400.1	GRF
At1g19700	SALK	AT1G19700.1	HB
At1g20700	SALK	AT1G20700.1	HB
At1g26960	SALK	AT1G26960.1	HB
At1g62990	SALK	AT1G62990.1	HB
At1g69780	SALK	AT1G69780.1	HB
At1g75410	SALK	AT1G75410.1	HB
At1g79840	SALK	AT1G79840.1	HB
At2g22430	SALK	AT2G22430.1	HB
At2g23760	SALK	AT2G23760.1	HB
At2g33880	SALK	AT2G33880.1	HB
At2g35940	SALK	AT2G35940.1	HB
At3g01220	SALK	AT3G01220.1	HB
At3g19510	SALK	AT3G19510.1	HB
At3g61890	SALK	AT3G61890.1	HB
At4g16780	SALK	AT4G16780.1	HB
At4g17460	SALK	AT4G17460.1	HB
At4g32040	SALK	AT4G32040.1	HB
At4g32880	SALK	AT4G32880.1	HB
At4g35550	SALK	AT4G35550.1	HB
At4g36870	SALK	AT4G36870.1	HB
At4g40060	SALK	AT4G40060.1	HB
At5g47370	SALK	AT5G47370.1	HB
At1g32330	SALK	AT1G32330.1	HSF
At3g02990	SALK	AT3G02990.1	HSF
At3g22830	SALK	AT3G22830.1	HSF
At3g24520	SALK	AT3G24520.1	HSF
At3g51910	SALK	AT3G51910.1	HSF
At4g11660	SALK	AT4G11660.1	HSF
At4g13980	SALK	AT4G13980.1	HSF
At4g18880	SALK	AT4G18880.1	HSF
At5g45710	SALK	AT5G45710.1	HSF
At5g62020	SALK	AT5G62020.1	HSF
At2g38950	SALK	AT2G38950.1	JUMONJI
At4g20400	SALK	AT4G20400.1	JUMONJI
At1g71692	SALK	AT1G71692.1	MADS
At3g57390	SALK	AT3G57390.1	MADS

At1g06180	SALK	AT1G06180.1	MYB
At1g09770	SALK	AT1G09770.1	MYB
At1g14350	SALK	AT1G14350.1	MYB
At1g17520	SALK	AT1G17520.1	MYB
At1g18330	SALK	AT1G18330.1	MYB
At1g19000	SALK	AT1G19000.1	MYB
At1g22640	SALK	AT1G22640.1	MYB
At1g49950	SALK	AT1G49950.1	MYB
At1g73410	SALK	AT1G73410.1	MYB
At1g74430	SALK	AT1G74430.1	MYB
At2g16720	SALK	AT2G16720.1	MYB
At2g23290	SALK	AT2G23290.1	MYB
At2g31180	SALK	AT2G31180.1	MYB
At2g37630	SALK	AT2G37630.1	MYB
At2g38090	SALK	AT2G38090.1	MYB
At2g46830	SALK	AT2G46830.1	MYB
At2g47460	SALK	AT2G47460.1	MYB
At3g09370	SALK	AT3G09370.1	MYB
At3g11440	SALK	AT3G11440.1	MYB
At3g16350	SALK	AT3G16350.1	MYB
At3g23250	SALK	AT3G23250.1	MYB
At3g49690	SALK	AT3G49690.1	MYB
At3g50060	SALK	AT3G50060.1	MYB
At3g55730	SALK	AT3G55730.1	MYB
At4g05100	SALK	AT4G05100.1	MYB
At4g09460	SALK	AT4G09460.1	MYB
At4g39160	SALK	AT4G39160.1	MYB
At5g02840	SALK	AT5G02840.1	MYB
At5g04760	SALK	AT5G04760.1	MYB
At5g05790	SALK	AT5G05790.1	MYB
At5g08520	SALK	AT5G08520.1	MYB
At5g14750	SALK	AT5G14750.1	MYB
At5g26660	SALK	AT5G26660.1	MYB
At5g40330	SALK	AT5G40330.1	MYB
At5g47390	SALK	AT5G47390.1	MYB
At5g52260	SALK	AT5G52260.1	MYB
At5g52660	SALK	AT5G52660.1	MYB
At5g58900	SALK	AT5G58900.1	MYB
At5g65790	SALK	AT5G65790.1	MYB
At5g67300	SALK	AT5G67300.1	MYB
At5g67580	SALK	AT5G67580.1	MYB
At1g01010	SALK	AT1G01010.1	NAC
At1g12260	SALK	AT1G12260.1	NAC

At1g25580	SALK	AT1G25580.1	NAC
At1g33060	SALK	AT1G33060.1	NAC
At1g34190	SALK	AT1G34190.1	NAC
At1g52880	SALK	AT1G52880.1	NAC
At1g52890	SALK	AT1G52890.1	NAC
At1g69490	SALK	AT1G69490.1	NAC
At1g77450	SALK	AT1G77450.1	NAC
At3g10500	SALK	AT3G10500.1	NAC
At3g15500	SALK	AT3G15500.1	NAC
At3g29035	SALK	AT3G29035.1	NAC
At3g49530	SALK	AT3G49530.1	NAC
At4g27410	SALK	AT4G27410.2	NAC
At4g35580	SALK	AT4G35580.1	NAC
At5g04410	SALK	AT5G04410.1	NAC
At5g13180	SALK	AT5G13180.1	NAC
At5g14000	SALK	AT5G14000.1	NAC
At5g18270	SALK	AT5G18270.1	NAC
At5g22290	SALK	AT5G22290.1	NAC
At5g24590	SALK	AT5G24590.2	NAC
At5g46590	SALK	AT5G46590.1	NAC
At5g62380	SALK	AT5G62380.1	NAC
At5g63790	SALK	AT5G63790.1	NAC
At5g66300	SALK	AT5G66300.1	NAC
At1g20640	SALK	AT1G20640.1	NIN-LIKE
At1g64530	SALK	AT1G64530.1	NIN-LIKE
At1g05380	SALK	AT1G05380.1	PHD
At2g36720	SALK	AT2G36720.1	PHD
At1g69170	SALK	AT1G69170.1	SBP
At1g76580	SALK	AT1G76580.1	SBP
At3g60030	SALK	AT3G60030.1	SBP
At5g18830	SALK	AT5G18830.1	SBP
At5g43270	SALK	AT5G43270.1	SBP
At1g58100	SALK	AT1G58100.1	TCP
At3g27010	SALK	AT3G27010.1	TCP
At3g47620	SALK	AT3G47620.1	TCP
At5g08330	SALK	AT5G08330.1	TCP
At5g51910	SALK	AT5G51910.1	TCP
At1g33240	SALK	AT1G33240.1	TRIHELIX
At1g54060	SALK	AT1G54060.1	TRIHELIX
At1g76880	SALK	AT1G76880.1	TRIHELIX
At1g76890	SALK	AT1G76890.2	TRIHELIX
At2g44730	SALK	AT2G44730.1	TRIHELIX
At3g14180	SALK	AT3G14180.1	TRIHELIX
At3g24490	SALK	AT3G24490.1	TRIHELIX

At3g54390	SALK	AT3G54390.1	TRIHELIX
At5g01380	SALK	AT5G01380.1	TRIHELIX
At1g25280	SALK	AT1G25280.2	TUB
At1g53320	SALK	AT1G53320.1	TUB
At1g76900	SALK	AT1G76900.1	TUB
At2g18280	SALK	AT2G18280.1	TUB
At2g47900	SALK	AT2G47900.1	TUB
At3g06380	SALK	AT3G06380.1	TUB
At5g18680	SALK	AT5G18680.1	TUB
At1g69810	SALK	AT1G69810.1	WRKY
At1g80840	SALK	AT1G80840.1	WRKY
At2g23320	SALK	AT2G23320.1	WRKY
At2g24570	SALK	AT2G24570.1	WRKY
At2g30250	SALK	AT2G30250.1	WRKY
At2g30590	SALK	AT2G30590.1	WRKY
At2g46400	SALK	AT2G46400.1	WRKY
At2g47260	SALK	AT2G47260.1	WRKY
At3g56400	SALK	AT3G56400.1	WRKY
At3g58710	SALK	AT3G58710.1	WRKY
At4g01250	SALK	AT4G01250.1	WRKY
At4g23810	SALK	AT4G23810.1	WRKY
At4g24240	SALK	AT4G24240.1	WRKY
At4g26640	SALK	AT4G26640.1	WRKY
At4g30935	SALK	AT4G30935.1	WRKY
At4g31550	SALK	AT4G31550.1	WRKY
At5g15130	SALK	AT5G15130.1	WRKY
At5g22570	SALK	AT5G22570.1	WRKY
At1g14440	SALK	AT1G14440.1	ZF-HD
At1g75240	SALK	AT1G75240.1	ZF-HD
At3g28920	SALK	AT3G28920.1	ZF-HD
At5g15210	SALK	AT5G15210.1	ZF-HD
At5g39760	SALK	AT5G39760.1	ZF-HD
At3g63350	TIGR	AT3G63350.1	HSF
At3g58780	TIGR	AT3G58780.1	MADS
At4g28500	TIGR	AT4G28500.1	NAC
At2g43500	TIGR	AT2G43500.1	NIN-LIKE
At2g02540	TIGR	AT2G02540.1	ZF-HD

The 653 transcription factors in the stele collection, their pENTR vector sources, gene models, and transcription factor family.

Supplementary Table 2

Transcription Factor	Interaction	Target
AT1G07640	PD	AT1G30490
AT1G12610	PD	AT1G30490
AT1G47870	PD	AT1G30490
AT1G61730	PD	AT1G30490
AT1G76880	PD	AT1G30490
AT3G19290	PD	AT1G30490
AT3G45610	PD	AT1G30490
AT3G55370	PD	AT1G30490
AT3G61850	PD	AT1G30490
AT5G05410	PD	AT1G30490
AT5G63790	PD	AT1G30490
AT1G12610	PD	AT1G62990
AT4G01680	PD	AT1G62990
AT5G18560	PD	AT1G62990
AT5G52020	PD	AT1G62990
AT4G28500	PD	AT1G64620
AT1G04880	PD	AT1G71930
AT1G12610	PD	AT1G71930
AT1G47870	PD	AT1G71930
AT1G52880	PD	AT1G71930
AT1G61730	PD	AT1G71930
AT1G68360	PD	AT1G71930
AT1G76510	PD	AT1G71930
AT4G37260	PD	AT1G71930
AT5G18560	PD	AT1G71930
AT5G61590	PD	AT1G71930
AT5G61600	PD	AT1G71930
AT5G63790	PD	AT1G71930
AT1G03840	PD	AT2G34710
AT1G07640	PD	AT2G34710
AT1G10480	PD	AT2G34710
AT1G12610	PD	AT2G34710
AT1G13960	PD	AT2G34710
AT1G61730	PD	AT2G34710
AT1G64620	PD	AT2G34710
AT1G66140	PD	AT2G34710
AT1G68360	PD	AT2G34710
AT1G71930	PD	AT2G34710
AT2G01570	PD	AT2G34710
AT2G28810	PD	AT2G34710
AT2G33880	PD	AT2G34710
AT2G37590	PD	AT2G34710
AT2G38340	PD	AT2G34710
AT2G47890	PD	AT2G34710
AT3G01970	PD	AT2G34710
AT3G19580	PD	AT2G34710

AT3G23690	PD	AT2G34710
AT3G28920	PD	AT2G34710
AT3G45610	PD	AT2G34710
AT3G50410	PD	AT2G34710
AT3G55370	PD	AT2G34710
AT3G61850	PD	AT2G34710
AT4G14560	PD	AT2G34710
AT5G14000	PD	AT2G34710
AT5G15210	PD	AT2G34710
AT5G43270	PD	AT2G34710
AT5G60850	PD	AT2G34710
AT5G63790	PD	AT2G34710
AT1G04880	PD	AT3G25710
AT1G68360	PD	AT3G25710
AT1G76580	PD	AT3G25710
AT2G36400	PD	AT3G25710
AT2G46400	PD	AT3G25710
AT3G11580	PD	AT3G25710
AT3G20310	PD	AT3G25710
AT4G32800	PD	AT3G25710
AT4G36730	PD	AT3G25710
AT5G05410	PD	AT3G25710
AT5G61590	PD	AT3G25710
AT1G02220	PD	AT3G43430
AT1G04880	PD	AT3G43430
AT1G12610	PD	AT3G43430
AT1G68360	PD	AT3G43430
AT2G28810	PD	AT3G43430
AT4G28500	PD	AT3G43430
AT4G29230	PD	AT3G43430
AT4G34610	PD	AT3G43430
AT5G24590	PD	AT3G43430
AT1G04880	PD	AT4G37650
AT1G12610	PD	AT4G37650
AT1G29160	PD	AT4G37650
AT1G54060	PD	AT4G37650
AT1G61730	PD	AT4G37650
AT3G20770	PD	AT4G37650
AT4G32800	PD	AT4G37650
AT5G63790	PD	AT4G37650
AT1G04880	PD	AT5G12870
AT1G49720	PD	AT5G12870
AT1G61730	PD	AT5G12870
AT1G68360	PD	AT5G12870
AT1G76580	PD	AT5G12870
AT1G76880	PD	AT5G12870
AT2G26150	PD	AT5G12870
AT2G46270	PD	AT5G12870
AT4G36730	PD	AT5G12870
AT5G10510	PD	AT5G12870

AT5G43700	PD	AT5G12870
AT1G05805	PD	AT5G25160
AT1G12610	PD	AT5G25160
AT1G71930	PD	AT5G25160
AT1G76880	PD	AT5G25160
AT2G44730	PD	AT5G25160
AT3G11580	PD	AT5G25160
AT4G32800	PD	AT5G25160
AT5G52020	PD	AT5G25160
AT5G63790	PD	AT5G25160
AT1G12610	PD	AT5G53980
AT2G36400	PD	AT5G53980
AT3G25730	PD	AT5G53980
AT4G36730	PD	AT5G53980
AT5G04340	PD	AT5G53980
AT5G05610	PD	AT5G53980
AT5G63790	PD	AT5G53980
AT1G12610	PD	AT5G60200
AT1G47870	PD	AT5G60200
AT1G67030	PD	AT5G60200
AT1G73410	PD	AT5G60200
AT3G45610	PD	AT5G60200
AT3G49930	PD	AT5G60200
AT5G15210	PD	AT5G60200
AT5G63790	PD	AT5G60200
AT1G01260	PD	AT5G60690
AT1G07640	PD	AT5G60690
AT1G12610	PD	AT5G60690
AT1G13960	PD	AT5G60690
AT1G20700	PD	AT5G60690
AT1G21910	PD	AT5G60690
AT1G24625	PD	AT5G60690
AT1G51700	PD	AT5G60690
AT1G52880	PD	AT5G60690
AT1G53320	PD	AT5G60690
AT1G61730	PD	AT5G60690
AT1G64620	PD	AT5G60690
AT1G66140	PD	AT5G60690
AT1G67030	PD	AT5G60690
AT1G68360	PD	AT5G60690
AT1G71930	PD	AT5G60690
AT2G26150	PD	AT5G60690
AT2G28810	PD	AT5G60690
AT2G37590	PD	AT5G60690
AT2G38340	PD	AT5G60690
AT2G41940	PD	AT5G60690
AT3G15510	PD	AT5G60690
AT3G21270	PD	AT5G60690
AT3G22760	PD	AT5G60690
AT3G45610	PD	AT5G60690
AT3G50410	PD	AT5G60690

AT3G55370	PD	AT5G60690
AT3G61850	PD	AT5G60690
AT4G29230	PD	AT5G60690
AT4G34610	PD	AT5G60690
AT5G10510	PD	AT5G60690
AT5G14000	PD	AT5G60690
AT5G25160	PD	AT5G60690
AT5G60200	PD	AT5G60690
AT5G63790	PD	AT5G60690
AT5G65210	PD	AT5G60690
AT5G66730	PD	AT5G60690

Identified transcription factor-promoter interactions.

Supplementary Table 3

AT1G01260
AT1G02220
AT1G03840
AT1G04880
AT1G05805
AT1G07640
AT1G10480
AT1G12610
AT1G13960
AT1G20700
AT1G21910
AT1G24625
AT1G29160
AT1G47870
AT1G49720
AT1G51700
AT1G52880
AT1G53320
AT1G54060
AT1G61730
AT1G64620
AT1G66140
AT1G67030
AT1G68360
AT1G71930
AT1G73410
AT1G76510
AT1G76580
AT1G76880
AT2G01570
AT2G26150
AT2G28810
AT2G33880
AT2G36400
AT2G37590
AT2G38340
AT2G41940
AT2G44730
AT2G46270
AT2G46400
AT2G47890
AT3G01970
AT3G11580
AT3G15510
AT3G19290

AT3G19580
AT3G20310
AT3G20770
AT3G21270
AT3G22760
AT3G23690
AT3G25730
AT3G28920
AT3G45610
AT3G49930
AT3G50410
AT3G55370
AT3G61850
AT4G01680
AT4G14560
AT4G28500
AT4G29230
AT4G32800
AT4G34610
AT4G36730
AT4G37260
AT5G04340
AT5G05410
AT5G05610
AT5G10510
AT5G14000
AT5G15210
AT5G18560
AT5G24590
AT5G25160
AT5G43270
AT5G43700
AT5G52020
AT5G60200
AT5G60850
AT5G61590
AT5G61600
AT5G63790
AT5G65210
AT5G66730

Interacting transcription factors.

Supplementary Notes

Identification of Transcription Factors Expressed in the Root Stele

Seven hundred and eight transcription factors are expressed in the stele as determined by microarray expression data that profile expression in 10 GFP marker lines covering one or multiple cell types of the stele (APL, JO121, J2661, S17, S18, S4, S32, SUC2, WOL, J2501)[1]. We selected transcription factors (annotated in one of three databases) that were at least expressed in one of these marker lines. The expression presence threshold was determined as described before [2].

Comparison of Direct Transformation vs. Mating and the High Copy Number[3] and Low Copy Number AD Vector

We tested interaction differences between direct transformation and mating with a promoter (*REV*) that previously had multiple transcription factor interactors. Using the mating platform with the AD vector we identified 40% (2 of 5) interactions that were obtained using direct transformation. This was a consistent finding when using other promoters. Specifically, interactions obtained using the *HIS3* reporter were weak or inconsistent. Using the direct transformation method with the AD-2μ vector, 100% overlap in interactions was observed as compared to the low copy AD vector. We obtained a 44% overlap of interactions between mating with AD-2μ vector and the direct transformation assays. Two of the four positives which did not overlap were obtained as positives with only one reporter, *LacZ*, in the direct transformation assays with both vectors. This suggests that the mating assay has increased sensitivity as compared to direct transformation. The weak and inconsistent results observed with the *HIS3* reporter were rectified in mating with the AD-2μ vector. Increased sensitivity of the *LacZ* reporter was also observed, both in terms of the number of interactions and the length of time to observe a positive interaction.

Hand Replicator

Additionally, we tested the use of a hand replicator to perform this pipeline. We screened the *REV* promoter with the robot and with a hand replicator against the same 94 transcription factor prey described in the Supplementary Note "*Comparison of Direction Transformation vs. Mating and the High Copy Number and Low Copy Number AD Vector"*. We obtained 8 interactions with robot and 9 interactions from hand replicator. This demonstrates the flexibility of the eY1H pipeline.

Comparison of Efficiency in Labor, Cost and Time

Labor and Time

For direct transformation, we can reasonably transform 2 promoters against 7 transcription factor plates with no technical replicates in one day (1, 330 interactions). This will comprise 40 days of labor and 33 days for results for the full transcription factor resource. For mating, we can reasonably mate 20 promoters against 7 transcription factor plates with 4 technical replicates in one day (51,520 interactions; 2 days labor and 8 days for results). For 10 promoters, accounting for diploid selection and reporter plates, this will take 10 days for screening. For the robotic mating, a program can be set and run without constant supervision.

Costs

Automation allows us to screen 384 interactions in a single transcription factor plate against multiple promoters in a day. It is more cost-effective to screen multiple promoters at once; this reduces ethanol and plate costs.

1 Brady, S. M. *et al.* A High-Resolution Root Spatiotemporal Map Reveals Dominant Expression Patterns. *Science* **318**, 801-806, doi:10.1126/science.1146265 (2007).

2 Levesque, M. P. *et al.* Whole-Genome Analysis of the SHORT-ROOT Developmental Pathway in Arabidopsis. *PLoS Biology* **4**, e143 (2006).

3 Reece-Hoyes, J. S. *et al.* Enhanced yeast one-hybrid (eY1H) assays for high-throughput gene-centered regulatory network mapping. *Nature Methods* (In Press).