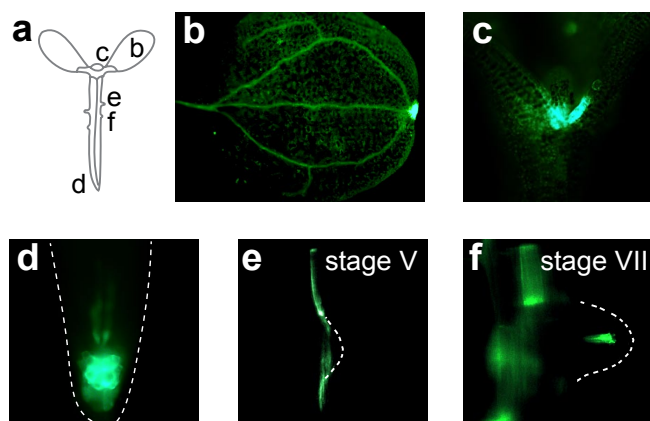
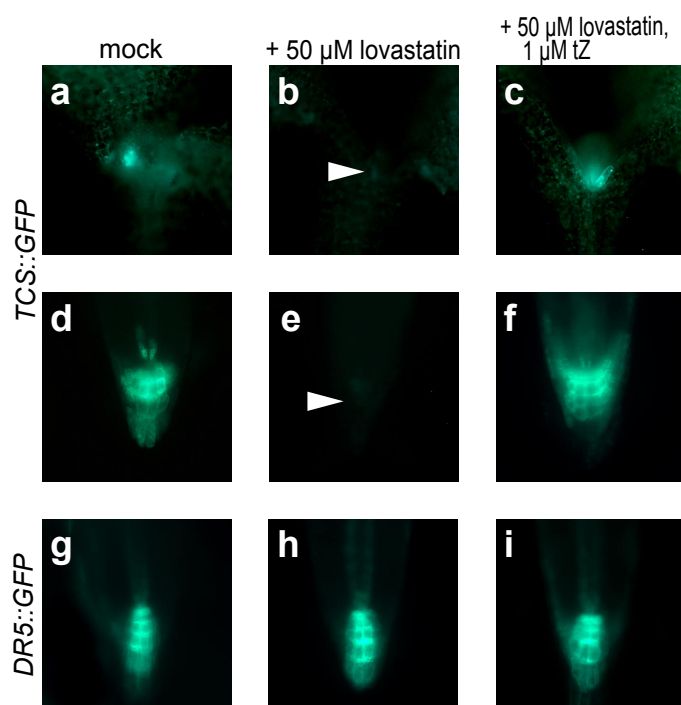


SUPPLEMENTARY INFORMATION



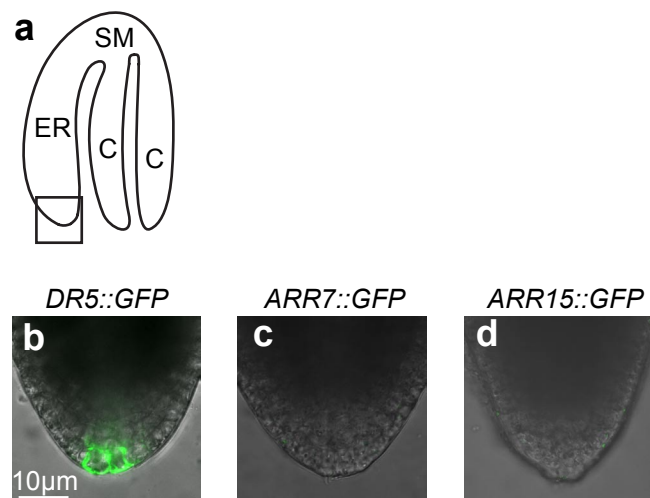
Supplementary Figure 1 | Typical *TCS::GFP* activity at the seedling stage

a, Schematic drawing of a seedling. Labels refer to the different organs shown in following panels. **b**, cotyledon; **c**, shoot apical meristem; **d**, root primodium; **e**, lateral root stage V; **f**, lateral stage VII. **e**, **f**, Emerging primodium outlined. Stages according to ref. 20.



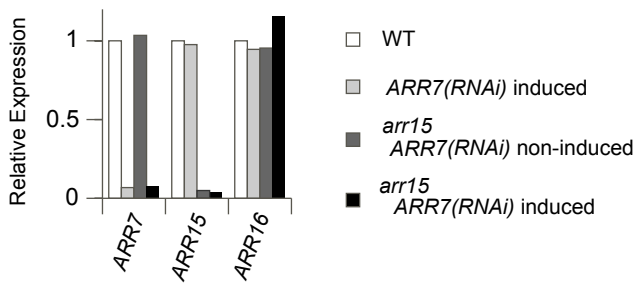
Supplementary Figure 2 | *TCS::GFP* expression depends on endogenous cytokinin production

a–c, *TCS::GFP* expression in shoot meristem. **d–f**, *TCS::GFP* expression in root meristem. **g–i**, *DR5::GFP* expression in root meristem. Addition of lovastatin, a cytokinin synthesis inhibitor^{34,35}, caused downregulation of *TCS::GFP* expression (**b**, **e**), but had no effect on *DR5::GFP* expression (**h**). Expression of *TCS::GFP* could be restored by co-administration of the cytokinin tZ (**c**, **f**). Seedlings were germinated and grown in liquid culture for 4 d. Chemicals as indicated were added. Expression of GFP was analysed 18 h later. Experiments were repeated three times with similar results. For each condition, >40 seedlings were analysed of which at least 80% showed a phenotype as displayed.



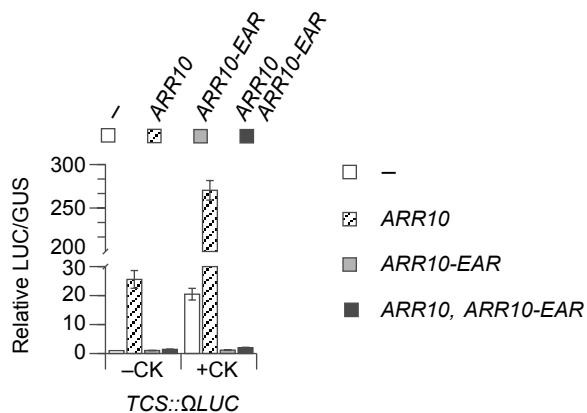
Supplementary Figure 3 | Auxin signalling, and *ARR7* and *ARR15* transcription at the embryonic upturned-U stage

a, A schematic drawing of a late-stage embryo (upturned-U stage). The square denotes the root tip that is shown enlarged below in (**b**, **c**, **d**). **b**, Auxin signalling visualised by *DR5::GFP* was active. **c**, **d**, *ARR7* and *ARR15* transcription was not detected in the same location. C, cotyledon; SM, shoot meristem; ER embryonic root.



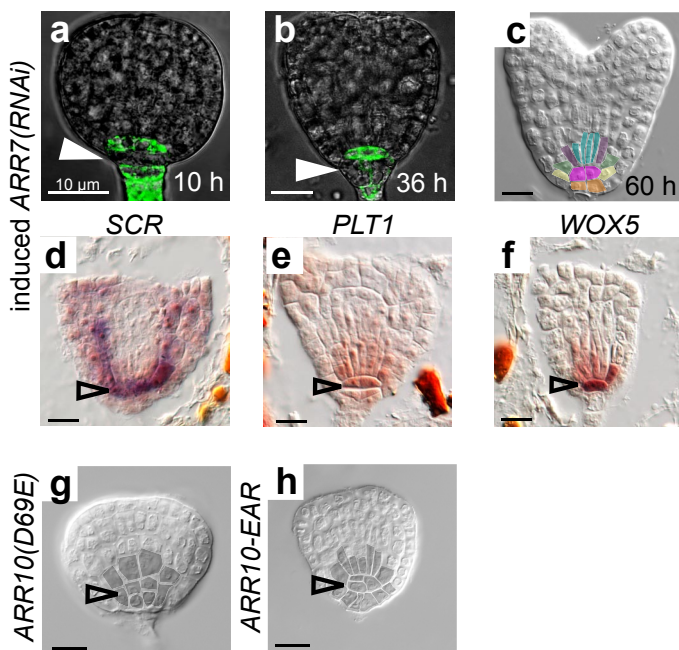
Supplementary Figure 4 | Efficiency and specificity of the inducible *ARR7(RNAi)*

ARR7, *ARR15* and *ARR16* mRNA levels in ethanol-treated and untreated embryos of different genotypes were measured by qRT-PCR relative to wild type embryos. Expression of *EIF4A* was used as a standard.



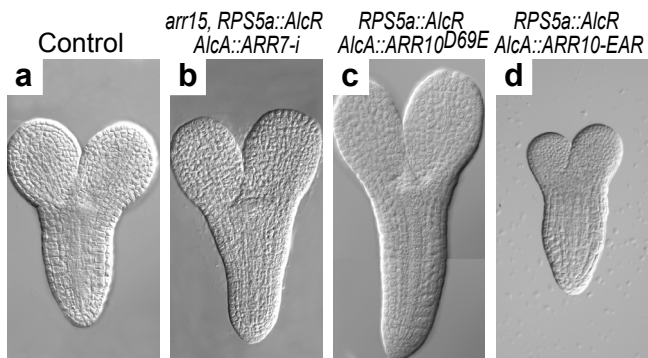
Supplementary Figure 5 | B-type *ARR10-EAR* acts dominantly as a transcriptional repressor in cytokinin signalling

Transient expression of *ARR10-EAR* abolished cytokinin (CK)- and *ARR10*-dependent induction of *TCS::QLUC*. Error bars s.d. (n=3).



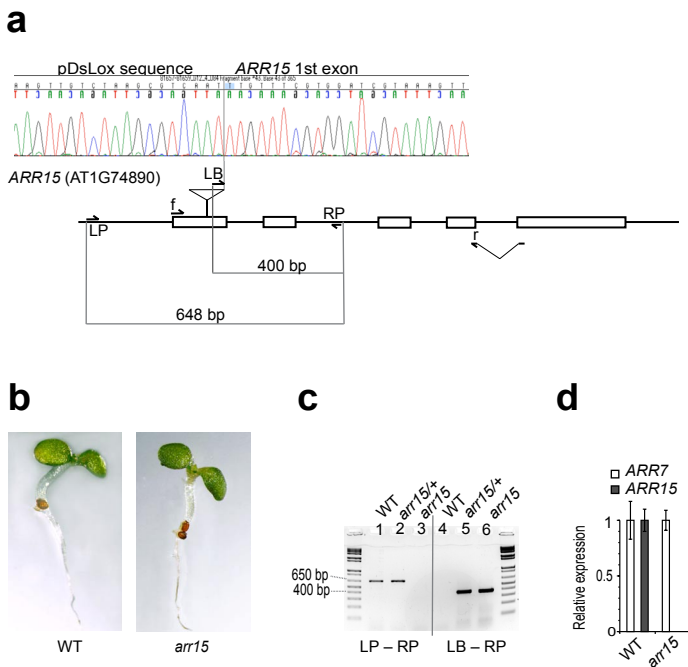
Supplementary Figure 6 | Function of differential phosphorelay output for root-stem cell establishment

a–f, Control embryos after ethanol induction of *ARR7(RNAi)* expression, in wild-type background. *RPS5a::AlcR* for ubiquitous inducible expression of *AlcA::ARR7(RNAi)* (**a–f**) and *AlcA::ARR10-EAR* (**h**). **g**, *DR5::AlcR* for conditional expression of *AlcA::ARR10(D69E)* in auxin-signalling cells. **d–f**, *In situ* hybridizations. Artificial colours in (**c**) refer to stem-cell identity, with qc in pink. Cells shaded in grey (**g**, **h**) have unclear identity. Filled arrowheads (**a**, **b**) denote basal cells, open arrowheads quiescent centre (**d–f**), or missing quiescent centre (**g**, **h**). **h** indicate time of ethanol exposure prior to mounting, (**d–h**) were mounted 60 h after ethanol induction.



Supplementary Figure 7 | Two component signalling after embryonic heart stage is not essential for root stem cell organisation

a–d, Transgene-inducing ethanol was added at early heart stage for 60 h. **a**, No transgene-induction control. **b**, Increased root size in the inducible *arr7 arr15* double mutants. **c**, Enlarged embryonic shoot and root after ubiquitous phosphorelay activation by expressing *ARR10(D69E)*. **d**, Reduced shoot and root size after dominant-negative interference with phosphorelay signalling by expressing *ARR10-EAR*.



Supplementary Figure 8 | Molecular characterisation of the WISCDSLOX334D02 T-DNA insertion line

a, Overview of the *ARR15* locus. The WISCDSLOX334D02 T-DNA is inserted in the first exon of the *arr15* gene, after bp 73 relative to the start codon. The position of primers RB, LB and RB, used for the PCR analysis (**c**), and primers f and r used for qRT-PCR (**d**), is depicted. **b**, Pictures of a Columbia wild type seedling, and a homozygous *arr15* seedling. Both seedlings shown were randomly selected from a group of 20 that was subsequently used for PCR and qRT-PCR analysis (**c**, **d**). **c**, PCR analysis performed on genomic DNA isolated from seedlings of genotypes as indicated. The 400bp DNA fragment obtained from homozygous *arr15* seedlings was sequenced to fine-map the T-DNA insertion site (**a**). **d**, non-detectable *ARR15* mRNA levels in *arr15* seedlings, using primers f and r, as indicated in (**a**). Primer sequences are: LB: AACGTCCGCAATGTGTTATTAAGTTGTC, RP: TCCACATTGTGAAACCTTAGATTCTT, LP: ATCCTCCAGATTCTCTTCTGTGATTT, f: AATGGCTCTCAGAGATTTATCTTC r: TCATACATTGTTCTATACGAGGTTG

Supplementary Table 1 | Oligonucleotides used for qRT-PCR

| <i>Gene name</i> | <i>Accession¹</i> | <i>Forward primer</i> | <i>Reverse primer</i> |
|-----------------------|------------------------------|---------------------------|-----------------------------|
| eIF4A | AT3G13920 | TCATAGATCTGGTCCTTGAACCC | GGCAGTCTCTTCGTGCTGAC |
| AHK1 | AT2G17820 | GGTCTCGTGGTTATGCTTTCC | CAGCTCGTTCCAGTCTGATG |
| AHK2 | AT5G35750 | GGAATTGAGTTGGCCGTTTATG | TGTAATGATCTGCCGGAACC |
| AHK3 | AT1G27320 | GGTGGAGTTGGCAGTATACATATC | CGAAACCTCCAGGATCAC |
| AHK4/ CRE1/ WOL | AT2G01830 | ATGGACGGATTTGAAGCAAC | ATCGGTAAATGCCATTCGAG |
| AHK5/ CKI2 | AT5G10720 | TCGACATCGGAGAATGAACAAG | CCGTCATCGCGATTATAGGC |
| CKI1 | AT2G47430 | ACTGCCAAATGCCAGAAATG | TCTCCACTTTCCTAATCTCTCTAGTTG |
| ARR1 | AT3G16857 | GCAAGTCACCTCCAGAAATACC | ATCCTGACCCGTCATAAACG |
| ARR2 | AT4G16110 | GTGTGCGATTGGTAACATTGTGG | GTTGCGATACTCCTCCAAGC |
| ARR3 | AT1G59940 | GACTCGTATCGACAGATGTCTTG | CGTCTCTCGTCAAGTAACTCCTC |
| ARR4 | AT1G10470 | CAGAATCGACAGATGCCTTG | CTTCCGTTTGTTCGTTG |
| ARR5 | AT3G48100 | GGTTGGATTTGAGGATCTGAAG | TCCAGTCATCCCAGGCATAG |
| ARR5 | AT3G48100 | CATCAGCTTTCAGAGAAATACCAG | CAGCTCCTTCTTCAAGACAtcTATC |
| ARR6 | AT5G62920 | TCTTCTTGCAAAGTTACTGTTGTTG | CCTCAACATCCAAACCAAGG |
| ARR7 | AT1G19050 | CCTCGTATACAAGAATGTCTCAAAG | CTGCTAGCTTCACCGGTTTTC |
| ARR8 | AT2G41310 | CATCGCCACAAATTCATCAG | GCCGCTGATTCTTAACCTTTC |
| ARR9 | AT3G57040 | TCACCAGGTAGTTGAAGTGAATC | TCTCTGAGGACATGATAACTACTGG |
| ARR10 | AT4G31920 | GACACAGGAACAGAGCCAATC | TATGCATGTTCCGAGTGAGC |
| ARR11 | AT1G67710 | TCGTGATCAGCGATGTGAAC | TCCACCGACATCATTATTACAGG |
| ARR12 | AT2G25180 | CACGATGAAGCAGGAACAGA | TTCTGAGTGAACATAACCCTCCA |
| ARR13 | AT2G27070 | ATCAGAATCAGGGACAAGCTG | AAACGCCTAGGCTGCTGAT |
| ARR14 | AT2G01760 | GTCATTTACAGAAGTTCCGATTGT | GAGGAAACCAGAGCTTGAATG |
| ARR15 | AT1G7489 | CAGCACTCAGAGAAATCCCAGT | TCATACATTGTTCTATACGAGGTTG |
| ARR16 | AT2G40670 | CCTGTAACGTTATGAAGGTGAGTC | GACTCCTTCACTTTCTTGAGTAGC |
| ARR17 | AT3G56380 | TTCTTGCAAAGTGACAAGTGC | TCAATGAATCAGTCTGTTGTGG |
| ARR18 | AT5G58080 | CCAGTCATTTGCAGAAGTACAGAC | AGTCCATCAAGTTGAGCCATC |
| ARR18 | AT5G58080 | GAAGTCGGGAAAGCCAGAG | AGTAGCCACTGGCACCATTC |
| ARR19 | AT1G49190 | AATTGGTGCTAACCCAAAGG | CGGTGTTTCTGAAGATGACTTG |
| ARR20 | AT3G62670 | CATCTCCAGAAGTACCGTCAAAG | GGCTGCAAGAGTGACATCTG |
| ARR21 | AT5G07210 | TTGGATCCACAGGAATTGG | CATGTCATTGTTGAACAGAGAGTTC |
| ARR22 | AT3G04280 | GGATGGAGTTTCGACAATAAG | CGCTCTTCTTCTTGGTCAGC |
| ARR23 | AT5G62120 | GCCATAGAATGAACGGAGAAAC | AGCCAATCATCTGGATCGTC |
| ARR24 | AT5G26594 | GCCATCATGAACGGTATTC | TCCTTCGTTAGCTCGTGTGG |

¹TAIR, The Arabidopsis Information Resource, www.arabidopsis.org

Supplementary Table 2 | Name and sequence of oligonucleotides or insert sequences

Plasmid name with parent vector in brackets. Small letters indicate linker DNA, italicised letters denote restriction sites used for cloning, small and underlined letters mark mutagenised residues.

hbt95::ARR14-HA (pUC18)

BamH1_ARR14_f *cgcgatcc*ATGCCGATCAACGATCAGTTTC, StuI_ARR14_r *aaggcct*TCTTTGAAGATCTTTCC

hbt95::ARR15-HA (pUC18)

Nco1_ARR15_f *catgcc*ATGGCTCTCAGAGATTTATCTTC, Stu1_ARR15_r *ataggcct*ACCCCTAGACTCTAATTTGATC-CCTCT

hbt95::ARR20-HA (pUC18)

BamH1_ARR20_f *catggatcc*ATGTCAGTTTTTTTCGAACATAC, StuI_ARR20_r *aaggcct*ATTGTGACCAATCTGATCG

hbt95::ARR22-HA (pUC18)

Nco1_ARR22_f *aaagaaatcgacc*ATGGCAACAAAATCC, Stu1_ARR15_r *taggcct*AGCATCGAAGAGGTGGCTAA

ARR7::Ω eGFP-ER (pCB302)

HindIII_pARR7_f *tgtggattttattgaagctt*CTACTAC, Apall_pARR7_r *acctaccaaccggtgcac*TCAAACCTCAG

ARR7m::Ω eGFP-ER (pCB302)

Used for mutagenesis: GATCTGGTCTCGAGcCAATGgCTCTCGCATATCAG, TCTTTACAAAATGTTTTTgCAATG-gCCTCTCACAATCGGCTAAAAGA, AAGATTTTACTCTCTTCCAGATTTTgGCCAAAAAGGACTCACAAAATCA GAT-GTTATGATTGAGgCAAAAGGATCCACC, AATACTTTGgCCATATTTTCTCTCAAGATTTTgGCCAAAAGCAAA, ATAAAAAATAAAAAATGgCCCTTTTgGCTTTAAATAAAATAAT, ACGAGGAGTAGTCGCTGgCGGATTGAAAGAAACAGAC-CA, ACCTTTGAAGcCACATTTGATTATTTGTGTTTTCTGcCACTTCCCAAACA

ARR15::Ω eGFP-ER (pCB302)

HindIII_pARR15_f *tgTTTTgaaaaaagCTTTTTAAGTTGACTTCTTG*, XbaI_pARR15_r *actctaga*GTTTTCTCTCGGGAAAG-TAAACAAC

ARR15m::ΩeGFP-ER (pCB302)

Used for mutagenesis: AGAGGAGAGTAGTCATTGgCAGATAGAAAGAAAGAGCAGcCAAAAGGATCGTA, ATGACCGT-GAAGcCACAATATTACGTTTTCTGgCAAGgCATAAACACATCACT

RPS5a::AicR/AicA::ARR7(RNAi) (pDM7)

sequence of *ARR7-i* TTTGACAATGGCGGTTGGTGAGGTCATGAGGATGGAGATCCCGCCGGTGGAGATTTGACT-GTTACTACTCCGGAGTTGCATGTTCTTGCCGTCGATGATAGTATTGTGGATCGTAAAGTCATCGAGAGGTTGCT-TAGAATCTCTTCTTGTAAGGTA AAAACTCAAACCAATCTTTTTCTGCTCTGTTTTTGTGGTTTTAACTCTCCTCT-GTTTTTCTTCTTCATTAACAACTCTCCTCTGTTTTTGTAGTTACAAGTGTCTTTTTTTTTTGTATTTCAGTggatc-tACTTTACAAGAAGAGATTCTAAGCAACCTCTCGATGACTTTACGATCCACAATACTATCATCGACGGCAAGAACATG-CAACTCCGGAGTAGTAACAGTCAAATCTCCACCGGCGGGAATCTCCATCCTCATGACCTACCAACCGCCATTGTCAA

RPS5a::AicR/AicA::ARR10-EAR-GFP (pDM7)

Sequence of *ARR10-EAR-GFP*: ATGACTATGGAGCAAGAAATTGAAGTCTTGGACCAGTTTCCGGTTGGGATGAGAGT-TCTTGCTGTTGACGATGACCAGACTTGTCTCCGTATTCTCCAGACTTTGCTTCAGCGCTGCCAATATCACGTTACAA-CAACGAATCAGGCACAGACCGCATTGGAGTTGTTGAGGGAGAACAAGAATAAGTTTGATCTTGTTATTAGCGATGTC-GACATGCCAGACATGGATGGTTTCAAGCTGCTTGAGCTTGTGGTCTTGAAATGGACTTACCTGTCATAATGTTATCT-GCGCATAGCGATCCAAAGTATGTGATGAAAGGAGTCAAGCACGGTGCCTGTGATTATCTGCTTAAACCGGTTCGAAT-TGAGGAGCTTAAGAACATATGGCAACATGTTGTGAGAAAGAGCAAACCTTAAGAAGAATAAGAGCAATGTGAGTAAT-GGTTCAAGAACTGTGATAAAGCAAACAGAAAACGTAAGAACAGTATGAAGAGGAGGAAGAGGAAGAAAGAGG-GAATGATAATGATGATCCAACGGCGCAGAAGAAGCCTCGTGTCTTTGGACGCATGAGCTGCACAATAAATTCCTAG-CAGCTGTTGATCATTTAGGCGTTGAGAGAGCTGTTCCAAAAAAGATTCTAGATCTGATGAATGTTGACAAACTCACTA-GAGAGAATGTTGCAAGCCACCTTCAGAAATTCCGCGTTGCTCTGAAGAAGGTGTCTGATGACGCCATTCAACAAG-CTAACAGGGCGGCTATTGACTCACATTTTATGCAAATGAATTCTCAGAAAGGACTTGGTGGCTTCTACCACCACCAC-CGCGGAATACCTGTTGGATCCGGTCAGTTCCATGGTGAACCACAATGATGAGGCATTACTCTTCAAATAGGAATCT-TGGTCGTCTGAATTCCCTTGGAGCAGGAATGTTCCAACCAGTCTCATCATGTTTTCTCGTAACCATAATGATGGAG-

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RPS5a::AicR/AicA::ARR10(D69E)-GFP (pDM7)

sequence of *ARR10(D69E)-GFP*: ATGACTATGGAGCAAGAAATTGAAGTCTTGGACCAGTTTCCG-
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 CCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACT-
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CATGGCCGACAAGCAGAAGAACGGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG-
CAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCT-
GAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGC-
CGCCGGGATCACTCACGGGCATGGACGAGCTGTACAAGTAA

DR5:: Ω AicR/AicA::ARR10(D69E)-GFP (pDM7)

sequence of *ARR10(D69E)-GFP*: see above