AFGC Microarray Control Set

Distributed by the Nottingham Arabidopsis Stock Centre
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AFGC Microarray Control Set (Distributed by the Nottingham Arabidopsis Stock Centre [http://arabidopsis.org.uk])

The AFGC Microarray Control Set is currently composed of 8 transgene controls and 10 spiking controls assembled to provide access to a uniform set of clones for use in plant DNA microarray experiments. Each tube contains one microgram of desiccated plasmid DNA that can be resuspended in TE buffer to your desired concentration. Instructions for amplification and use of the spiking controls are attached on subsequent sheets.

This set of clones was assembled by the Michigan State University DNA Microarray Facility, part of the Arabidopsis Functional Genomics Consortium funded by NSF grant 9872638.
Any questions or comments regarding this set can be addressed to:

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Quality Control

Each plasmid was prepared in bulk with the QIAGEN® Plasmid Maxi Kit at NASC from donated DNA.

Purity was further tested at NASC by amplifying individual clones with universal primers. All amplifications produce a single product of expected length.
Amplification conditions using the universal primer pair indicated on the clone information sheet were as follows:

Amplification mix

10mM Tris (pH 8.3), 50mM KCl, 2mM $MgCl_2$, 0.2mM each dNTPs, 0.4mM each primer, 10ng plasmid template, 2U Taq DNA Polymerase.

Cycling parameters

1 min	@ 94°C	
30 sec	@ 94°C	Cycle 25X
30 sec	@ 60°C	
1 min	@ 72°C	
2 min	@ 72°C	

Information For Spiking Controls

All spiking controls contained in this set were selected as showing low cross-hybridization signals to Arabidopsis RNA isolated from a number of different tissues grown in different environmental conditions. mRNA derived from these clones can be spiked into experimental RNA in order to serve as normalization controls, determine sensitivity of the array, establish the linear range of the array, etc…

Spiking controls Sp1-4 are cloned into the pBluescript vector and contain polyA tails long enough to be useful as spiking controls (see clone information sheet). The inserts are oriented such that the plasmid can be cleaved with XhoI and transcribed from the T3 promoter. The resulting transcript will, however, contain about 68 bases of the polylinker sequence which may contribute to unwanted background in some cases. Spiking controls Sp5-10 are cloned into pSE936 (Elledge et al., PNAS, 88:1731-1735 (1991)) which contains no promoter sequences. Furthermore only one clone, Sp9, contains a strong polyA tail.

In order to efficiently transcribe spiking mRNA-s which both lack vector sequence and contain sufficient polyA tails, we have designed primers which directly splice the T3 promoter and a poly $(dT)_{22}$ tail to the specific gene sequence.

Listed below are tested primer sequences used to amplify a 300bp DNA fragment for use as an in vitro transcription template for clones Sp1-10. The asterisk separates the gene specific sequence from the T3 promoter in the case of the forward primers, and from the poly $(dT)_{22}$ in the case of the reverse primers. Primer pairs were tested as follows:

PCR amplification was performed under conditions indicated for amplification of control clones. Amplification products were purified with a QIAquick® PCR purification kit (Qiagen). Transcription was carried out using a Riboprobe® System – T3 in vitro transcription kit (Promega). Transcription products were purified using an RNeasy® mini kit (Qiagen) and quantified by A_{260} measurement.

Spiking control Sp1 B-cell receptor-associated protein (AF126021)

Sp1-F 5'-CAATTAACCCTCACTAAAGGG*CGCGAATCTGTGTTCACC

Sp1-R 5'-TTTTTTTTTTTTTTTTTTTTTT*AATGGACGGCAACACTCG

Spiking control Sp2 Myosin heavy chain (X13988)

Sp2-F 5'-CAATTAACCCTCACTAAAGGG*GCTCCACCTGGATGATGCC

Sp2-R 5'-TTTTTTTTTTTTTTTTTTTTTT*CTCCTCAGCGTTCCTTGCATC

Spiking control Sp3 Myosin light chain 2 (M21812)

Sp3-F 5'-CAATTAACCCTCACTAAAGGG*TCGGGGAGAAGCTCAAGGG

Sp3-R 5'-TTTTTTTTTTTTTTTTTTTTTT*AGGTCGGGCCGAACAGAAG

Spiking control Sp4 Insulin-like growth factor II (X07868)

Sp4-F 5'-CAATTAACCCTCACTAAAGGG*GATGCCATAGCAGCCACC

Sp4-R 5'-TTTTTTTTTTTTTTTTTTTTTT*CAGGCCAATGTGGGTTCC

Spiking control Sp5 FLJ10917fis (AK001779)

Sp5-F 5 '-CAA TT AACCCTCACT AAAGGG*GGCAGACAAATGTGTGAATCC

Sp5-R 5' TTTTTTTTTTTTTTTTTTTTTT*CCCGGGTCAA TCAGACA T AC

Spiking control Sp6 HSPC120 (AF161469)

Sp6-F 5 '-CAA TT AACCCTCACT AAAGGG*TGCCAGAAGAAGTTGAAGCA

Sp6-R 5' TTTTTTTTTTTTTTTTTTTTTT* AAAGTGCCTTTTCAGCTCCA

Spiking control Sp7 Beta2 microglobulin (NM-004048)

Sp7-F 5 '-CAA TT AACCCTCACT AAAGGG*TGACTTTGTCACAGCCCAAG

Sp7 -R 5 , TTTTT*CTCTGCTCCCCACCTCT AAG

Spiking control Sp8 Phosphoglycerate kinase (pgkl) (NM-000291)

Sp8-F 5'-CAATT AACCCTCACT AAAGGG* AAGGTGCTCAACAACATGG

Sp8-R 5' TTTTTTTTTTTTTTTTTTTTT*CCA TTCAAAT ACCCCCACAG

Spiking control Sp9 Tyrosine phosphatase (pac-l) (Ll1329)

Sp9-F 5 '-CAA TT AACCCTCACT AAAGGG*GACCTGGTGCTCTTCTGCTG

Sp9-R 5' TTTTTTTTTTTTTTTTTTTTT* ACACACGCAACA TGACACAC

Spiking control Spl0 G10 homolog (edg-2) (Ul1861)

Sp 10-F 5 '-CAA TT AACCCTCACT AAAGGG* A TGGCTGGGAGTTGA TTG

Sp 10-R 5' TTTTTTTTTTTTTTTTTTTTT* AGTTGGTGTCCCGTGTCTG

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Clone Information

Clone	ID	Accession #	Coordinates[a]	Vector	Amplification Primers	PCR (bp)[b]	
	Transgenes:						
BT	B. thuringiensis cry1Ac	U89872	415-2225	pBluescript II SK	unM13F/unM13R	2084	
GFP	Green Fluorescent Protein	AF078810	1603-2319	pGEM-T	unM13F/unM13R	990	
Globin	Globin	NM_000518	50-523	pBluescript II SK	unM13F/unM13R	651	
GUS	beta-glucuronidase (uidA)	A00196	284-2151	pBluescript II SK	unM13F/unM13R	2045	
HPH	hygromycin B phosphotransferase	K01193	199-1251	pBluescript II SK	unM13F/unM13R	1230	
Luc	Luciferase	X65316	102-1754	pBluescript II SK	unM13F/unM13R	1926	
BAR	Phosphinothricin acetyl transferase	X17220	94-535	pGEM-T Easy	unM13F/unM13R	715	
nptII	kanamycin/neomycin phosphotransferase	V00618	142-953	pGEM-T Easy	unM13F/unM13R	1085	
	Spiking Controls:						PolyA[c]
Sp1	B-cell receptor prot. (IMAGE:1420858)	AF126021	270-1416	pBluescript II SK	unM13F/unM13R	1324	18
Sp2	Myosin heavy chain (IMAGE:1593605)	X13988	5049-6032	pBluescript II SK	unM13F/unM13R	1180	19
Sp3	Myosin reg. light chain 2 (IMAGE:1592600)	M21812	295-662	pBluescript II SK	unM13F/unM13R	560	16
Sp4	Insulin-like growth factor (IMAGE:1576490)	X07868	3259-4037	pBluescript II SK	unM13F/unM13R	1028	18
Sp5	FLJ10917fis	AK001779	164-1520	pSE936	pSE936F/unM13R	1562	11(1)
Sp6	HSPC120	AF161469	448-1699	pSE936	pSE936F/unM13R	1451	14 (4)
Sp7	Beta2 microglobulin	NM_004048	9-925	pSE936	pSE936F/unM13R	1122	7
Sp8	Phosphoglycerate kinase (pgk1)	NM_000291	717-1739	pSE936	pSE936F/unM13R	1228	4
Sp9	Tyrosine phosphatase (pac-1)	L11329	651-1675	pSE936	pSE936F/unM13R	1230	19 (2)
Sp10	G10 homolog (edg-2)	U11861	272-992	pSE936	pSE936F/unM13R	926	8

[a]Coordinates indicate the bases within the sequence file (indicated by the Accession #) which are actually present in the control clone.

[b]PCR (bp) indicates the expected size of the PCR product when amplified with the indicated primers.

[c]Poly A indicates the length of the polyA tail present in the clone with the number of mismatches (if any) in parentheses.

Primer sequences for amplification:
univM13F 5'-GTTTTCCCAGTCACGACGTTG
univM13R 5'-TGAGCGGATAACAATTTCACACAG
pSE936F 5'-ATACCTCTATACTTTAACGTCAAGG