Arabidopsis Plasmid Information Sheet

Concentration: 0.5 μg/μL
Volume: 5 μL
Store pARAB at –20°C

The UHN Microarray Centre is willing to provide our customers with *one* free aliquot of pARAB plasmid per lab. As such, it is highly recommended that customers transform the plasmid into *E.coli* so that it can be amplified. As little as 50 ng can be used for transformation. Any standard protocol for transformation can be used.

The plasmid contains a fragment of the *Arabidopsis Thaliana* chlorophyll synthetase gene (as found by sequencing/BLAST search; see attached sheet for partial sequence of the insert) and thus should not be homologous to any human, yeast, or mouse sequences. The insert size is approximately 1.3-1.4 kb. This fragment is identical to the one that we spot on our cDNA microarrays. The clone is prepared in such a manner that an artificial poly A tail will be introduced during T7 run off transcription. This polyA tail allows the RNA to be converted into labeled cDNA when added to your labeling reaction as a positive control.

In order to obtain the RNA, you will need to perform an *in vitro* transcription reaction. The plasmid includes an SstI restriction site that can be used to linearize the plasmid and a T7 promotor (located at the end of the plasmid opposite to the restriction site). The plasmid also encodes ampicillin resistance to allow for selection during transformation.

Thus, in order to use the pARAB as a control for your microarray experiments:

1. Transform competent *E.coli* cells using 50 ng (or amount suggested by the protocol you choose to follow) of the pARAB and grow an overnight culture to amplify the plasmid. From this culture, reserve a small aliquot and make a glycerol stock for future use.
2. Isolate plasmid DNA.
3. Linearize the plasmid using SstI (SacI, which has the same recognition sequence as SstI, can also be used). When run on an agarose gel, the linearized plasmid is approximately 4 kb.
4. Perform *in vitro* transcription using a commercially available T7 *in vitro* transcription kit (in the past we have used the Sp6/T7 transcription kit from Roche and the MEGAscript™ T7 kit from Ambion, following the manufacturer's protocol).
5. Determine the concentration (Abs. 260). If you run the artificial *Arabidopsis* transcripts on an agarose gel, you should get a strong band at approximately 500 bp and several other weaker bands between 500 bp and 1 kb in size.
6. Aliquot in working concentrations (2-5 ng/μL) and use for spiking labeling reactions as outlined in the Direct Labeling protocol.

If you have any additional questions, please do not hesitate to email us at:

μA
UHN Microarray Centre

help@microarrays.ca

Partial sequence of the *Arabidopsis* insert (fragment of the chlorophyll synthetase gene):

GAATTGGGTACCGGGCCCCCCCTCGAGGTCGGCGGCCGCCAGTGTGAGTAATTTAGGTC
GATTCTTTGCATGATGATGTCTGGTCCTTGTCTTACTGGCTATACACAGACAATCAACG
ACTGGTATGATAGAGATATCGACGCAATTAATGAGCCATATCGTCCAATTCCATCTGGA
GCAATATCAGAGCCAGAGGTTATTACACAAGTCTGGGTGCTATTATTGGGAGGTCTTGG
TATTGCTGGAATATTAGATGTGTGGGCAGGGCATACCACTCCCACTGTCTTCTATCTTG
CTTTGGGAGGATCATTGCTATCTTATATATACTCTGCTCCACCTCTTAAGCTAAAACAA
AATGGATGGGTTGGAAATTTTGCACTTGGAGCAAGCTATATTAGTTTGCCATGGTGGGC
TGGCCAAGCATTGTTTGGCACTCTTACGCCAGATGTTGTTGTTCTAACACTCTTGTACA
GCATAGCTGGGTTAGGAATAGCCATTGTTAACGACTTCAAAAGTGTTGAAGGAGATAGA
GCATTAGGACTTCAGTCTCTCCCAGTAGCTTTGGCACGAAACTGCAAATGGATATGCGT
TGGGCTATAGACATTACCAGCTTCTG

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