**Supplemental Section**

# Arabidopsis Actin Depolymerizing Factor *ADF9* Participates in Cytoplasmic and Nuclear Processes

Brunilís Burgos-Rivera[1,2], Daniel R. Ruzicka[1,2], Roger B. Deal[3], Elizabeth C. McKinney[1], Lori King-Reid[1,], and Richard B. Meagher[1,4]

[1]Department of Genetics, University of Georgia, Athens, GA 30602, USA

[3]Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. North, Seattle, WA, 98109, USA

**Nucleosomal DNA preparation** – This protocol for nucleosome preparation was modified from Vega-Palas and Ferl (1995). The nuclear scanning assay of nucleosome occupancy uses PCR amplification of nested products of MNase digested nucleosomal DNA as first reported by Sekinger et al. (2005).

1. Collect 0.75 grams of leaf or seedling tissue and freeze in liquid nitrogen
2. Grind frozen tissue with mortar/pestle in liquid nitrogen, transfer to fresh mortar/pestle with 5 ml HBM buffer (below) and regrind thawed tissue.
3. Filter through two layers of Miracloth into 15 ml Falcon Tube on ice.
4. Spin at 2000g 4°C for 10 minutes
5. Carefully remove and discard supernatant, and resuspend pellet in 1 mL of HBB buffer (below).
6. Spin at 200g 4° C for 2 min
7. Carefully remove and discard supernatant, and resuspend crude nuclear pellet in 300 $\mu$L TNE Buffer.
8. Add 15 units of Micrococcal nuclease (MNase) per 300 □l reaction and digest at 37° C for 3 minutes. Using nucleosomes from leaf tissue this condition produced the nearly complete digestion to mononucleosomes presented in Figure 8A. (MNase from Roche Scientific, Inc., #10107921001 from *Staphylococcus aureus*, 1 mg/15,000 units, Stock 15 u/ul in 50% glycerol and PBS)
9. Add 2.5 $\mu$L 0.5M EDTA to stop the reaction and spin at max for 3 minutes

10. Keep supernatant and discard pellet, add 1 $\mu$L RNaseA (boiled) to solution and incubate at room temp for 20 minutes.

11. Add 1 vol. phenol/chloroform/isoamyl alcohol to solution, vortex, and spin max 3 minutes

12. Remove aqueous phase to a fresh tube and add 1/10 vol. 3M sodium acetate (pH 5.2), 2 vol. 95% ethanol, and 2 $\mu$L glycogen (20 mg/ml in d-water, Roche Cat. 901393). Chill at -80° C for at least 20 minutes.

13. Spin at 4° C max speed for 15 minutes, wash pellet with cold 1 ml 75% ethanol, and spin at 4° C max speed for 15 minutes.

14. Carefully remove ethanol and dry on bench for 5 minutes. Resuspend pellet in 40μL $dH_2O$.

15. Run 10 μL on 2% agarose gel to confirm mono-nucleosome purity.

**Real Time qPCR amplifications**

1. Quantitative PCR control reactions for primer amplification efficiency on purified non-nucleosomal DNA.
    a. DNA was purified using a CTAB protocol (Doyle et al., 1990).
    b. DNA concentration (1 ng/reaction) and primer concentration of 0.5 μM following the protocol for SYBR green detection chemistry recommended by ABI.
2. Quantitative PCR reactions on nucleosomal DNA
    a. From the 40 μl of resuspended nucleosomal DNA dilute the DNA 1/25 to 1/50 fold for qPCR reactions. Use 5 μl of that dilution per reaction. Primer concentrations of 0.5 μM. Again follow SYBR green detection chemistry.

**Real Time qPCR calculations of the Relative Quantity (RQ) of nucleosome-protected DNA in *adf9-1* vs wild-type**.

1. We will consider the PCR primer product #5 within the *FLC* locus in Figure 8 in the text as an example: where the product for #5 is P5 and for actin *ACT2* is A2;

where plant samples for wild-type nucleosomal DNA is WT and for *adf9-1* is *a9*; where genomic wild-type DNA is gDNA; Nucleosomal is Nuc.; and where CT is the cycle threshold value.

2. Relative Quantity calculation for P5 amplification based on a calculation of the ddCT of dCT values.
    a. dCT for gDNA of P5 is measured relative to actin A2
        i. dCT of $P5_{gDNA} = CT_{gDNAp5}\text{-}CT_{gDNAA2} = 24.216\text{-}23.949 = 0.267$
    b. dCT for nucleosomal P5 DNA is measured relative to actin in the WT sample and the experimental a9 sample.
        i. dCT of $P5_{WT} = CT_{WTp5}\text{-}CT_{WTA2} = 24.601\text{-}21.418 = 3.183$
        ii. dCT of $P5_{a9} = CT_{a9p5}\text{-}CT_{a9A2} = 22.801\text{-}21.32 = 1.481$
3. RQ is estimated from the ddCT
    a. gDNA $P5_{gDNA}$ $RQ_{ddCT} = 2^{-ddCT} = 2^{-(dCT_{gDNA}\text{-}dCT_{gDNA})} = 2^{-(0.267\text{-}0.267)} = 1.0$
    b. Nuc. $P5_{WT}$ $RQ_{ddCT} = 2^{-ddCT} = 2^{-(dCT_{WTP5}\text{-}dCT_{gDNA})} = 2^{-(3.183\text{-}0.267)} = 0.132$
    c. Nuc. $P5_{a9}$ $RQ_{ddCT} = 2^{-ddCT} = 2^{-(dCT_{a9P5}\text{-}dCT_{gDNA})} = 2^{-(1.481\text{-}0.267)} = 0.431$
    d. These $RQ_{ddCT}$ values for P5 in WT and a9 samples are the same as those shown in Figure 8 in the main text. The normalized RQ for all gDNA products = 1 and are not shown.

**Buffers:**

**HBM**
25 mM Tris pH 7.6
0.44 M Sucrose
10 mM $MgCl_2$
0.1% Triton-X
2 mM Spermidine
10 mM B-mercaptoethanol

**HBB**
Same as HBM except no spermidine and increase Triton-X to 0.5%

**TNE**
10 mM Tris pH 8.0
100 mM NaCl
5 mM $MgCl_2$

1 mM EDTA
4 mM $CaCl_2$

**PCR Primer Design**

To obtain primers with the specific spacing and specificity needed for the nucleosomal scanning assay, oligonucleotide were designed to have estimated $tm_{1/2}$ values of 58 to 62ºC based on the summation of 2ºC/AT bp and 4ºC/GC bp suggested for short oligonucleotides (Maniatis et al., 1989) or estimated $tm_{1/2}$ values of 50 to 54ºC following the primer design program at Oligo Analyzer (www.IDTDNA.com). When possible the primer locations were moved up or downstream a few nucleotides to position A or T residues on the 3´end of each primer following the observation that this improves target specificity and lowers background amplification of inappropriate products (Crameri and Stemmer, 1993).

**Bibliography**

**Crameri A, Stemmer WP** (1993) 10(20)-fold aptamer library amplification without gel purification. Nucleic Acids Res **21:** 4410

**Doyle JJ, Doyle JL, Brown AHD, Grace JP** (1990) Multiple origins of polyploids in the *Glycine tabacina* complex inferred from chloroplast DNA polymorphism. Proc. Natl. Acad. Sci. **87:** 714-717

**Maniatis T, Fritsch EF, Sambrook J** (1989) Calculating melting temperatures for perfectly matched hybrids between oligonucleotides and their target sequences. *In* Molecular cloning: A laboratory Manual, Vol 2. Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York, p 11.46

**Sekinger EA, Moqtaderi Z, Struhl K** (2005) Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. Mol Cell **18:** 735-748

**Vega-Palas MA, Ferl RJ** (1995) The Arabidopsis Adh gene exhibits diverse nucleosome arrangements within a small DNase I-sensitive domain. Plant Cell **7:** 1923-1932