Supplementary Data

A fluorescence-based assay suitable for quantitative analysis of deadenylase enzyme activity

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Figure S1

(A) Comparison of probes containing a 3’ TAMRA or a black hole quencher (BHQ)-1 moiety. The Flc-labelled RNA substrate (1.0 μM) was incubated for 60 min at 30 °C in the presence of the indicated amount of Caf1/CNOT7 enzyme. Reactions were stopped by the addition of SDS (final concentration of 0.5%) and a five-fold molar excess of the indicated probe.

(B) Optimisation of probe:substrate ratio. The 5’ Flc-labelled RNA substrate (1.0 μM) was incubated for 60 min at 30 °C in the presence of Caf1/CNOT7 enzyme (0.4 μM). Reactions were stopped by the addition of SDS (final concentration of 0.5%) and the indicated molar excess of a probe containing either a TAMRA or BHQ-1 modification of the 3’ end of the oligonucleotide. Error bars indicate the standard error of the mean.
Figure S2

(A) Stability of fluorescence intensity measured up to 72 h after the completion of reactions. Reactions containing Flc-labelled substrate were incubated for 60 min at 30 °C before the addition of a solution containing SDS (final concentration of 0.5%) and a five-fold excess of 3’ TAMRA labelled probe. Fluorescence was measured at regular intervals 0-72 h after addition of the probe mixture. Reactions were kept at room temperature in the dark. (B) The fluorescence signal remains stable up to 7 days after addition of the probe mixture. Reactions containing Flc-labelled substrate were incubated for 60 min at 30 °C before the addition of a solution containing SDS (final concentration of 0.5%) and a five-fold excess of 3’ TAMRA labelled probe. Fluorescence was measured immediately after addition of the probe mix and at 24 h intervals from day 3 until day 7. Reactions were kept at constant temperature (20 °C) in the dark. Error bars indicate the standard error of the mean.