Electronic Supplementary Material (ESI) for Analytical Methods.

In-Gel Detection of Biotin-Protein Conjugates with a Green Fluorescent Streptavidin Probe

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Abbreviations

biotin protein ligase (BirA); streptavidin (Stv); 2-(4-hydroxyazobenzene) benzoic acid (HABA); protein of interest (POI)

Keywords

Biotinylation detection; SDS-PAGE; avidin; green fluorescent protein; biotin protein ligase

SUPPLEMENTARY FIGURE LEGENDS

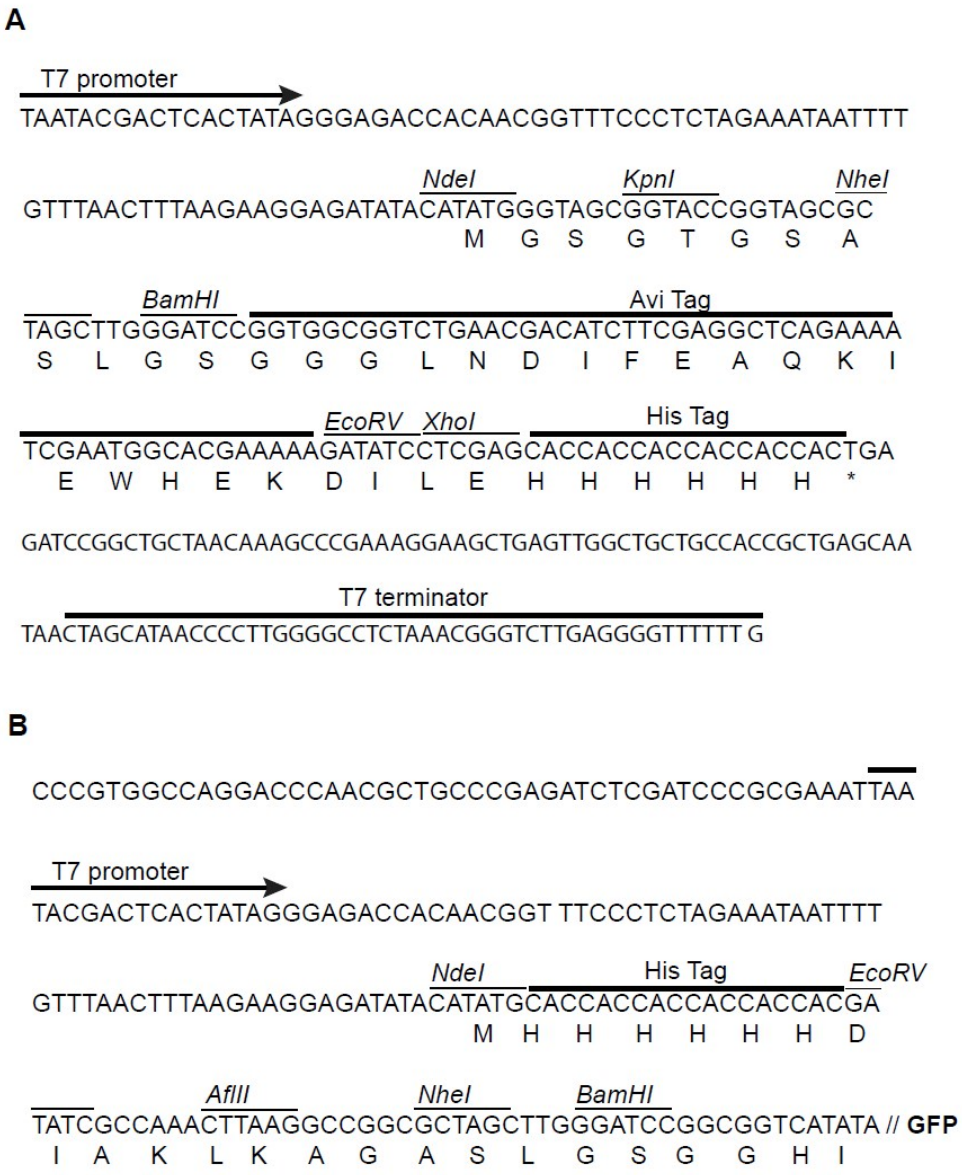

Figure S1. Details of the multiple cloning site regions of the AviTag and GFP vectors. A) AviTag vector (pSA236) and **B)** GFP vector (pET-uvGFP) [21].

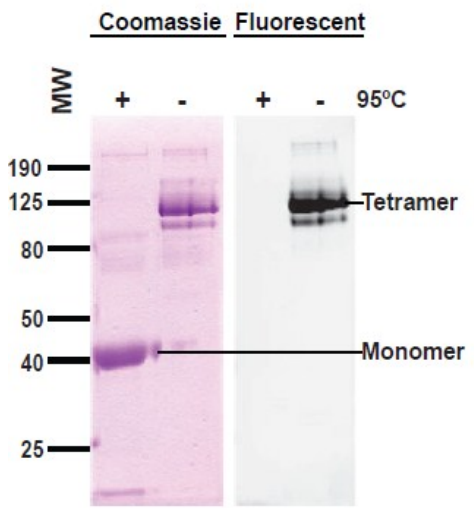

Figure S2. Electrophoretic behaviour of native and heat-denatured Stv-GFP by SDS-PAGE. The electrophoretic mobility of Stv-GFP heated at 95°C for 15 min in SDS-PAGE sample buffer (+) was compared to the same sample prior heat treatment (-) in 10% polyacrylamide gel. Formation of monomeric Stv-GFP and loss of fluorescence was confirmed following heat denaturation of the tetrameric complex. Stv-GFP (7.5 µl, 5 µM) were mixed with 7.5 µl 2x SDS sample buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue), subjected to 10% SDS-PAGE and visualised over a UV transilluminator (right gel in inverted colours), then stained with Coomassie Blue (left gel).

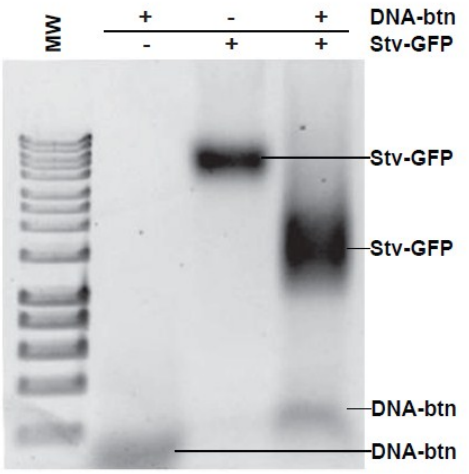

Figure S3. GFP-EMSA analysis of Stv-GFP:biotinylated-DNA complexes. Free biotinylated DNA (DNA-btn) and Stv-GFP with and without DNA-btn were separated by agarose (1%) gel electrophoresis. Migration of DNA-btn was retarded in presence of Stv-GFP demonstrating that Stv-GFP is fully functional for biotin binding.

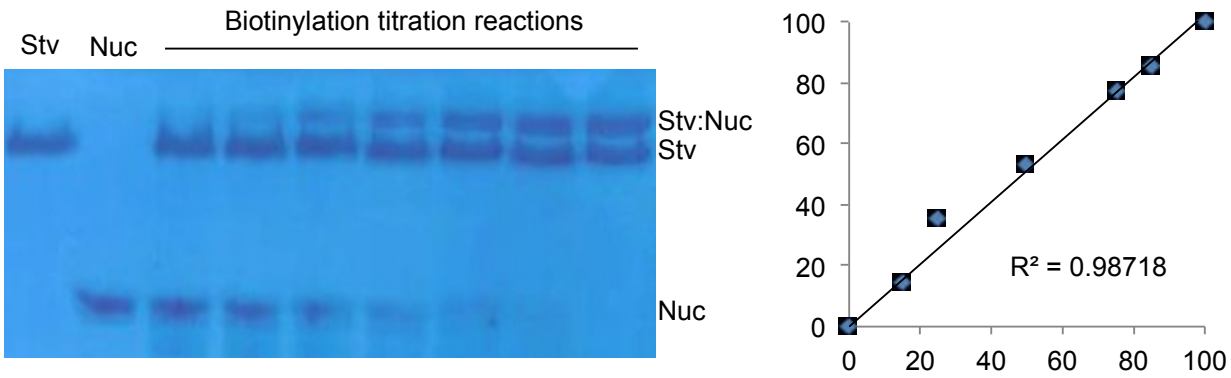

Figure S4. Biotinylation titration reactions. Biotinylation reactions and purification process were performed as per usual. The non-biotinylated reactions were treated identically in the absence of BirA. Seven different ratios of biotinylated over non-biotinylated Nuc were tested: 0%, 15%, 25%, 50%, 75%, 85% and 100% and the reactions were separated in a15% SDS-PAGE gel. lane 1 = stv alone, lane 2 = Nuc alone, lanes 3-9 proportions as above. The lower bands corresponding to non-biotinylated Nuc were integrated as per usual and all results were normalised with the value of the 0% Nuc-biotin (lane 3) expressed in % and compared to the respective theoretical ratio (Nuc-bio/Nuc) expressed in % to demonstrate the linearity of the assay. Measured ratio in % (y-axis); theoretical ratio in % (x-axis).