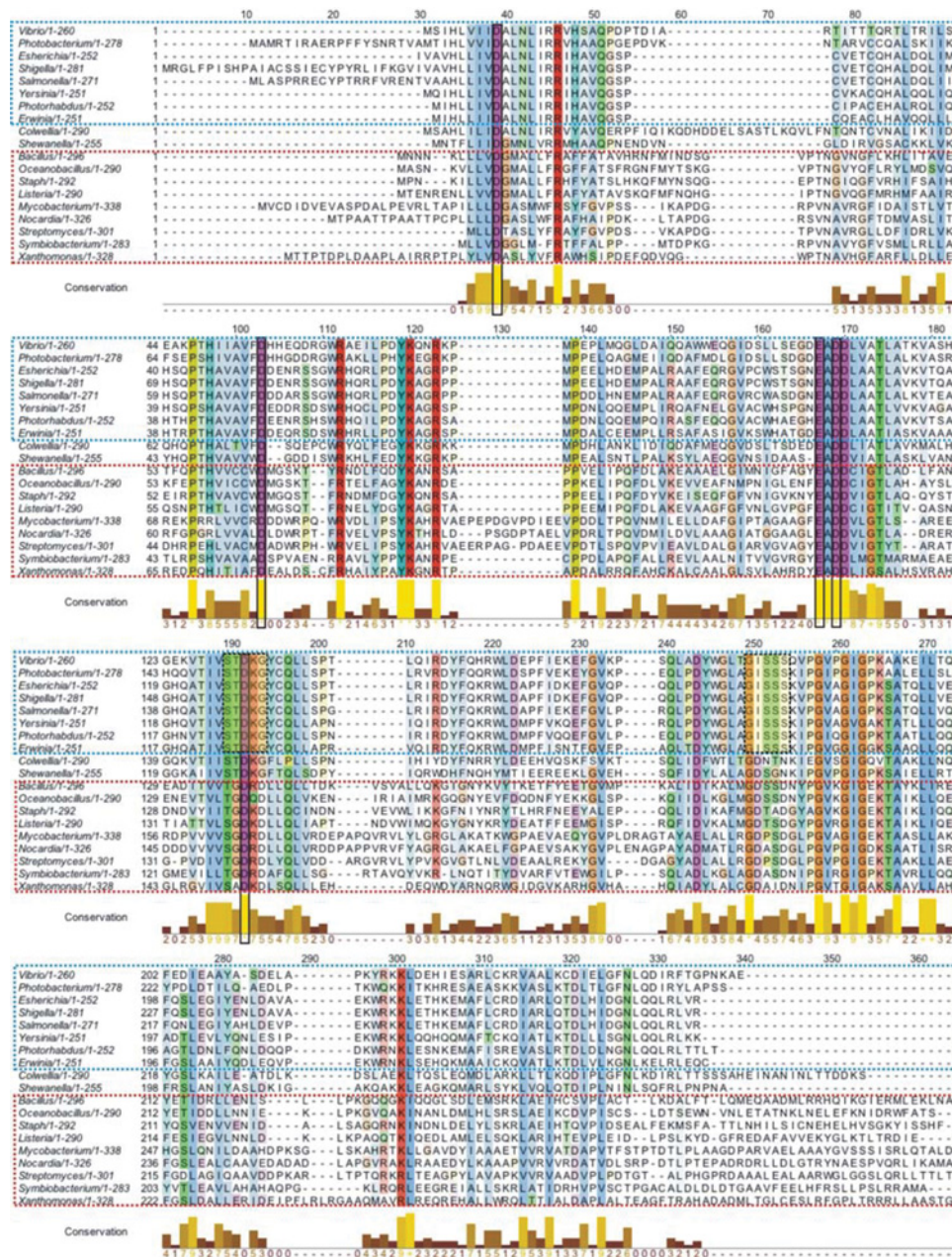


SUPPLEMENTARY ONLINE DATA

# Active site substitutions delineate distinct classes of eubacterial flap endonuclease

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**Figure S1** Multiple sequence alignment of FEN-like paralogues

We examined available sequence depositions with GenBank, based on local sequence alignment (TBLASTN), restricted to organisms recently identified as carrying a Poll FEN paralogue [1]. ClustalW alignments were prepared and coloured using ClustalX annotation [2]. Sequence conservation is depicted by histograms, where yellow represents most highly conserved residues, with strictly conserved residues denoted by asterisks. The absolute conservation of carboxylate ligands at metal site I are highlighted, additionally boxed in black. The two sub-classes are defined by dashed boxes; ExoI family members in blue and FEN family members in red. A double motif was identified to define ExoI, based around the active site substitutions: STDKG (position 190) and GISSS (position 250), with randomised probabilities of  $8.7 \times 10^{-7}$  and  $1.4 \times 10^{-6}$  respectively [3].

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Received 14 August 2008/29 October 2008; accepted 11 November 2008

Published as BJ Immediate Publication 11 November 2008, doi:10.1042/BJ20081637