Figure S6 R_09-02, as overexpressed in the active form in E. coli at low temperatures. The quantification of the activity level (A) and optical density (B) of cells expressing R_09-02 was performed at 37, 28 and 22 ºC at the indicated time points. Please refer to the Materials and Methods for details of the activity quantification (using pNPβX as the substrate). (C, D) A Coomassie-stained SDS-PAGE gel showing the purification of the R_09-02 protein. Only R_09-02, which represents the most atypical enzyme in terms of its biochemical characteristics, is shown; the other enzymes derived from the R library were also found to be more than 98% pure (data not shown). (C) SDS-PAGE gel showing the gene expression at 37 ºC and the presence of inclusion bodies. (D) SDS-PAGE gel showing the gene expression at 20 ºC. As shown, a high percentage of protein is produced in a soluble form, which resulted in a purity higher than 98% after a single His6-tag purification step.