

Table S1. Overview of all tested combinations (A-domain vs. substrate) by biochemical assay.

Protein	L-proline	EPL	PPL	BuPL	PePL	OH-L-proline	L-alanine	L-valine	L-tyrosine
CcbC	+	-	-	NT	NT	+	+	+	-
LmbC	+	+	+	+	+	+	+	+	-
LmbC G308V	+	+	+	NT	NT	NT	NT	NT	NT

EPL - (2*S*,4*R*)-4-ethyl-L-proline; PPL - (2*S*,4*R*)-4-propyl-L-proline; BuPL - (2*S*,4*R*)-4-butyl-L-proline; PePL - (2*S*,4*R*)-4-pentyl-L-proline and OH-L-proline - trans-4-hydroxy-L-proline. Adenylation activity is expressed by number of “+”, when “+ + +” corresponds to the activity of the native A-domain with its natural substrate; “-“ not detectable; NT - not tested.