	LeuRS	lleRS
	*	*
E. coli	35 YYCLSMLPYPSG-RLHMGHVRNYT 57	7 52 ILHDGPPY-ANGCIHIGHSVNKILK 75
B. subtilis	33 FYALDMFPYPSGAGLHVGHPEGYT 56	5 51 VLHDGPPY-ANGDIHMGHALNKILK 74
H. pylori	31 KYILSMLPYPSG-EIHMGHVRNYT 53	3 52 TLHDGPPY-AN <mark>GHLHLGHALNK</mark> IL <mark>K</mark> 75
S. aureus	33 FYALDMFPYPSGAGLHVGHPEGYT 56	6 36 VFYDGPPT-ANGLPHAGHVLGRVIK 59
T. thermophilus	35 QYVLVMFPYPSG-DLHMGHLKNYT 57	7 41 VVYEGPPT-ANGLPHVGHAQA <mark>R</mark> SYK 64
H. salinarum	36 EYVLAMFPYTSG-QLHMGHVRNYA 58	8 44 FFV <mark>DGPPY-TSG</mark> AAHM <mark>GTTWNK</mark> TL <mark>K</mark> 67
M. vulcanius	32 FFITAAFPYLNG-VLHAGHLRTFT 54	4 41 FV- <mark>DGPPY-CSGAIHLGTAWNK</mark> II <mark>K</mark> 63
M. thermautotrophicus	27 IFLTVAYPYPSG-AMHIGHGRTYT 49	9 43 SFL <mark>DGPPY</mark> -C <mark>SG</mark> RIHLGTAWNKIMK 66
P. horikoshii	36 FYITVAFPYLSG-HLHVGHARTYT 58	8 43 YFL <mark>DGPPY-VSG</mark> AIHL <mark>GT</mark> AWN <mark>K</mark> IIK 66
S. solfataricus	29 FFTTVAFPYPNS-PWHIGHGRTYV 5	1 46 LFIDGPPYPSSPTPHIGTIWNKVIK 70
G. lamblia	42 FFVTFPYPYMNG-RLHLGHLFSAT 64	4 52 NFYDGPPF-ATGLPHYGHLLAGTIK 75
H. sapiens	46 YFVTFPYPYMNG-RLHLGHTFSLS 68	8 46 T <mark>FYDGPPF-ATGLPHYG</mark> HILA <mark>G</mark> TI <mark>K</mark> 69
M. musculus	48 YFVTFPYPYMNG-RLHLGHTFSLS 70	0 42 TFYDGPPF-ATGLPHYGHILAGTIK 65
S. cerevisiae	59 FMSSMAYPYMNG-VMHAGHCFTLS 8	1 41 SFF <mark>DGPPF-ATGTPHYG</mark> HILASTI <mark>K</mark> 64
S. pombe	67 FFGNMPYPYMNG-ALHLGHAFTLS 85	9 36 TFF <mark>DGPP</mark> F-A <mark>TG</mark> R <mark>PHHG</mark> HLLASTI <mark>K</mark> 59

Figure S5. Sequence alignment of various LeuRS or IleRS enzymes from representative bacteria, archaea and eukaryotes.

Both LeuRS (left panel) and IleRS (right panel) contain aligned sequences from five bacterial enzymes: *Escherichia coli, Bacillus subtilis, Helicobacter pylori, Staphylococcus aureus* and *Thermus thermophilus*; five archaeal enzymes: *Halobacterium salinarum, Methanocaldococcus vulcanius, Methanothermobacter thermautotrophicus, Pyrococcus horikoshii* and *Sulfolobus solfataricus*; and five eukaryotic cytosolic enzymes: *Giardia lamblia, Homo sapiens, Mus musculus, Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Methionine at position 40 (*E. coli* numbering; the position is marked with an asterisk) is strictly conserved in bacterial LeuRSs, while most archeal enzymes have alanine instead of methionine, and eukaryotic cytosolic enzymes typically have proline (left panel). Homologous position in IleRS (right panel; the position is marked with an asterisk) has a strictly conserved glycine.