

RNA Tagging plasmid list:

Amplify active PUP-2 out of RT1 or RT3. Amplify catalytically inactive PUP-2 out of RT2.

RT-1: pBSII/PUP2/3HA/URA3

Description: Base construct used to create PUP2/3HA fusion proteins in *S. cerevisiae*. This is the catalytically active PUP-2 enzyme with a 3-HA epitope tag. This construct was used to create BFR1/PUP2/3HA and PUF5/PUP2/3HA fusion protein strains.

Resistance: ampicillin

RT-2: pBSII/PUP2-DADA/3HA/URA3

Description: Base construct used to create PUP2-DADA/3HA fusion proteins in *S. cerevisiae*. This is the catalytically inactive PUP-2 enzyme (D185A and D187A substitutions) with a 3-HA epitope tag. This construct was used to create catalytically inactive fusion protein strains.

Resistance: ampicillin

RT-3: pCS2/3HA/PUM1_RBD/PUP2/URA3

Description: Base construct used to create the PUF3/PUP2 tagging strain. The PUP2/URA3 sequences were amplified out of this plasmid for integration. It lacks a 3HA epitope tag. The PUM1_RBD refers to the RNA-binding domain of *X. laevis* Pum1 and was not included in the integration.

Resistance: ampicillin

For yeast labs:

RT-4: pBSII/PUF3 5' flank/PUP2/3HA/URA3/PUF3 3' flank

Description: Construct used to generate the PUF3/PUP2/3HA tagging strains. Surrounding the PUP2/3HA/URA3 are large regions of homology to integrate at the 3' end of the PUF3 gene.

Resistance :ampicillin

RT-5: pBSII/PUF3 5' flank/PUP2-DADA/3HA/URA3/PUF3 3' flank

Description: Construct used to generate the PUF3/PUP2-DADA/3HA tagging strains. Surrounding the PUP2/3HA/URA3 are large regions of homology to integrate at the 3' end of the PUF3 gene.

Resistance: ampicillin