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## Data Sheet

# pYSG-IBA123

Cat. No.: 5-4723-001

Version: 3.0  
Revision Date: 27.07.2021

|                         |   |
|-------------------------|---|
| <b>Description</b>      | <b>StarGate Acceptor Vector for high-level expression of target proteins in yeast. It carries the the copper-inducible promoter (CUP1) for controlled high-level expression, the URA3 auxotrophy marker for selection after transformation (do not use URA3 for selection during expression), the LEU2d auxotrophy marker for selection to increase plasmid copy number for expression (do not use LEU2d for selection after transformation), and the 2 micron ori for episomal replication in yeast.</b> |
| <b>Affinity tag</b>     | Twin-Strep-tag <sup>®</sup> is fused to the C-terminus and GST-tag is fused to the N-terminus of the recombinant protein. GST-tag can be removed by digesting with PreScission <sup>™</sup> Protease.   |
| <b>Cloning Strategy</b> | Cloning into StarGate Acceptor Vectors has to be done with the restriction enzyme Esp3I. There is no Multiple Cloning Site (MCS) available that can be used for the integration of the gene of interest instead (see manual).   |
| <b>Yeast Expression</b> | Cultivate transformed yeast cells under LEU2d selection until OD <sub>600</sub> reaches 0.8-1.2. Induce protein expression by addition of copper sulphate to a final concentration of 0.5 mM.   |
| <b>Form</b>             | 5 µg, dissolved in 20 µl TE buffer, pH 8.0: 10 mM Tris/HCl, 1 mM EDTA   |
| <b>Concentration</b>    | 250 ng/µl   |
| <b>Stability</b>        | 12 months after shipping  |
| <b>Storage</b>          | recommended: 2-8 °C for frequent usage, -20 °C for long-term storage  |
| <b>Shipping</b>         | room temperature  |
| <b>Hazards</b>          | Product is not classified as hazardous according to (EC) No 1272/2008 [CLP].<br>A Material Safety Data Sheet is provided.   |

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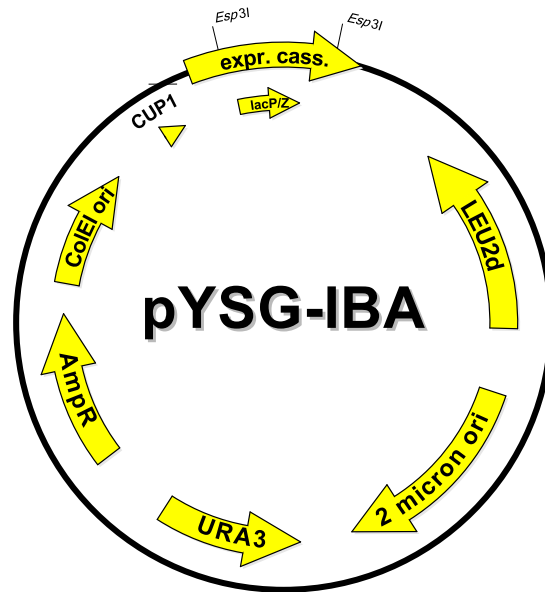
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# Expression cassette of pYSG-IBA123



## Expression cassette of pYSG-IBA123, continued

LacP/Z cassette = contains LacZ alpha fragment under control of a separate promoter, which allows alpha complementation of *LacZ* mutations such as *LacZ* $\Delta$ M15 as in *E. coli* DH5 $\alpha$  or TOP10.  
 your protein = after StarGate cloning using *Esp3I* your gene of interest will be located here



| Features                         | from bp | to bp | Sequencing primer                 |
|----------------------------------|---------|-------|-----------------------------------|
| LEU2d                            | 1668    | 574   | YSG-Primer-for                    |
| 2 micron ori                     | 2032    | 3194  |                                   |
| URA3                             | 4293    | 3490  | 5' - CAATATCATATAGAAAGTCATCGA -3' |
| Ampicillin resistance gene       | 4725    | 5585  | YSG-Primer-rev                    |
| ColEI ori                        | 5756    | 6345  |                                   |
| CUP1 promoter                    | 6873    | 6925  | 5' - GCAGCTACCCACATTGGCATTGGC -3' |
| forward primer binding site      | 6939    | 6961  |                                   |
| GST-tag                          | 7049    | 7702  |                                   |
| PreScission™ Protease site (PCS) | 7703    | 7750  |                                   |
| LacZ alpha fragment              | 7979    | 8380  |                                   |
| Twin-Strep-tag®                  | 8444    | 8536  |                                   |
| reverse primer binding site      | 8577    | 8599  |                                   |
| total vector length              |         | 8600  |                                   |