

# The Laboratories for Reproductive Biology

## PLASMID INFORMATION FORM

NAME: GSTAR400-449

DESCRIPTION : Digest pGBT8AR400-449 with EcoR2 and Sal2 to get the AR400-449 fragment  
the ligate AR400-449 to pGEX-4T-1 (digested with EcoR2 and Xho2)

ORIGINATOR: Suxia Bai

VECTOR: pGEX-4T-1

VECTOR SIZE: 4969 bp

INSERT: AR400-449

INSERT SIZE: 150 bp

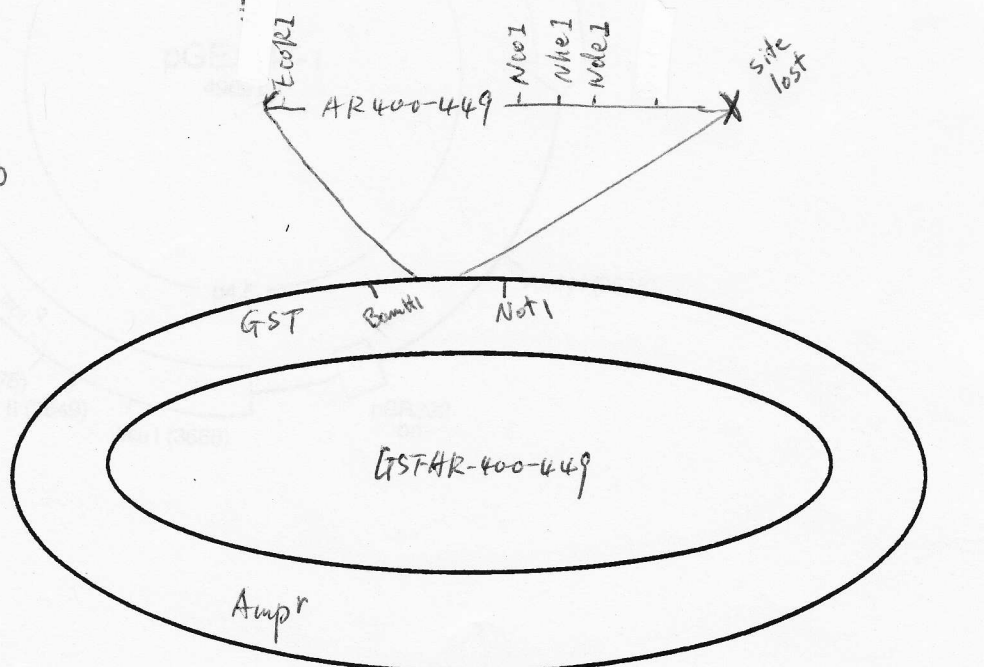
ANTIBIOTIC RESISTANCE: Amp

HOST: XL-1 blue

RELEVANT INFORMATION:

RNA CONCENTRATION:

REFERENCES:



## E coli BL21 Cells

### GENOTYPE:

The BL21 strain is F-ompT,  $r_B^-$ ,  $m_B^-$ .

### GROWTH CONDITIONS:

The lyophilized culture should be resuspended in 1 ml of L-broth. Grow overnight before plating onto L-broth media plates.

### LONG TERM STORAGE:

Mix an equal volume of stationary phase culture (growth in L-broth) with glycerol and store at  $-70^\circ\text{C}$ . BL21 can be revived by streaking onto L-broth media plates.

### RECOMMENDED USAGE:

The BL21 strain is recommended for expression of GST fusion proteins. Since it does not transform well, an alternate strain is recommended for maintenance of the plasmid.

### REFERENCE:

Studier, F. W., et al., *Methods in Enzymology*, **185**: 60.

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### 5. Structural elements on pGEX-4T-1

**Glutathione S-transferase gene region:** *tac* promoter: -10: 205-211; -35: 183-188; *tac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for thrombin cleavage: 918-935; Primer region for double-stranded sequencing: 874-890  
MCS: 930-966

**$\beta$ -lactamase gene region:** Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235

***lacI*<sup>d</sup> gene region:** Start codon (GTG): 3318; Stop codon (TGA): 4398

**Plasmid replication region:** Site of replication initiation: 2995; Region necessary for replication: 2302-2998.

### References

1. Smith, D. B. and Johnson, K. S., *Gene* **67**, 31 (1988).
2. Eaton, D., et al., *Biochemistry*, **25**, 505 (1986).
3. Marston, F. A. O., *Biochem J.* **240**, 1 (1986).
4. Schein, C. H. and Noteborn, M. H. M., *Bio/Technology* **6**, 291 (1988).
5. Smith, D. B. and Corcoran, L. M., *Current Protocols*, pg. 16.7.1 (1990).

Lambda ZAP<sup>®</sup> is a registered trademark of Stratagene Cloning Systems.

MAP:

