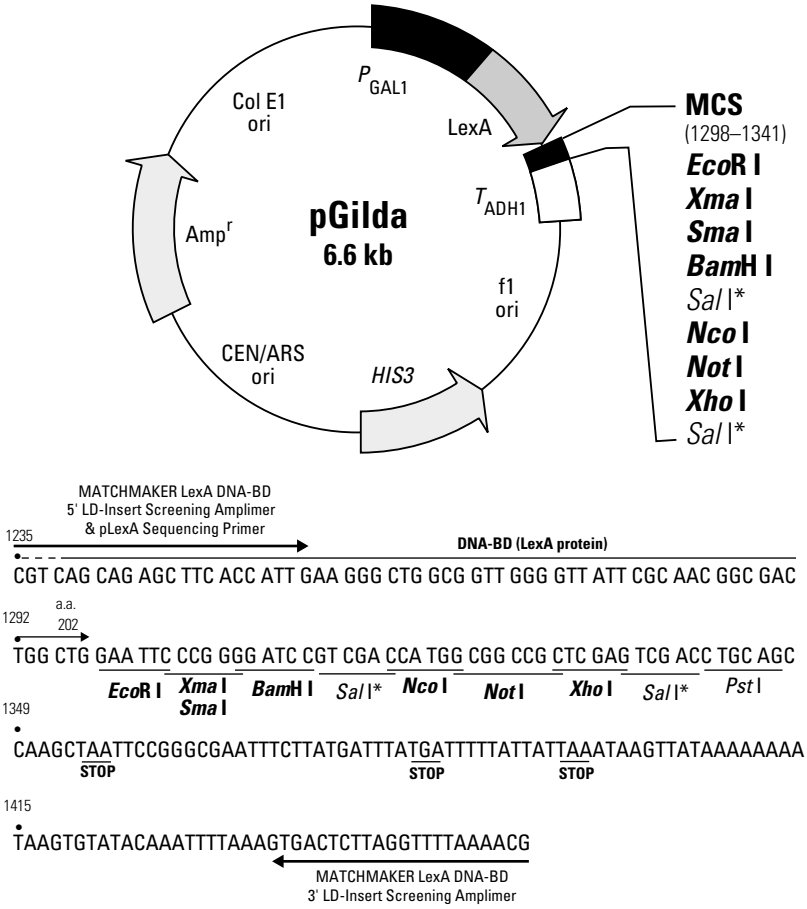
GenBank Accession #: Submission in progress. Catalog #6183-1



Restriction map and multiple cloning site (MCS) of pGilda. Unique restriction sites are in bold. The *Sal* I sites (*) may be used as a unique site for cloning. The MCS of pGilda is identical to that of pLexA, witrh the exception of the *Xma* I/ *Sma* I site, which are not unique in pLexA.

Description:

pGilda (1, 2) is a cloning vector used to generate fusions of a bait protein with the 202-residue LexA protein, which acts as a DNA-binding domain in the MATCHMAKER LexA Two-Hybrid System (#K1609-1). The hybrid protein is expressed in yeast cells from the tightly regulated *GAL1* promoter (P_{GAL1}); transcription is repressed in the presence of glucose and induced in the the absence of glucose and presence of galactose (3). pGilda carries the *HIS3* gene for selection in His^- auxotrophic yeast strains and is compatible with MATCHMAKER LexA Libraries. This vector replicates autonomously in both *E. coli* (using the Col E1 ori) and in *S. cerevisiae* (using the CEN/ARS ori). Amp^r is used for selection in *E. coli*.

Note: pGilda can also be used with other LexA two-hybrid systems, as long as the system utilizes a His^-, $GAL4^+$, $GAL80^+$ yeast host strain having an appropriate reporter gene under the control of LexA operators.

Location of features
- GAL1 promoter: 2–671
  GAL4 binding sites: 224–240, 243–259, 261–277, 325–341
  Transcription start point: 616
- LexA protein (a.a. 1–202): 692–1297
- Multiple cloning site (MCS): 1298–1341
- *ADH1* terminator: 1363–1676
- f1 bacteriophage origin of replication: 1882–2320
- Yeast *HIS3* gene: Start codon (ATG): 3257–3255; stop codon (TAG): 2600–2598
- CEN/ARS plasmid replication origin: 3859–4377
- Ampicillin resistance gene: 4460–5334
  β-lactamase coding sequences: Start codon (ATG): 4474–4476; stop codon (TAA): 5332–5334
- Fragment containing the Col E1 plasmid replication origin: 5394–6274

Primer locations
- pLexA Sequencing Primer (included in the MATCHMAKER LexA Two-Hybrid System): 1235–1256
- MATCHMAKER LexA DNA-BD LD-Insert Screening 5' Amplimer (#9109-1): 1235–1256
- MATCHMAKER LexA DNA-BD LD-Insert Screening 3' Amplimer (#9109-1): 1457–1436

Propagation in *E. coli*
- Recommended host strain: DH5α or other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1

Propagation in *S. cerevisiae*
- Recommended host strain: EGY48 (2)
- Yeast replication origin: CEN/ARS
- Selectable marker: plasmid confers to His^- yeast hosts the ability to grow on synthetic minimal medium lacking histidine (SD/–His)
- To keep expression of the LexA fusion protein repressed, grow pGilda-transformed yeast cells in SD/–His containing 2% dextrose (glucose). To induce fusion protein expression, eliminate glucose from the medium and instead use high-quality 2% galactose*; include 1% raffinose for improved growth. If you are planning to perform a Western blot, grow the culture to saturation in SD/Glucose/–His liquid medium. Then, pellet the cells by centrifuging, wash cells with SD/Gal/Raff/–His, and resuspend at the same density in SD/Gal/Raff/–His. Typically, 20 hours of growth in induction medium is required to be able to see a strong fusion protein band on a Western blot using the LexA Monoclonal Antibody (#5397-1) as probe.
  * The galactose must be highly purified and contain <0.01% glucose; the presence of of glucose contaminants in the medium will effectively repress fusion protein expression.
- Minimal SD Base and Minimal SD Agar Base, either with dextrose or galactose + raffinose, and 10X Dropout (DO) Supplements are available from CLONTECH. Alternatively, prepare SD medium according to the instructions in the CLONTECH Yeast Protocols Handbook (PT3024-1). If you need a copy, please call our Literature Request Line (800-662-2566, extension 2).

Related Products:
- MATCHMAKER LexA Two-Hybrid System (#K1609-1)
- MATCHMAKER LexA Libraries (many)
- LexA Monoclonal Antibody (#5397-1)
- MATCHMAKER B42AD LD-Insert Screening Amplimer Set (#9108-1)
- Minimal SD Base (contains glucose; #8602-1)
- Minimal SD Base/Gal/Raf (contains galactose & raffinose; #8611-1)
- –His DO Supplement (#8606-1)

References:
1. Golemis, E. A., *et al.* (1996) *Current Protocols in Molecular Biology* (John Wiley & Sons, Inc), Ch. 20.0 and 20.1.
2. Gimeno, R. E., *et al*. (1996) *Mol. Biol. Cell.* **7**:1815–1823.
3. Guthrie, C. & Fink, G. R. (1991) In *Methods in Enzymology* (Academic Press, San Diego) **194**:1–932.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.