pFUSE2ss-CLIg-ml1

Plasmid featuring the constant region of the mouse Ig lambda 1 light chain and the IL2 signal sequence

Catalog # pfuse2ss-mcll1

For research use only

Version # 14J28-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pFUSE2ss-CLIg-ml1** plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar)

Storage and stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20 ˚C.
- Resuspended DNA should be stored at -20 ˚C and is stable for up to 1 year.
- Store *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable for 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

Materials required for antibody generation & isotype switching

- pFUSE2ss-CLIg plasmid that features the constant region of the kappa or lambda light chains. pFUSE2-CLIg plasmids are selectable with blasticidin.

- pFUSEss-CHIg plasmid for the constant region of the heavy chain, this plasmid is selectable with Zeocin™ (sold separately, see RELATED PRODUCTS).

GENERAL PRODUCT USE

pFUSE-CHIg and pFUSE2-CLIg plasmids are designed to change a monoclonal antibody from one isotype to another, therefore, enabling the generation of antibodies with the same antigen affinity but with different effector functions (increased or reduced ADCC and CDC). Furthermore, they can be used to produce entire IgG antibodies from Fab or scFv fragments that are either chimeric, humanized or fully human depending on the nature of the variable region.

pFUSE-CHIg and pFUSE2-CLIg express the constant regions of the heavy (CH) and light (CL) chains, respectively. They contain a multiple cloning site (MCS) upstream of these constant regions to enable the cloning of the variable (VH and VL) regions of a given antibody. Transfection of mammalian cell lines with the recombinant pFUSE-CHIg and pFUSE2-CLIg pair allows to generate an IgG antibody that can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.

pFUSE2ss plasmids contain the versatile hIL2 signal sequence for the generation of secretable immunoglobulin polypeptides, when the native Ig signal sequence is not available (such as VL derived from phage display library). Coexpression of the pFUSE2ss-CLIg (cloned with the VL region) with a pFUSEss-CHIg (cloned with the VH region) will allow for the expression of recombinant antibodies.

Features of pFUSEss-CHIg and pFUSE2ss-CLIg plasmids

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **MCS:** The multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **CMV enh/hFerL prom:** This composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer and the core promoter of the human ferritin light chain gene. This ubiquitous promoter drives the expression of the blasticidin-resistance gene in mammalian cells.
- **IL2 ss:** The human IL2 signal sequence contains 20 amino acids (MYRMQLLSCIALSLALVTNS) and share common characteristics with signal peptides of other secretory proteins. The intracellular cleavage of the IL2 signal peptide occurs after Ser20 and leads to the secretion of the immunoglobulin chain.
- **EM2KC** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*. EM2KC is located within an intron and is spliced out in mammalian cells.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

pFUSE2ss-CLIg-ml1 specific features

- **Mouse IgLC1 (Ig Lambda 1 Light constant domain):** When cloning your VL of choice in the MCS, care must be taken to preserve the integrity of the lambda 1 light chain constant region and reading frame.
- **Bsr (blasticidin resistance gene):** Resistance to blasticidin is conferred by the bsr gene from Bacillus cereus. The same resistance gene confers selection in both mammalian cells and *E. coli*.

References:

1. Kim DW. *et al.* 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. 91(2):217-23. **2. Takebe Y. *et al.* 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol. 8(1):466-72. **3. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. **4. Yu J. & Russell JE. 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

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PROTOCOL

Obtaining VH and VL sequences

To obtain the cDNA sequence of the VH and VL regions from an antibody producing hybridoma, total RNA or mRNA is extracted and reverse transcribed to cDNA. PCR is performed with 5' degenerate primers to anneal to the unknown VH and VL regions and the 3' primers designed to anneal to the "known" CH and CL regions. Alternatively 5' RACE can be used. The resulting amplicons must be sequenced.

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 μg/μl, resuspend the DNA in 20 μl of sterile H_2O. Store resuspended plasmid at -20°C.

Cloning into pFUSEss-CHIg and pFUSE2ss-CLIg

Once the VH and VL sequence are known, inserts for cloning into the plasmids can be generated. In pFUSE2ss-CLIg-ml1, the constant region of the mouse lambda 1 light chain is preceded by a multiple cloning site containing four restriction sites: EcoRI, EcoRV, NcoI, and AvrII. Using EcoRI as the 5' cloning site ensures that the cloned VL will follow the hIL2 signal sequence without unwanted additional amino-acids. In pFUSE2ss-CLIg-ml1, use AvrII as the 3' cloning site for the VL in order to preserve the lambda 1 constant domain amino acid sequence.

Note: When generating the insert for VH, use Eco47III (blunt-end cloning) as the 3' cloning site in order to preserve the IgG constant amino acid sequence. Using EcoRI as the 5' cloning site ensures that the cloned VH will follow the hIL2 signal sequence without unwanted additional amino-acids.

Choice of strategies for the transfection

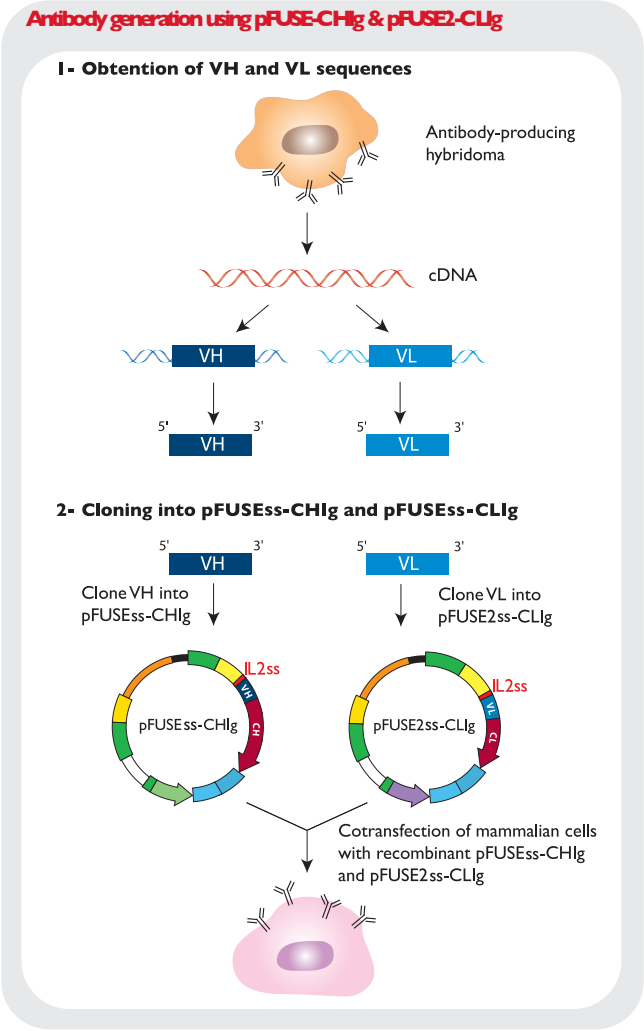
Cotransfect mammalian cells, such as 293 and CHO cells, with the recombinant plasmids pFUSE2ss-CLIg encoding the light chain and pFUSEss-CHIg encoding the heavy chain. Antibody production depends greatly on the ratio of heavy chain and light chain expression. Typically, pFUSEss-CHIg to pFUSE2ss-CLIg ratio of 2:3 is used to cotransfect mammalian cells. Since both plasmids share the same plasmid backbone, the appropriate heavy chain to light chain ratio can be easily determined by varying the quantities of plasmids.
OR
Transfect cells using a transfection agent, such as LyoVec™, with the plasmid coding for light chain and select the best clone. Following selection of the best clone, the plasmid coding for the heavy chain clone can be transfected into this clone.

Use blasticidin and Zeocin™ to select pFUSE2-CLIg and pFUSE-CHIg respectively.

Antibody production can be analyzed by different techniques including SDS-PAGE, flow cytometry, ELISA, or a bioactivity assay.

Antibody purification

The resulting IgG antibody can be purified from the supernatant using the appropriate Protein A, Protein G or Protein L affinity chromatography.



RELATED PRODUCTS

Product	Catalog Code
Blasticidin	ant-bl-1
Fast-Media® Blas Agar	fas-bl-s
Fast-Media® Blas TB	fas-bl-l
LyoVec™	lyec-12
pFUSEss-CHIg-mG1	pfusess-mchg1
pFUSEss-CHIg-mG2a	pfusess-mchg2a
pFUSEss-CHIg-mG2b	pfusess-mchg2b
pFUSEss-CHIg-mG3	pfusess-mchg3
Protein L / Agarose	gel-protl-2
Protein G / Agarose	gel-agg-5

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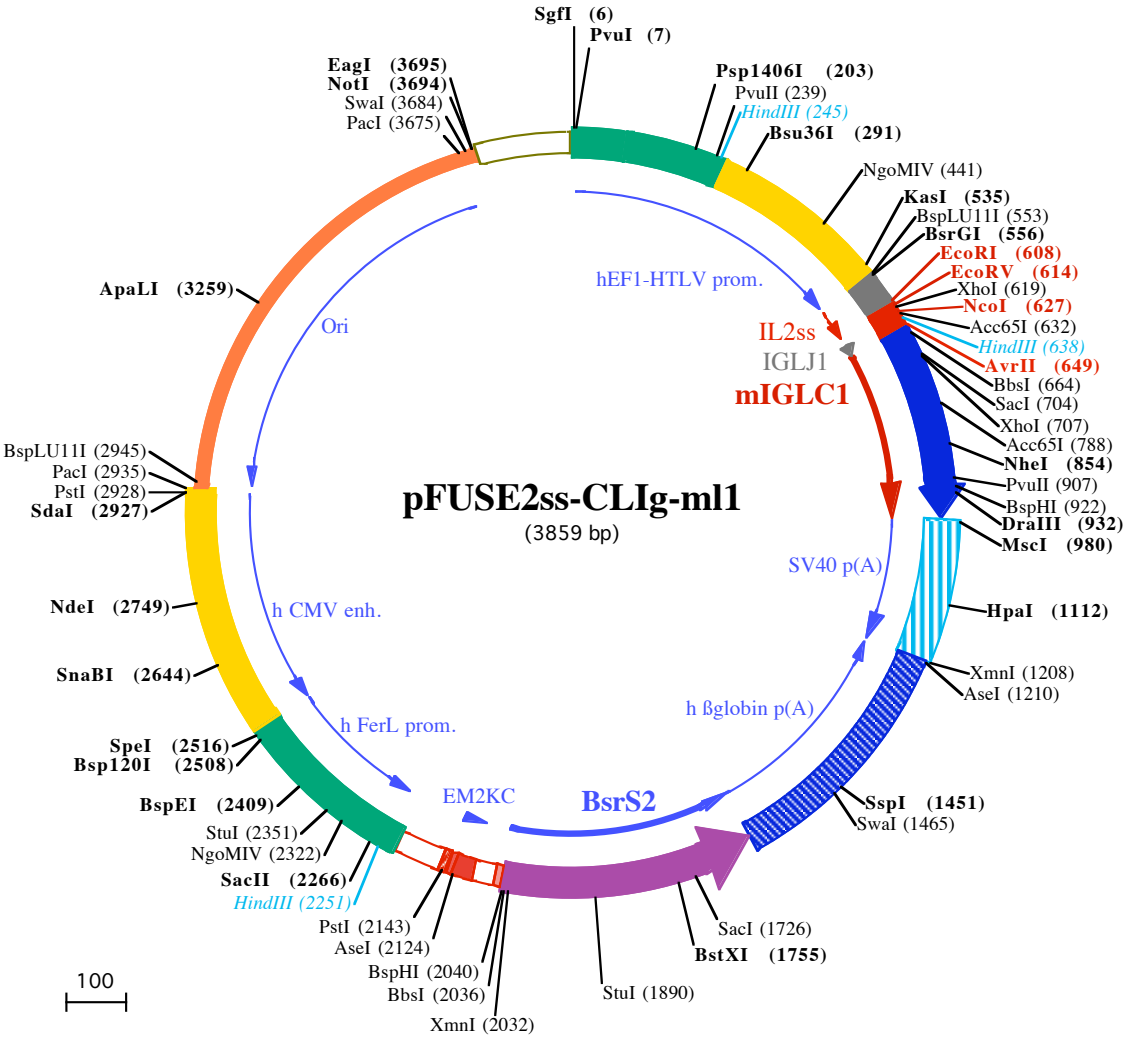
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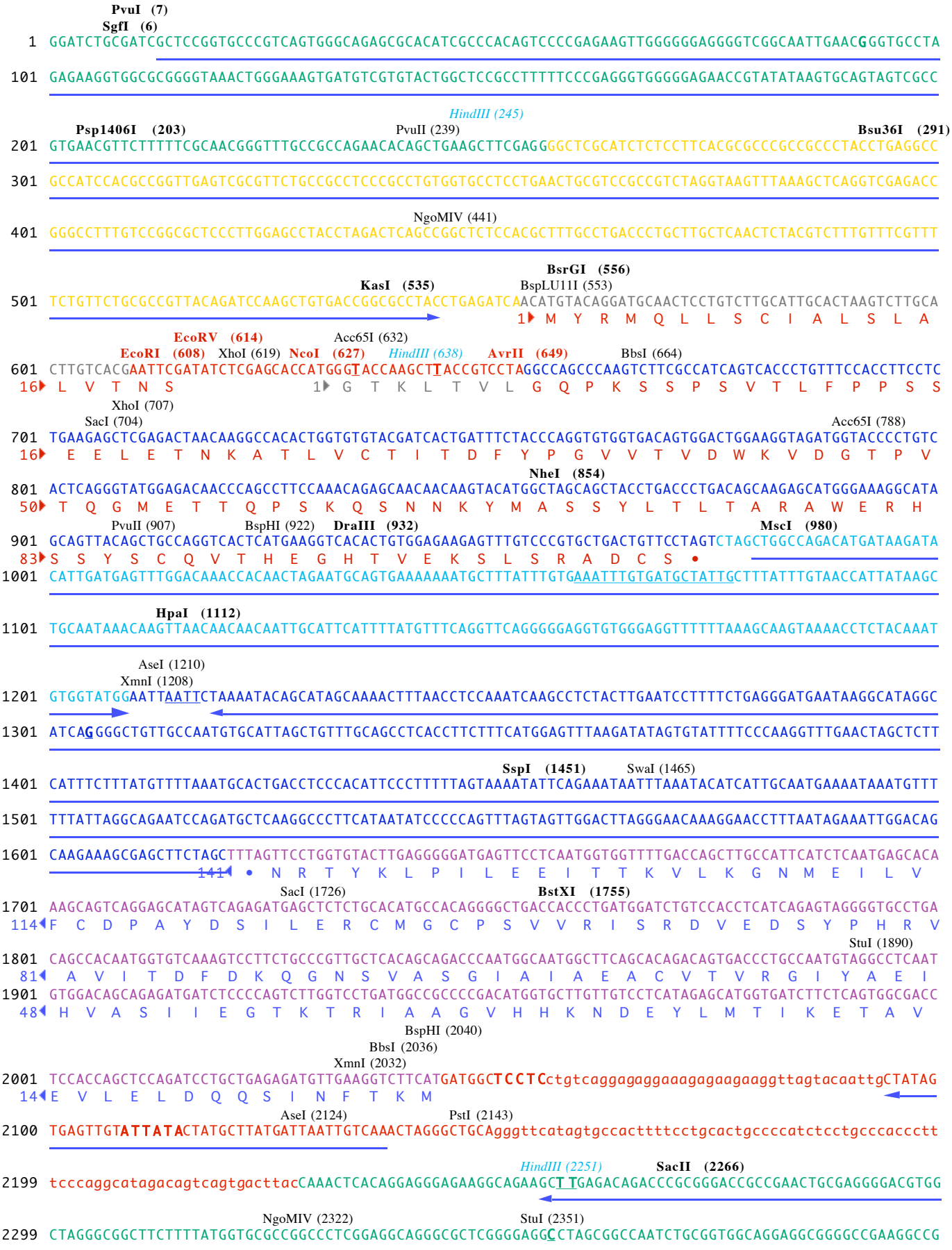
PvuI (7)
Psp1406I (203)
PvuII (239)
HindIII (245)
Bsu36I (291)
NgoMIV (441)
KasI (535)
BspLU11I (553)
BsrGI (556)
EcoRI (608)
EcoRV (614)
XhoI (619)
NcoI (627)
Acc65I (632)
HindIII (638)
AvrII (649)
BbsI (664)
SacI (704)
XhoI (707)
Acc65I (788)
NheI (854)
PvuII (907)
BspHI (922)
DraIII (932)
MscI (980)
HpaI (1112)
XmnI (1208)
AseI (1210)
SspI (1451)
SwaI (1465)
SacI (1726)
BstXI (1755)
StuI (1890)
XmnI (2032)
BbsI (2036)
BspHI (2040)
AseI (2124)
PstI (2143)
HindIII (2251)
SacII (2266)
NgoMIV (2322)
StuI (2351)
BspEI (2409)
Bsp120I (2508)
SpeI (2516)
SnaBI (2644)
NdeI (2749)
SdaI (2927)
PstI (2928)
PacI (2935)
BspLU11I (2945)
ApaLI (3259)
PacI (3675)
SwaI (3684)
NotI (3694)
EagI (3695)
hEF1-HTLV prom.
IL2ss
IGLJ1
mIGLC1
SV40 p(A)
h ßglobin p(A)
BsrS2
EM2KC
h FerL prom.
h CMV enh.
Ori
100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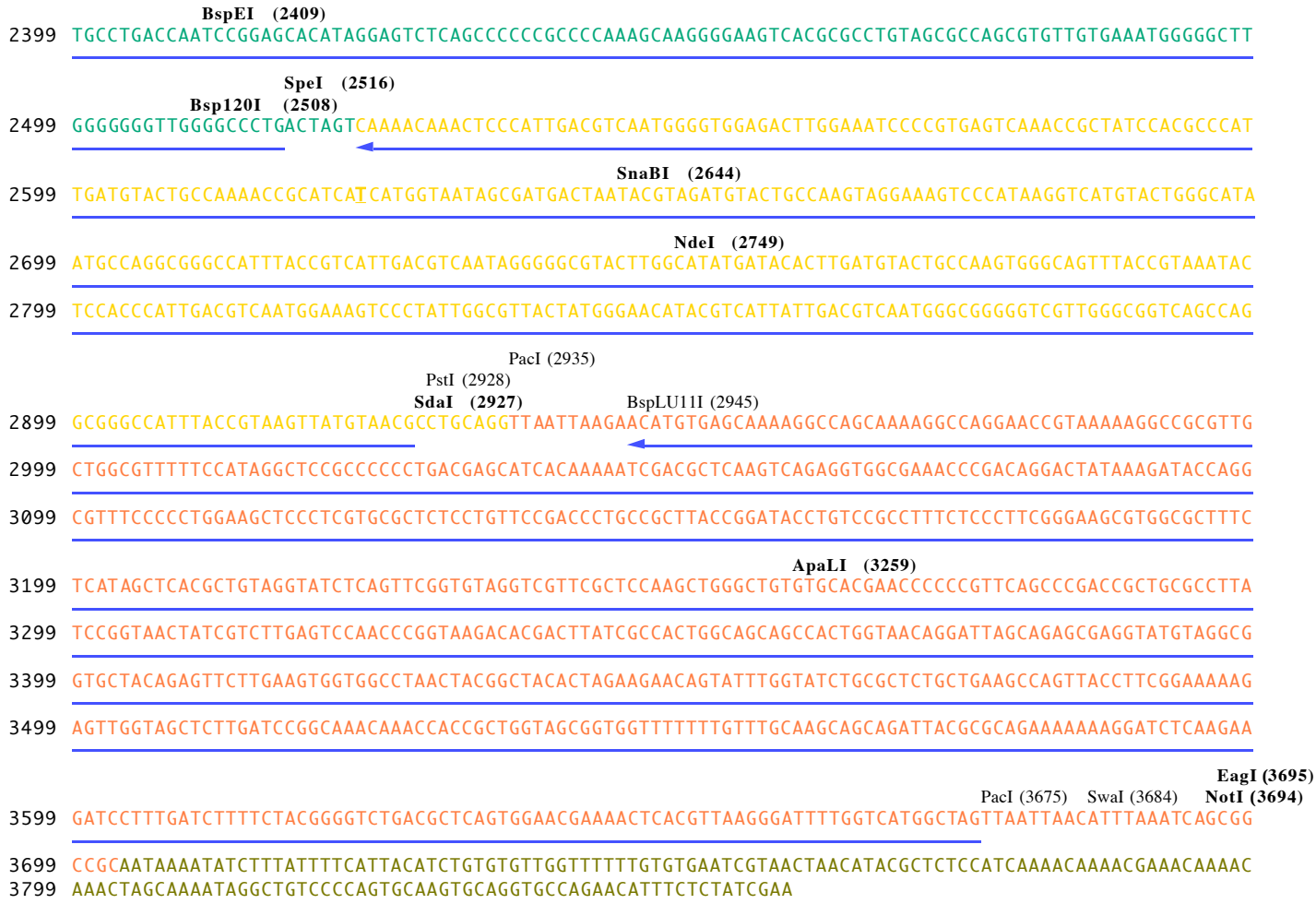


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2699 ATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATAC

2799 TCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAG

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3199 TCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA

3299 TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCG

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3799 AAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
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Fast-Media®

Microwaveable media for selection and propagation of *E. coli* transformants

Catalog # fas-xx-l, fas-xx-s, fas-xx-xgal

For research use only

Version # 10G07-MM

PRODUCT INFORMATION

Contents:

E. coli **Fast-Media®** are prepared as individual sealed pouches containing the necessary amount of powder for preparation of 200 ml of selective liquid or agar medium.

30 pouches are supplied for each order of TB or Agar and 20 pouches are supplied for each order of XGal Agar.

Storage and stability:

Fast-Media® are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.

When properly prepared, **Fast-Media®** plates or broths are stable for 4 weeks at 4˚C, and remain sterile and selective**.**

Quality control:

The high quality and performance of each formulation has been tested with some widely used and proprietary *E. coli* K12 derived strains*. These include DH5α, Top10, MC1061, XL1 blue, JM 109, TB1, GT100, GT110, GT115, GT116.

The adequate plasmids carrying the appropriate *E. coli* resistance genes are used as positive control.

**E. coli* recipient strains carrying the Tn5 transposon are resistant to Kanamycin and Zeocin™.

GENERAL PRODUCT USE

E. coli **Fast-Media®** are microwaveable ready-to-use solid or liquid media, supplied with a selective antibiotic, and chromogenic substrates (for five references), therefore designed for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies.

- Fast-Media® Agar formulation is LB based agar medium supplemented with selective antibiotic, it is used for selection of resistant *E. coli* colonies after transformation by vectors carrying a selection resistance gene.

- Fast-Media® X-Gal formulation is a LB based agar medium supplemented with selective antibiotic, X-Gal and IPTG. It is used for detection of blue/white resistant colonies after transformation by a vector carrying *LacZ* gene.

- Fast-Media® TB formulation is a Terrific Broth based liquid medium supplemented with selective antibiotic. It's used for high cell density culture of transformed bacteria, and extraction of high quantity and quality of required plasmid.

FAST-MEDIA® FEATURES

E. coli **Fast-Media®** offer researchers a quick and convenient way to prepare 200 ml of liquid culture medium, or 8-10 agar plates in about five minutes USING A MICROWAVE INSTEAD OF AN AUTOCLAVE.

E. coli **Fast-Media®** are available with a large variety of prokaryotic selective agents including Ampicillin, Blasticidin S, Hygromycin B, Kanamycin, Puromycin and Zeocin™ (see table below). **Fast-Media®** is also available with no selective agent (Base) that can be prepared with or without antibiotics.

	Agar	X-Gal	TB
Base	√		√
Ampicillin	√	√	√
Blasticidin	√	√	√
Hygromycin	√	√	√
Kanamycin	√	√	√
Puromycin	√		√
Zeocin™	√	√	√

SPECIAL HANDLING

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breath the dust**.**

METHOD

For customer convenience, procedure is directly printed on each pouch.

1- Pour the pouch contents into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled or deionized water.
3- Mix thoroughly by swirling the glass bottle or flask.
4- Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).
Do not heat in a closed container.
5- Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6- Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7- Allow the medium to cool to 50-55 °C, use directly for liquid medium, or pour plates for solid medium.
Caution: *Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.*
Note*: Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.*

For preparation of supplemented Fast-Media® Base.

- Follow the instructions above and when media has cooled to 50-55 ˚C add the antibiotic at the appropriate concentration for selection of *E. coli*.

TECHNICAL SUPPORT
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