SYNTHETIC GENOMICS®

Vmax™ Express Electrocompetent Cells

User Guide

Catalog Numbers CL1100-05, CL1100-10, CL1100-20

REV. 1.6 10.04.17
Part number 40026

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techservices@sgidna.com or

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Table of Contents

Product Information

Included products and storage information

Vmax™ Electrocompetent Cells					
Component	Quantity			Volume	Storage Temperature*
	Cat. No. CL1100-05	Cat. No. CL1100-10	Cat. No. CL1100-20		
Vmax™ Express Electrocompetent Cells	5 vials	10 vials	20 vials	40 µL/vial	**–80°C**
Vmax™ Recovery Medium	5 vials	10 vials	20 vials	500 µL/vial	**2°C to 8°C***
Positive control pACYC/Chlor plasmid	1 vial	1 vial	1 vial	25 µL at 10 ng/µL	**–20°C***

*All components may be stored at –80°C if it is more convenient. If desired, unopened vials of Vmax Recovery Medium can be stored at room temperature (20 to 24°C).

Accessory products

- Vmax™ Enriched Growth Media (SGI-DNA Cat. No. CL1500-1000)

Required materials not included

- Gene Pulser® Xcell™ Microbial System or equivalent electroporation system
- Gene Pulser®/MicroPulser™ Cuvettes, 0.1 cm gap
- LB-Miller Plates with appropriate antibiotic selection

  Note: For best results, use only LB-Miller agar plates. Do not use LB-Lennox or LB-Luria plates (Vmax™ growth will be suboptimal).
- Vmax™ Enriched Growth Media (SGI-DNA Cat. No. CL1500-1000) or compatible culture media listed in Table 2 (page 8)

Guidance and recommendations

- Use cells within 6 months of receipt.
- Store cells at –80°C and avoid temperature fluctuations.
- Thaw Vmax™ cells on ice.
- If cells need to be resuspended, do so by gently flicking the tubes.
- Thoroughly pre-chill electroporation cuvettes on ice before use.
- Use the provided recovery medium for best results. Pre-warm media to room temperature or 30°C prior to use.
- Use DNA that has been purified and resuspended in nuclease-free water or TE.
- Only use cuvettes with the recommended gap width.
- Electroporation conditions may vary with different electroporation instruments. If you are using an electroporator other than that specified in the protocol, you many need to optimize electroporation conditions.

Overview

Vmax™ Express Electrocompetent Cells

Vmax™ Express cells are an engineered *Vibrio natriegens* strain containing a major extracellular nuclease knockout and insertion of an IPTG-inducible T7 RNA polymerase cassette for expression of genes under a T7 promoter.

Introduction

Vmax™ Express is a novel bacterial strain for recombinant protein expression. This fast growing and easily manipulated strain was developed by SGI-DNA and Synthetic Genomics, Inc. The Vmax™ Express system is designed for fast growth and high yields of recombinant proteins using plasmids and workflows similar to those used for *E. coli*. Unlike other commonly used prokaryotic recombinant protein expression systems, Vmax™ Express is derived from the marine microorganism, *Vibrio natriegens*[1,2]. This gram-negative, non-pathogenic bacterium exhibits the fastest growth rate of any known organism with a doubling time of less than 14 minutes under ideal conditions, a growth rate that is twice as fast as that of *E. coli*[3].

Researchers at Synthetic Genomics, Inc. extensively studied different isolates of *V. natriegens*, developed a suite of genomic tools, and engineered *V. natriegens* to create Vmax™ Express, a next-generation platform for protein expression[4]. The naturally occurring properties of Vmax™, such as a strong transcription system to support its fast growth rate[5], make it ideal for the rapid expression of large amounts of soluble recombinant protein. Vmax™ Express competent cells are engineered to allow exogenous protein expression and have been demonstrated to produce high yields of recombinant proteins under a tightly controlled, inducible T7 promoter system. Similar to other historically used bacterial expression strains such as *E. coli* BL21(DE3), Vmax™ Express can be cultured with routine growth medium such as Luria Broth (LB) supplemented with salt, 2xYT, Terrific broth (TB) and other commercial auto-inducible media. However, Vmax™ Enriched Growth Media is recommended for best results.

To allow seamless transfer of your *E. coli*-based expression constructs, Vmax™ Express is compatible with the pET-derived series of vectors that use the phage T7 expression system regulated by the addition of Isopropyl β-D-1-thiogalactopyranoside (IPTG). Unlike *E. coli*, the induction of Vmax™ Express is independent of growth phase and has been performed at a variety OD_{600} readings (0.5 to 1). Protein expression induced over this range remains stable with overnight induction and no negative impacts of expression have been observed over this range. This flexibility in induction time gives you added convenience, reducing the need to closely monitor OD. Other advantages of Vmax™ Express include:

- Typical protein yields 2- to 4-fold higher than *E. coli* due to Vmax's high biomass production and speed of growth.
- Efficient protein production with no restrictions on an optimal harvest point within a 24-hour period.

Figure 1 (on the following page) depicts the workflow comparison of *E. coli* and Vmax™ Express.

The Vmax™ Express workflow can be completed in 3 days

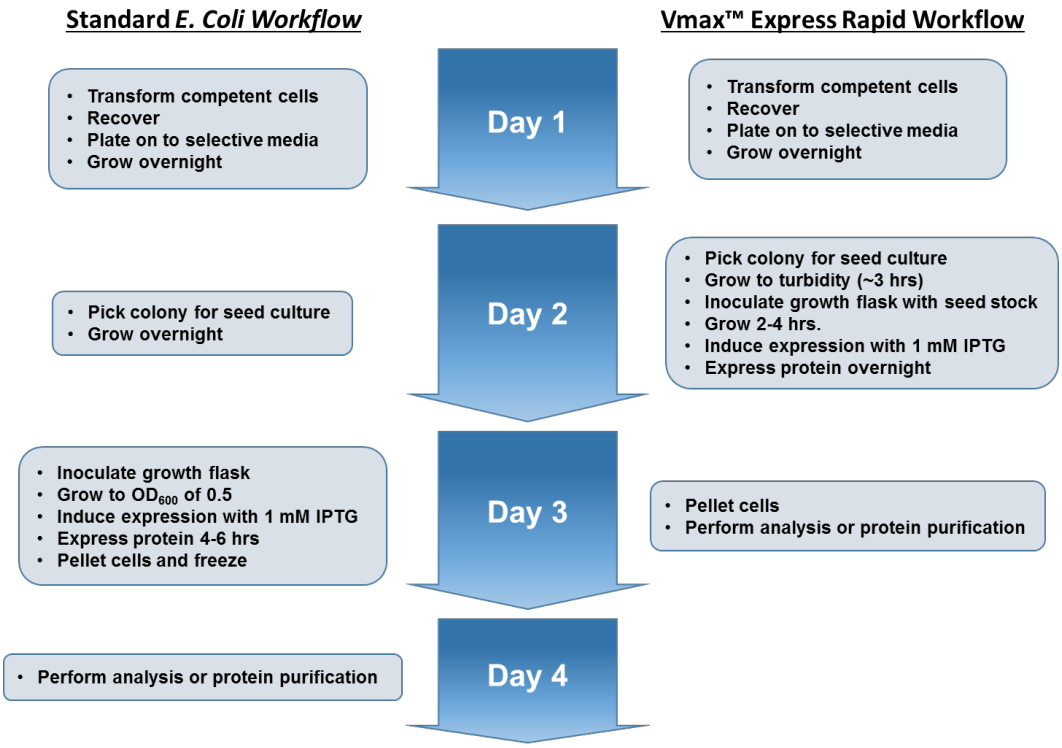

Figure 1. Comparison of protein expression workflows.

Table 1. Vmax™ Express Key Features and Advantages

Key Feature	Vmax™ Express	*E. coli*	Vmax™ Advantages over *E. coli*
Doubling Time	<14 minutes	~30 minutes	• Fast growth rate • Saves time
Growth Requirements	• Optimal at 30°C • Rapid growth from 20°C to 37°C	• Optimal at 37°C • Slow growth from 20°C to 24°C	• Potentially omit temperature-controlled shaker • Lower temperature aids solubility of some overexpressed proteins
Biomass	>14 OD	8–10 OD	• Faster and more cost-effective production of proteins • Up to twice the biomass per volume of media
Protein Expression	Produces more soluble protein with less optimization	Soluble protein expression is dependent on optimal induction OD and temperature	• More convenient • Potential for higher rate of success with difficult to express proteins • Higher soluble protein yields

Handling and storing transformed Vmax™ Express cells

Vmax™ Express Electrocompetent Cells are provided ready-to-use. Each vial of cells can be transformed with plasmid DNA and recovered using the single aliquots of Vmax™ Recovery Media provided. After recovery, single colonies on a solid growth plate may be isolated in as few as 6–8 hours[4]. Once a colony is visible, it can be used for downstream workflows such as protein expression in liquid culture. While Vmax™ Express can be used in a manner similar to *E. coli*, observe the following handling differences and best practices.

Vmax™ Express handling practices
• Protect cells from temperature fluctuations during storage. • Thaw cells on ice just prior to use. • Perform recovery at 37°C or 30°C. • Store agar plates with established Vmax™ Express cells at room temperature. Do not store agar plates at 2–8°C as this can adversely impact re-proliferation. • You may leave agar plates at room temperature for up to 72 hours. Single colonies will remain well isolated since Vmax™ Express cells exhibit contact inhibition. • For long-term storage, we recommend preparing glycerol stocks with 25% glycerol and storing at –80°C.

Growing and maintaining transformed Vmax™ Express cells

Vmax™ Growth Media Recommendations

A variety of media commonly used for *E. coli* are compatible with Vmax™ Express. However, Vmax™ Express grows best in higher osmotic conditions and some media salt supplements may be required. For optimal growth, we recommend Vmax™ Enriched Growth Media (SGI-DNA Cat. No. CL1500-1000), which has been formulated specifically for Vmax™ and allows for fast doubling times.

Table 2. Vmax™ Express Growth Condition in Different Media

Media	Format	25°C	30°C	37°C	Recommendation*
Vmax™ Enriched Growth Media	Liquid culture	Growth	Growth	Growth	++++ (Preferred)
Enhanced 2xYT media	Liquid Culture	Growth	Growth	Growth	+++ (Recommended)
Brain heart infusion broth + v2 salts‡	Liquid culture	Growth	Growth	Growth	+++ (Recommended)
LB-Miller†	Agar plate	Slow growth	Growth	Growth	+++ (Recommended)
Brain heart infusion agar + v2 salts‡	Agar Plate	Growth	Growth	Growth	++
MagicMedia™ *E. coli* Expression Media	Liquid Culture	Not tested	Growth	Growth	++
LB-Miller†	Liquid culture	Growth	Growth	Supplement with v2 salt‡	+
Terrific broth	Liquid Culture	Not tested	Growth	Growth	+

*Recommendation Key: ++++ = Preferred media, +++ = Recommended, ++ = Acceptable, + = Acceptable, but other media may support better growth

†LB-Miller media contains 10 g/L of NaCl. Other LB variants (e.g. LB-Lennox and LB-Luria) contain less salt and will not support optimal growth.

‡v2 salt: 204 mM NaCl, 4.2 mM KCl, and 23.14 mM $MgCl_2$ (these are in addition to salts present in base media)

Antibiotic Selection Using Vmax™ Express

E. coli expression vectors and their corresponding antibiotic selection markers are compatible with Vmax™ Express. However, plasmid copy number and the minimum inhibitory concentration (MIC) may be different between Vmax™ and *E. coli*. Recommended concentrations for maintaining antibiotic selection using Vmax™ Express culture on solid or liquid culture are listed in the following table.

Table 3. Maintaining Antibiotic Selection in Vmax™ Express

Antibiotic Marker	Concentration	
	Solid Media	Liquid Culture
Ampicillin/Carbenicillin*	2-50 µg/mL	2-25 µg/mL
Kanamycin	100 µg/mL	200 µg/mL
Tetracycline	2.5 µg/mL	
Chloramphenicol	5–12.5 µg/mL	12.5–25 µg/mL

*Vmax cells are more sensitive to ampicillin/carbenicillin than E. coli. When using solid media, amp/carb concentrations above 12.5ug/mL typically do not decrease transformation efficiency. However, higher antibiotic concentrations tend to result in colony size heterogeneity.

Kanamycin and Vmax™ Express Cells

Vmax™ Express has natural resistance to Kanamycin. Perform selection of Kanamycin-resistant (Kan^R) colonies on plates with 100 µg/mL Kanamycin. This concentration slows the growth of cells lacking a plasmid with the Kanamycin-resistance gene. Using a lower concentration of Kanamycin will result in an increase of background colonies, making it difficult to identify transformed cells. Representative images in Figure 2 on page 16 provide an example of how to identify Kan^R positive clones when compared to naturally resistant background colonies.

Vmax™ Express Compatible Plasmid Origins of Replication

A variety of commonly used plasmid origins of replication and antibiotic selection markers are known to be compatible with Vmax™ Express. The optimal recovery temperature can vary with different combinations of origins of replication and antibiotic selection markers used. As shown in Table 4, most combinations of post-transformation growth on agar plates can be performed at either 30°C or 37°C. However, for cells with a kanamycin resistance marker, optimal recovery occurs solely at 30°C (see Figures 2 and 3 on page 16). The following table shows different plasmid origins of replications tested. If you do not know the origin of replication for the plasmid you are using we recommend performing duplicate reactions and recovering at both 30°C and 37°C or determining plasmid copy origin using an online resource such as **wishart.biology.ualberta.ca/PlasMapper**.

Table 4. Transformation Guidelines for Introducing Plasmid into Vmax™ Express

Plasmid Origin of Replication	Plasmid Backbone	Compatible?	Growth Plate Recommendation
pMB1	pBR322 pET vectors	Yes	37°C recommended (30°C acceptable)
ColE1	pCDNA3.0 pGEM5zf	Yes	37°C recommended (30°C acceptable)
pUC	pUC19 **Selection with Kanamycin**	Yes	Grow plates at 30°C only
	pUC19 **Selection with all other antibiotics**	Yes	37°C recommended (30°C acceptable)
p15A	pACYC184	Yes	37°C recommended (30°C acceptable)
RK2	N/A	Maybe	Incompatible with electroporation. Can be introduced by conjugation.

Procedures

Before starting

If this is your first time using Vmax™ Express Electrocompetent Cells, we recommend reading through the entire protocol before starting. While the Vmax™ Express workflow is similar to that of *E. coli*, there are minor differences in the handling of Vmax™ cells that are important to understand prior to starting your work.

Materials

Required materials provided

- Vmax™ Express Electrocompetent Cells
- Vmax™ Recovery Media
- Positive control pACYC/Chlor plasmid (*optional*)

Materials and equipment not supplied

- Ice bucket with ice
- Sterile culture tubes (e.g. Falcon® 14-mL Round-Bottom Polypropylene Tubes, Corning Cat. No. 352006)
- Plasmid DNA for transformation (minimum concentration of 1 ng/µL in TE buffer or water)
- Electroporation cuvette (e.g. Gene Pulser®/MicroPulser™ Cuvettes, 0.1 cm gap
- Electroporation system (e.g. Gene Pulser® Xcell™ Microbial System or equivalent system)
- LB-Miller Agar plates (e.g. Teknova Cat. No. L1100)
- Antibiotic for plasmid selection (see Table 2 on page 8 and Table 3 on page 9)
- Vmax™ Enriched Growth Media (SGI-DNA Cat. No. CL1500-1000) or other bacterial growth media (see Table 2 on page 8 and Table 3 on page 9)
- Antibiotics for selection
- Sterile glycerol and cryopreservation tubes (*optional*, for creation of glycerol stocks for long-term storage)
- Incubators and orbital shakers
- Pipettors and plastics
- Fernbach baffled growth flask for large-scale protein expression
- Rotating Shaker incubator
- IPTG
- Centrifuge and centrifuge tubes capable of >4000 × *g*

Electroporation protocol

1. For each transformation reaction,
   - Thaw one vial of Vmax™ Express Electrocompetent cells on ice for 5 minutes.
   - Warm one vial of Vmax™ Recovery Media to room temperature (20°C to 24°C).
   - Pre-chill a 0.1-cm electroporation cuvette on ice.
2. Transfer 500 µL Vmax™ Recovery Media to a Falcon® 14-mL Round-Bottom Tube. Allow media to sit at room temperature until needed in Step 8.
   Note: Vmax™ recovery medium is rich and prone to contamination. Discard any remaining medium or return it to –20°C for storage.
3. As soon as the electrocompetent cells are thawed, add 1 ng – 100 ng of plasmid DNA (in TE buffer or water only) to the competent cells on ice. Briefly mix by flicking tubes.
   Note: Add no more than 2 µL of plasmid DNA to the competent cells. Concentrate the plasmid DNA if necessary.
4. Incubate the DNA and cell mixture on ice for 3–5 minutes.
5. During incubation, verify the Gene Pulser® Xcell System is set to the following electroporation parameters: 900 V, 25 uF, 200 Ohms, 0.1-cm cuvette. If further optimization is desired, voltage changes in the range of +/- 200V in 50 V increments can be used. **Note:** These are the optimized electroporation settings for the Gene Pulser® Xcell™ Microbial System using a cuvette with a 0.1-cm gap. If other instruments and/or cuvettes are used, you may need to optimize the conditions for best results.
6. Transfer cells to a chilled cuvette using a pipette. Verify that the cells completely occupy the bottom of the cuvette and that no air remains between walls of the cuvette and the cells.
7. Transfer the cuvette to the shock pod and perform electroporation.
   Note: Time constants typically range from 3.3–3.7 ms.
8. Transfer cells to a room temperature Falcon® 14-mL Round-Bottom Tube containing recovery media immediately after pulse.
9. To transfer any remaining cells, add 200 µL of Vmax™ Recovery Media to the cuvette. Dispense the media to the same round-bottom tube from Step 8.
10. Place the Falcon® 14-mL Round-Bottom Tubes in a tube rack in an orbital shaker (250 rpm) at 30°C for 2 hours at to allow recovery of cells and expression of antibiotic resistance marker.
    Note: Recovery can be performed at 37°C unless kanamycin is used for selection.
11. During cell recovery, pre-warm LB-Miller agar plates prepared with antibiotics (see Table 3 on page 9 for recommended concentrations) at the appropriate incubation temperature.
12. Proceed to "Plating instructions" on page 13.

Plating instructions

Important notes

- We recommend incubating plates at 25°C to 30°C overnight.
- For plates containing kanamycin, plates must be incubated at 25°C to 30°C.
- Colonies typically appear after 12–16 hours at 25°C to 30°C and after ~8 hours at 37°C.
- Store agar plates with established Vmax™ Express at room temperature for up to 72 hours. Due to contact inhibition single colonies will remain well-isolated.
- Do not store Vmax™ agar plates at 2–8°C as this can adversely impact propagation and growth.
- For long-term storage, we recommend preparing glycerol stocks with 25% glycerol and storing at –80°C.

Plating protocol

1. Plate 200 µL of the transformation reaction per plate.
2. *(Optional)* For the control plasmid, dilute the transformation reaction 1:200 with Vmax™ Recovery Media. Plate 50–100 µL of the diluted reaction on an LB agar plate supplemented with 12.5 µg/mL Chloramphenicol.
3. Incubate plates at 25°C, 30°C, or 37°C for at least 8 hours.

Preparing seed cultures

Before starting

- Pre-warm media before use if it has been stored at 4°C. Inoculating cold media with Vmax™ Express will result in poor or no growth of the culture.
- For optimal results, we recommend using **Vmax™ Enriched Growth Media** (SGI-DNA Cat. No. CL1500-1000) or enhanced 2xYT medium (see "Media recipes" on page 19). Refer to "Table 2. Vmax™ Express Growth Condition in Different Media" on page 8 for additional information and media options.
- Add appropriate antibiotics for maintaining plasmid selection to the media.
- For selection with kanamycin, refer to the representative images in Figure 2 on page 16 for guidance before picking colonies.

Seed culture protocol

1. Pick the first/largest colonies to appear.
2. Inoculate a culture tube containing growth media that is at room temperature or at 30°C or 37°C.
3. Culture cells at 30°C or 37°C at 250 rpm for ~3 hours for rapid protein expression or for 6–16 hours if the culture will be used as a standard seed culture or for long-term storage at –80°C.

   Note: Refer to the following table for additional information regarding rapid protein expression and standard seed/glycerol stock-compatible cultures.

	Rapid expression protocol	Standard protocol
Incubation duration	~3 hours **Note:** 3 hours for 25 mL of media	6–16 hours
OD_{600} post-incubation	≥0.3	2–7
Seed stock use	For large-scale expression culture	For large-scale expression culture
Can be used for preparing glycerol stocks?	No	Yes

Glycerol cultures

For critical clones, we recommend preparing multiple glycerol culture aliquots to avoid freeze/thaw cycles.

1. To prepare a glycerol stock, choose from one of the following options:
   - Add 250 µL of sterile 100% glycerol to a 1-mL cryopreserve vial or other –80°C compatible sterile tube. Add 750 µL of bacterial seed culture (prepared as described in "Preparing seed cultures" on page 14) and mix for a final concentration of 25% glycerol.
   - Alternatively, spin down 1 mL of bacterial seed culture and resuspend in 1 mL sterile media supplemented with 25% glycerol.
2. Place vials in –80°C freezer for storage.
3. To recover culture from glycerol stock, use a sterile inoculation loop to scoop frozen culture and transfer to liquid or solid growth medium with appropriate antibiotics and incubate at 30°C or 37°C.

Protein expression protocol

The following protocol is a standard protocol for protein expression using Vmax™ Express cells. This protocol has been used to successfully express multiple proteins with high yields. For some proteins, optimization of expression temperature and/or expression times may improve yield, solubility, or activity. For a rapid expression assay, pick a single colony, inoculate seed culture in selective media, and grow to an OD_{600} of 0.3 (~3 hours in 25 mL of media) before inoculating a growth flask (see "Preparing seed cultures" on page 14 for additional details).

Before starting

- To allow sufficient aeration, ensure the expression culture will not exceed ¼ of flask volume (e.g, for a 500-mL Fernbach baffled flask, grow a maximum of 125 mL of expression culture).
- Optimal expression media are Vmax™ Enriched Growth Media or Enhanced 2xYT media. Other liquid media listed in Table 2 on page 8 can also be used (see "Media recipes" on page 19).

Protocol

1. On the day of expression, inoculate a seed culture (OD_{600} of 2–7) into growth media containing appropriate antibiotics. Use 1/100 volume of seed culture relative to the total amount of media to inoculate the growth flask (e.g. for 100 mL of growth media, add 1 mL of seed culture).
2. Grow expression culture at 30°C for ~1-2 hours.
   Note: Expression can be performed at 37°C, but protein expression is optimal when cells are grown at 30°C on a rotating shaker incubator at 250 rpm.
3. After ~1 to 2 hours, induce protein expression by adding IPTG to a final concentration of 1 mM. **Note:** Although induction is typically performed 2 hours after inoculation, Vmax™ Express cells can be induced within a wide range of time points during growth phase. The optical density at induction can be between 0.5–1 OD_{600} with no apparent difference in expression. If auto-induction medium is used, expression will initiate during overnight culture.
4. Incubate induced cells for 4–24 hours.
   Note: For most proteins, longer post-induction incubation times will allow for increased biomass, which typically results in higher protein yields. Although some proteins may express high levels sooner, overnight induction is recommended to maximize yield. Vmax™ Express has been shown to maintain soluble protein expression after overnight culture.
5. Harvest cells by transferring to a centrifugation tube and spinning at 4000–5000 × *g* for 10 minutes at 4°C. Remove supernatant.
6. Store the cell pellet at –80°C or proceed to protein purification or lysis and protein purification.
   Note: If you are using 96-well plates, apply an adhesive plate seal.

Appendix

Expected results

Representative Expression Data Using Vmax™ Express

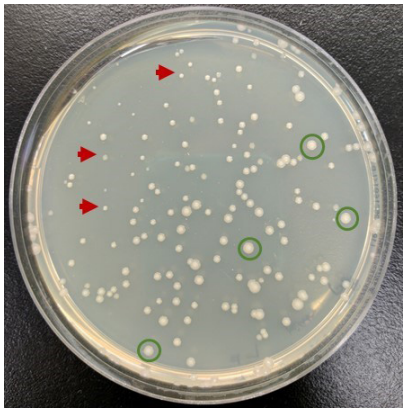

Figure 2. Morphology of positive Kan^R clones and naturally resistant clones. Vmax™ Express has a naturally high minimum inhibitory concentration (MIC) to kanamycin selection. Using kanamycin selection with Vmax™ Express, positive clones will appear larger (circled in green) and naturally resistant clones will appear smaller (red arrows) after incubation at 30°C overnight. Use only positive clones (green circles) exhibiting the correct morphology and larger size for downstream applications. In the image shown to the left, Vmax™ Express was transformed with 5 ng of a pET28a-derived plasmid and recovered for 2 hours at 30°C in Vmax™ Recovery Media. 200 µL of recovered cells were plated on agar containing 100 µg/mL of kanamycin and incubated for 16 hours at 30°C.

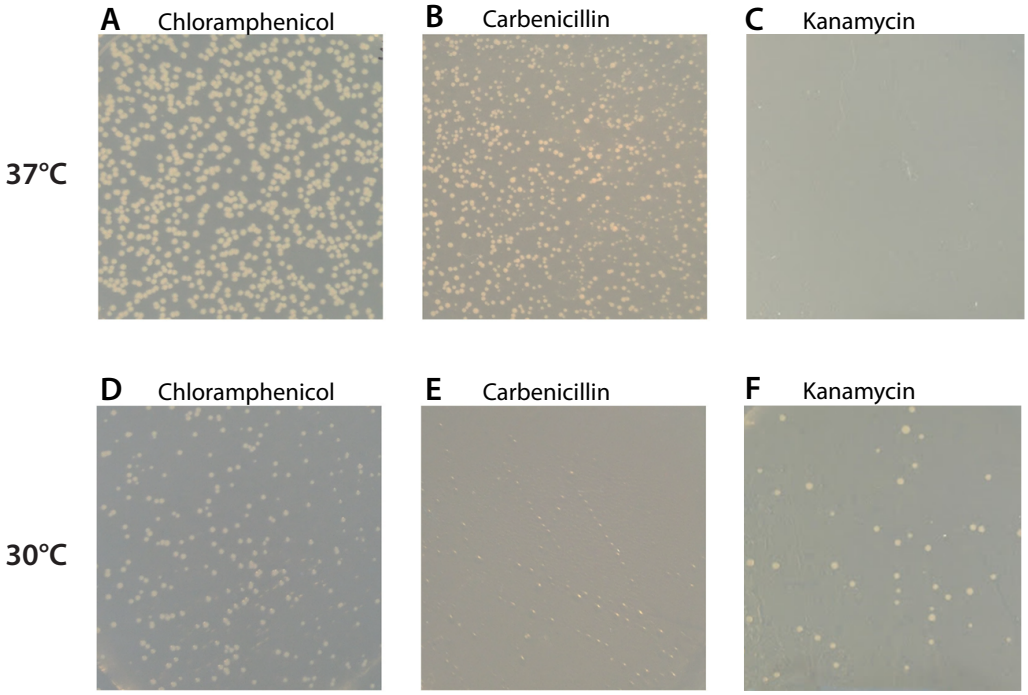

Figure 3. Colony output of Vmax™ Express at 37°C and 30 °C. Vmax™ Express can be recovered at 37°C or 30°C for the majority of vector backbones and antibiotic selection media. With the exception of kanamycin selection, growth at 37°C recovery can increase colony output. When kanamycin is used for selection, grow Vmax™ Express only at 30°C. In the image above, Vmax™ Express was transformed with **(A, D)** pBR325 (12 ng), **(B, E)** pGEM-p53 plasmid (20 ng), or **(C, F)** pET28a-746 (10 ng) and recovered at 37°C or 30°C in Vmax™ Recovery Media for 2 hours. 200 µL of recovered cells were plated on **(A, D)** 12.5 µg/mL chloramphenicol, **(B, E)** 50 µg/mL carbenicillin, or **(C, F)** 100 µg/mL kanamycin. Plates were incubated 16 hours at 37°C or 30°C.

Vmax™ Express exhibits high protein expression levels

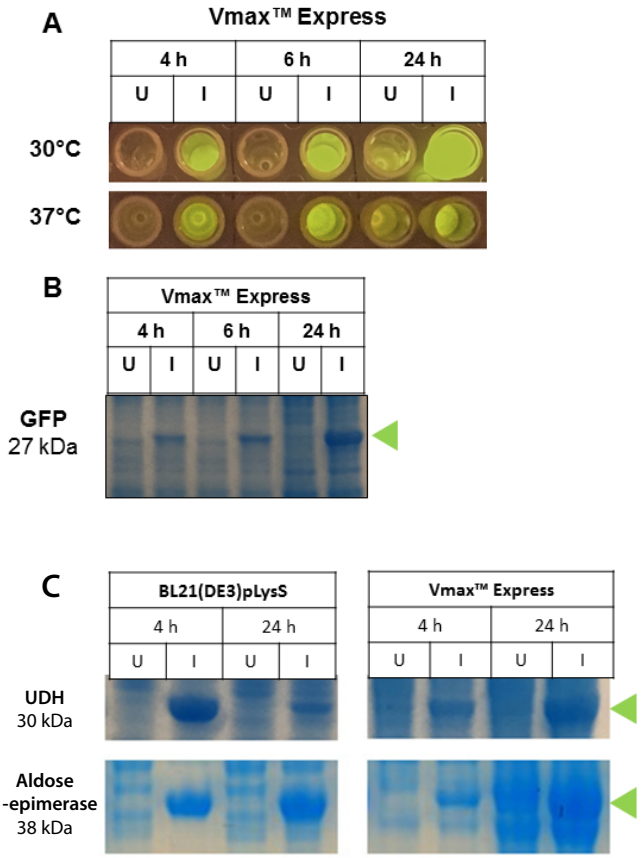

Figure 4. High levels of protein express in Vmax™ Express. (**A**) To evaluate protein expression over time, a GFP expression plasmid was introduced into Vmax™ Express cells. Expression was performed at 30°C and 37°C and analyzed at 4, 6, and 24 hour time points. Images of tubes showing the luminescence of GFP in induced cells (I) and uninduced cells (U) under UV lamp are shown. **(B)** Gel analysis of proteins from uninduced and induced Vmax™ Express cells grown at 30°C. **(C)** To evaluate protein expression in Vmax™ Express and *E. coli*, uronate dehydrogenase (UDH) or aldose 1-epimerase expression vectors were introduced into *E. coli* BL21(DE3)pLysS and Vmax™ Express. The two metabolic proteins are more highly expressed in Vmax™ Express and demonstrate significant stability over 24 hours of expression. In all panels shown, expression was analyzed by the amount of soluble protein in equal volumes of culture at the indicated time points.

Vmax™ Express supports induction over a wide range of optical densities

Cell	BL21pLysS			Vmax™ Express					
OD	0.5	0.5		0.5	0.5	0.5	0.5	0.9	1.3
IPTG (mM)	-	1		-	0.5	1	2	1	1

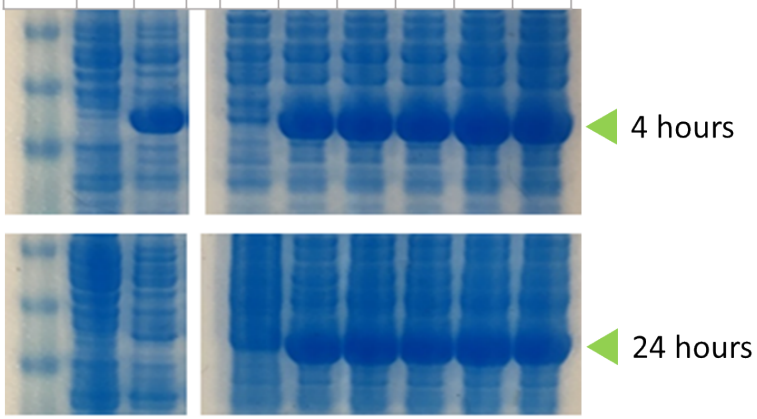

Figure 5. Vmax™ Express supports induction over a wide range of optical densities and can yield greater amounts of soluble protein. Vmax™ Express cells and *E. coli* BL21(DE3)pLysS cells containing an expression construct for uronate dehydrogenase were used for protein expression. BL21 cells were grown in LB media at 37°C to an OD of 0.5 and induced with 1 mM IPTG and grown for an additional 4 or 24 hours. Vmax™ Express were grown in enhanced 2xYT medium at 30°C to variable ODs and induced with 0.5, 1, and 2 mM IPTG and grown for an additional 4 or 24 hours. Vmax™ Express cells generate more soluble protein per cell and greater biomass. Protein expressed by Vmax™ Express remained stable after 24 hours while proteins in *E. coli* formed insoluble inclusion bodies.

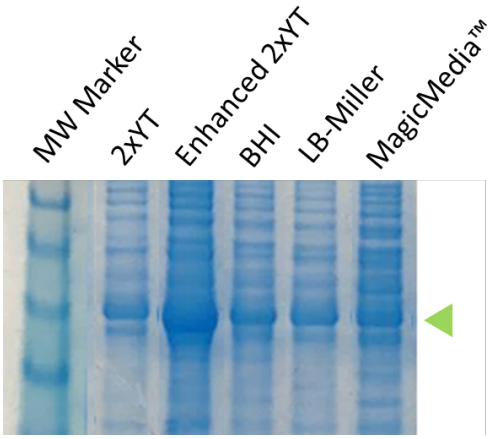

Table 5. OD_{600} of Vmax™ Express cells after 2 hours of growth at 30°C.

Media	OD_{600} at induction
2xYT	0.9
Enhanced 2xYT	1.0
BHI	0.8
LB	0.7
MagicMedia™ *E. coli* Expression Media	N/A

Figure 6. Vmax™ Express exhibits high expression levels using different types of growth media. Vmax™ Express containing an expression construct for Aldose 1-epimerase were cultured in 2xYT medium (Lane 1), enhanced 2xYT medium (Lane 2), LB medium (Lane 3), BHI medium (Lane 4) and MagicMedia™ *E. coli* Expression Media (Lane 5). After 2 hours of growth, OD_{600} levels were measured (Table 5) and cells were induced overnight with 1 mM IPTG.

Media recipes

Media	Contents
LB-Miller	10.0 g/L Tryptone 5.0 g/L Yeast Extract 10.0 g/L NaCl
LB-Miller + v2 salts	LB broth supplemented with additional salts: 204.0 mM NaCl 4.2 mM KCl 23.14 mM $MgCl_2$
LB-Miller agar	LB-Miller broth + 1.5% agar
Brain heart infusion broth + v2 salts	37 g/L Brain Heart Infusion Broth Dry Media (Teknova Cat. No. B9500) supplemented with additional salts: 204.0 mM NaCl 4.2 mM KCl 23.14 mM $MgCl_2$
Brain heart infusion + v2 salts agar	Brain Heart Infusion Broth + v2 salts + 1.5% agar
Terrific broth	12 g/L Tryptone 24 g/L Yeast Extract 4 g/L Glycerol 1X Phosphate Buffer (17 mM KH_2PO_4, 72 mM K_2HPO_4)
2xYT media	16 g/L Tryptone 10 g/L Yeast Extract 5 g/L NaCl
Enhanced 2xYT medium	20 g/L Yeast Extract 32 g/L Tryptone 17 g/L NaCl supplemented with 0.2% Glucose and 17.6 mM Na_2HPO_4 adjusted to pH 7.4

Troubleshooting

Observation	Potential Cause	Recommendation
Few or no colonies	Transformation efficiency in Vmax™ Express is dependent on the vector backbone	• Vmax™ Express transformation efficiencies can be 1 x 10^5. Ensure that you are adhering to the guidelines in "Table 4. Transformation Guidelines for Introducing Plasmid into Vmax™ Express" on page 10 for best results. • After transformation, recover for 2 hours. • Plate entire transformation reaction.
Size of colonies is heterogeneous	Vmax™ has natural resistance to kanamycin	Use recommended antibiotic selection concentrations listed in "Table 3. Maintaining Antibiotic Selection in Vmax™ Express" on page 9.
Lawn of colonies	Transformation efficiency in Vmax™ Express is dependent on the vector backbone	• Ensure that you are adhering to the guidelines in "Table 4. Transformation Guidelines for Introducing Plasmid into Vmax™ Express" on page 10. • After transformation, recover for 2 hours. • Dilute the transformation reaction with recovery medium before plating.
No or poor growth in liquid culture	No viable colonies on plate	Store Vmax™ Express clones on solid media at room temperature for up to 72 hours. For long-term storage, maintain clones in glycerol stock. Do not store Vmax™ Express cells on solid media at 2°C to 8°C.
	Antibiotic concentration is too high	The minimal inhibitory concentrations of antibiotics for Vmax™ Express may not mirror *E. coli*. Use recommended antibiotic concentrations listed in "Table 3. Maintaining Antibiotic Selection in Vmax™ Express" on page 9.
No or low protein expression	Expressed protein may be toxic to cells	• Codon optimization may be required. • Contact SGI-DNA technical service for further recommendations on optimal construct design.
	Suboptimal culture condition	Vmax™ Express is compatible with many types of commercially available media. However, protein expression levels may vary between different formulations. Refer to "Table 2. Vmax™ Express Growth Condition in Different Media" on page 8.
	IPTG concentration too low or low IPTG efficacy	• Use IPTG at a final concentration of 1 mM • Use a fresh aliquot or newly prepared IPTG. Repeated freeze thawing results in reduced IPTG efficacy.

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Technical Services: For technical assistance, contact customer support at **techservices@sgidna.com**

Synthetic Genomics, Inc.