

**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 1 of 18**

Supersedes

1.0

**Released by / Effective Date:**

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**Written by:**

Printed Name:	Title:	Signature/Date:

**Approved by:**

Printed Name:	Title:	Signature/Date:

**QA Approved by:**

Printed Name:	Title:	Signature/Date:

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 2 of 18**

Supersedes

1.0

## 1. PURPOSE

- 1.1. The purpose of this procedure is to prepare the plasmid bacterial stock for storage at -80°C in glycerol stock.

## 2. SCOPE

- 2.1. This procedure applies to the Human Papillomavirus (HPV) Serology Laboratory located at the Advanced Technology Research Facility (ATRF), Room C2007.

## 3. REFERENCES

- 3.1. Addgene plasmid long-term storage Instructions
- 3.2. HSL\_EQ\_001: Biosafety Cabinet (BSC) Use and Maintenance
- 3.3. HSL\_EQ\_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory
- 3.4. HSL\_EQ\_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory
- 3.5. HSL\_EQ\_010: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath
- 3.6. HSL\_EQ\_011: Use and Maintenance of the Forma Scientific Orbital Shaker
- 3.7. HSL\_EQ\_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.8. HSL\_EQ\_017: Use and Maintenance of a Laboratory Convection Oven
- 3.9. HSL\_EQ\_019: Use and Maintenance of the Milli-Q Integral 3 Water System
- 3.10. HSL\_GL\_001: Waste Disposal at the Advanced Technology Research Facility
- 3.11. HSL\_LAB\_004: Plasmid Purification Using a QIAGEN Kit

## 4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred as analyst, is responsible for reviewing and following this procedure.
- 4.2. The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 3 of 18**

Supersedes

1.0

- 4.3. The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

## 5. DEFINITIONS

Term	Definition
Amp	Ampicillin
Blas	Blasticidin
Kan	Kanamycin
LB	Luria broth
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
TB	Terrific Broth
TBD	To be determined
Type I Water	Ultrapure/Reagent Grade/Critical applications
Zeo	Zeocin

## 6. REAGENTS, MATERIALS AND EQUIPMENT

### 6.1. Reagents

- 6.1.1. Addgene Plasmid Bacterial Stab
- 6.1.2. HPV Serology Laboratory transformed plasmids in DH5a bacteria
- 6.1.3. Fast-Media® Amp Media (Invivogen, Cat # fas-am-b or equivalent)
- 6.1.4. Fast-Media® Kan Media (Invivogen, Cat # fas-kn-b or equivalent)
- 6.1.5. Fast-Media® Blas TB (Invivogen, Cat # fas-bl-l or equivalent)
- 6.1.6. Fast-Media® Zeo TB (Invivogen, Cat # fas-zn-l or equivalent)
- 6.1.7. Fast-Media® Amp Agar (Invivogen, Cat # fas-am-s or equivalent)
- 6.1.8. Fast-Media® Kan Agar (Invivogen, Cat # fas-kn-s or equivalent)
- 6.1.9. Fast-Media® Blas Agar (Invivogen, Cat # fas-bl-s or equivalent)
- 6.1.10. Fast-Media® Zeo Agar (Invivogen, Cat # fas-zn-s or equivalent)
- 6.1.11. 5x Terrific Broth with Kanamycin (Teknova, Cat # T8211-12 or equivalent)

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 4 of 18**

Supersedes

1.0

- 6.1.12. 10x Terrific Broth (Teknova, Cat # T7009 or equivalent)
- 6.1.13. Ampicillin Solution, 100 mg/ml (Teknova, Cat # A9626 or equivalent)
- 6.1.14. LB Broth, 1 L Bottle (Teknova, Cat # L8000-12 or equivalent)
- 6.1.15. Kanamycin Solution, 100 mg/ml (Teknova, Cat # K2135 or equivalent)

6.2. Equipment

- 6.2.1. -80°C Freezer
- 6.2.2. 2-8°C Refrigerator
- 6.2.3. Orbital Shaker
- 6.2.4. Convection Oven
- 6.2.5. Water Bath
- 6.2.6. Microwave
- 6.2.7. Pipettes
- 6.2.8. Class II Biosafety Cabinet (BSC)

6.3. Consumables

- 6.3.1. Petri Dishes (Warehouse, Cat# 66401175)
- 6.3.2. Baffled Glass Flask (Thomas Scientific, Cat # 1234D77 or equivalent)
- 6.3.3. Pipette Tips
- 6.3.4. Serological Pipettes
- 6.3.5. Cryovial (Fisher Scientific, Cat # 12-565-163N or equivalent)
- 6.3.6. Glycerol (Sigma-Aldrich, Cat # G7893-500mL)
- 6.3.7. Flask (Thomas Scientific, Cat # 1234D77)

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 5 of 18**

Supersedes

1.0

- 6.3.8. Type I Water (Milli-Q water system (HSL\_EQ\_019, Q-POD with BioPak cartridge) or Distilled water (Life Technologies, Cat # 15230204 or equivalent))
- 6.3.9. Nalgene 0.2 µm PES membrane 1000 mL filter bottle (Thomas Scientific, Cat # 1234K59 or equivalent)
- 6.3.10. Nalgene 0.2 µm PES membrane 250 mL filter bottle (Thomas Scientific, Cat # 1234K60 or equivalent)
- 6.3.11. Sterile aluminum foil

## 7. HEALTH AND SAFETY CONSIDERATIONS

- 7.1. Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.
- 7.2. Refer to the respective SDS when working with any chemicals.
- 7.3. Refer to "HSL\_GL\_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

## 8. PROCEDURAL PRINCIPLES

- 8.1. All process relevant information is recorded on "HSL\_LAB\_002.01: Preparation of Plasmid Bacterial Stock for Storage in Glycerol Form."
- 8.2. All procedures in this SOP must be performed in the BSC identified for bacterial work per "HSL\_EQ\_001: Biosafety Cabinet (BSC) Use and Maintenance."
- 8.3. To obtain sterile Type I water using the Milli-Q, sterile filter Type I water from the Milli-Q (see "HSL\_EQ\_019: Use and Maintenance of the Milli-Q Integral 3 Water System") using a 0.2µm PES filter.
- 8.4. Sterile filtered Type I water from the Milli-Q or purchased Distilled water from vendor may be used interchangeably as sterile Type I water throughout the procedure.

## 9. PREPARING AGAR PLATE

**Note:** Procedure in section 9 is only performed when using agar powder.

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 6 of 18**

Supersedes

1.0

- 9.1. Pour the pouch contents into a clean, autoclaved 1L borosilicate glass flask or borosilicate glass bottle.

**Note:** Pouch refers to “Fast-Media® Amp/Kan/Blas/Zeo Agar” powder. Make sure to use the appropriate antibiotic additive for bacteria being used; see Attachment 1 for reference.

- 9.2. Add 200 mL of sterile Type I water.
- 9.3. Mix thoroughly by swirling the glass bottle or flask.
- 9.4. Place flask in the microwave and set for 1 minute at full power.
- 9.5. Repeat steps 9.3 and 9.4 two (2) additional times.
- 9.6. Make sure the medium is completely dissolved. Repeat step 9.3 and 9.4 if necessary.
- 9.7. Replace the autoclaved foil cover on the flask and allow the medium to cool to 50-55°C before use. At this temperature, the flask will still be warm to the touch, but cool enough to touch for several seconds.

**Note:** May use water bath set at 50°C per “HSL\_EQ\_010: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath” to maintain temperature.

- 9.8. Place petri dishes (8-10) on flat surface.
- 9.8.1. Remove the lid to a single petri dish, swirl the flask vigorously for several seconds to mix media then remove the foil cover of the flask.
- 9.8.2. Transfer approximately 20 mL LB agar into each petri dish to completely cover the bottom of the dish. Replace the lid of each petri dish as it is poured.
- Note:** May use Serological Pipette.
- 9.8.3. Let the petri dish sit at room temperature until the agar has solidified.
- 9.8.4. Wrap petri dishes with parafilm.
- 9.8.5. Label the plates with date of preparation and antibiotic additive. See Attachment 2 for label example. Dishes should be stored UPSIDE DOWN at 2-8°C and given an expiration date of FOUR (4) WEEKS.

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 7 of 18**

Supersedes

1.0

## 10. PREPARING LIQUID MEDIA

10.1. Preparing Liquid Media from pouch such as Invivogen Fast-Media®.

10.1.1. Pour the pouch contents into a clean, autoclaved 1L borosilicate glass flask or borosilicate glass bottle.

**Note:** Pouch refers to “Fast-Media® Amp/Kan/Blas/Zeo” powder. Make sure to use appropriate antibiotic additive for bacteria being used; see Attachment 1 for reference.

10.1.2. Add 200 mL of Type I water.

10.1.3. Mix thoroughly by swirling the glass bottle or flask.

10.1.4. Place flask in the microwave and set for 1 minute at full power.

10.1.5. Repeat steps 10.1.3 and 10.1.4 two (2) additional times.

10.1.6. Make sure the medium is completely dissolved. Repeat step 10.1.3 and 10.1.4 if necessary.

10.1.7. Replace the autoclaved foil cover on the flask and allow the medium to cool to 25-37°C before use.

**Note:** May use water bath set at 37°C per HSL\_EQ\_010 to maintain temperature.

10.2. Preparing Liquid Media from sterile liquid source.

10.2.1. Prepare fresh 200 mL growth medium with antibiotic in a clean, autoclaved 1 L flask; see Attachment 1 for reference.

10.2.1.1. Dilute stock media as needed using sterile Type I water.

For example, Teknova LB media is used neat without addition of Type I water. Teknova 10x TB media is prepared by mixing 20 mL 10x TB media with 180 mL sterile Type I water.

10.2.1.2. If Kanamycin is required, add to growth media at a final concentration of 50 µg/mL (1:2000 dilution of 1 mg/mL stock concentration).

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 8 of 18**

Supersedes

1.0

For example, 200  $\mu$ L of 1 mg/mL solution into 400 mL growth media.

- 10.2.1.3. If Ampicillin is required, add to growth media at a final concentration of 100  $\mu$ g/mL (1:1000 of 1 mg/mL stock concentration).

For example, 400  $\mu$ L of 1 mg/mL solution into 400 mL growth media.

- 10.2.2. Cool the medium to at least 37°C, but not less than room temperature before use.

## 11. PREPARATION OF 50% GLYCEROL

**Note:** If using previously prepared "Autoclaved 50% Glycerol," N/A "50% Glycerol Preparation" section on HSL\_LAB\_002.01.

- 11.1. Mix equal amount of sterile Type I water and 100% Glycerol in an autoclavable container.
- 11.2. Follow liquid autoclave procedure per HSL\_GL\_001 and autoclave the 50% Glycerol preparation.
- 11.3. Label the bottle as "Autoclaved 50% Glycerol" with Data Reference, preparation date, analyst initials and one (1) year expiration date. Store at room temperature.

**Note:** Data Reference is the Logbook number with page number as a three-digit number. For example, *LAB12345001* where *LB12345* is the Logbook number and *001* is page 1.

## 12. STREAKING A PLATE FROM A PLASMID BACTERIAL STAB CULTURE

- 12.1. Obtain one agar plate with appropriate antibiotic (section 9). Prior to streaking the plate, pre-warm the plate for at least 20 minutes at  $37 \pm 2^\circ\text{C}$  in the Oven per "HSL\_EQ\_017: Use and Maintenance of a Laboratory Convection Oven" or Orbital Shaker per "HSL\_EQ\_011: Use and Maintenance of the Forma Scientific Orbital Shaker" to ensure evaporation of any residual condensation.
- 12.2. Label the bottom of the plate with the plasmid name, antibiotic resistance, date and analyst initials.
- 12.3. Using a sterile pipette tip, touch the bacteria growing within the punctured area of the stab culture. See Figure 1 below.

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 9 of 18**

Supersedes

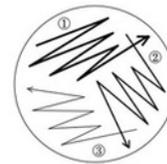
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Figure 1: Bacterial Stab



Figure 2: Plate Streak

Streak Diagram



Single Colony on Streaked Plate



- 12.4. Run the tip lightly over a section of the plate to spread the bacteria over approximately one-third of the surface area of the plate, as shown in Figure 2, to create streak #1.
- 12.5. Using a fresh sterile pipette tip or sterile loop, pass through streak #1 and spread the bacteria over the next one-third section of the plate, to create streak #2.
- 12.6. Using a third sterile pipette tip or sterile loop, pass through streak #2 and spread the bacteria over the last one-third section of the plate, to create streak #3.
- 12.7. Incubate the plate overnight (12-18 hours) at the designated growth temperature using the Oven per HSL\_EQ\_017 or Orbital Shaker per HSL\_EQ\_011.

**Note:** Although the growth temperature is often 37°C, some plasmids require a different temperature or growth condition. Refer to manufacturer plasmid information page for specific growth conditions.

**Note:** DO NOT use a CO<sub>2</sub> culture incubator for this incubation.

- 12.8. Check for single colonies. A single colony should appear as a white dot growing on the solid medium. See Figure 2.
- 12.9. Once single colonies are obtained, proceed to Section 13, Inoculating an Overnight Liquid Culture.

### 13. INOCULATING AN OVERNIGHT LIQUID CULTURE

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 10 of 18**

Supersedes

1.0

- 13.1. Obtain appropriate liquid media for the bacteria desired (section 10).
- 13.2. Label a sterile tube or flask with the HPV type information, date, and analyst initials.
- 13.3. Add 10 mL liquid media to the labeled sterile tube or flask.
- 13.4. Using a sterile pipette tip or loop, select a single colony from the streaked agar plate (section 12).
- 13.5. Drop the tip or loop into the liquid media then swirl.
- 13.6. Loosely cover the culture with sterile aluminum foil or a cap that is not air tight.
- 13.7. Incubate bacterial culture at  $37 \pm 2^{\circ}\text{C}$  at  $250 \pm 10$  RPM for 12-18 hours in the Orbital Shaker per HSL\_EQ\_011.

#### 14. CREATING GLYCEROL STOCK OF THE PLASMID

- 14.1. Label cryovials with the following information: Plasmid name, Lot number, Preparation date, Analyst Initials, Volume, and Liquid Media composition. See Attachment 2 for label example.

**Note:** The Lot number is the Logbook number with page number as a three-digit number. For example, *LB12345001* where *LB12345* is the logbook number and *001* is page 1.

- 14.2. When bacterial growth is observed, add equal volume of 50% glycerol (section 11) in the tube with bacteria. Gently mix by inversion.
- 14.3. Aliquot 1 mL into each labeled cryovial.
- 14.4. Place cryovials into a box labelled per Attachment 2 and freeze at  $-80 \pm 10^{\circ}\text{C}$ . The stock is stable when stored at  $-80 \pm 10^{\circ}\text{C}$  (see Table 1 below).

Table 1: Approximate Time Bacterial Cultures Remain Stable

Condition	Temperature ( $^{\circ}\text{C}$ )	Time (approximate)
Agar plates	4	4 weeks
Stab cultures	4	2 weeks
Glycerol Stock	-80	1-10 years

- 14.5. To recover bacteria from bacterial stock, thaw the vial on wet ice and follow the procedure in Section 10.2 of "HSL\_LAB\_004: Plasmid Purification using a Qiagen Kit."

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 11 of 18**

Supersedes

1.0

**Note:** Vials are single use.

## 15. ATTACHMENTS

- 15.1. Attachment 1: List of Plasmids and Characteristics
- 15.2. Attachment 2: Petri Dish Labels, Vial Label and Box Label
- 15.3. Attachment 3: HSL\_LAB\_002.01: Preparation of Plasmid Bacterial Stock for Storage in Glycerol Form

## 16. REVISION HISTORY

Version	Change	Reason
1.0	Create new SOP for plasmid bacterial stab in glycerol stock preparation.	Currently no SOP
2.0	<ol style="list-style-type: none"> <li>1. Transfer SOP to new template, form HSL_LAB_002.01 as separate form</li> <li>2. Remove HSL_GL_002, HSL_GL_003, HSL_GL_004, HSL_GL_006, HSL_GL_007, HSL_GL_008, HSL_GL_009, HSL_GL_010, HSL_EQ_009 from References section.</li> <li>3. Split Equipment and Consumables section into two subsections.</li> <li>4. Added additional media and consumables.</li> <li>5. Removed FME, HPV, HSL, and added TB and TBD to definitions section.</li> <li>6. Added Attachment 1 containing media for each plasmid type.</li> <li>7. Added Attachment 2 containing label templates.</li> <li>8. Grammar and formatting corrections throughout document.</li> </ol>	<ol style="list-style-type: none"> <li>1. Consistency between procedures.</li> <li>2. SOPs not referenced in procedure.</li> <li>3. Consistency between procedures, clarification.</li> <li>4. Reflect current practice.</li> <li>5. FME, HSL not listed in procedure. HPV captured in Purpose. TB and TBD added to procedure.</li> <li>6. Clarification.</li> <li>7. Clarification.</li> <li>8. Clarification.</li> </ol>

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 12 of 18**

Supersedes

1.0

**Attachment 1: List of Plasmids and Characteristics**

<b>COMPANY</b>	<b>PLASMID</b>	<b>BACTERIA STRAIN</b>	<b>ANTIBIOTIC</b>	<b>GROWTH MEDIA</b>
ADDGENE	PVITRO-HPV6 L1L2	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	PVITRO-HPV52 L1L2	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	PVITRO-HPV31 L1L2	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	PVITRO-HPV18 L1L2	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	PVITRO-HPV11 L1L2	E.COLI_DH5A	KANAMYCIN	TB
ADDGENE	PVITRO-HPV33 L1L2	E.COLI_DH5A	KANAMYCIN	LB
ADDGENE	P11L2W	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	P58SHELL	E.COLI_DH5A	AMPICILLIN	LB
ADDGENE	P18SHELL	E.COLI_DH5A	AMPICILLIN	TB
ADDGENE	P31SHELL	E.COLI_DH5A	AMPICILLIN	LB
ADDGENE	P52SHELL	E.COLI_DH5A	AMPICILLIN	TB
ADDGENE	P11L1W	E.COLI_DH5A	KANAMYCIN	TBD
ADDGENE	P45SHELL	E.COLI_DH5A	AMPICILLIN	LB
ADDGENE	P16SHELL	E.COLI_DH5A	AMPICILLIN	TB
ADDGENE	P6SHELLR	E.COLI_DH5A	AMPICILLIN	TB
SCHILLER	PFWB (EGFP)	E.COLI_DH5A	BLASTICIDIN	LB
SCHILLER	PYSEAP	E.COLI_DH5A	BLASTICIDIN	TBD

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

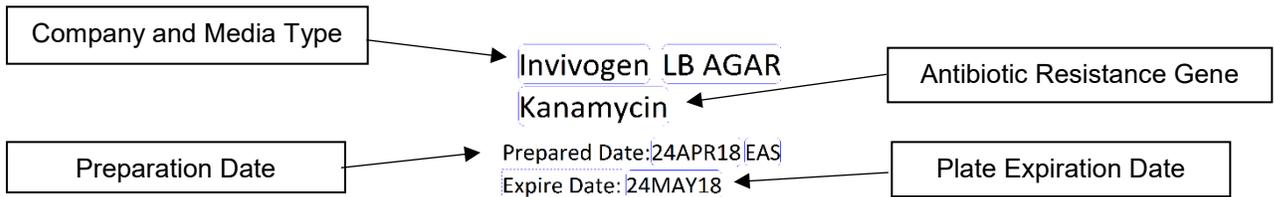
**Page 13 of 18**

Supersedes

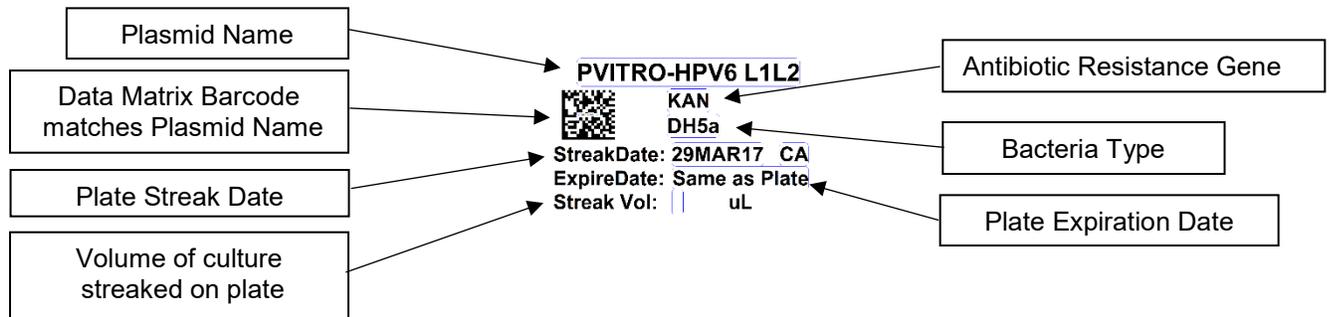
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**Attachment 2: Petri Dish Labels, Vial Label and Box Label**

AGAR ONLY PETRI DISH LABEL



AGAR AND BACTERIA WITH TRANSFORMED PLASMID PETRI DISH LABEL



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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

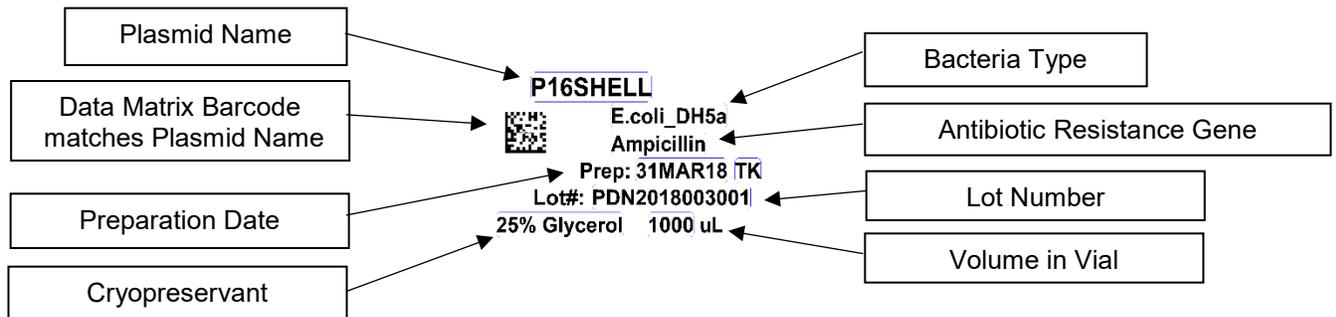
2.0

**Page 14 of 18**

Supersedes

1.0

GLYCEROL PLASMID BACTERIAL STOCK VIAL LABEL



GLYCEROL PLASMID BACTERIAL STOCK BOX LABEL

Study: *E. coli (DH5a) HPV16 pShell*  
 Sample Type: *Glycerol- Bacteria*  
 Date: *27AUG17*  
 Initials: *TK*  
 Box 1 of 1

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**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 15 of 18**

Supersedes

1.0

**Attachment 3: HSL\_LAB\_002.01: Preparation of Plasmid Bacterial Stock for Storage in Glycerol Form**

<p><b>Frederick National Laboratory for Cancer Research</b> <i>sponsored by the National Cancer Institute</i></p>		<p>HPV Serology Laboratory Standard Operating Procedure Form</p>	
<p><b>Form Title:</b> Preparation of Plasmid Bacterial Stock for Storage in Glycerol Form</p>			
<p><b>Document ID:</b> HSL_LAB_002.01</p>		<p>Version:</p>	<p>2.0</p>
<p>Associated SOP: HSL_LAB_002</p>		<p>Effective Date:</p>	
<p>Supersedes Version:</p>	<p>1.0</p>	<p><b>Page 1 of 4</b></p>	

**Equipment**

Equipment Name	Equipment ID	Calibration Due Date
BSC	<input type="checkbox"/> HSL_007 <input type="checkbox"/> HSL_008 <input type="checkbox"/> HSL_009 <input type="checkbox"/> Other _____	
-80°C Freezer	<input type="checkbox"/> HSL_022 <input type="checkbox"/> Other _____	
2-8°C Refrigerator	<input type="checkbox"/> HSL_029 <input type="checkbox"/> Other _____	
Water Bath	<input type="checkbox"/> HSL_010 <input type="checkbox"/> Other _____	
Orbital Shaker	<input type="checkbox"/> HSL_011 <input type="checkbox"/> Other _____	
Convection Oven	<input type="checkbox"/> HSL_025 <input type="checkbox"/> Other _____	
Microwave	<input type="checkbox"/> HSL_053 <input type="checkbox"/> Other _____	
Pipette _____ μL	PIP_	
Pipette _____ μL	PIP_	

**Reagents**

	Lot Number	Expiration Date
<input type="checkbox"/> N/A Fast-Media Amp Media		
<input type="checkbox"/> N/A Fast-Media Kan Media		
<input type="checkbox"/> N/A Fast-Media Blas TB		
<input type="checkbox"/> N/A Fast-Media Zeo TB		
<input type="checkbox"/> N/A Fast-Media Amp Agar		
<input type="checkbox"/> N/A Fast-Media Kan Agar		
<input type="checkbox"/> N/A Fast-Media Blas Agar		
<input type="checkbox"/> N/A Fast-Media Zeo Agar		
<input type="checkbox"/> N/A LB		
<input type="checkbox"/> N/A TB		
<input type="checkbox"/> N/A Kanamycin		
<input type="checkbox"/> N/A Ampicillin		
<input type="checkbox"/> N/A Autoclaved 50% Glycerol		
<input type="checkbox"/> N/A 100% Glycerol		

<p>Performed by/date:</p>	
<p>Reviewed by/date:</p>	

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**Frederick National Laboratory  
for Cancer Research**

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**HPV Serology Laboratory  
Standard Operating Procedure**

**SOP Title:** Preparation of Plasmid Bacterial Stock for Storage in Glycerol

**Document ID:** HSL\_LAB\_002

Version

2.0

**Page 17 of 18**

Supersedes

1.0

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<b>Form Title:</b> Preparation of Plasmid Bacterial Stock for Storage in Glycerol Form			
<b>Document ID:</b> HSL_LAB_002.01	Version:	2.0	
Associated SOP: HSL_LAB_002	Effective Date:		
Supersedes Version:	1.0	<b>Page 3 of 4</b>	

**Agar Plate Incubation:**

Temperature (°C):		Analyst Initials:	
Start Time / Date:			
End Time / Date:		Analyst Initials:	

**Liquid Media Preparation:**

Volume of Type I Water (mL)	Fast-Media or LB/TB Media Used (Amp/Kan/Bias)	# Fast-Media Pouches Added or Volume of LB/TB (mL)
Performed by/date:		

**Overnight Culture Inoculation:**

Temperature (°C):		Analyst Initials:	
Incubation Start Time / Date:			
Incubation End Time / Date:		Analyst Initials:	

**50% Glycerol Preparation:**  N/A

Volume of Type I Water (mL)	Volume of 100% Glycerol (mL)	Date Prepared and Autoclaved
Performed by/date:		

**Comments:**

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N/A

Reviewed by/date:	
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