

**SOP Title:** Bacterial Culture Maintenance

**Document ID:** 20000

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**Effective Date:** 06Jun22

## 1. PURPOSE

1.1 The purpose of this procedure is to describe the maintenance of bacteria cultures.

## 2. SCOPE

2.1 This procedure applies to all bacterial plasmid culture being used for HPV VLP production.

## 3. REFERENCES

3.1 15000: Waste Disposal at the Advanced Technology Research Facility

3.2 26000: Biosafety Cabinet (BSC) Use and Maintenance

3.3 26005: Use and Maintenance of a 2-8°C Refrigerator

3.4 26007: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath

3.5 26008: Use and Maintenance of the Orbital Shaker

3.6 26009: Use and Maintenance of Pipettes

3.7 26014: Use and Maintenance of a Laboratory Convection Oven

3.8 26016: Use and Maintenance of the Water Purification Systems

3.9 26030: Use and Maintenance of -80°C Freezers

## 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.

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

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## 5. DEFINITIONS

- 5.1 Amp – Ampicillin
- 5.2 Blas – Blasticidin
- 5.3 Kan – Kanamycin
- 5.4 LB - Luria broth
- 5.5 SDS - Safety Data Sheets
- 5.6 SOP - Standard Operating Procedure
- 5.7 TB - Terrific Broth
- 5.8 TOC – Total Oxidizable Carbon
- 5.9 Type I Water – Ultrapure/Reagent Grade/critical applications (Resistivity >18 MΩ-cm and TOC ≤ 50 ppb)
- 5.10 Zeo - Zeocin

## 6. REAGENTS, MATERIALS AND EQUIPMENT

- 6.1 Reagents
  - 6.1.1 HPV Serology Laboratory transformed plasmids in DH5a bacteria
  - 6.1.2 Fast-Media® Amp Media (Invivogen, Cat # fas-am-b or equivalent)
  - 6.1.3 Fast-Media® Kan Media (Invivogen, Cat # fas-kn-b or equivalent)
  - 6.1.4 Fast-Media® Blas TB (Invivogen, Cat # fas-bl-l or equivalent)
  - 6.1.5 Fast-Media® Zeo TB (Invivogen, Cat # fas-zn-l or equivalent)
  - 6.1.6 Fast-Media® Amp Agar (Invivogen, Cat # fas-am-s or equivalent)
  - 6.1.7 Fast-Media® Kan Agar (Invivogen, Cat # fas-kn-s or equivalent)

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- 6.1.8 Fast-Media® Blas Agar (Invivogen, Cat # fas-bl-s or equivalent)
- 6.1.9 Fast-Media® Zeo Agar (Invivogen, Cat # fas-zn-s or equivalent)
- 6.1.10 5x Terrific Broth with Kanamycin (Teknova, Cat # T8211-12 or equivalent)
- 6.1.11 10x Terrific Broth (Teknova, Cat # T7009 or equivalent)
- 6.1.12 Ampicillin Solution, 100 mg/ml (Teknova, Cat # A9626 or equivalent)
- 6.1.13 LB Broth, 1 L Bottle (Teknova, Cat # L8000-12 or equivalent)
- 6.1.14 Kanamycin Solution, 100 mg/ml (Teknova, Cat # K2135 or equivalent)
- 6.1.15 Zeocin Solution, 100 mg/ml (Invivogen, Cat # ant-zn-05 or equivalent)
- 6.1.16 Bleach, Concentrated (FNLCR Warehouse, Cat # 68100251 or equivalent)
- 6.1.17 Primary Disinfectant (Cavicide, FNLCR Warehouse, Cat # 79300360 or equivalent)
- 6.1.18 Secondary Disinfectant (Ster-ahol, VWR, Cat # 14003-358 or equivalent)

## 7. Equipment

- 7.1.1 -80°C (Range: -65 to -90°C) Freezer
- 7.1.2 2-8°C Refrigerator
- 7.1.3 Erlenmeyer filter flask
- 7.1.4 Orbital Shaker
- 7.1.5 Convection Oven
- 7.1.6 Water Bath
- 7.1.7 Microwave
- 7.1.8 Pipettes

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7.1.9 Serological Pipettor

7.1.10 Class II Biosafety Cabinet (BSC)

7.1.11 Silicone Hand Protector (Thomas Scientific Cat # 1150H33 or equivalent)

7.1.12 Heat Resistant Gloves (Thomas Scientific Cat # 1176R24 or equivalent)

7.2 Consumables

7.2.1 Baffled Glass Flask (Thomas Scientific, Cat # 1234D77 or equivalent)

7.2.2 Pipette Tips

7.2.3 Sterile Beaker or Graduated Cylinder

7.2.4 Serological Pipettes

7.2.5 Sterile filtered Type I Water (water purification system (26016, Q-POD with BioPak cartridge) or Distilled water (Life Technologies, Cat # 15230204 or equivalent))

7.2.6 Nalgene 0.2 µm PES membrane 1000 mL filter bottle (Thomas Scientific, Cat # 1234K59 or equivalent)

7.2.7 Nalgene 0.2 µm PES membrane 250 mL filter bottle (Thomas Scientific, Cat # 1234K60 or equivalent)

7.2.8 Sterile aluminum foil

7.2.9 Wipe, Low-Lint, Wypalls (Warehouse, Cat # 79300335 or equivalent)

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## 8. HEALTH AND SAFETY CONSIDERATIONS

- 8.1 Proper safety precautions must 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.
- 8.2 Refer to the respective SDS when working with any chemicals.
- 8.3 Refer to “15000: Waste Disposal at the Advanced Technology Research Facility” regarding waste disposal processes at the ATRF.

## 9. PROCEDURAL PRINCIPLES

- 9.1 All process relevant information is recorded on “20000-01: Bacterial Culture Maintenance Form.”
- 9.2 When available, all steps are performed in the BSC “26000: Biosafety Cabinet (BSC) Use and Maintenance.”
- 9.3 To obtain sterile Type I water using the Water Purification Systems, sterile filter Type I water (see “26016: Use and Maintenance of Water Purification Systems”) using a 0.2µm PES filter. Reagent expires 2 months from date of preparation and is stored at room temperature.
- 9.4 Sterile filtered Type I water from the Water Purification Systems or purchased Distilled water from vendor may be used interchangeably as sterile Type I water throughout the procedure.

## 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 flask or glass bottle.
  - 10.1.2 **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.
  - 10.1.3 Add 200 mL of Type I water.

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10.1.4 Mix thoroughly by swirling the glass bottle or flask.

10.1.5 Remove foil and place flask in the microwave and heat on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes)

10.1.6 Repeat steps 10.1.4 and 10.1.5 until medium is completely dissolved. Do not overboil. Use heat resistant gloves or silicone hand protectors to hold flask.

10.1.7 Replace the autoclaved foil cover on the flask and allow the medium to cool to 20-37°C before use.

**Note:** May use water bath set at  $37 \pm 2^\circ\text{C}$  per "26007: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath" 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 flask; see Attachment 1 for reference.

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

10.2.1.2 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.

10.2.1.3 If Kanamycin is required, add to growth media at a final concentration of 50 µg/mL (1:2000 dilution of 100 mg/mL stock concentration).

10.2.1.4 For example, 200 µL of 100 mg/mL solution into 400 mL growth media.

10.2.1.5 If Ampicillin is required, add to growth media at a final concentration of 100 µg/mL (1:1000 of 100 mg/mL stock concentration).

For example, 200 µL of 100 mg/mL solution into 200 mL growth media.

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## 11. INOCULATING AN OVERNIGHT LIQUID STARTER CULTURE FROM A GLYCEROL STOCK

- 11.1 To recover bacteria from glycerol stock, thaw the vial on wet ice.
- 11.2 **Note:** Vials are single use.
- 11.3 Thaw antibiotic selection on wet ice or in 2-8°C refrigerator.
- 11.4 Label a sterile tube or flask with the HPV type information, date, and analyst initials. See Attachment 2 for reference.
- 11.5 Add 0.1 mL of glycerol stock to flask with 200 mL of liquid media with appropriate antibiotic selection for plasmid and swirl to mix. (See Attachment 1, and Section 9)
- 11.6 Loosely cover the culture with sterile aluminum foil or cap.
- 11.7 Incubate bacterial culture at  $37 \pm 2^\circ\text{C}$  at  $250 \pm 10$  RPM for 12-18 hours in the Orbital Shaker per "26008: Use and Maintenance of the Orbital Shaker."
- Note:** Certain plasmids may require different incubation time and temperature, consult with Scientific Manager.
- 11.8 Starter cultures can be stored at 2-8°C for 1 week after incubation and can be passaged no more than 4 times.

## 12. INOCULATING AN OVERNIGHT STARTER CULTURE FROM A PREVIOUS STARTER CULTURE

- 12.1 Bacterial starter cultures should be maintained for use no more than 4 passages. Any deviations require approval by the Scientific Manager.
- 12.2 Thaw antibiotic on wet ice.
- 12.3 Label a sterile tube or flask with the HPV type information, date, and analyst initials. See Attachment 2 for reference.
- 12.4 Add 0.1 mL of Starter Culture to flask with 200 mL of liquid media with appropriate antibiotic selection for plasmid and swirl to mix. (See Attachment 1, and Section 9)
- 12.5 Loosely cover the culture with sterile aluminum foil or a cap.

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- 12.6 Incubate bacterial culture at  $37 \pm 2^\circ\text{C}$  at  $250 \pm 10$  RPM for 12-18 hours in the Orbital Shaker per 26008.

**Note:** Certain plasmids may require different incubation time and temperature, consult with Scientific Manager or designee.

Table 1: Approximate Time Bacterial Cultures Remain Stable

Condition	Temperature ( $^\circ\text{C}$ )	Time (approximate)
Glycerol Stock	-65 to -90	1-10 years
Starter Culture	2-8	1 week

### 13. FORM REVIEW

- 13.1 Analyst fills out data capture form during testing and dates and initials form.
- 13.2 A second review of the data capture form is completed by the Scientific Manager or designee (Group Leader) for completeness and dates and initials form.

### 14. ATTACHMENTS

- 14.1 Attachment 1: List of Plasmids and Characteristics
- 14.2 Attachment 2: Starter Culture Flask Label
- 14.3 Attachment 3: 20000-01 Bacterial Culture Maintenance Form

### 15. REVISION HISTORY

Version	Change	Reason
1.0	New SOP	Currently no SOP

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Version	Change	Reason
2.0	<ol style="list-style-type: none"> <li>1. Updated Fast-Media preparation to reflect product instructions in section 9.1</li> <li>2. Added heat resistant glove and silicone hand protectors to Equipment in section 6.2</li> </ol>	<ol style="list-style-type: none"> <li>1. Reflect current practices</li> <li>2. Reflect current practices</li> </ol>
3.0	<ol style="list-style-type: none"> <li>1. Change in Form 20000-01: Orbital shaker temperature range, temperature recording at time of start of incubation and temperature recording at the end of incubation</li> <li>2. Corrected reference to form as 20000-01 throughout SOP.</li> <li>3. Removed 35°C incubation temperature.</li> <li>4. Include in Form 2000-01: Water bath temperature for media preparation</li> </ol>	<ol style="list-style-type: none"> <li>1. As per requirements to document all temperatures</li> <li>2. Accurate reference.</li> <li>3. Reflect current practices</li> <li>4. As per requirements to document all temperatures</li> </ol>

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

<i>COMPANY</i>	<i>PLASMID</i>	<i>BACTERIA STRAIN</i>	<i>ANTIBIOTIC</i>	<i>ANTIBIOTIC CONC.</i>	<i>GROWTH MEDIA</i>
ADDGENE	PVITRO-HPV6 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	PVITRO-HPV52 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	PVITRO-HPV31 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	PVITRO-HPV18 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	PVITRO-HPV11 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	PVITRO-HPV33 L1L2	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	P11L2W	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	P58SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
ADDGENE	P18SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
ADDGENE	P31SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
ADDGENE	P52SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB

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<i>COMPANY</i>	<i>PLASMID</i>	<i>BACTERIA STRAIN</i>	<i>ANTIBIOTIC</i>	<i>ANTIBIOTIC CONC.</i>	<i>GROWTH MEDIA</i>
ADDGENE	P11L1W	E.COLI_DH5A	KANAMYCIN	50 µg/mL	LB or TB
ADDGENE	P45SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
ADDGENE	P16SHELL	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
ADDGENE	P6SHELLR	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB
SCHILLER	PFWB (EGFP)	E.COLI_DH5A	ZEOCIN	25 µg/mL	LB or TB
SCHILLER	PYSEAP	E.COLI_DH5A	BLASTICIDIN	75 µg/mL	LB or TB
SCHILLER	PHPV16L1H	E.COLI_DH5A	AMPICILLIN	100 µg/mL	LB or TB

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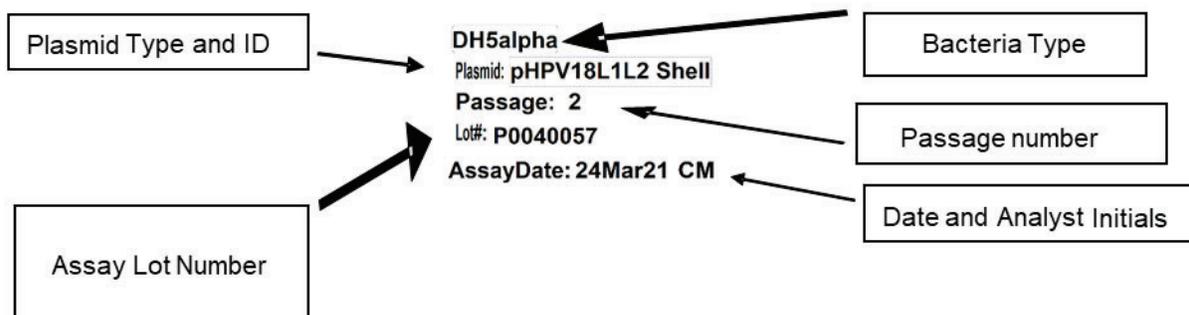
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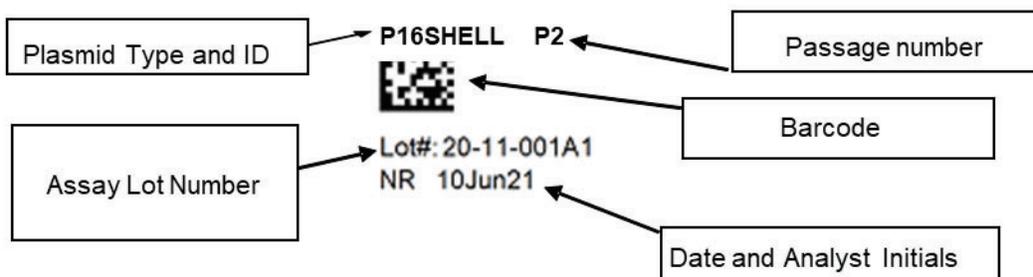
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**Attachment 2: Starter Culture Flask Label**

Starter Culture Flask Label (Manual Label)



Starter Culture Flask Label (LIMS Label)



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Standard Operating Procedure Form

**Form Title:** Bacterial Plasmid Culture Form

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Plasmid Being Purified: \_\_\_\_\_ Passage #: \_\_\_\_\_

**Equipment**

Equipment Name	Equipment ID	Calibration Due Date
BSC	<input type="checkbox"/> HSL_009 <input type="checkbox"/> Other:	
Orbital Shaker	<input type="checkbox"/> HSL_011 <input type="checkbox"/> HSL_050 <input type="checkbox"/> Other:	
<input type="checkbox"/> N/A Water Bath (Range: 37±2°C)	<input type="checkbox"/> HSL_010 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Microwave	<input type="checkbox"/> HSL_053 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A 2-8°C Refrigerator	<input type="checkbox"/> HSL_043 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Pipette:                      μL	PIP_	
<input type="checkbox"/> N/A Pipette:                      μL	PIP_	
<input type="checkbox"/> N/A Water Purification System	HSL_	

**Reagents**

	Lot Number	Expiration Date
<input type="checkbox"/> N/A Distilled water	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Fast-Media Amp Media	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Fast-Media Kan Media	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Fast-Media Blas Media	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Fast-Media Zeo Media	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A LB	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A TB	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Ampicillin	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Kanamycin	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Zeocin	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Glycerol Stock		<input type="checkbox"/> N/A
<input type="checkbox"/> N/A Starter Culture		
<input type="checkbox"/> N/A Filter Unit	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
<input type="checkbox"/> N/A	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

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**Liquid Media Preparation for Starting Culture:**

Fast-Media Pouches  N/A Section, LB or TB used

Type I Water Sterile Filtered	Volume of Water (mL)	# of Fast-Media Pouches Added	Total # of Flasks Prepared
<input type="checkbox"/> Yes <input type="checkbox"/> N/A			

LB or TB  N/A Section, Fast-Media Pouches used

Volume of LB or TB (mL)	Volume of Type I water Sterile Filtered (mL) <input type="checkbox"/> N/A	Volume of Prepared Antibiotic Added ( $\mu$ L)	Total # of Flasks prepared
Water Bath Temperature	<input type="checkbox"/> N/A	Performed by/date	<input type="checkbox"/> N/A

**Starting Culture Preparation:**

Volume of Glycerol Stock per Flask (mL)	Volume of Liquid Media per Flask (mL)

**Overnight Incubation of Starting Culture:**

Incubation Temperature 37 $\pm$ 2°C:		Incubation Start Time / Date:	
Performed by/date:			
Incubation Temperature 37 $\pm$ 2°C:		Incubation End Time / Date:	
Total Hours incubated		Performed by/date	
Expiration Date of Starter Culture		Storage Location of Starter Culture	
Reviewed by/date:			

Comments:  N/A

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