

Frederick National Laboratory for Cancer Research <small>sponsored by the National Cancer Institute</small>	HPV Serology Laboratory Standard Operating Procedure	
Plasmid Purification Using a QIAGEN HiSpeed Plasmid Maxi Kit		
Document ID: HSL_LAB_004	Version 2.0	Page 1 of 14

Released by/Date Effective:

Author Name	Title	Signature/Date

Approver Name	Title	Signature/Date

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1. PURPOSE

- 1.1. The purpose of this procedure is to describe how to use the QIAGEN HiSpeed Maxiprep kit for plasmid purification.

2. SCOPE

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

3. REFERENCES

- 3.1. QIAGEN Plasmid Purification Handbook
- 3.2. HSL_LAB_004.02: Plasmid Purification Form: Maxi Prep
- 3.3. HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility
- 3.4. HSL_GL_003: Good Documentation Practices for the HPV Serology Laboratory
- 3.5. HSL_GL_004: Laboratory Notebook Control and Use for the HPV Serology Laboratory
- 3.6. HSL_GL_006: Reagent Preparation for the HPV Serology Laboratory
- 3.7. HSL_GL_007: Reagent and Chemical Expiry in the HPV Serology Laboratory
- 3.8. HSL_GL_008: Laboratory Flow and Gowning Procedures for the HPV Serology Laboratory
- 3.9. HSL_GL_009: HPV Serology Laboratory BSL-2 Procedures
- 3.10. HSL_GL_010: Control and Request of Documents in the HPV Serology Laboratory
- 3.11. HSL_EQ_001: Biosafety Cabinet (BSC) Use and Maintenance
- 3.12. HSL_EQ_003: Use and Maintenance of the Thermo Fisher Sorvall Legend XTR Centrifuge in the HPV Serology Laboratory
- 3.13. HSL_EQ_007: Use and Maintenance of a 2-8°C Refrigerator in the HPV Serology Laboratory
- 3.14. HSL_EQ_008: Use and Maintenance of -80°C Freezers in the HPV Serology Laboratory
- 3.15. HSL_EQ_010: Use and Maintenance of the Fisher Scientific Isotemp GDP10 Water Bath
- 3.16. HSL_EQ_011: Use and Maintenance of the Forma Scientific Orbital Shaker

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- 3.17. HSL_EQ_012: Use and Maintenance of Pipettes in the HPV Serology Laboratory
- 3.18. HSL_EQ_016: Use and Maintenance of -20°C Freezers in the HPV Serology Laboratory
- 3.19. HSL_EQ_017: Use and Maintenance of a Laboratory Convection Oven
- 3.20. HSL_EQ_019: Use and Maintenance of the Milli-Q Integral 3 Water System
- 3.21. HSL_EQ_020: Use and Maintenance of the Eppendorf Centrifuge
- 3.22. HSL_EQ_021: Use and Maintenance of NanoDrop 1000 Spectrophotometer
- 3.23. HSL_LAB_008: Bacterial Cell Culture Measurement Using the GeneQuant Calculator
- 3.24. HSL_LAB_016: DNA Quantitation using the NanoDrop
- 3.25. HSL_TRN_001: Training Program for the HPV Serology Laboratory

4. RESPONSIBILITIES

- 4.1. The Research Associate, hereafter referred to 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.

5. REAGENTS, CHEMICALS AND EQUIPMENT

- 5.1. Reagents
 - 5.1.1. QIAGEN Plasmid Maxi Kit (QIAGEN, Cat # 12162)
 - 5.1.2. Fast-Media® Amp Media (LB Media) (InvivoGen, Cat # fas-am-b or equivalent)
 - 5.1.3. Fast-Media® Kan Media (LB Media) (InvivoGen, Cat # fas-kn-b or equivalent)
 - 5.1.4. Fast-Media® Blas Media (TB Media) (InvivoGen, Cat # fas-bl-l or equivalent)
 - 5.1.5. 5x Terrific Broth with Kanamycin (Teknova, Cat # T8211-12 or equivalent)

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- 5.1.6. 10x Terrific Broth (Teknova, Cat # T7009 or equivalent)
- 5.1.7. Ampicillin Solution, 100mg/ml (Teknova, Cat # A9626 or equivalent)
- 5.1.8. LB Broth, 1 L Bottle (Teknova, Cat # L8000-12 or equivalent)
- 5.1.9. Kanamycin Solution, 100mg/ml (Teknova, Cat # K2135 or equivalent)
- 5.1.10. Distilled water (Life Technologies, Cat # 15230204 or equivalent)
- 5.1.11. Ethanol, 200% proof (Sigma, Cat # E-7023-500ml or equivalent)
- 5.1.12. Isopropanol (Sigma, Cat # PX1827-7 or equivalent)
- 5.1.13. Sodium Acetate (Fisher Scientific, Cat # AM9740 or equivalent)
- 5.1.14. TE Buffer (Sigma, Cat # 93283 or equivalent)
- 5.1.15. Bleach (Warehouse, Cat # 68100251 or equivalent)
- 5.1.16. Glycogen (Thomas Scientific, Cat # C913F62 or equivalent)
- 5.1.17. Cavicide (Warehouse, Cat # 79300360)
- 5.2. Equipment and Consumables
 - 5.2.1. QIARack (QIAGEN, Cat # 19015 or equivalent)
 - 5.2.2. XTR Centrifuge
 - 5.2.3. Eppendorf Centrifuge
 - 5.2.4. Biosafety Cabinet II (BSC)
 - 5.2.5. Orbital Shaker
 - 5.2.6. Convection Oven
 - 5.2.7. GeneQuant Calculator
 - 5.2.8. Nanodrop 1000 Spectrophotometer
 - 5.2.9. Water bath
 - 5.2.10. Microwave
 - 5.2.11. -80°C Freezer
 - 5.2.12. 2-8°C Refrigerator

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- 5.2.13. 50 mL Conical Tubes (Warehouse, Cat # 66401493 or equivalent)
- 5.2.14. Pipettes and Pipette Tips
- 5.2.15. 1 L Flask (VWR, Cat # 32645-056 or equivalent)
- 5.2.16. Nalgene Bottles (VWR, Cat # 21020-028 or equivalent)
- 5.2.17. Wet ice
- 5.2.18. 2" Box and 81 positions insert (Warehouse, Cat # 81150001 and 81150004 or equivalent)
- 5.2.19. 1.5 mL Tubes (Thomas Scientific, Cat # 1228H20 or equivalent)
- 5.2.20. 500 mL Centrifuge Tube (Corning, Cat # 431123 or equivalent)
- 5.2.21. Cell Strainer (Thomas Scientific, Cat # 1198D11 or equivalent)
- 5.2.22. Milli-Q water system
- 5.2.23. Nalgene 0.2 µm PES membrane 1000 mL filter bottle (Thomas Scientific, Cat # 1234K59 or equivalent)

6. HEALTH AND SAFETY CONSIDERATIONS

- 6.1. 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.
- 6.2. Refer to the respective SDS when working with any chemicals.
- 6.3. Refer to "HSL_GL_001: Waste Disposal at the Advanced Technology Research Facility" regarding waste disposal processes at the ATRF.

7. DEFINITIONS

Term	Definition
Amp	Ampicillin
FME	Facilities, Maintenance and Engineering
HPV	Human Papilloma Virus
HSL	HPV Serology Laboratory
Kan	Kanamycin
LB	Luria broth
RCF (g)	Relative Centrifugal Force
RPM	Revolutions per minute
SDS	Safety Data Sheets
SOP	Standard Operating Procedure
Type I Water	Ultrapure/Reagent Grade/Critical applications

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8. QIAGEN MAXI KIT PROCEDURE

8.1. Bacteria Culture Growth

8.1.1. Starter Culture

8.1.1.1. Colony/Stab: If starting post-transformation or post-bacterial stab, pick a single colony from a freshly-streaked selective plate or use a stabbing and inoculate a starter culture of 2–5 ml growth medium containing the appropriate selective antibiotic. Incubate the starter culture overnight at 37±2°C with shaking at 250 RPM.

8.1.1.2. Glycerol Stock: If inoculating via glycerol stock, take 0.1 mL glycerol stock or volumes as needed for optimal plasmid growth and place into 200 mL growth medium that contains the appropriate selective antibiotic. Incubate overnight at 37±2°C in a shaking incubator set at 250 RPM to achieve an optimal OD range between 0.8 to 1.4. Record the OD on Form HSL_LAB_004.02.

Note: Typically, 16-18 hours of incubation will achieve the optimal OD range; however, certain plasmids may require longer incubation.

8.2. Culture Growth

8.2.1. Prepare fresh 400 mL growth medium with antibiotic in a clean, autoclaved 1 L flask. Cool the medium to at least 37°C, but not less than room temperature before use.

Note: Number of flasks to prepare will vary depending on the needs of the experiment. It may range from 1- 13 flasks.

Note: If Kanamycin is required, it should be added to the growth media at final concentration of 50 µg/mL (1:2000 dilution of 1 mg/mL stock concentration). Ex. 200 µL of 1 mg/mL solution into 400 mL growth media.

Note: If Ampicillin is required, it should be added to the growth media at final concentration of 100 µg/mL (1:1000 dilution of 1 mg/mL stock concentration). Ex. 400 µL of 1 mg/mL solution into 400 mL growth media.

Note: Water used to prepare growth media may be either Distilled water purchased from manufacture or obtained as sterile filtered Type I water from Milli-Q system.

8.2.2. Dispense 2 mL of the starter culture into the freshly prepared 400 mL selective growth medium or volumes as needed for optimal plasmid growth.

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8.2.3. Incubate the flasks overnight at 37±2°C with shaking at 250 RPM to achieve an optimal OD range between 0.7 to 1.2. Record the OD on Form HSL_LAB_004.02.

Note: Typically, 16-18 hours of incubation will achieve the optimal OD range; however, certain plasmids may require longer incubation.

8.3. QIAGEN Maxi Kit Preparation

8.3.1. Vortex briefly one vial of RNase A solution, and briefly centrifuge the vial to ensure that all the liquid is at the bottom of the tube. Using a pipette, add the solution to Buffer P1 for a final concentration of 100 µg/mL.

8.3.2. Add one vial of LyseBlue reagent to Buffer P1, for a final dilution of 1:1000.

8.3.3. Label Buffer P1 with initial and date to identify the addition of RNase A and LyseBlue reagents. This must be stored at 2-8°C once the RNase A has been added.

8.3.4. Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37±2°C in a water bath.

8.3.5. Pre-chill Buffer P3 bottle provided in the kit prior to starting the procedure at 2-8°C and keep on ice at the start of the procedure. This will also need to be stored at 2-8°C so that is chilled and ready for later experiments. Label bottle with open date and assign an expiration date of one year.

8.3.6. Prepare the needed volume of 70% proof ethanol and store it on ice or at 2-8°C to allow it to chill (5 mL per maxi column).

8.4. Plasmid Purification

8.4.1. Read the OD of a sample of the bacterial culture using the GeneQuant Calculator. Refer to SOP# HSL_LAB_008 (Bacterial Cell Culture Measurement Using the GeneQuant Calculator).

Note: If the OD of the bacteria culture is 1.2 or greater, the culture may require 25 mL of P1, P2, and P3 to optimally lyse the bacteria.

8.4.2. Transfer 400 mL of the bacterial culture into a 500 mL centrifuge tube.

8.4.3. Harvest the bacterial cells by centrifugation at 4000 x g for 10 minutes at 4°C using the XTR centrifuge.

8.4.4. Decant the medium into a waste container with bleach (10% final concentration), and ensure residual growth medium has been removed (for example, put the bottle up-side down on a piece of paper towel to absorb excess media).

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- 8.4.5. Re-suspend the bacterial pellet in 20 mL Buffer P1 and mix either by vortex or manually pipetting. Ensure that no clumps are visible.
- 8.4.6. Add 20 mL Buffer P2 and mix thoroughly by inverting the sealed tube until lysate is entirely blue. Incubate at room temperature for a total of five minutes.
- Note:** Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 minutes.
- 8.4.7. Add 20 mL of chilled Buffer P3, mix immediately and thoroughly by vigorously inverting approximately 4–6 times or until the solution becomes white. Incubate on ice for at least 20 minutes.
- 8.4.8. Centrifuge at 4000 x g using the XTR centrifuge for 10 minutes at 4°C.
- 8.4.9. During centrifugation, equilibrate a HiSpeed Maxi Tip by adding 10 mL of Buffer QBT to the HiSpeed Maxi Tip, and allow the HiSpeed Maxi Tip to empty completely by gravity flow.
- 8.4.10. After centrifugation, using a cell strainer over a column, pour the supernatant evenly into two HiSpeed Maxi Tips (approximately 30 mL each).
- Note:** This alleviates the use of the QIAFilter.
- 8.4.11. Allow the clear lysate to enter the resin by gravity flow.
- 8.4.12. Once flow stops, wash the HiSpeed Maxi Tip with 60 mL Buffer QC.
- Note:** Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove all contaminants in most plasmid DNA preparations.
- 8.4.13. Once the Buffer QC has moved through the QIAGEN-tip, place the QIAGEN-tip over a 50 mL conical tube to collect eluate.
- 8.4.14. Elute DNA with 15 mL Buffer QF and collect the eluate in a 50 mL conical tube.
- 8.4.15. Once Buffer QF has stopped flowing, discard QIAGEN-tip and precipitate DNA by adding 10.5 mL (0.7 volumes) room-temperature isopropanol to the eluted DNA and incubate at room temperature for up to five minutes.
- Note:** After this step, perform the remaining procedure in the BSC identified for bacterial work.
- 8.4.16. During the incubation, remove the plunger from a 30 mL syringe and attach the QIAprecipitator Maxi Module onto the outlet nozzle (white side attaches to syringe).

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Note: DO NOT use excessive force, bending or twisting to attach the QIAprecipitator.

- 8.4.17. Place the QIAprecipitator/30 mL syringe over a waste bottle (50 mL conical tube), transfer the eluate/isopropanol mixture into the 30 mL syringe, and insert the plunger. Filter the eluate/isopropanol mixture through the QIAprecipitator using constant pressure.

Note: Complete the QIAprecipitator procedure within **10 minutes**. To prevent detachment of the QIAprecipitator and subsequent loss of DNA and alcohol, do not use excessive force when pushing liquid through the QIAprecipitator.

- 8.4.18. Remove the QIAprecipitator from the 30 mL syringe and pull out the plunger.

Note: Always remove the QIAprecipitator before removing plunger.

- 8.4.19. Re-attach the QIAprecipitator and add 5 mL 70% ethanol (cold, stored at 2-8°C) to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitator using constant pressure.

- 8.4.20. Remove the QIAprecipitator from the 30 mL syringe and pull out the plunger.

- 8.4.21. Attach the QIAprecipitator to the 30 mL syringe again, insert the plunger, and dry the membrane by pressing air through the QIAprecipitator quickly and forcefully. Repeat this step.

- 8.4.22. Dry the outlet nozzle of the QIAprecipitator with absorbent paper to prevent ethanol carryover.

- 8.4.23. Remove the plunger from a 5 mL syringe and attach the QIAprecipitator onto the outlet nozzle. Hold the outlet of the QIAprecipitator over a labeled collection tube.

- 8.4.24. Add 1 mL of TE to the 5 mL syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.

- 8.4.25. Remove the QIAprecipitator from the 5 mL syringe, pull out the plunger and reattach the QIAprecipitator to the 5 mL syringe.

- 8.4.26. Add 500 µL of TE to the 5 mL syringe and elute into the same labeled collection tube.

- 8.4.27. Pass the full volume of pDNA through a syringe filter (0.2 micron) into one properly labeled tube, and place DNA plasmid samples in designated box and store the samples in -80°C freezer. Record final aliquot storage location on HSL_LAB_004.02: Plasmid Purification Form: Maxi Prep.

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Note: A tube and box label template file is available for generating labels. See Attachment 1: DNA Plasmid Labels and Attachment 2: Box Label Example for details.

8.4.28. Document DNA Plasmid storage information on HSL_LAB_004.02: Plasmid Purification Form: Maxi Prep.

8.4.29. Document sample location in the O:\HSL\HSL_Freezer Inventory\HSL_022\ -80 freezer_C134285_Inventory_Shelf5_Rack16_20.xlsm file.

9. ADDITIONAL ETHANOL PRECIPITATION OF PLASMID DNA (IF NEEDED):

- 9.1. Transfer 375 µL of 1.5 mL of DNA solution into three 1.5 mL tubes for a total of 4 tubes with 375 µL each.
 - 9.2. Add 37.5 µL (1/10 volume) of Sodium Acetate (3M, pH 5.2) to each tube. Mix by inverting tubes.
 - 9.3. Add Glycogen to achieve a final concentration of approximately 100 ng/µL.
 - 9.4. Add 900 µL of 200 proof chilled ethanol to each tube. Mix by inverting tubes, and incubate at -20±5°C overnight.
 - 9.5. Centrifuge the tubes (keep the same orientation for all the tubes) using the Eppendorf centrifuge at 15,000 x g at 4°C for 15 minutes.
 - 9.6. Remove the tubes, aspirate supernatant carefully as not to disturb the DNA pellet. Be aware that the pellet can be very small and barely visible.
 - 9.7. Wash DNA pellet by adding 500 µL 70% chilled ethanol.
 - 9.8. Centrifuge using Eppendorf centrifuge at 15,000 x g at 4°C for 10 minutes.
 - 9.9. Aspirate supernatant as before. Dry pellet at room temperature or at 37±2 °C using the convection oven until ethanol has fully evaporated.
- Note:** Be careful to not over dry the DNA pellet, as it will be difficult to dissolve in solution.
- 9.10. Add 50 µL of TE buffer to plasmid DNA pellet and let sit for at least 30 minutes at room temperature to help dissolve pellet.
 - 9.11. Combine all DNA into one properly labeled tube, and place DNA plasmid samples in designated box and store the samples in -80°C freezer. Record final aliquot storage location on HSL_LAB_004.02: Plasmid Purification Form: Maxi Prep.

Note: A tube and box label template file is available for generating labels. See Attachment 1: DNA Plasmid Labels and Attachment 2: Box Label Example for details.

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9.12. Document DNA Plasmid storage information on HSL_LAB_004.02: Plasmid Purification Form: Maxi Prep.

9.13. Document sample location in the O:\HSL\HSL_Freezer Inventory\HSL_022\ -80 freezer_C134285_Inventory_Shelf5_Rack16_20.xlsm file.

10. DETERMINATION OF YIELD.

10.1. To determine the yield, DNA concentration should be measured using the Nanodrop 1000 Spectrophotometer (refer to HSL_LAB_016 DNA Quantitation using the NanoDrop).

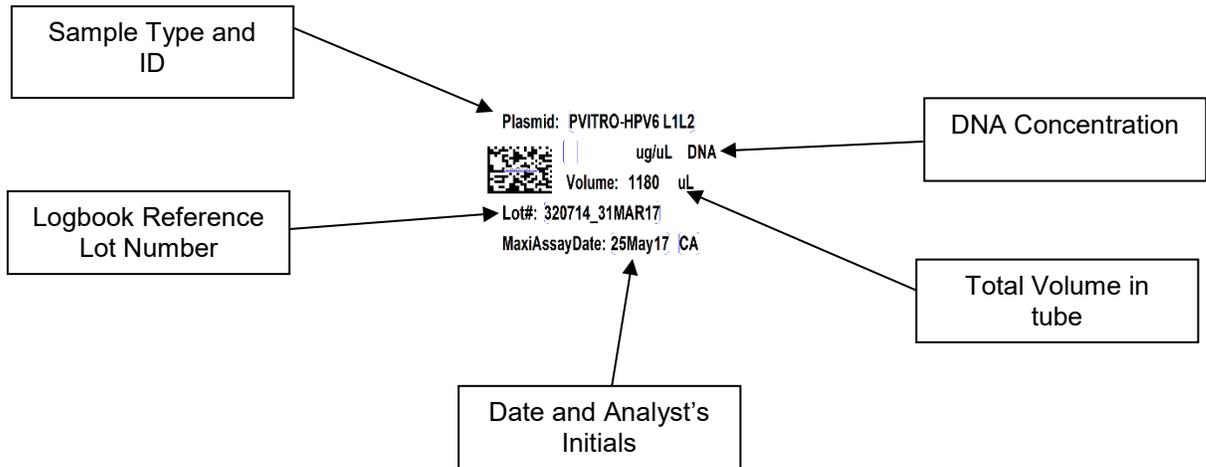
10.1.1. The 260/280 ratio should be at approximately 1.8 and the 260/230 ratio should be in the general range of 2.0-2.2. This will help determine the purity of the isolated DNA.

11. ATTACHMENTS

11.1. Attachment 1: DNA Plasmid Labels

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Attachment 1: DNA Plasmid Labels



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Attachment 2: Box Label Example

Study: *HPV16 pShell DNA*
 Sample Type: *Plasmid DNA in TE*
 Date: *14AUG17*
 Initials: *TK*
 Box 1 of 2



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12. REVISION HISTORY

Revision Start Date	Version #	Changes	Reasons
03Apr17	New	Create new SOP for Plasmid purification using QIAGEN Kit	New SOP
20Oct17	1.0	Added new antibiotic Kanamycin and instructions for use. Updated formatting.	Harmonize/update laboratory practices and make formatting flow.

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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:	<input type="checkbox"/> N/A
Eppendorf Centrifuge	<input type="checkbox"/> HSL_006 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
XTR Centrifuge	<input type="checkbox"/> HSL_033 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Orbital Shaker	<input type="checkbox"/> HSL_011 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Microwave	<input type="checkbox"/> HSL_021 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
-80°C Freezer	<input type="checkbox"/> HSL_022 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
-20°C Freezer	<input type="checkbox"/> HSL_034 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
2-8°C Refrigerator	<input type="checkbox"/> HSL_029 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
GeneQuant Calculator	<input type="checkbox"/> HSL_051 <input type="checkbox"/> Other:	<input type="checkbox"/> N/A
Pipette: μL	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Pipette: μL	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

Reagents

Equipment Name	Lot Number	Expiration Date
QIAGEN Plasmid Maxi Kit	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Growth Media:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Antibiotic:	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
TE Buffer	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
200 Proof Ethanol	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A
Isopropanol	<input type="checkbox"/> N/A	<input type="checkbox"/> N/A

Comments:

N/A

Analyst/Date:	
Review By/ Date:	

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Plasmid Being Purified: _____

Culture Progression

Starting Material (Type)
<input type="checkbox"/> Colony <input type="checkbox"/> Bacterial Stab <input type="checkbox"/> Glycerol Stock <input type="checkbox"/> Culture
Starting Material Information (ex. Lot#, Prep. Date, End Culture OD):

Inoculation of Starting Material (Type)
<input type="checkbox"/> N/A <input type="checkbox"/> Starter Culture (Small Volume) <input type="checkbox"/> Large Culture
<input type="checkbox"/> N/A Volume of Starting Material:
<input type="checkbox"/> N/A Volume of Starter Culture(s):
<input type="checkbox"/> N/A Inoculation Date:

Culture Growth
Volume of Growth Media per Flask:
Volume of Inoculate per Flask:
Total Number of Flask(s):
Inoculation Date:

Culture Growth/Incubation Times (FIO)			
	Date / Time	Total Hours	Culture O.D. (End)
Start			
End			

Comments:
<input type="checkbox"/> N/A

Analyst/Date:	
Review By/ Date:	

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<input type="checkbox"/> N/A Reagent Preparation
<input type="checkbox"/> Add 1 vial of RNase A to Buffer P1
<input type="checkbox"/> Add 1 vial of LyseBlue reagent to Buffer P1
<input type="checkbox"/> Initial and date Buffer P1 Bottle

Plasmid Purification (Check box when performed)

<input type="checkbox"/> N/A Harvesting Bacterial Cells
<input type="checkbox"/> Centrifuge for 10 mins at 4000 x g at 4°C
<input type="checkbox"/> Add ____ mL of Buffer P1
<input type="checkbox"/> Add ____ mL of Buffer P2
<input type="checkbox"/> Incubate at room temperature for up to 5 minutes
<input type="checkbox"/> Add ____ mL of chilled Buffer P3
<input type="checkbox"/> Incubate on ice for at least 20 mins
<input type="checkbox"/> Centrifuge for 10 mins at 4000 x g at 4°C
<input type="checkbox"/> Add 10 mL of Isopropanol and incubate for 5±2 minutes

<input type="checkbox"/> N/A Ethanol Precipitation of Plasmid DNA
<input type="checkbox"/> Add 900 µL of 200 proof ethanol (stored at -20±5°C) and incubate at -20±5°C overnight
<input type="checkbox"/> Centrifuge at 15,000 x g 4°C for 30 minutes
<input type="checkbox"/> Add 500 µL of 70% ethanol (-20±5°C) and centrifuge at 15,000 x g 4°C for 10 minutes
<input type="checkbox"/> Add 50 µL of TE Buffer and incubate for at least 30 minutes

Equipment Name	Equipment ID	Calibration Due Date
-80°C Storage Freezer	<input type="checkbox"/> HSL_022 <input type="checkbox"/> Other:	
	Shelf #:	Rack #:

NanoDrop Raw Data Reference: _____

Comments:	<input type="checkbox"/> N/A
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Analyst/Date:	
Review By/ Date:	