

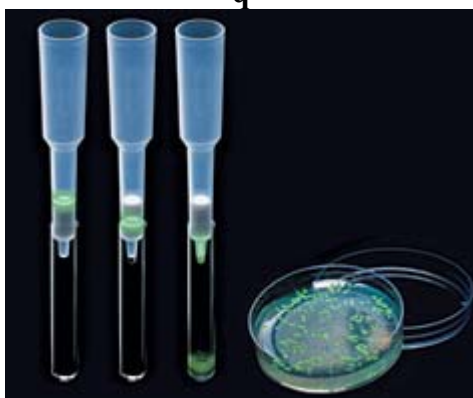
Title: Column Chromatography of Green Fluorescent Protein

Approvals:

Preparer Date 07Oct06 Reviewer: Mary Jane Kurtz Date 09Jul13

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Part I Crude Isolation of GFP from Lysed Cells



1. **Purpose:**
 - 1.1 The purpose of this SOP is to purify green fluorescent protein (GFP) from lysed *E. coli* cells using column chromatography.
2. **Scope:**
 - 2.1 This SOP will be executed whenever the purification of GFP is needed.
 - 2.2 This SOP will be executed after the performance of pGLO Bacterial Transformation.
3. **Responsibility:**
 - 3.1 It is the responsibility of the Instructor to aid the students in the performance of all the procedures described in this SOP.
 - 3.2 It is the responsibility of the Instructor to update this SOP as needed.
4. **References:**
 - 4.1 Instructions from Biotechnology Explorer Green Fluorescent Protein (GFP) Purification Kit Instruction Manual (Bio-Rad catalog number 166-0005EDU)
 - 4.2 GFP Chromatography Kit. (Bio-Rad catalog number 166-0005EDU)
5. **Definitions:**
 - 5.1 Ion Exchange Chromatography

- 5.2 Hydrophobic Interaction Chromatography
- 5.4 Elution
- 5.5 Eluate
- 5.6 Column Volume
- 5.7 Supernatant

6. Hazard Communication

- 6.1 Wear personal protection equipment
- 6.2 Wear eye protection.
- 6.3 For Disposal of Biohazard Waste use an autoclavable bag and follow proper sterilization techniques.

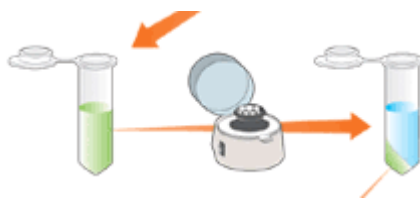
7. Materials

- 7.1 Safety glasses or goggles
- 7.2 Microfuge tubes
- 7.3 Pipettes
- 7.4 Microtube rack
- 7.5 Marker
- 7.6 *E. coli* GFP cell culture from Bacterial Transformation and Upstream Processing SOP
- 7.7 Incubator to grow *E. coli* transformed with GFP at 35°C
- 7.8 Freezer
- 7.9 Microfuge centrifuge
- 7.10 Vortex
- 7.11 TE Buffer
- 7.12 Lysozyme in 1ml of TE Buffer
- 7.13 UV Lamp

8. Procedure:

8.1 Preliminary Procedures before Chromatography

- 8.1.1 Take 2ml of pGLO transformed *E. coli* culture from the 20ml culture tube, centrifuge at 2,000 RPM for twenty minutes. The cell pellet should fluoresce when viewed by a UV lamp.



- 8.1.2 While waiting, rehydrolyze the lysozyme with 1 ml of TE buffer.
- 8.1.3 Pour off the *E. coli* supernatant into a 10% Clorox solution.
- 8.1.5 Add 750 μ l of TE buffer and resuspend the pellet by pipetting the fluid up and down many times to make a homogenous mixture. Vortex if necessary.
- 8.1.6 Add 1 drops of the reconstituted lysozyme solution. Mix the contents

gently. Freeze contents at -20°C for 1 day at least.

- 8.1.7 Thaw the microfuge tube containing bacterial cell lysate to hand warmth temperature.

- 8.1.8 Centrifuge for 10 minutes at maximum speed.
- 8.1.9 Observe the bacterial cell supernatant with the UV light. It should fluoresce.
- 8.1.10 Remove the bacterial cell lysate supernatant and place into a clean microfuge tube. This is the fraction we will use to purify GFP.

Part II

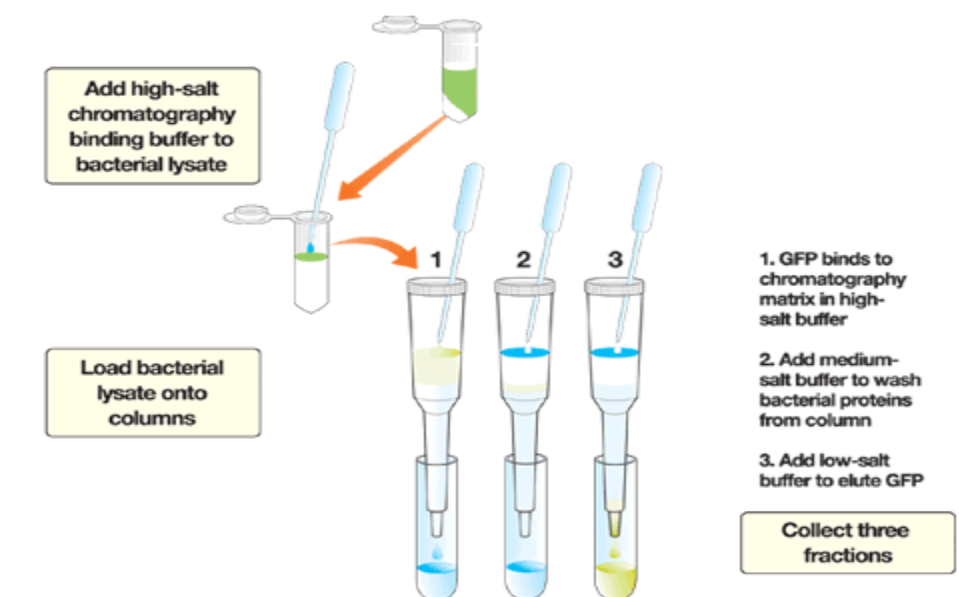
Hydrophobic Interaction Chromatography

9.0 Materials

- 9.1 250ul of bacterial cell lysate
- 9.2 Pre-packed Hydrophobic Interaction Chromatography Column
- 9.3 Waste tube or beaker
- 9.4 HIC Buffers:
 - 9.4.1 Binding buffer (= 4.0M ammonium sulfate in TE Buffer pH 8)
 - 9.4.2 Equilibration buffer (= 2.0M ammonium sulfate in TE Buffer pH8)
 - 9.4.3 Wash buffer (= 1.3M ammonium sulfate in TE Buffer pH8)
 - 9.4.4 Elution buffer (= TE buffer)
- 9.5 Test tube rack
- 9.6 Magic marker
- 9.7 3 test tubes (5 ml volume)
- 9.8 disposable pipettes ~ 3 ml
- 9.9 Pipetman 1000ul with tips
- 9.10 UV black light

10.0 Procedure (Illustrated Overview):

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- 10.1 Remove caps from the top and bottom of the HIC column and then allow the fluid within to drain.

- 10.2 Add 2 ml of Equilibration buffer to the top of the column. Allow the column to drain into the waste beaker. Cap the bottom of the column.
- 10.3 Transfer 250 μ l of bacterial cell lysate to a 1.0ml microfuge tube and add 250 μ l of binding buffer to it.
- 10.4. Label three 5ml plastic test tubes in sequence from 1-3.
 - 10.4.1 Place test tube number 1 under the column. Remove the cap from the bottom of the column and add 250 μ l of the bacterial cell lysate supernatant/ Binding buffer used in 10.3. to the top of the column. Allow the solution to drain completely into test tube 1.
 - 10.4.2 Place test tube number 2 under the column. Add 250 μ l of Wash buffer. Allow the buffer to drain to the top of the column into test tube 2.
 - 10.4.3 Place test tube number 3 under the column. Add 750 μ l of Elution buffer to the column. Allow the buffer to drain completely into test tube 3. This tube should have pure GFP in it. It can be seen glowing green under the long range UV lamp.
- 10.5 Note the GFP positive test tube(s) and save for future analysis by SDS-PAGE.

Part III

Ion Exchange (Anion Ion Exchange) Chromatography (IEX)

11.0 Materials:

- 11.1 250 μ l of bacterial cell lysate supernatant
- 11.2 Pre-packed MacroPrep High Q for Anion Exchange Chromatography column
- 11.3 Waste tube or beaker
- 11.4 Anion Ion Exchange Chromatography buffers:
 - 11.4.1 Equilibration buffer (=50 mM Tris pH 8.3)
 - 11.4.2 Elution buffer 1 (50 mM Tris, pH 8.3, 130 mM NaCl)
 - 11.4.3 Elution buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl)
 - 11.4.4 Elution buffer 3 (50 mM Tris pH 8.3, 300 mM NaCl)
 - 11.4.5 Elution buffer 4 (50mM Tris pH 8.3, 500 mM NaCl)
- 11.5 Test tube rack
- 11.6 Marker
- 11.7 6 test tubes (5 ml volume)
- 11.8 Disposable pipettes ~ 3 ml
- 11.9 Pipetman 1000ul with tips
- 11.10 UV black light
- 11.11 Lab marker
- 11.12 Microfuge tube

12. Procedure:

- 12.1 Label 5, 5ml test tubes in sequence from 1-5.

12.2
Remove cap from the
top and then the
bottom of the IEX
column

- 12.3 Equilibrate the column by adding 2ml of 50mM Tris pH 8.3. Collect and discard into the waste beaker.
- 12.4 Load 250µl of the bacterial cell lysate supernatant onto the top of the column and collect the flow through fraction in test tube 1. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.
- 12.5 Immediately add 250ul of Elution Buffer 1 (50 mM Tris, pH 8.3, 130 mM NaCl) to the top of the column and collect the eluate in test tube 2. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.
- 12.6 Add 250ul of Elution Buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl) to the top of the column and collect the eluate in test tube 3. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.
- 12.7 Add 750ul of Elution Buffer 3 (50 mM Tris, pH 8.3, 300 mM NaCl) to the top of the column and collect the eluate in test tube 4. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.
- 12.8 Add 250ul of Elution Buffer 4 (50 mM Tris, pH 8.3, 500 mM NaCl) to the top of the column and collect the eluate in test tube 5. Examine with UV light. Save 10ul of the flow through in a microfuge tube for future use.
- 12.9 Using the UV light select the test tube(s) containing GFP for analysis by SDS-PAGE as well as any additional samples that were collected.

Part III

Ion Exchange (Cation Ion Exchange) Chromatography (IEX)

13.0 Materials

- 13.1 Bacterial lysate or previous isolated sample, 250 µl
- 13.2 Pre-packed IEX column (cation) Macro-Prep High S
- 13.3 Waste tube or beaker
- 13.4 Cation Ion Exchange Chromatography buffers:
 - 13.4.1 Equilibration buffer (= 50 mM Tris pH 8.3)
 - 13.4.2 Elution Buffer 1 (50mM Tris, pH 8.3, 130 mM NaCl)
 - 13.4.3 Elution Buffer 2 (50 mM Tris, pH 8.3, 200 mM NaCl)
 - 13.4.4 Elution Buffer 3 (50 mM Tris, pH 8.3, 300 mM NaCl)
 - 13.4.5 Elution Buffer 4 (50 mM Tris, pH 8.3, 500 mM NaCl)
- 13.5 Test tube rack
- 13.6 Marker
- 13.7 5 test tubes (5 ml volume)
- 13.8 Disposable pipettes ~3ml
- 13.9 Pipetman 1000 µl with tips
- 13.10 UV black light
- 13.11 Microfuge tube

14.0 Procedure:

- 14.1 Label 5 (5 ml) test tubes in sequence from 1-5 and place into test tube rack.
- 14.2 Remove cap from the top and then the bottom of the IEX column.

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- 14.3 Equilibrate the column by adding 2 ml of 50mM Tris pH 8.3. Collect and discard

- into a waste beaker.
- 14.4 Load 250 μ l of the bacterial cell lysate supernatant onto the top of the column and collect the flow through fraction (eluate) into test tube 1. Examine with UV light; if GFP is present it will glow.
Save 10 μ l of flow through liquid in a microfuge tube for future use.
 - 14.5 Immediately add 250 μ l of Elution Buffer 1 (50mM Tris, pH 8.3, 130mM NaCl) to the top of the column and collect the eluate in test tube 2. Examine with UV light again as in 14.4. Save 10 μ l of the flow through liquid in a microfuge tube for future use.
 - 14.6 Add 250 μ l of Elution Buffer 2 (50mM Tris pH 8.3, 200mM NaCl) to the top of the column and collect the eluate in test tube 3. Save 10 μ l of the flow through in a microfuge tube for future use.
 - 14.7 Add 750 μ l of Elution Buffer 3 (50 mM Tris pH 8.3, 300mM NaCl) to the top of the column and collect the eluate in test tube 4. Examine with UV light. Save 10 μ l of the flow through in a microfuge tube for future use.
 - 14.8 Add 250 μ l of Elution Buffer 4 (50 mM Tris, pH 8.3, 500mM NaCl) to the top of the column and collect the eluate in test tube 5. Examine with UV light. Save 10 μ l of the flow through to a microfuge tube for future use.
 - 14.9 Using the UV light select the test tube(s) containing GFP for analysis by SDS-PAGE or include all samples if you wish.