Phycocyanobilin (PCB) Purification from *Spirulina*

Obtain cyanobacteria, *Spirulina* (Seltzer Chemical(Spirulina Powder , 191775-C, 1-800-735-8137)

(http://www.seltzerchemicals.com/Products/All/)

Day 1

1 –Suspend 50 g *Spirulina* powder in 1.5 liter H_2O (30 ml/g) in a 2.8-liter flask covered with aluminum foil. Stir for 10 minutes then divide into six 250 ml centrifuge bottles and centrifuge 8000 RPM, GS3 rotor, 4° C for 1 hr.

2 – Decant supernatant into another 2.8 liter flask and add 15g TCA - final concentration of 1% (w/v). Stir for 1 hr in the dark (aluminum foil cover) at 4° C. Centrifuge 8000 RPM, GS3 rotor, 4° C for 10 min. Discard supernatant.

3 - Add 25 ml MeOH to each bottle; resuspend green pellets completely using a tissue homogenizer and/or vortex – keep pellets on ice during this process. After pellets are fully resuspended, add MeOH to each bottle to a final volume of 250 ml and repeat centrifugation (8000 RPM, 4° C / 10 min.) Discard supernatant.

4 – Repeat step (3) 4X – or until MeOH wash is colorless.

5 – Store final washed pellets at -20° C, wrapped in foil overnight.

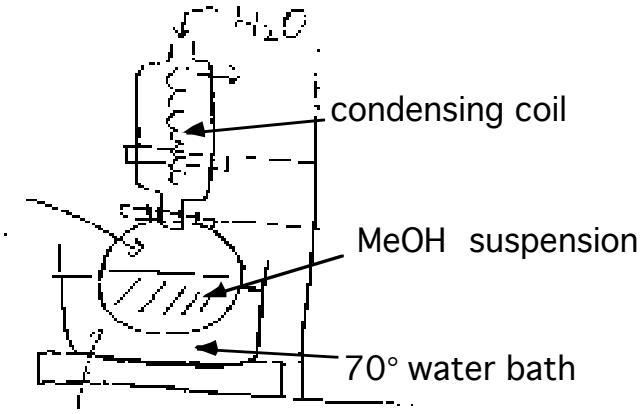
****It is critical that all steps from this point on are performed under low intensity, green 'safelight' conditions in a darkroom (see below for safelight specifications - a photographic darkroom, with "orange" safelights turned off, is fine); and/or that samples are shielded from light with foil. Avoid exposure to bright, white light. This is because the free chromophore(once

released form its protein-bound state) is very susceptible to photobleaching. The aim is to exclude/minimize photodamage.

Day 2 - **Methanolysis**

6 – Resuspend and pool pellets in a final volume of 500 ml MeOH.

7 – Heat suspension in a 1-liter flask in a water bath at 70 – 75 °C with a condensing coil cooled with tap water (see below) for 5-8 hrs (very slow boil).



8 – Transfer suspension to two 250 ml bottles and centrifuge 8000 RPM, GS3 rotor, 4° C / 20 min.

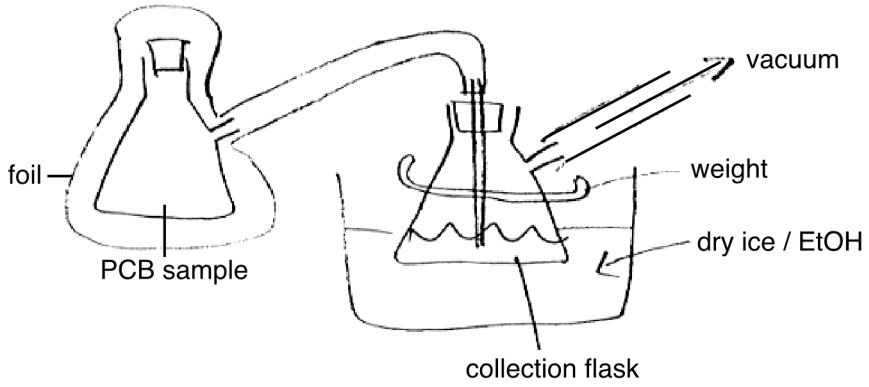
9 – Decant and filter supernatant through miracloth directly into a 1-liter side-armed flask and store, foil-wrapped at –20 °C (1st methanolysis supernatant). Store firm pellet from centrifugation, foil-wrapped at -20 °C.

Day 3

10 - Set up 2nd methanolysis of pellets from step 9 as in steps 6-9. (Alternatively, set up at the end of Day 2 and run methanolysis overnight). (2nd methanolysis supernatant).

Day 4

11 – Remove 1st methanolysis supernatant (step 9) from freezer and connect to another side-armed flask which is attached to a vacuum pump and sitting in a dry ice / EtOH bath (cold trap collection flask). Monitor volume of MeOH in collection flask until it reaches 400 ml. This process will take 9-10 hrs depending on vacuum. Store concentrated PCB/MeOH remaining in the original flask(~50 ml) foil-wrapped, at -20 °C.



Day 5

Chloroform extraction of phycocyanobilin (PCB)

12 - In a large separatory funnel – mix concentrated PCB/MeOH (~50 ml) with 50 ml chloroform and 100 ml H_2O. Shake and let stand for 5 min.

13 – Collect chloroform (bottom phase) in a beaker. This contains the PCB.

14 - Add another 50 ml chloroform to remaining aqueous phase and repeat steps 13 and 14. Continue with chloroform extraction until solution is almost colorless (usually 2-3X)

15 – Pool these several chloroform/PCB fractions and transfer to a 1 liter round bottom flask. Evaporate chloroform with a stream of nitrogen gas.

16 – Dissolve dried PCB in 4 X 0.75 ml DMSO – Final volume ~3 ml. This is the stock solution. Remove samples to determine PCB concentration

(see below), then aliquot remaining DMSO/PCB and store, foil-wrapped, at –80 ºC.

17 – Repeat steps 11-16 on 2nd methanolysis supernatant.

Determination of PCB concentration

Dilute DMSO/PCB stock (1:100, 1:1000) into 1 ml MeOH:HCl, 95%:5% solution for the determination of [PCB] in a spectrophotometer (HCl is 37.5%)

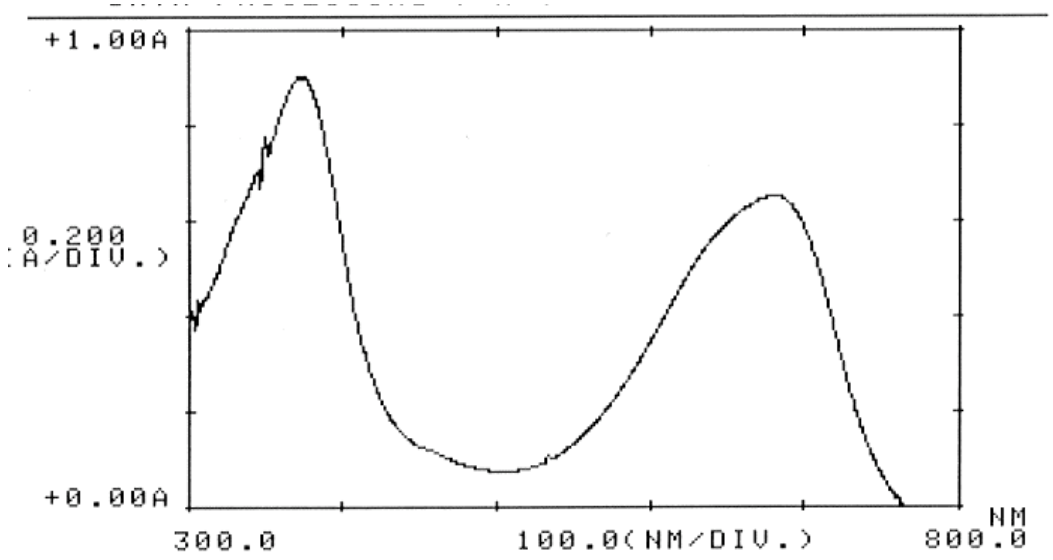
Aliquot DMSO/PCB stock and store, foil-wrapped, at –80º C

Read Absorbance at 680 nm.

$$C(mM) = \frac{A_{680}}{37.9} \times dil.factor$$

Scan sample 300 – 800 nm for Absorbance Spectrum.

A sample spectrum of a good PCB prep (1:1000) is shown below:



***Safelight Specifications**

Fluorescent tubes: any green fluorescent tube. Example: Sylvania F40G
(www.sylvania.com)

Filters: Roscolene 877 (medium blue green)
Roscolene 874 (meduium green)
Thin, flexible colored plastic sheets.
(www.rosco.com)

Green tubes must be wrapped completely with both filters – one layer of each.

Note: This safelight is also used 9in darkroom space for manipulations during experiments with the gene-promoter system in cells.

For questions on this protocol, contact:

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510-559-5935

Plasmids for Light-induced Transactivation

PhyA-Full Length and phyB-N-terminal fusions to the GAL4 – binding domain (GBD).

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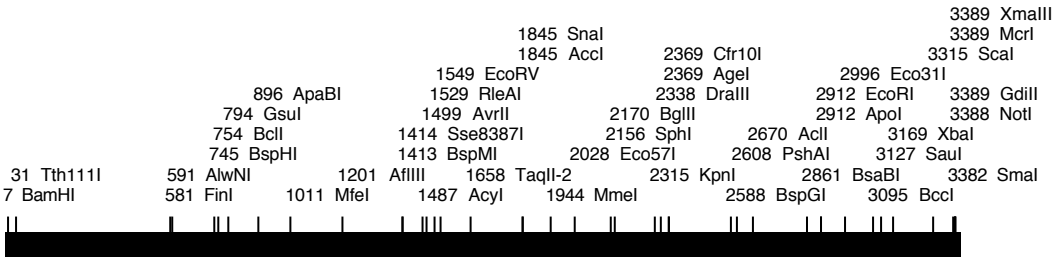
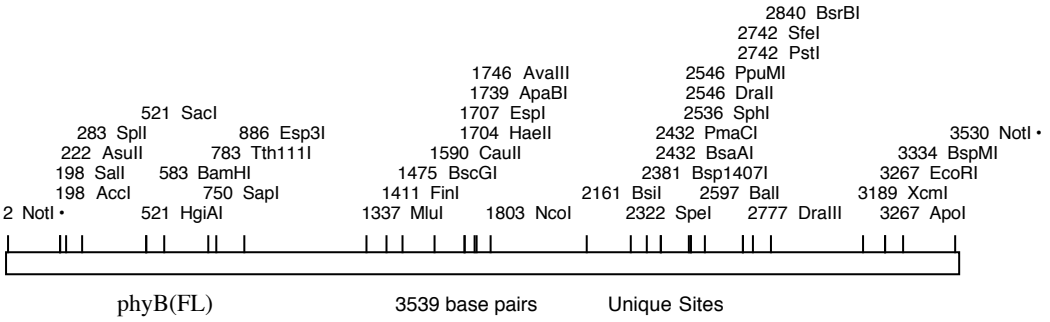
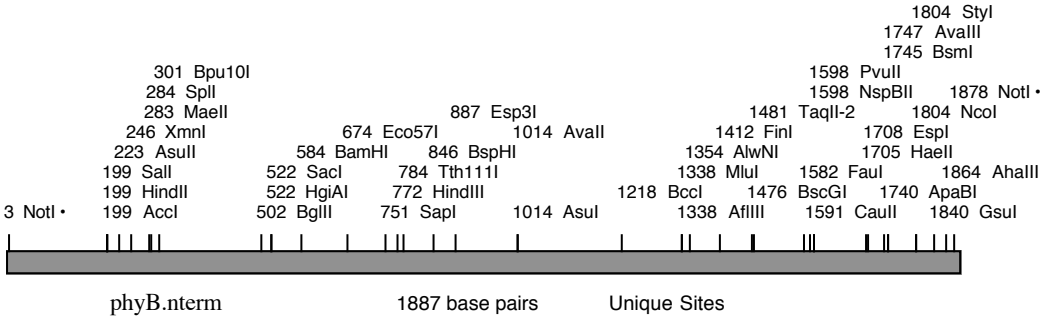


BamHI:NotI fragment in D153 (see below)





Both phyB(FL) and phyB(nterm) are cloned as NotI:NotI fragments in D153 (see below)

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D153 Date 7/30/94 Entered Rob

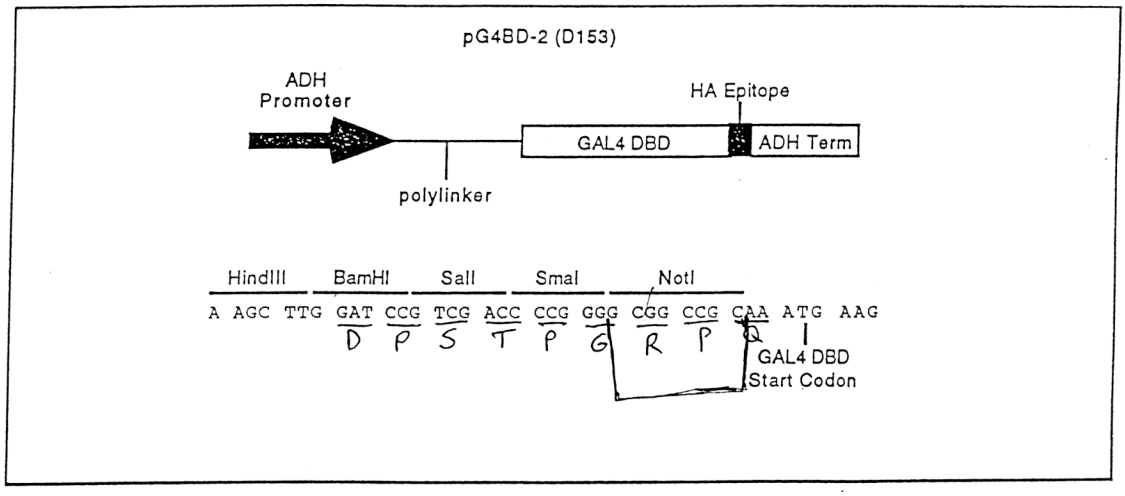
Plasmid Name
Two Hybrid Bait-GAL4 DNA Binding Domain Fusion Vector -2
(pBG4D-2)

Description
D15 (pAS2, pAS1-CYH2) was digested with SacI and HindIII and the approximately 2.1 kb band containing the CYH2 gene and the ADH1 promoter was gel purified and ligated into D150 which had been cut with SacI and HindIII also. The correct clone was identified by SacI and HindIII digestion. The backbone of this plasmid is YEp112 (TRP1). This plasmid does not contain an ATG in the polylinker. Therefore, the bait sequences you clone into the polylinker must contain there own ATG, which will serve as the translation start site.

Selection Amp/TRP1
Vector Backbone YEplac112
Strider File yes
Purpose Two Hybrid
Keywords Bait GAL4 DNA binding

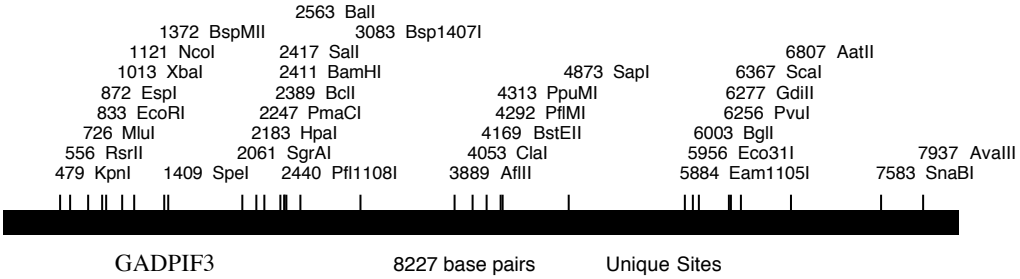
Origin
RB 7/30/94
D150+D15
SacI-HindIII #1

DNA preps
boiling mini
Qiagen 1.1 mg/ml



Please note: Unfortunately we do not have detailed sequence information for the D153 plasmid.

GADPIF3 plasmid:



PIF3 ORF: EcoRI (833) – BamHI (2411)
vector; pGAD424 Clontech.
http://www.clontech.com/techinfo/vectors/vectorsF-I/pGAD424.shtml