

## **Horwitz lab Protocol for screening libraries encoding cDNAs fused to GFP**

The following is the protocol that the Horwitz lab uses for screening libraries encoding cDNA fused to GFP. In brief, bacteria expressing plasmids are cloned into microtiter plates; the result is that each well contains bacteria expressing a single gene. Replica wells are then used for transfection of cells growing in glass bottom microtiter plates. Each well is then visualized and scored for either gene localization or perturbation of morphology. The screen is presented in: Manabe R, Whitmore L, Weiss JM, Horwitz AR. Identification of a novel microtubule-associated protein that regulates microtubule organization and cytokinesis by using a GFP-screening strategy. *Curr Biol.* 2002. 12:1946-51.

### **Solutions for Gene Screening:**

REF growth media:

low glucose DMEM (Gibco #11855-084)  
10% FBS  
1:100 Pen/strep  
1:100 NEAA

Solution I: 30mM Glucose  
15mM Tris-HCl pH8  
30mM Na<sub>2</sub>EDTA  
60ug/ml RNAase A

Solution II: 0.2N NaOH  
1% SDS

Solution III: 14ml MilliQ H<sub>2</sub>O  
14ml glacial acetic acid  
72ml 5M potassium acetate  
(Final Concentration: 3.6M Potassium/6M Acetate)

DNA Binding Solution (Bind solution): 6.1 M KI (make fresh)

2X Luria Broth (LB): with 1X NaCl

Tris-EDTA (TE) Buffer:  
10mM Tris-HCl pH8.0  
1mM Na EDTA

### **Screening**