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A Random-Library GFP Based Screen For Crystallization Targets. Robert E. Collins*, Alec Hodel, Mary Hodel, Gary Ratner*, Krista Hennig, Xing Zhang, Xiaodong Cheng, Emory Univ. Dept. of Biochemistry; *Emory Univ. Graduate Div. of Biological and Biomedical Science.

The formation of the GFP fluorophore is dependent on the properties of proteins fused N-terminal to it. This observation has led to its use as an *in-vivo* reporter for the soluble expression and folding of proteins expressed in bacteria. To identify soluble fragments of large and otherwise unworkable proteins, we have generated and refined a random-library GFP screen. The screen has allowed for biochemical and crystallographic work to proceed on several novel domains of the histone methyltransferase, G9a, and the autocatalytic domain of nucleoporin 98.

cDNAs of interest are sonicated, ends blunted, ligated to a GFP-containing vector, and plated on autoinduction media. Fluorescent colonies are selected by eye. Typically, about .05%-.1% of the library are positive clones, reflecting the highly selective nature of this screen (If one estimates $\frac{1}{2}$ of the clones to be in the right orientation, and $\frac{1}{3}$ to be in frame at each end, about 5% of the library should produce an in-frame fusion protein). All clones isolated express at high levels (>20mg/L), with hexahistidine tags replacing GFP for crystal trials.