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Evaluating the Function of an Understudied Family of Antiviral Proteins

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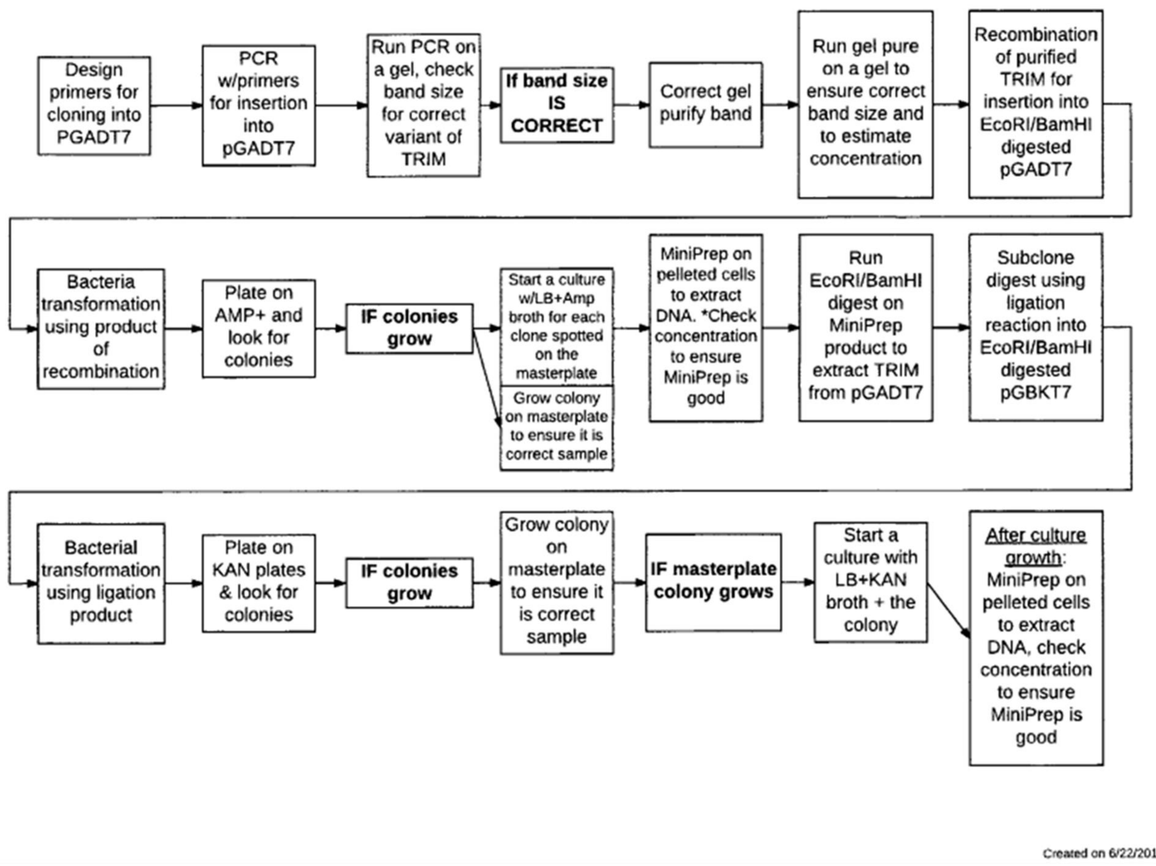
Abstract

Human immune responses to viral infections are associated with the interferon system, which induces the expression of as many as 300 proteins. Many of these proteins are believed to have antiviral functions, yet many of the interferon stimulated genes (ISGs) remain poorly characterized. The goal of this research is to identify the functional role of ISGs that are members of the tripartite motif (TRIM) family of proteins. Here, we report the progress of TRIM gene cloning into plasmids used in yeast two-hybrid assays. We hypothesize that protein-protein interactions identified in these assays will provide unbiased insights into the functional role of these putative antiviral proteins. The yeast-two hybrid assay is the cornerstone for a research pipeline designed to more fully characterize the TRIM proteins.

TRIM Cloning Flow Chart

The flow diagram describes the initial steps taken when cloning TRIM genes into plasmids for performing the yeast two-hybrid protocol.



Y2H Flowchart

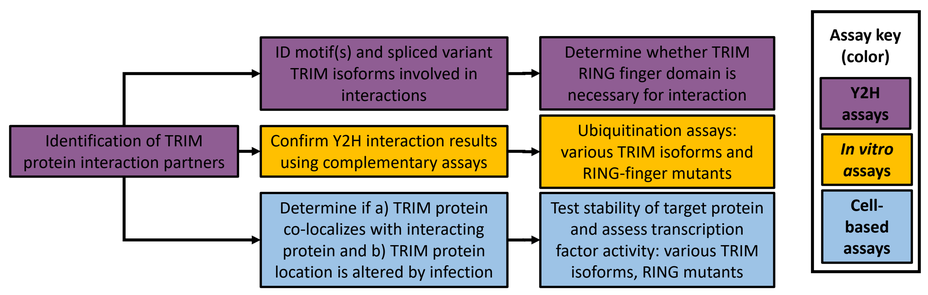
The following spreadsheet is a depiction of the first few sets of yeast transformations.

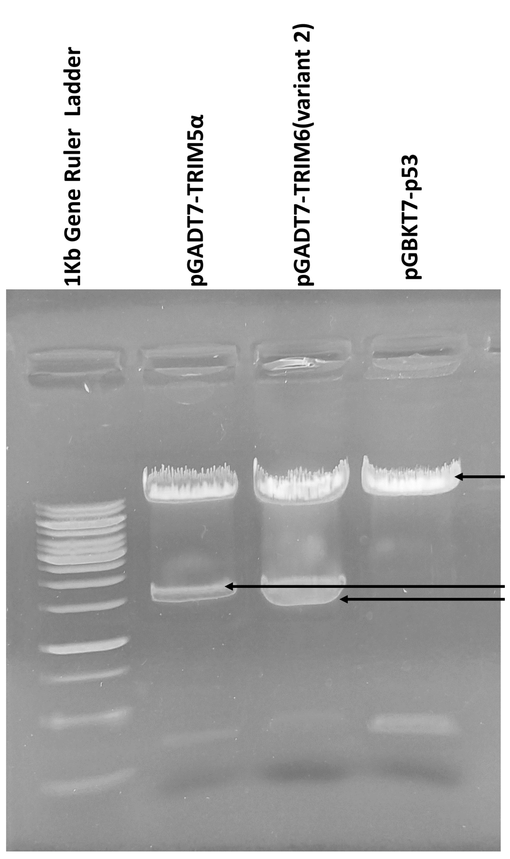
pGBKT7 \ pGADT7	None	Empty	T Ag	TRIM5a	TRIM6(v2)
None					
Empty		Set 2			
p53		Set 3			
Lam	Set 1	Set 4			

Future Plans

Once TRIM-interacting proteins are identified, a series of experiments summarized in the flow chart will be performed. Additional Y2H assays will evaluate the motifs and/or isoforms important for the interaction. Mutations to the RING finger of the TRIM proteins will also be made to evaluate whether this E3 ligase-related motif is involved in the interaction.

Alternative protein-protein interaction assays will be performed. Some will involve in vitro analysis and others will evaluate co-localization of TRIM proteins and their binding partners in living cells.





Subcloning TRIM Genes from pGADT7 into pGBKT7

Three plasmids were digested with EcoRI-HF and XhoI. The image shows the results of gel electrophoresis. The arrows indicate the bands that were cut out of the gel for use in ligation reactions.

Acknowledgements

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