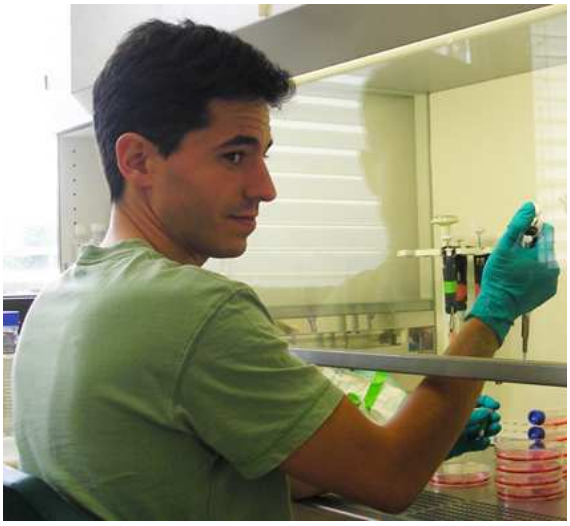
# System-wide identification of RNA-binding proteins by interactome capture



Alfredo Castello, Rastislav Horos, Claudia Strein, Bernd Fischer, Katrin Eichelbaum, Lars M. Steinmetz, Jeroen Krijgsveld and Matthias W. Hentze

European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, 69117 Heidelberg, Germany

Corresponding author: Alfredo Castello , alfredo.castello@embl.de
Publication Date: 14 Oct. 2013

## Introduction

mRNA interactome capture is a novel and unbiased technique to identify the active RBPs of cultured cells. Making use of in vivo UV-crosslinking of RBPs to polyadenylated RNAs, covalently bound proteins are captured with oligo(dT) magnetic beads. Following stringent washes, the mRNA interactome is determined by quantitative mass spectrometry.

The protocol takes three working days for analysis of single proteins by western blot and about two weeks for the determination of complete cellular mRNA interactomes by mass spectrometry. The most important advantage of interactome capture over other in vitro and in silico approaches is that only RBPs bound to RNA in a physiological environment are identified. Applied to HeLa cells, interactome capture revealed hundreds of novel RBPs. Interactome capture can also be broadly used to define the mRNA interactome of different cell lines and to compare different biological states.

## Protocol

Please use the link below to access the protocol.
http://www.hentze.embl.de/protocols.html

Published in Nature Protocols. 2013 Mar;8(3):491-500.

PMID: 23415593 (Nature Publishing Group, a division of Macmillan Publishers Limited)

http://www.nature.com/nprot/journal/v8/n3/full/nprot.2013.020.html