A Hybridization Chain Reaction-based Method for Amplifying Immunosignals

Minmin Luo (luominmin@nibs.ac.cn)
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Rui Lin
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Qiru Feng
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Peng Li
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Ping Zhou
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Ruiyu Wang
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Zhe Liu
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Zhiqiang Wang
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Xiangbing Qi
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Nan Tang
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Feng Shao
National Institute of Biological Sciences (NIBS), Beijing, 102206, China

Method Article

Keywords: Immunofluorecence, Neuroscience, Cell Biology, Hybridization Chain Reaction, Multiplexed Labeling

Posted Date: January 26th, 2018

DOI: https://doi.org/10.1038/protex.2018.014

Abstract

We describe the development of immunosignal HCR \(isHCR), which combines antibody-antigen interactions with Hybridization Chain Reaction technology for amplifying immunofluorescence by up to 2-3 orders of magnitude, and with low background. We further optimized its use in multiplexed imaging and in state-of-the-art tissue expansion and clearing techniques. With its highly modular and easily adaptable design, isHCR can be applied broadly in basic research and clinical diagnostics.

Introduction

Antibody-based immunoassays remain the most popular methods for detecting and identifying the location of proteins and other biomolecules in biological samples. A major limitation in the use of immunoassays is that the low abundance of a given target molecule in a sample often necessitates signal amplification before detection is possible. Amplification can be achieved using conjugated enzymes. Current amplification methods have several drawbacks: 1) high background, 2) reduced spatial resolution, 3) lack of multiplexity, and 4) unsuitable for use with large-volume samples in new tissue expansion and clearing techniques. To overcome many of these limitations, we adapt hybridization chain reaction \(HCR) technology to amplify immunosignals. We here describe immunosignal HCR \('isHCR') and demonstrate the use of HCR in a variety of immunoassays. The streptavidin-biotin interaction can be utilized for the typical isHCR application. isHCR uses label-free streptavidin, which allows the attachment of synthesized 5'- biotinylated DNA HCR initiators to the biotinylated antibodies. These initiators can then be elaborated with fluorescent DNA HCR amplifiers for signal detection. Alternatively, the DNA HCR initiators can be conjugated directly onto antibodies, therefore omitting the streptavidin-biotin interaction.

Reagents

1x Phosphate Buffered Saline \(PBS) TritonX-100 5× sodium chloride citrate \(SCC buffer) 2% Paraformaldehyde \(PFA) in PBS Tween-20 dextran sulfate BSA Graphene Oxide Streptavidin DNA HCR initiator DNA HCR amplifiers \(the sequences and modifications can be found in the associated publication)

Equipment

thermal cycler, fluorescent microscopy

Procedure

Tissue section preparation 1. Anesthetize mice with an overdose of pentobarbital and perfuse intracardially with PBS, followed by paraformaldehyde \(PFA, 4% wt/vol in PBS). 2. Dissected out tissues and postfix them in 4% PFA for 4 h at room temperature or 1 d at 4°C. 3. Dehydrate tissue samples in 30% sucrose solution. 4. Prepare thin tissue sections on a Cryostat microtome. Immunostaining 1. Wash and

permeablize sections in 0.3% Triton X-100 in PBS \(PBST) and blocked them in 2% BSA in PBST at room temperature for 1 h. 2. Incubate sections with primary antibodies for various times according to the efficiency of the antibodies. 3. Wash samples for three times in PBST. \(if DNA HCR initiator conjugated secondary antibodies are used, incubate samples in HCR amplification buffer \[5× sodium chloride citrate \(SCC buffer), 0.1% vol/vol Tween-20, and 10% wt/vol dextran sulfate in ddH2O] for 30min at room temperature) 4. Sections are incubated with biotinylated secondary antibodies or DNA HCR initiator conjugated secondary antibodies. 5. Wash samples for three times in PBST. isHCR amplification \(All reagents were dissolved in HCR amplification buffer \[5× sodium chloride citrate \(SCC buffer), 0.1% vol/vol Tween-20, and 10% wt/vol dextran sulfate in ddH2O].) \(For biotinylated secondary antibodies, perform the following four steps first: 1. Incubate samples in 1 μg/mL streptavidin at room temperature for 30 min. 2. Wash three times in PBST. 3. Incubate samples with 0.5 μM DNA-biotin HCR initiators at room temperature for 30 min. 4. Wash three times in PBST.) 1. Snap-cooled a pair of DNA-fluorophore HCR amplifiers separately in 5× SSC buffer by heating at 95°C for 90s and cooling to room temperature over 30 min. 2. Both of these amplifiers were then added to amplification buffer to a final concentration of 12.5 nM \(Optional: graphene oxide \(GO) can be added for applications that demands background suppression. Add GO to the buffer from step 2 to 20 μg/mL and vortex thoroughly) 3. Incubate samples with the buffer from step 2 overnight at room temperature. 4. Wash three times in PBST. 5. mount sections on slides and observe.

Acknowledgements

M.L. is supported by China MOST \(2012YQ03026005, 2013ZX0950910, 2015BAI08B02), NNSFC \(91432114, 91632302), and the Beijing Municipal Government.