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Illustration inspired by the work of Dorothy Hodgkin

接触世界顶尖实验技术，提高实验效率与成功率

——Springer Protocols资源介绍

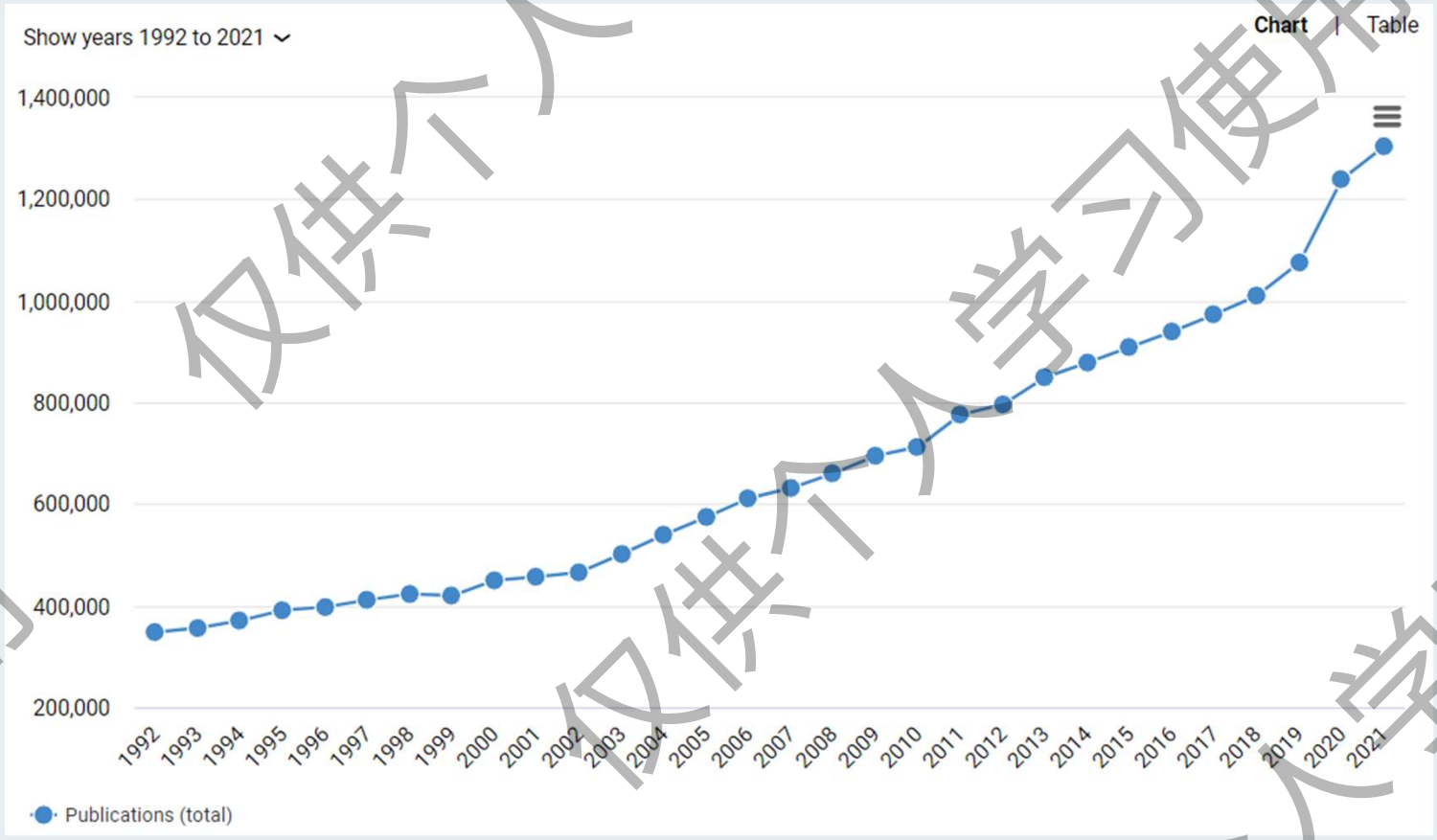
张宏伟 博士
高级解决方案专家
数据库科研学术顾问

April 2023

ADVANCING DISCOVERY

目录

生命科学领域研究大幅增长



科研产出每年增长8-9%

预计科学产出每9年翻一番

1) All documents indexed by **Dimensions** for all life science areas, 1992–2021.
2) “Global scientific output doubles every nine years”. Nature News Blog. May 7, 2014

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- **The Global Scientific Research [1-3] Landscape in the past some year:**

8,000,000 active researchers

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1,600,000 journal articles published

- **And *<u>many other</u>* data sources:**
  - Research data repositories[3]: ≥ 2,000
  - Dark data[4]: ???

1) Research publications indexed by Incites & Web of Science
2) "The STM Report, 4th Edition". International Association of STM Publishers, Feb. 20, 2015.
3) "Key IP5 Statistical Indicators 2017". IP5 Offices, Mar. 2018.
4) Registry of Research Data Repositories, re3data.org.
5) "Dark analytics: Illuminating opportunities hidden within unstructured data", Deloitte Insights Feb. 7, 2017

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如何浏览数据洪流


KQED FUTURE OF YOU

Explosion of Big Data, But Scientists Can't Keep Up

By **Richard Harris**
NPR Shots
NOVEMBER 29, 2016

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News Article

NATURE | NATUREJOBS | FEATURE

Scientific literature: Information overload

Esther Landhuis

Nature 535, 457-458 (2016) doi:10.1038/nj7612-457a

Published online 20 July 2016

This article was originally published in the journal *Nature*

How to manage the research-paper deluge? Blogs, colleagues and social media can all help.

信息过载[1-4]**:** aka *冗余、信息处理、信息过剩、数据烟雾，但也有过滤器故障*

挑战 需要特殊的工具来快速发现跨越多个来源的高质量科学数据

1) "Information Overload." Wikipedia Entry. June 10, 2018.
2) "Explosion of Big Data, But Scientists Can't Keep Up". KQED, Nov. 29, 2016
3) "Scientific literature: Information overload". Nature Jobs. Jul. 20, 2016.
4) "The big data explosion sets us profound challenges - how can we keep up?". The Guardian. Jul. 2, 2016.

我们了解科学家在实验室实施新方案时面临的挑战

- 快速**找到**相关和可靠的实验室指南
- 需要多种方法来**评估**实验室指南
- 在自己特有的环境中**实施**实验步骤

解决方法 开发专门针对实验室指南和方法优化的研究型解决方案

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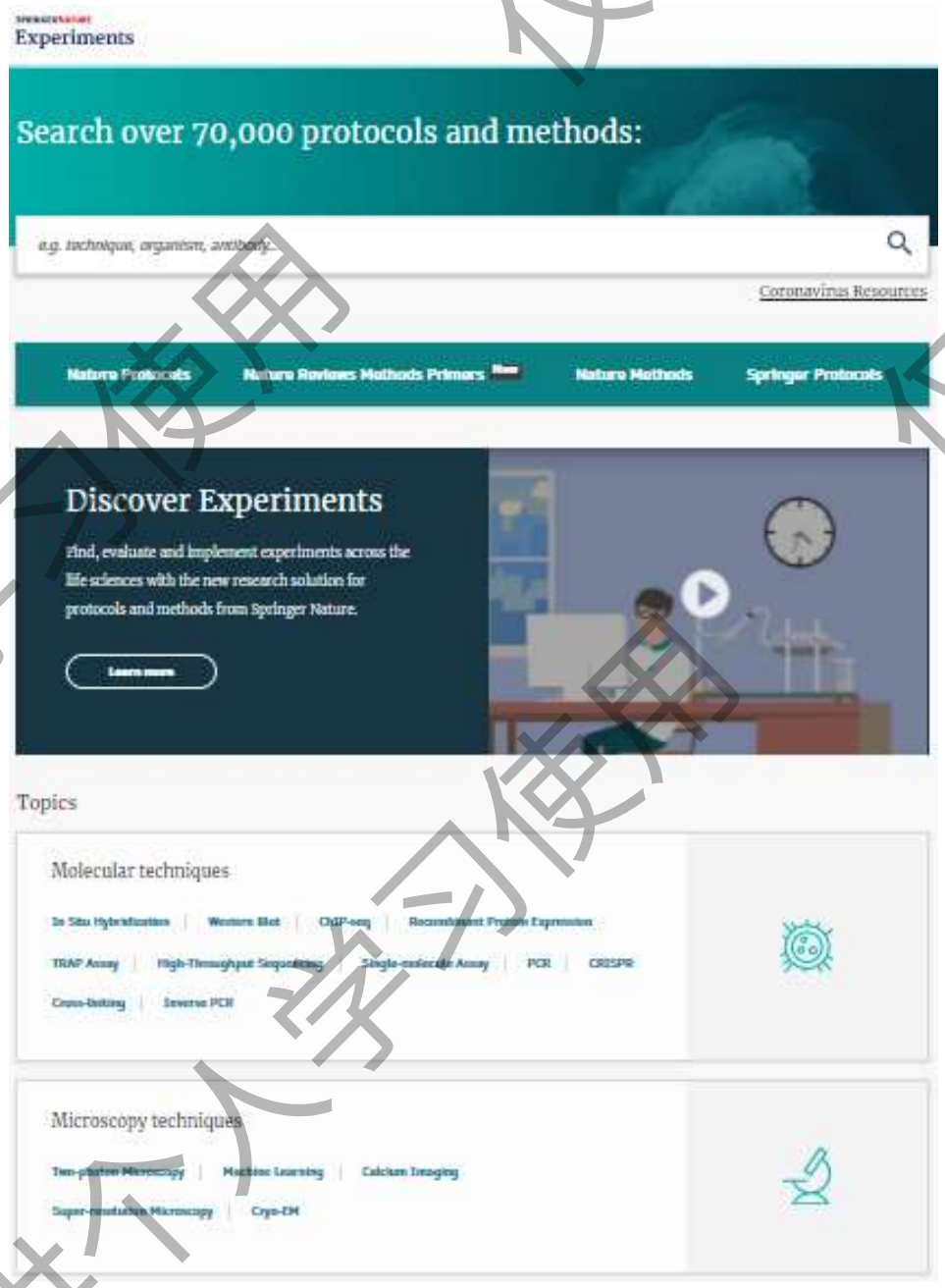

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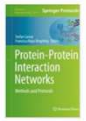

Protein-Protein Interaction Networks pp 67-80 | Cite as

A Web-Based Protocol for Interprotein Contact Prediction by Deep Learning

Authors | Authors and affiliations

Xiaoyang Jing, Hong Zeng, Sheng Wang, Jinbo Xu

Protocol
First Online: 04 October 2019

460 Downloads

Part of the Methods in Molecular Biology book series (MIMB, volume 2074)

Abstract

Identifying residue–residue contacts in protein–protein interactions or complex is crucial for understanding protein and cell functions. DCA (direct-coupling analysis) methods shed some light on this, but they need many sequence homologs to yield accurate prediction. Inspired by the success of our deep-learning method for intraprotein contact prediction, we have developed RaptorX-ComplexContact, a web server for interprotein residue–residue contact prediction. Given a pair of interacting protein sequences, RaptorX-ComplexContact first searches for their sequence homologs and builds two paired multiple sequence alignments (MSA) based on genomic distance and phylogeny information, respectively. Then, RaptorX-ComplexContact

1 Introduction

Proteins play various roles in cellular and biochemical processes by physically interacting with other proteins or forming protein complexes [1, 2]. Studying protein–protein interactions (PPIs) at residue level is crucial for understanding protein functions in organisms. Experimental techniques have been greatly improved to determine protein complex structure, but they are still low throughput and costly [3, 4]. Therefore, developing effective computational methods to elucidate the 3D structure of a PPI or complex from its sequence is

2 Materials

The following are required and optional materials for the use of RaptorX-ComplexContact server:

1. A personal computer with Internet connection and a web browser with JavaScript enabled. RaptorX-ComplexContact server is compatible with three popular web browsers: Google Chrome, Firefox, and Internet Explorer. Nevertheless, the former two browsers may be slightly better than the third one in visualizing the prediction results.
2. The amino-acid sequences or multiple sequence alignments (MSAs) of the query protein pair in FASTA format. Only the MSAs generated by HHblits are systematically tested although in principle any MSAs shall work.
3. The amino-acid sequences or multiple sequence alignments (MSAs) could also be uploaded to the server as text files.
4. The job name and email address are optional, but a valid email address is strongly recommended since it can facilitate job management and result retrieval.

3 Methods

3.1 Job Submission

1. Open the hyperlink http://raptorx.uchicago.edu/ComplexContact/ in the web browser.
2. From the menu at the top of the page, select “New job.”

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1) Shown publication metrics refer to the data is since January 2018

Research paper

Introduction
Describes the research question and the hypothesis to be explored

Results
Finding reports with experimental data compiled into charts or figures

Discussion
Results are interpreted in relation with published evidence

Method
Experimental design of the study and list of procedures performed (protocols)

Research protocol

Introduction
Describes the protocol and its range of applications

Materials
List the compositions of all buffers, solutions and equipment needed, with quantities used and operation instructions

Method
Detailed and chronological explanation of the individual stages of the technique, with timings and critical steps

Notes
Recommendations and details to implement the protocols

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Illustrative example based on the paper CRISPR/Cas9 system targeting regulatory genes of HIV-1 inhibits viral replication in infected T-cell cultures, Youdiil Ophinni, Mari Inoue, Tomohiro Kotaki2 & Masanori Kameoka, Scientific Reports (2018) and protocol Beads-on-a-String on a Bead: Reconstitution and Analysis of Chromatin on a Solid Support, Raphael Sandaltzopoulos and Peter B. Becker, Chromatin Protocols, Methods in Molecular Biology, vol. 1288 (2015)

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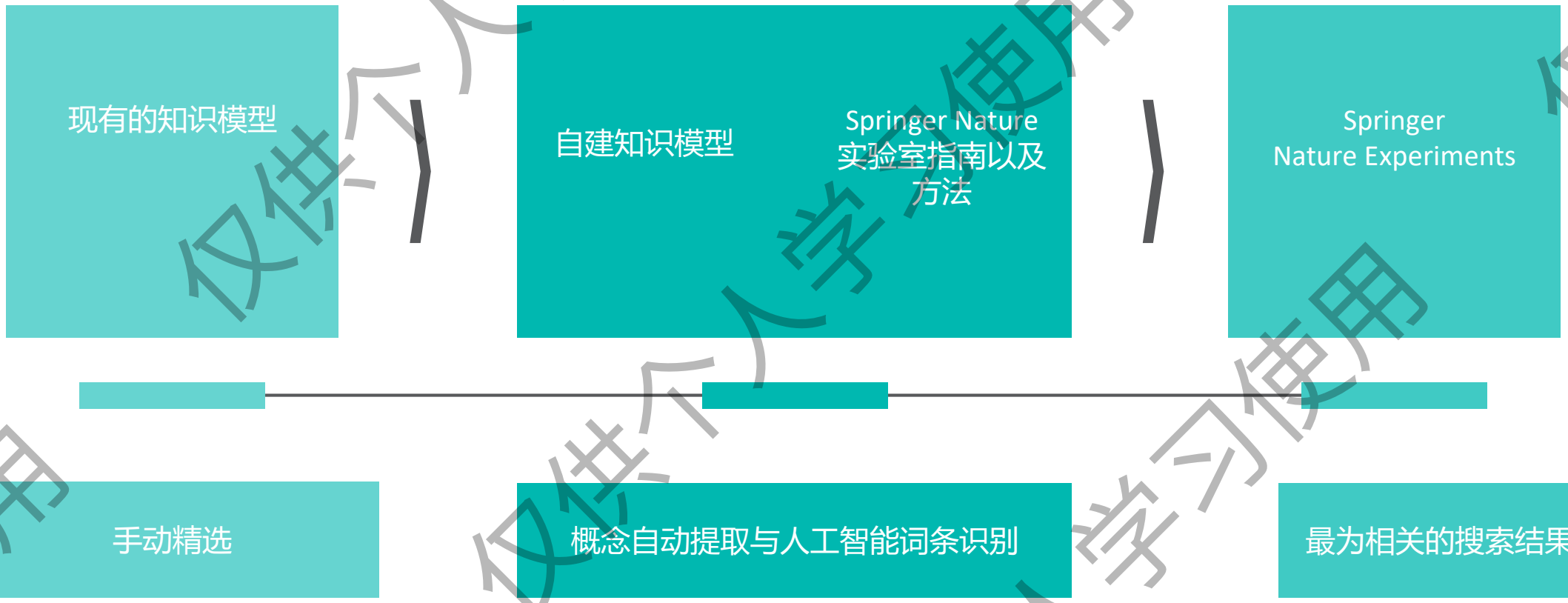

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- 生物化学
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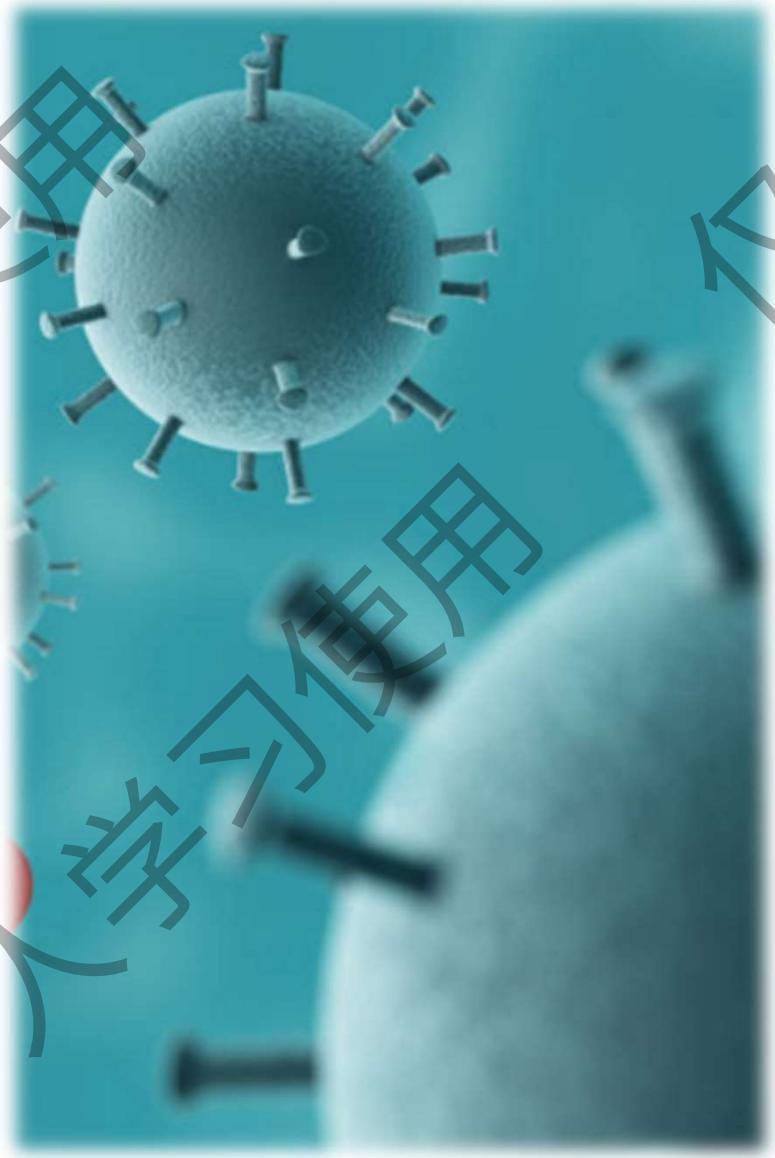
相关信息精选

- Biochemical Characterization of Middle East Respiratory Syndrome Coronavirus Spike Protein Proteolytic Processing
- A Multiplex Polymerase Chain Reaction for Differential Detection of Turkey Coronavirus from Chicken Infectious Bronchitis Virus and Bovine Coronavirus
- Expression of the Severe Acute Respiratory Syndrome Coronavirus 3a Protein and the Assembly of Coronavirus-Like Particles in the Baculovirus Expression System
- Evaluation of Activation and Inflammatory Activity of Myeloid Cells During Pathogenic Human Coronavirus Infection
- Development of a Mouse-Adapted MERS Coronavirus



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- 多元回归分析的应用Regression analysis[4]
- 分子流行病学Molecular Epidemiology[5]
- 遗传流行病学Genetic Epidemiology[6]
- 空间信息技术在流行病学中的应用Spatial Technology[7]

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- 公共卫生保健建模方法Modelling epidemics[24]
- 动物疫情防控Animal epidemic prevention and control[25]
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* *Find references 1-26 in Springer Nature Experiments platform.*

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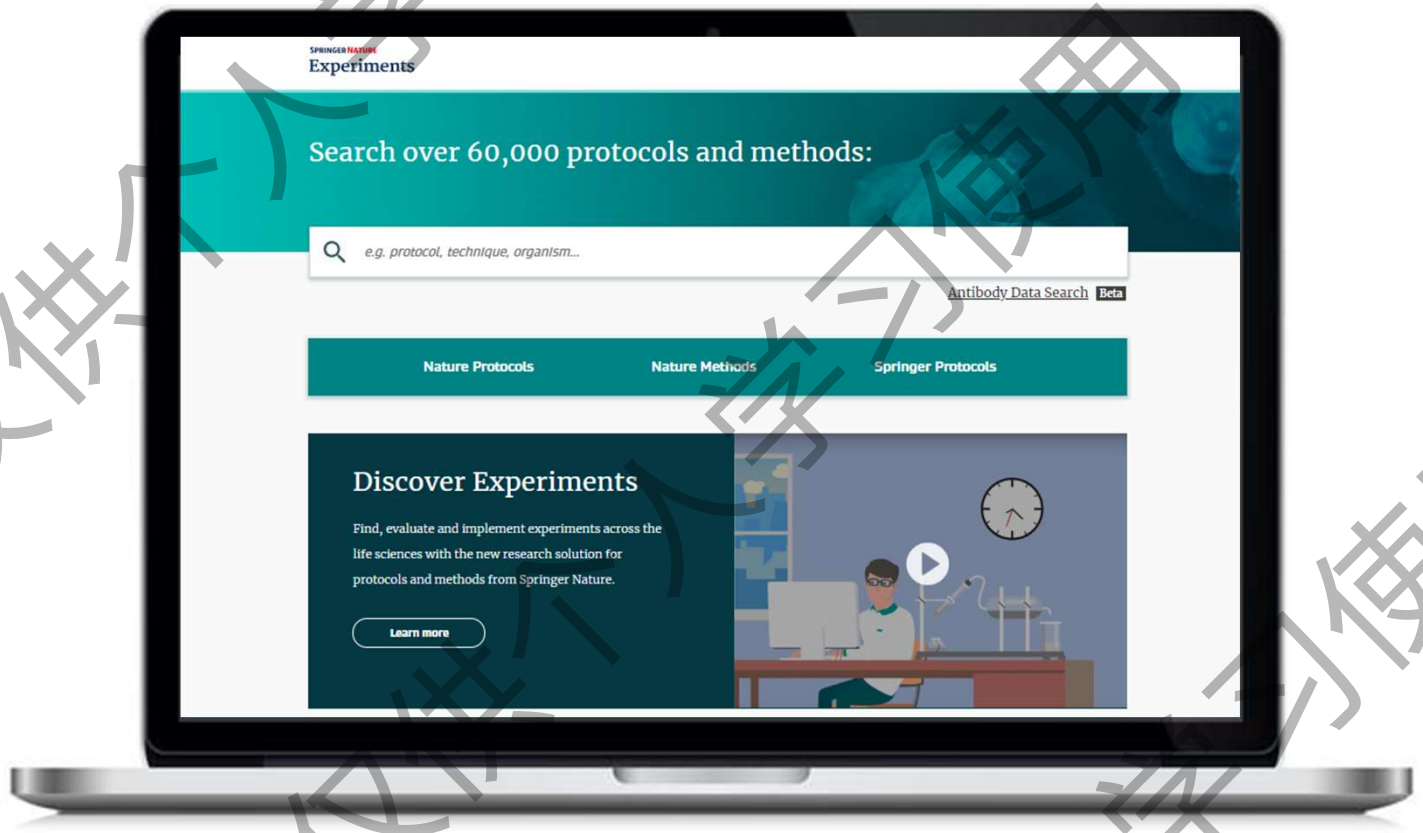

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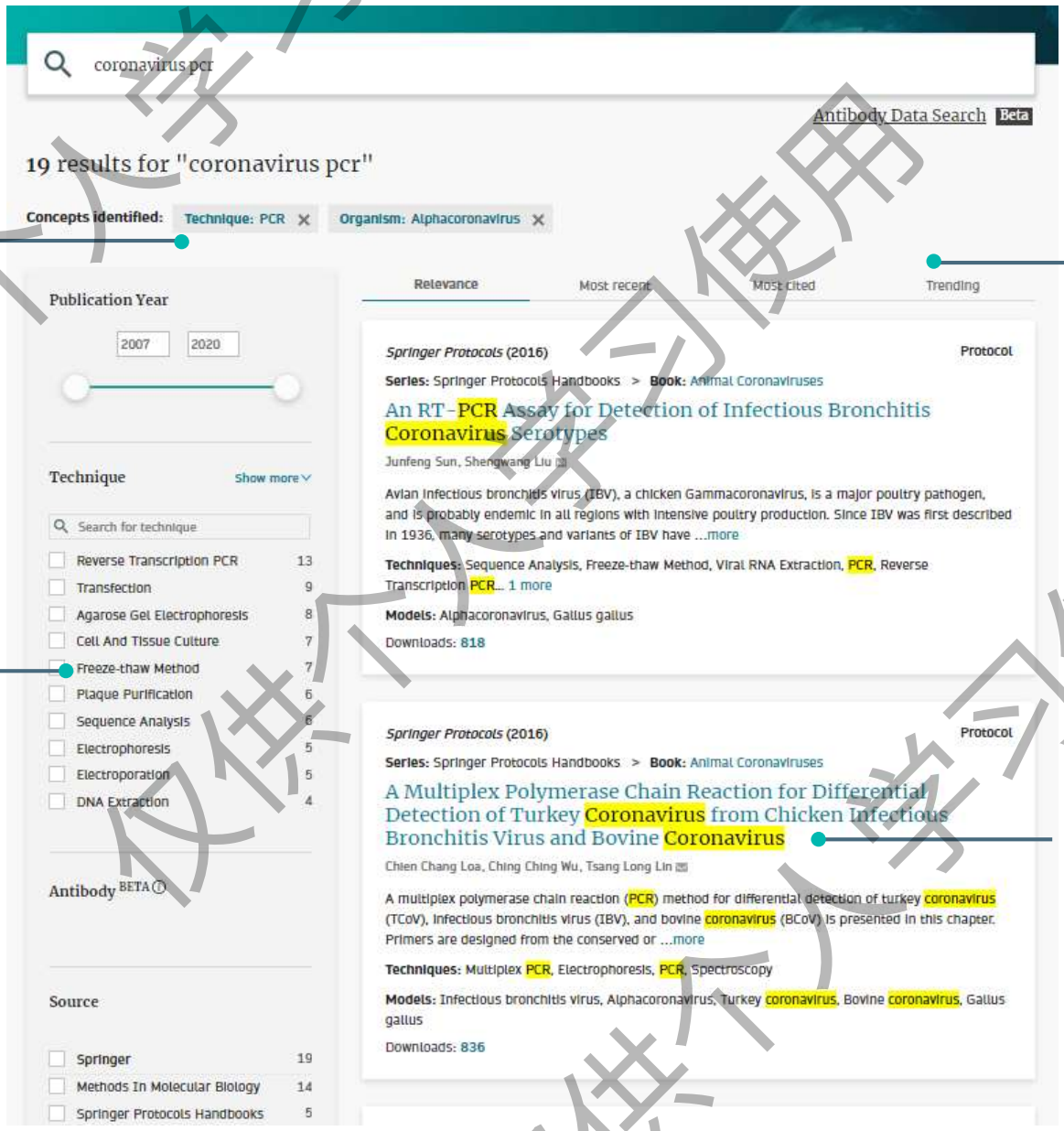

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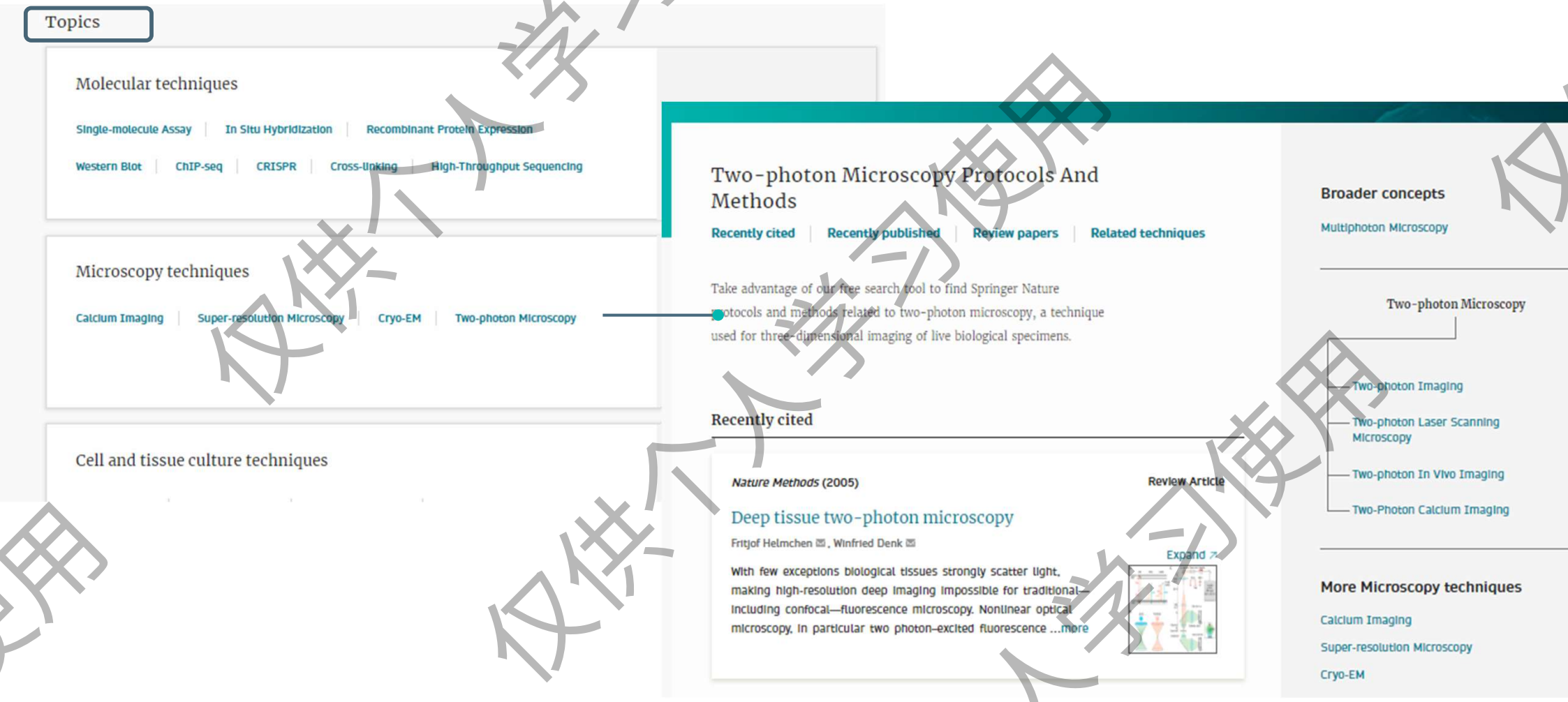

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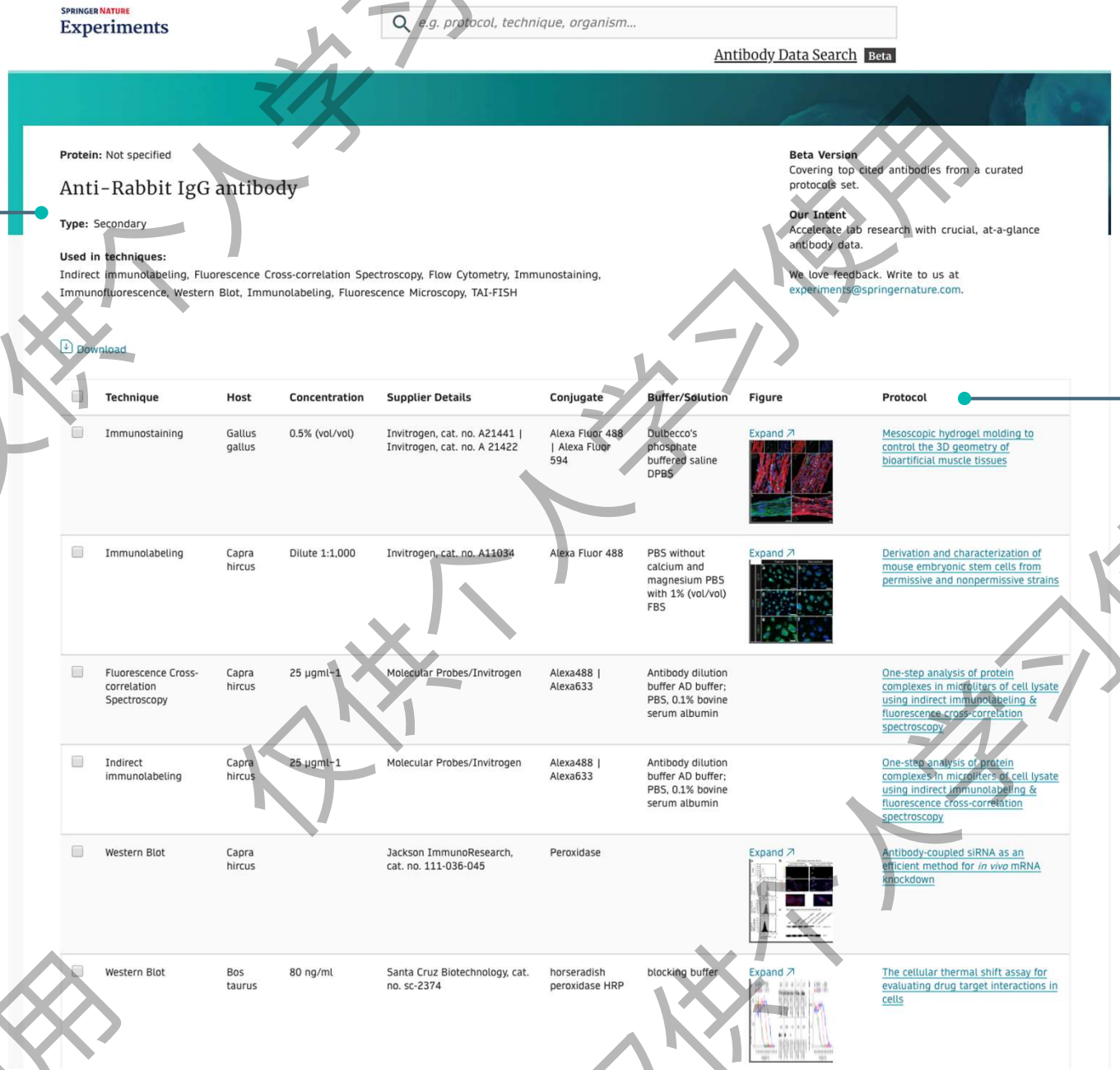

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e.g. protocol, technique, organism...

Antibody Data Search Beta

Protein: Not specified

Anti-Rabbit IgG antibody

Type: Secondary

Used in techniques:
Indirect immunolabeling, Fluorescence Cross-correlation Spectroscopy, Flow Cytometry, Immunostaining, Immunofluorescence, Western Blot, Immunolabeling, Fluorescence Microscopy, TAI-FISH

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Technique	Host	Concentration	Supplier Details	Conjugate	Buffer/Solution	Figure	Protocol
Immunostaining	Gallus gallus	0.5% (vol/vol)	Invitrogen, cat. no. A21441 | Invitrogen, cat. no. A 21422	Alexa Fluor 488 | Alexa Fluor 594	Dulbecco's phosphate buffered saline DPBS	Expand	Mesoscopic hydrogel molding to control the 3D geometry of bioartificial muscle tissues
Immunolabeling	Capra hircus	Dilute 1:1,000	Invitrogen, cat. no. A11034	Alexa Fluor 488	PBS without calcium and magnesium PBS with 1% (vol/vol) FBS	Expand	Derivation and characterization of mouse embryonic stem cells from permissive and nonpermissive strains
Fluorescence Cross-correlation Spectroscopy	Capra hircus	25 µgml−1	Molecular Probes/Invitrogen	Alexa488 | Alexa633	Antibody dilution buffer AD buffer; PBS, 0.1% bovine serum albumin		One-step analysis of protein complexes in microliters of cell lysate using indirect immunolabeling & fluorescence cross-correlation spectroscopy
Indirect immunolabeling	Capra hircus	25 µgml−1	Molecular Probes/Invitrogen	Alexa488 | Alexa633	Antibody dilution buffer AD buffer; PBS, 0.1% bovine serum albumin		One-step analysis of protein complexes in microliters of cell lysate using indirect immunolabeling & fluorescence cross-correlation spectroscopy
Western Blot	Capra hircus		Jackson ImmunoResearch, cat. no. 111-036-045	Peroxidase		Expand	Antibody-coupled siRNA as an efficient method for *in vivo* mRNA knockdown
Western Blot	Bos taurus	80 ng/ml	Santa Cruz Biotechnology, cat. no. sc-2374	horseradish peroxidase HRP	blocking buffer	Expand	The cellular thermal shift assay for evaluating drug target interactions in cells

MERS Coronavirus pp 21-37 | Cite as

Biochemical Characterization of Middle East Respiratory Syndrome Coronavirus Spike Protein Proteolytic Processing

Authors Authors and affiliations

Gary R. Whittaker, Jean K. Millet

Protocol
First Online: 28 December 2019

3.2k Downloads

Part of the Methods in Molecular Biology book series (MIMB, volume 2099)

Abstract

The coronavirus spike envelope glycoprotein is an essential viral component that mediates virus entry events. Biochemical assessment of the spike protein is critical for understanding structure–function relationships and the roles of the protein in the viral life cycle. Coronavirus spike proteins are typically proteolytically processed and activated by host cell enzymes such as trypsin-like proteases, cathepsins, or proprotein-convertases. Analysis of coronavirus spike proteins by western blot allows the visualization and assessment of proteolytic processing by endogenous or exogenous proteases. Here, we present a method based on western blot analysis to investigate spike protein proteolytic cleavage by transient transfection of HEK-293 T cells allowing expression of the spike protein of the highly pathogenic Middle East respiratory syndrome coronavirus in the presence or absence of a cellular trypsin-like transmembrane serine protease, matriptase. Such analysis enables the characterization of cleavage patterns produced by a host protease on a coronavirus spike glycoprotein.

Key words

Coronavirus Spike protein Virus entry Middle East respiratory syndrome (MERS) Proteolytic processing Host cell protease Matriptase Western blot Transient transfection

2 Materials

All cell culture materials should be kept sterile and manipulated within a biosafety cabinet. When not in use they should be stored at 4 °C. All liquid and solid waste materials should be discarded and/or properly inactivated in appropriate disposable waste containers. Solutions diluted in water should be prepared with ultra-purified water with a resistivity of 18.2 MΩ·cm at 25 °C.

2.1 Plasmids and Antibodies

1. pcDNA3.1-OPT-MERS-wt-S-C9. This plasmid encodes a full-length, wild-type (wt), mammalian codon-optimized sequence of the MERS-CoV spike gene from the EMC/2012 strain fused with a C9 bovine rhodopsin epitope tag at the C-terminus.
2. pcDNA3.1-hMatriptase. This plasmid contains the coding sequence of the human matriptase gene.
3. pcDNA3.1. This plasmid is used as an empty vector control plasmid.
4. Rabbit polyclonal antibody against MERS-CoV strain EMC/2012 spike protein.
5. Mouse monoclonal antibody (IgG_1) against the extracellular domain of human matriptase (clone D-7).
6. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibodies.
7. HRP-conjugated goat anti-mouse IgG antibodies.

2.2 Cell Culture Reagents and Materials

1. Dulbecco's phosphate buffered saline (DPBS) with calcium and magnesium.
2. Dulbecco's Modified Eagle Medium (DMEM).
3. Heat-inactivated fetal calf serum (FCS).
4. 1 M N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES).
5. 100× penicillin-streptomycin (PS) solution.
6. Human embryonic kidney (HEK) HEK-293 T/17 cells were obtained from the American Type Culture Collection. The/17 numbering refers to a clone that has been specifically selected to obtain higher transfection efficiencies. Cells were cultured in a 37 °C, 5% CO_2 incubator in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (vol/vol) FCS, 10 mM HEPES, 100 IU/mL penicillin, and 100 μg/mL streptomycin. For long-term storage, the cells can be frozen and stored in liquid nitrogen.
7. 1× Trypsin solution. 0.25% trypsin, 2.21 mM ethylenediaminetetraacetic acid (EDTA).
8. Cell counting slide with 10 counting grids.
9. Gibco™ Opti-minimal essential medium (Opti-MEM™) reduced serum medium (for transfections).

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Evan Zhang, Senior Solutions Specialist
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