Video Article

September 2016-This Month in JoVE: Introducing JoVE Genetics, JoVE Biochemistry, and JoVE Cancer Research

Wendy Chao[1], Aaron Kolski-Andreaco[2]

[1]Department of Ophthalmology, , Massachusetts Eye and Ear

[2]JoVE Content Production

Correspondence to: Aaron Kolski-Andreaco at aaron.kolski-andreaco@jove.com

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Abstract

This month we are pleased to introduce three new sections of the JoVE family: Genetics, Biochemistry, and Cancer Research.

JoVE Genetics contains methodologies for exploring all aspects of genes and heredity-from human genetics to model organisms, epigenetics to evolutionary genetics, and gene editing to gene therapy. This section features a method for genotyping pufferfish species by liquid chromatography/mass spectrometry, described by Miyaguchi. This technique can aid in the appropriate identification and differentiation of toxic species, which is not only important for public health but also for forensics and investigations of food fraud. Also in JoVE Genetics, Yu *et al.* report methods for genetically engineering the unconventional yeast *Yarrowia lipolytica* with improved gene deletion efficiency. The engineered *Y. lipolytica* strains have potential applications in biofuel and biochemical production.

JoVE Biochemistry comprises methods that advance our understanding of biomolecule structure and function, as well as their interactions and transformations during biological processes. This month, Gunning *et al.* present a method of meat authentication using multiple reaction monitoring (MRM) mass spectrometry, which identifies peptides and gives relative quantitation for detecting adulterant species in meat mixtures. This method is sensitive enough to detect 1% horsemeat in beef products. Also in JoVE Biochemistry, Head and Liu describe a method for identifying small molecule-binding proteins using photoaffinity labeling. The target proteins are bound and covalently labeled within the live cellular environment, which helps preserve native protein structure and binding conditions.

JoVE Cancer Research encompasses a broad range of techniques used to advance the understanding and treatment of cancer. This includes methodologies for studying carcinogenesis, developing innovative diagnostics and therapeutics, and uncovering the mechanisms of drug resistance. This month in JoVE Cancer Research, Ansari *et al.* report a method of targeted cell isolation via glass surface functionalization. This method can identify biomarkers of resistance or susceptibility to anti-angiogenic therapies. Also in this section, Domogauer *et al.* present a mixed cell culture model that mimics the tumor microenvironment. With this model, the intercellular communication within the tumor microenvironment can be studied under various conditions.

You've just had a sneak peek of the articles in the newest sections of JoVE. Visit the website to see the full-length articles, plus many more, in JoVE: The Journal of Visualized Experiments.

Video Link

The video component of this article can be found at https://www.jove.com/video/5811/

Protocol

Identification of Small Molecule-binding Proteins in a Native Cellular Environment by Live-cell Photoaffinity Labeling

Sarah A. Head[1], Jun O. Liu[1,2]

[1]Department of Pharmacology and Molecular Sciences, **Johns Hopkins University School of Medicine**, [2]Department of Oncology, **Johns Hopkins University School of Medicine**

We describe here a method for identification of small molecule-binding proteins using photoaffinity labeling. The advantage of this technique is that binding and covalent labeling of the target proteins occurs within the live cellular environment, removing the risk of disrupting native protein structure and binding conditions upon cell lysis.

Improved Polymerase Chain Reaction-restriction Fragment Length Polymorphism Genotyping of Toxic Pufferfish by Liquid Chromatography/Mass Spectrometry

Hajime Miyaguchi

National Research Institute of Police Science

An improved polymerase chain reaction-restriction fragment length polymorphism method for genotyping pufferfish species by liquid chromatography/ mass spectrometry is described. A reverse-phase silica monolith column is employed for separating digested amplicons. This method can elucidate the monoisotopic masses of oligonucleotides, which is useful for identifying base composition.

A Mimic of the Tumor Microenvironment: A Simple Method for Generating Enriched Cell Populations and Investigating Intercellular Communication

Jason D. Domogauer, Sonia M. de Toledo, Edouard I. Azzam

Department of Radiology, New Jersey Medical School, **Rutgers University**

We adapted a permeable microporous membrane insert to mimic the tumor microenvironment (TME). The model consists of a mixed cell culture, allows simplified generation of highly enriched individual cell populations without using fluorescent tagging or cell sorting, and permits studying intercellular communication within the TME under normal or stress conditions.

A Method of Targeted Cell Isolation via Glass Surface Functionalization

Ali Ansari[1], Reema Patel[2], Kinsey Schultheis[1], Vesna Naumovski[3], P. I. Imoukhuede[1]

[1]Department of Bioengineering, **University of Illinois at Urbana-Champaign**, [2]Department of Liberal Arts & Sciences, **University of Illinois at Urbana-Champaign**, [3]Department of Biomedical Engineering, **Illinois Institute of Technology**

This protocol describes customizable surface functionalization of the desthiobiotin, streptavidin, and APTES system in order to isolate specific cell types of interest. In addition, this manuscript covers the applications, optimization, and verification of this process.

Genetic Engineering of an Unconventional Yeast for Renewable Biofuel and Biochemical Production

Ai-Qun Yu[1,2], Nina Pratomo[1,2], Tee-Kheang Ng[1,2], Hua Ling[1,2], Han-Saem Cho[1,2], Susanna Su Jan Leong[1,2,3], Matthew Wook Chang[1,2]

[1]Department of Biochemistry, Yong Loo Lin School of Medicine, **National University of Singapore**, [2]NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), Life Sciences Institute, **National University of Singapore**, [3]Food Science and Chemical Engineering, **Singapore Institute of Technology**

We herein report methods on the molecular genetic manipulation of the *Yarrowia lipolytica* Po1g strain for improved gene deletion efficiency. The resulting engineered *Y. lipolytica* strains have potential applications in biofuel and biochemical production.

Species Determination and Quantitation in Mixtures Using MRM Mass Spectrometry of Peptides Applied to Meat Authentication

Yvonne Gunning[1], Andrew D. Watson[1], Neil M. Rigby[2], Mark Philo[1], Joshua K. Peazer[1,3], E. Kate Kemsley[1]

[1]Analytical Sciences Unit, **Institute of Food Research**, [2]**Institute of Food Research**, [3]School of Chemistry, **University of East Anglia**

We present a protocol for identifying and quantifying the components in mixtures of species possessing similar proteins. Mass spectrometry detects peptides for identification, and gives relative quantitation by ratios of peak areas. As a tool food for fraud detection, the method can detect 1% horse in beef.

Disclosures

No conflicts of interest declared.

