

## 课程详述

### COURSE SPECIFICATION

以下课程信息可能根据实际授课需要或在课程检讨之后产生变动。如对课程有任何疑问，请联系授课教师。

The course information as follows may be subject to change, either during the session because of unforeseen circumstances, or following review of the course at the end of the session. Queries about the course should be directed to the course instructor.

1.	课程名称 <b>Course Title</b>	海洋分子生物学实验 <b>Marine Molecular Biology Lab</b>				
2.	授课院系 <b>Originating Department</b>	海洋科学与工程系 Department of Ocean Science and Engineering				
3.	课程编号 <b>Course Code</b>	OCE318				
4.	课程学分 <b>Credit Value</b>	2				
5.	课程类别 <b>Course Type</b>	专业选修课 Major Elective Courses				
6.	授课学期 <b>Semester</b>	秋季 Fall				
7.	授课语言 <b>Teaching Language</b>	中英双语 English & Chinese				
8.	授课教师、所属学系、联系方式（如属团队授课，请列明其他授课教师） <b>Instructor(s), Affiliation &amp; Contact</b> (For team teaching, please list all instructors)	郭静, 海洋科学与工程系, 创园 9 栋 602, guoj@mail.sustc.edu.cn Guo Jing, Department of Ocean Science and Engineering, Chuangyuan 9-602 Tel. 18126401114				
9.	实验员/助教、所属学系、联系方式 <b>Tutor/TA(s), Contact</b>	无 NA				
10.	选课人数限额(可不填) <b>Maximum Enrolment (Optional)</b>	10				
11.	授课方式 <b>Delivery Method</b>	讲授 <b>Lectures</b>	习题/辅导/讨论 <b>Tutorials</b>	实验/实习 <b>Lab/Practical</b>	其它(请具体注明) <b>Other (Please specify)</b>	总学时 <b>Total</b>
	学时数 <b>Credit Hours</b>			64		64

12. 先修课程、其它学习要求 <b>Pre-requisites or Other Academic Requirements</b>	
13. 后续课程、其它学习规划 <b>Courses for which this course is a pre-requisite</b>	
14. 其它要求修读本课程的学系 <b>Cross-listing Dept.</b>	

### 教学大纲及教学日历 SYLLABUS

#### 15. 教学目标 Course Objectives

本课程目标是为高年级海洋生物学、海洋科学专业学生提供有一点难度的现代海洋分子生物学基本实验技能训练的窗口，在全面介绍微生物海洋学的基本技术基础上，通过一系列综合性的实验操作，提高学生动手能力，引导并培养学生利用分子生物学实验手段实现海洋科学研究的基本思维和目的。要完成所有的课程任务，学生必须：

1、完成课前预习,对实验目的、实验原理、实验步骤、注意事项、试剂配方与作用以及实验设计的思路进行理解,鼓励带着问题上课。

2、按时上课,认真听讲,主动思考,积极回答问题,积极参与课堂讨论,遵守实验室规定,遵守课堂秩序,规范操作;仔细观察实验过程中每一步骤的实验现象,实事求是地记录并深入分析。通过动手操作能够加深对实验原理与设计思路的理解,有利于学生创新性思维及独立分析问题解决问题能力的提高。课堂表现、操作表现和出勤率是成绩考核的组成部分。

To complete all course tasks, students must:

1. Complete of pre-class preparation, understanding of the purpose of the experiment, experimental principles, experimental procedures, precautions, reagent formulation and function, and experimental design ideas, encourage the class with questions.

2. Attend the class on time, actively think and participate in class discussions, abide by laboratory regulations, observe classroom order, standardize operations; carefully observe the experimental phenomena at each step of the experimental process, realistically record and analyze in depth. Through hands-on operation, the understanding of experimental principles and design ideas can be deepened, which is conducive to students' innovative thinking and the ability to solve problems independently. Class performance, operational performance and attendance are part of the performance assessment.

#### 16. 预达学习成果 Learning Outcomes

到课程结束时, 学生应能:

- 1、了解海洋分子生物学实验技术体系和发展现状, 提升深入理解海洋分子生物学理论知识和技术原理的主观愿望, 更加积极地探究现代海洋分子生物学领域相关科学问题并利用现有技术解决实际问题;
- 2、能够掌握海洋古菌 SCM1 的培养与生理生化测定。利用流式细胞仪进行海洋微生物样品如 SCMI 的计数与活力分析。
- 3、学会海洋酵母菌的分离培养与鉴定等技能。
- 4、能够完成海水样品基因组 DNA 提取、PCR 扩增、qPCR、琼脂糖凝胶电泳等实验操作, 并能对实验现象进行观察与分析。
- 5、能够规范使用微量加样器、高压蒸汽消毒器、恒温摇床、超净工作台、高速冷冻离心机、制冰机、PCR 仪、qPCR 仪、水平电泳仪、凝胶成像系统等仪器, 熟练无菌操作技术, 养成分工协作的团队精神和习惯。

The goal of this course is to provide a high-level marine biology and marine science students with a difficult window of basic experimental skills training in modern marine molecular biology. Based on a comprehensive introduction to the

basic techniques of microbial oceanography, through a series of comprehensive The experimental operation enhances students' practical ability, guides and trains students to use molecular biology experiments to achieve the basic thinking and purpose of marine scientific research. By the end of the course, students should be able to:

1. Understand the marine molecular biology experimental technology system and development status, enhance the subjective desire to understand the theoretical knowledge and technical principles of marine molecular biology, and actively explore relevant scientific issues in the field of modern marine molecular biology and use existing technologies to solve practical problems. ;
- 2, Master the cultivation and physiological and biochemical determination of SCM1. Flow cytometry was used to perform counting and viability analysis of marine microbial samples, such as SCM1.
3. Learn the skills of separation, culture and identification of marine yeast.
4. Master the process of the genomic DNA extraction, PCR amplification, qPCR, agarose gel electrophoresis and other experimental operations of marine water samples, and can observe and analyze the experimental phenomena.
5. Regulate the use of micro-sampler, high-pressure steam sterilizer, constant temperature shaker, ultra-clean workbench, high-speed refrigerated centrifuge, ice machine, PCR instrument, qPCR instrument, horizontal electrophoresis instrument, gel imaging system and other instruments, Proficiency in aseptic technique and teamwork .

17. 课程内容及教学日历（如授课语言以英文为主，则课程内容介绍可以用英文；如团队教学或模块教学，教学日历须注明主讲人）

**Course Contents (in Parts/Chapters/Sections/Weeks. Please notify name of instructor for course section(s), if this is a team teaching or module course.)**

#### 一、海洋分子生物学实验简介及海水样品过滤(4 学时)

介绍实验室规则制度与课程安排；实验报告要求；实验课考核及评分标准；介绍海水样品采集、保存及运输等实验基础知识，进行海水样品过滤（不同滤膜孔径样品）。

#### 1. Introduction to marine molecular biology lab course and filtration of seawater sample (4 credit hours)

Introduce the laboratory rules system and curriculum arrangement; experimental report requirements; experimental class assessment and scoring standards; introduce the basic knowledge of seawater sample collection, preservation and transportation, and carry out filtration of seawater sample (different filter pore size samples).

#### 二、海水滤膜样品总 DNA 提取（4 学时）

海水和沉积物等海洋环境样品 DNA 的介绍（着重介绍 DNA 提取溶液成分与原理），利用手提法提取海水滤膜样品总 DNA，Nanodrop 测定提取 DNA 浓度和纯度。

#### 2. Total DNA extraction of seawater filter samples (4 credit hours)

Introduction of DNA from marine environmental samples such as seawater and sediments (emphasizing the composition and principle of DNA extraction solutions), using the portable method to extract total DNA from seawater filtration samples, and the Nanodrop to determine the concentration and purity of extracted DNA.

#### 三、利用 PCR 扩增海洋古菌基因片段（4 学时）

PCR 原理和多种 PCR 方法及应用介绍，学习引物的设计，利用 PCR 技术扩增已知的海洋古菌基因片段，了解琼脂糖凝胶电泳的原理；琼脂糖凝胶电泳制备；通过琼脂糖凝胶电泳检测 PCR 产物。

#### 3. Amplification of marine archaeal gene fragments by PCR (4 credit hours)

Introduce PCR principle and various PCR methods and application, learn primer design, using PCR technology to

amplify known marine archaeal gene fragments, understand the principle of agarose gel electrophoresis; agarose gel electrophoresis preparation; agarose gelation The PCR product was detected by gel electrophoresis.

#### 四、海洋古菌 qPCR 实验（8 学时）

qPCR 的原理、流程和应用的介绍（0.5 学时）。学会利用 384 孔板进行反应体系的配置（1 学时），反应体系的分装（1 学时），标准样品稀释和标准曲线的制作（1.5 学时），DNA 添加到孔板（1.5 学时），盖上孔板膜；去除气泡后离心，反复此步骤，直至完全去除气泡（1 学时），上机、命名样品和鉴定数据，判断数据是否可信（1.5 小时）。

#### 4. Marine archaeal qPCR experiment (8 credit hours)

Introduction to the principle, process and application of qPCR (0.5 credit hours). Learn to use 384-well plate for reaction system configuration (1 credit hour), reaction system dispensing (1 credit hour), standard sample dilution and standard curve preparation (1.5 credit hour), DNA added to well plate (1.5 credit hours) covered Orifice membrane; centrifuge after removing the bubbles, repeat this step until the bubbles are completely removed (1 credit hour), machine, name the sample and identify the data to determine whether the data is reliable (1.5 credit hours).

#### 五、氨氧化古菌 SCM1 培养基配制（7 学时）

古菌 SCM1 培养原理与培养基主要成分介绍（0.5 学时）。准备玻璃器皿（1 小时，需要用含有 1% 盐酸的超纯水冲洗配置过程中使用到的玻璃器皿（烧杯、搅拌棒、容量瓶、蓝盖瓶和量筒等），之后用超纯水冲洗 3 次）。复杂盐溶液的制备（1 小时），在无菌条件下，配备生长元素（3 个小时，包含 HEPE 缓冲液、乙二胺四乙酸铁钠溶液、微量元素混合溶液、磷酸氢钠溶液、磷酸二氢钾溶液、氯化铵溶液等），配备好的培养基高压灭菌锅灭菌、冷却（1.5 小时）。

#### 5. Preparation of ammonia-oxidizing archaea SCM1 medium (7 credit hours)

The principle of culture of SCM1 and the main components of the culture medium (0.5 credit hour). Prepare the glassware (1 credit hour, rinse the glassware (beaker, stir bar, volumetric flask, blue cap bottle and measuring cylinder, etc.) used in the configuration with ultrapure water containing 1% hydrochloric acid, then rinse with ultrapure water 3 Times). Preparation of complex salt solution (1 credit hour), under sterile conditions, with growth elements (3 credit hours, including HEPE buffer, sodium iron diamine tetraacetate solution, trace element mixed solution, sodium hydrogen phosphate solution, phosphoric acid II) Potassium hydrogen solution, ammonium chloride solution, etc., sterilized and cooled in an autoclave equipped with a medium (1.5 credit hours).

#### 六、氨氧化古菌 SCM1 生长曲线测定（9 学时）

SCM1 菌液生长测定原理介绍（0.5 学时，根据亚硝酸盐的浓度指示生长情况）。进行 SCM1 转接培养（0.5 学时），配制显色剂，利用紫外分光光度计测量其 538 nm 的吸收值（1 学时）。每隔 24h 测定 SCM1 菌液的吸收值，直至 SCM1 已经生长到了稳定期（每天取样测定吸收值，连续测 7 天，每天 1 学时，共 7 学时）

#### 6. Determination of growth curve of ammonia-oxidizing archaea SCM1 (9 credit hours)

The principle of SCM1 bacterial growth assay (0.5 credit hour, indicating growth according to the concentration of nitrite). The SCM1 transfer culture (0.5 credit hour) was performed, and a color developer was prepared, and the absorption value at 538 nm (1 credit hour) was measured by an ultraviolet spectrophotometer. The absorption value of SCM1 bacteria solution was measured every 24h until SCM1 had grown to a stable period (sampling value was measured every day, 7 days in a row, 1 credit hour per day, 7 credit hours in total)

#### 七、利用流式细胞仪进行稳定期 SCM1 计数（4 学时）

介绍流式细胞仪的工作原理，学会利用其进行 SCM1 活的和死的微生物准确的检测和计数，是海洋生物技术的重要的一步。了解海洋微生物的活性及生理状态对于理解微生物在自然界中的作用及其重要。

#### 7. Using the flow cytometer for SCM1 counting (4 credit hours)

Introduce the working principle of flow cytometry, and learning to use it to accurately detect and count live and dead microorganisms of SCM1 is an important step in marine biotechnology. Understand the activity and physiological state of marine microorganisms is important for understanding the role of microorganisms in nature.

#### 八、海洋酵母菌的分离培养（6 学时）

学习分离培养海洋酵母菌的方法（0.5 学时）。处理海泥样品（0.5 学时，加入无菌陈海水和吐温 80 溶液，震荡混匀），海泥过滤与浸出液处理（1 学时），滤膜贴到培养基培养 3 天，观察（1 学时），划线纯化（1 学时），培养 3 天，观察（1 学时），挑取单菌落保种（1 学时）。

#### 8. Separation and culture of marine yeast (6 credit hours)

Learn how to isolate and culture marine yeast (0.5 credit hour). Treatment of sea mud samples (0.5 credit hour, add sterile seawater and Tween 80 solution, shake and mix), sea mud filtration and leachate treatment (1 credit hour), filter membrane attached to the medium for 3 days, observation (1 credit hour) Purification by scribing (1 credit hour), culture for 3 days, observation (1 credit hour), single colony preservation (1 credit hour).

#### 九、海洋酵母菌的鉴定 I-目的基因片段的获得（7 学时）

海洋酵母菌 DNA 的微量提取（2 学时），海洋酵母菌的 18SrRNA 基因和 ITS 序列的 PCR 扩增（2.5 学时），PCR 扩增产物的回收（2.5 学时）

#### 9. identification of marine yeast I-target gene fragment acquisition (7 credit hours)

Micro-extraction of marine yeast DNA (2 credit hours), PCR amplification of 18SrRNA gene and ITS sequence of marine yeast (2.5 credit hours), recovery of PCR amplification products (2.5 credit hours)

#### 十、海洋酵母菌的鉴定 II-PCR 产物与载体连接及转化（5 学时）

掌握连接及转化的基本原理；将胶回收产物（目的片段及载体）进行连接；连接产物转化大肠杆菌。课堂实验 4 学时，第二天观察 1 学时。

#### 10. Identification of marine yeast II-PCR product and vector ligation and transformation (5 credit hours)

Master the basic principles of ligation and transformation; connect the gel recovery product (target fragment and vector); connect the product to *E. coli*. Classroom experiment 4 hours, observe 1 class hour the next day.

#### 十一、海洋酵母菌的鉴定 III-转化质粒提取和 PCR 检测（6 学时）

进行转化质粒提取和 PCR 检测，进行 18SrRNA 基因和 ITS 序列测序，送到测序公司进行测序，利用 NCBI 进行比对，确定鉴定菌株和相关菌株的相似度。

#### 11. Identification of marine yeast III-transformation plasmid extraction and PCR detection (6 credit hours)

The transforming plasmid was extracted and PCR was detected, and the 18SrRNA gene and ITS sequence were sequenced and sent to the sequencing company for sequencing. The NCBI was used for comparison to determine the similarity between the identified strain and the related strain.

### 18. 教材及其它参考资料 Textbook and Supplementary Readings

自编教材

参考资料：

1. 《Lewin's Genes XI》, Jocelyn E. Krebs; Elliott S. Goldstein; Stephen T. Kilpatrick, Jones & Bartlett Publishers, 2013

2. 《Molecular Biology》 Fifth Edition , Robert F. Weaver. McGraw-Hill Science/Engineering/Math, 2011
3. 《现代分子生物学》第4版, 朱玉贤 李毅 郑晓峰 郭红卫编, 高等教育出版社, 2013
4. 王祥红, 微生物与海洋微生物实验, 中国海洋大学出版社, 2011
5. Michael T. Madigan, John M. Martinko, Jack Parker, Brock's Biology of Microorganism 14TH, 2014

**课程评估 ASSESSMENT**

19. 评估形式 Type of Assessment	评估时间 Time	占考试总成绩百分比 % of final score	违纪处罚 Penalty	备注 Notes
出勤 Attendance		10		
课堂表现 Class Performance		30		
小测验 Quiz				
课程项目 Projects				
平时作业 Assignments		50		
期中考试 Mid-Term Test				
期末考试 Final Exam				
期末报告 Final Presentation		10		
其它 (可根据需要 改写以上评估方式) Others (The above may be modified as necessary)				

20. 记分方式 **GRADING SYSTEM**

- A. 十三级等级制 Letter Grading  
 B. 二级记分制 (通过/不通过) Pass/Fail Grading

**课程审批 REVIEW AND APPROVAL**

21. 本课程设置已经过以下责任人/委员会审议通过  
**This Course has been approved by the following person or committee of authority**

海洋科学与工程系本科教学委员会  
 Department of Ocean Science and Engineering Undergraduate Committee