南方科技大学 SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY

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 Course Title**	**现代生物技术实验 Advanced Biotechnology Laboratory**
2.	**授课院系 Originating Department**	生物系 Department of Biology
3.	**课程编号 Course Code**	BIO344
4.	**课程学分 Credit Value**	2
5.	**课程类别 Course Type**	专业选修课 Major Elective Courses
6.	**授课学期 Semester**	春季 Spring
7.	**授课语言 Teaching Language**	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） Instructor(s), Affiliation& Contact （For team teaching, please list all instructors）**	余春红，工程师，生物系 第一教学楼 231 室 yuch@sustc.edu.cn 0755-88018745 Yu Chunhong, Engineer, Department of Biology Rm.231, No.1 Teaching building
9.	**实验员/助教、所属学系、联系方式 Tutor/TA(s), Contact**	宋亚坤，实验员，生物系 第一教学楼 232 室 songyk@sustc.edu.cn Song Yakun, technician Rm.232, No.1 Teaching building 张敏，实验员，生物系 第一教学楼 232 室 zhangm6@sustc.edu.cn
10.	**选课人数限额(可不填) Maximum Enrolment （Optional）**	10

11. 授课方式 Delivery Method	讲授 Lectures	习题/辅导/讨论 Tutorials	实验/实习 Lab/Practical	其它(请具体注明) Other（Please specify）	总学时 Total
学时数 Credit Hours			56	8 (pre-and/or post-experimental treatment 8 hours)	64

12. 先修课程、其它学习要求 Pre-requisites or Other Academic Requirements	细胞生物学实验 Cell Biology Laboratory (BIO208)
13. 后续课程、其它学习规划 Courses for which this course is a pre-requisite	
14. 其它要求修读本课程的学系 Cross-listing Dept.	

教学大纲及教学日历 SYLLABUS

15. **教学目标 Course Objectives**

现代生物技术实验主要学习和运用近些年发展和流行使用的生命科学研究方法和技术，如 RNA 干扰、电穿孔、定量 PCR、基因编辑、细胞分选，等等，并学习如何开展项目研究。

The lab focuses on gene expression regulation and application of modern research techniques, such as RNAi, gene editing, electroporation, RT-qPCR, cell sorting and et al. Also students will learn how to perform research projects.

16. **预达学习成果 Learning Outcomes**

本课程完成后，学生将能够：

（1）掌握一些先进的生物科学研究技术

（2）培养对提出及开展生物课题研究的实验设计与操作能力

With the completion of this course, the students will

（1）gain deep understanding of some modern research techniques

（2）learn how to design and perform research projects

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.)

模块 I（实验 1）. 序列分析和 siRNA/sgRNA 设计 （4 学时）

介绍项目、课程安排和要求。学习怎么分析序列和设计 siRNA 或 sgRNA。

模块 II （实验 2-4）Crispr-cas9 载体构建（13 学时）

载体(Addgene ID: 48138) 含有 gRNA 框和 SpCas9 表达盒。gRNA 框由 U6 启动子驱动，且附近有两个

Bbs1 限制性内切酶切位点。学生需要将指导序列克隆到 gRNA 框。这个过程也叫 Crispr-cas9 载体构建。包含如下实验。

实验 2：引物退火和 Crispr-cas9 载体克隆(4 学时+0.5 h)

磷酸化和退火每对指导 RNA 寡核苷酸链，通过连接反应插入到线性化载体中。转化克隆 CRISPR-Cas9 载体，第二天观察结果。

实验 3：质粒 DNA 提取 (4 学时+ 0.5 h)

对于每对指导 RNA，提前一天挑 3-5 个单克隆，培养过夜。提取质粒 DNA。

实验 4：酶切分析 (4 学时)

通过酶切分析和测序筛选阳性克隆子。

模块 III（实验 5-6） 细胞转染（9.5 学时）

在测序验证正确的 CRISPR 载体构建后，因为内毒素会致死细胞，学生要分离去内毒素的质粒 DNA（实验 5），然后转染到哺乳动物细胞中（实验 6）。

实验 5：分离去内毒素的质粒 DNA (4 学时+0.5h)

对于每对指导 RNA，提前一天挑取 3-5 个单克隆，培养过夜。分离去内毒素的质粒 DNA。

实验 6：电转 (4 学时+ 1h)

分别转染 siRNA 和 CRISPR 载体到哺乳动物细胞中。

请注意：细胞转染后，需要不定期的观察细胞。

模块 IV （实验 7-10）指导 RNA 的功能验证（20 学时）

实验 7：细胞分选和培养基因编辑后的细胞克隆 (4 学时+1h)。

分选发生基因编辑的细胞。请注意：细胞分选后，需要不定期的观察和处理细胞。

实验 8：RNA 提取和检测，逆转录 (4 学时+1h)。

采用 Trizol 试剂提取 RNA，检测 RNA 质量，逆转录成 cDNA。

请注意：需要不定期的观察和处理细胞。

实验 9：相对定量 PCR (4 学时+1h)

请注意：需要不定期的观察和处理细胞。

实验 10：扩大培养基因编辑后的单克隆细胞株 (4 学时+1h)。

继续传代培养单细胞克隆。分析定量 PCR 结果。

请注意：需要不定期的观察和处理细胞。

模块 V（实验 11-13）测序验证基因编辑（13.5 学时）

首先提取基因组 DNA（实验 11），然后扩增含特殊位点的 DNA 片段（实验 12）。割胶纯化（实验 13），最后送出去测序。

实验 11：基因组 DNA 提取（4 学时+0.5h）

预先收集细胞，储存到-20 度。然后提取基因组 DNA。

实验 12：扩增含特殊位点的 DNA 片段（4 学时）。

实验 13：割胶回收扩增片段（4 学时+1 小时）。

割胶回收扩增片段，准备测序样品。如果测序样品不合格，可能要用 TA 克隆再准备测序样品。

模块 VI（实验 14）：总结和汇报（4 学时）

Module I (LAB 1). Sequence analysis and siRNA/sgRNA design (4 credit hours)

Introduce the project, course schedule and course requirement. Learn how to perform sequence analysis and design siRNA/sgRNA sequences.

Module II (LAB 2-4) Crispr-cas9 vector construction (13 credit hours)

The vector (Addgene ID: 48138) contains gRNA scaffold and SpCas9 expression cassette. The gRNA scaffold is driven by U6 promoter and flanked by two Bbs1 restriction sites. We will clone the guide sequence into the sgRNA scaffold in Module II. This process is also known as construct of CRISPR-Cas9 vectors, which is composed of the following experiments.

LAB 2: Oligo annealing and cloning into crispr-cas9 vectors (4credit hours+0.5 h)

Phosphorylate and anneal each pair of sgRNA oligos. Insert sgRNA duplex into the linearized vector by ligation reaction. Then transform/clone new CRISPR-Cas9 vectors containing sgRNA sequences. Observe result next day.

LAB 3: Plasmid DNA isolation from transformants (4 credit hours+ 0.5 h)

Pick 3-5 single colonies for each sgRNA insert and culture them overnight. Isolation Plasmid DNA from transformants.

LAB 4: Restriction analysis (4 credit hours)

Screen positive transformants by restriction analysis and sequencing.

Module III (LAB 5-6) Cell transfection (9.5 credit hours)

After sequence validation of correct CRISPR plasmid construct, we will isolate Plasmid DNA without endotoxin that may cause cell death (lab 5) and then transfect them into the mammalian cells (lab 6).

LAB 5: Plasmid DNAs isolation without endotoxin (4 credit hours+0.5h)

Pick 3-5 single colonies for each sgRNA insert and culture them overnight. Isolate plasmid DNAs without endotoxin.

LAB 6: Electroporation (4 credit hours+ 1h)

Transfect siRNA and correct CRISPR plasmid construct into mammalian cell lines, respectively..

Please note that aperiodic observation and treatment of cells are required after cell transfection.

Module IV (LAB 7-10) Functional validation of sgRNAs (20 credit hours)

LAB 7: Cell Sorting and clonal isolation of gene-edited cell lines (4 credit hours+1h)

Isolate gene-edited cells by cell sorting. Please note that aperiodic observation and treatment of cells are required after Cell Sorting.

LAB 8: RNA isolation and Detection, RT (4 credit hours+ 1h)

Isolate total RNAs from cells using Trizol reagent. Detect the quality of total RNAs and convert them into cDNA.

Please note that aperiodic observation and treatment of cells are required after Cell Sorting.

LAB 9 Relative quantitation (4 credit hours+ 1h)

Please note that aperiodic observation and treatment of cells are required after Cell Sorting.

LAB 10. Expanding gene-edited cell population derived from a single cell (4 credit hours+1)

Subculture cell population derived from a single cell. Analyze the result of Relative quantitation.

Please note that aperiodic observation and treatment of cells are required.

Module V (LAB 11-13) Detection of indel mutations in gene-edited cell lines by sequencing (13.5 credit hours)

Firstly, genomic DNA extraction will be performed (lab 11). Then, DNA fragments covering the specific target loci will be amplified (lab 12) and purified using gel extraction (lab 13). Finally, the purified DNA fragments will be sequenced by commercial corporations.

LAB 11: DNA extraction from mammalian cells (4 credit hours+0.5h)

Harvesting cells for DNA extraction in advance and store at -20°C. Then isolate genomic DNA from cells without red fluorescence.

LAB 12: Amplification of target gene (4 credit hours)

Amplify DNA fragments covering the specific target loci.

LAB 13: Gel extraction and sequencing (4 credit hours+1h)

Purify DNA fragments using gel extraction. Prepare qualified samples for sequencing. Use TA cloning if possible.

Module VI (LAB 14) Summarizing the projects and oral presentation (4 credit hours)

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

1.Lab mannual

2.Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc. 2013, 8(11):2281-2308.

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				
课堂表现 Class Performance		30		
小测验 Quiz				
课程项目 Projects		30		实验记录 Note
平时作业 Assignments		30		实验报告 Lab report
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

本课程经生物系本科教学指导委员会审议通过。 This Course has been approved by Undergraduate Teaching Steering Committee of Department of Biology.