

## 4-Week Offline Workshop on Molecular Cloning (TA Cloning)

For Undergraduate Students in Biology, Biotechnology, and Life Sciences

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### Workshop Overview

This **4-week hands-on offline workshop** focuses on **TA (Taq polymerase-mediated) cloning**, a widely used **ligation-independent cloning method**. Students will learn **PCR-based amplification, A-tailing, vector preparation, ligation, transformation, screening, plasmid DNA isolation, and sequencing analysis**. The workshop provides direct **wet-lab experience** along with discussions on troubleshooting and real-world applications.

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### Workshop Structure & Study Design

#### Week 1: Introduction to TA Cloning & Experimental Design

✦ **Objective:** Understanding the principles and workflow of TA cloning.

#### Topics Covered

- **Introduction to Molecular Cloning**
  - Overview of **traditional vs. TA cloning vs. restriction-based cloning**.
  - Applications of TA cloning in **gene expression, genetic engineering, and research**.
- **Principles of TA Cloning**
  - Role of **Taq polymerase** in A-tailing.
  - Selection of **TA vectors** (e.g., pGEM-T, pCR2.1-TOPO).
- **Experimental Planning & Sample Selection**
  - Choosing a **gene of interest** and designing an experiment.
  - Preparing genomic DNA or cDNA for amplification.

#### **Hands-on Activities:**

- ✓ **Plasmid vector preparation and restriction analysis.**
  - ✓ **DNA sample extraction & quantification using NanoDrop/spectrophotometry.**
  - ✓ **Assignment:** Design a TA cloning experiment for a selected gene.
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#### Week 2: PCR Amplification, A-Tailing & Vector Preparation

✦ **Objective:** Learning how to amplify DNA, generate A-overhangs, and prepare vectors for cloning.

#### Topics Covered

- **PCR Amplification for TA Cloning**
  - Primer designing with **Taq polymerase compatibility**.

- Setting up and optimizing **PCR reactions**.
- **A-Tailing of PCR Products**
  - Extension of A-overhangs at **3' ends** using Taq polymerase.
  - Checking **A-tailed products on agarose gel**.
- **Vector Preparation & Ligation Strategy**
  - Preparing **linearized TA vectors** with complementary T-overhangs.
  - **Ligation of PCR products into the TA vector using T4 ligase**.

 **Hands-on Activities:**


- ✓ **PCR amplification of a target gene with Taq polymerase.**
  - ✓ **Gel electrophoresis to check PCR & A-tailing efficiency.**
  - ✓ **Ligation reaction setup and incubation.**
  - ✓ **Assignment:** Troubleshoot a failed PCR reaction based on experimental data.
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### **Week 3: Transformation, Selection & Screening of Recombinant Clones**

✦ **Objective:** Understanding how recombinant DNA is introduced into cells and screened for successful clones.

#### **Topics Covered**

- **Transformation of Ligation Products into Competent Cells**
  - **Heat shock & electroporation** techniques for bacterial transformation.
  - Role of **competent cells (E. coli DH5α, TOP10)** in cloning.
- **Selection & Screening of Clones**
  - **Blue-white screening (LacZ selection method).**
  - Antibiotic selection using **ampicillin-resistant markers**.
- **Colony PCR & Restriction Digestion for Insert Confirmation**
  - Extracting plasmid DNA from selected colonies.
  - Digesting plasmid DNA with **restriction enzymes** to confirm insertion.

 **Hands-on Activities:**

- ✓ **Transformation of E. coli with ligated TA vector.**
  - ✓ **Screening colonies via blue-white selection & antibiotic resistance.**
  - ✓ **Colony PCR & restriction digestion of recombinant clones.**
  - ✓ **Assignment:** Interpret gel electrophoresis data for clone screening.
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### **Week 4: Plasmid DNA Isolation, Sequencing & Functional Analysis**

✦ **Objective:** Learning to extract and confirm the inserted gene sequence and analyze its function.

### Topics Covered

- **Plasmid DNA Isolation & Purification**
  - **Alkaline lysis method** for plasmid extraction.
  - Quantification using **NanoDrop & gel electrophoresis**.
- **Sequence Verification of Cloned Inserts**
  - Sending recombinant plasmids for **Sanger sequencing**.
  - Aligning sequences using **BLAST & Clustal Omega**.
- **Functional Applications of Cloned Genes**
  - Expressing **cloned genes for protein production**.
  - Applications in **biopharmaceuticals, genetic engineering & synthetic biology**.

### **Hands-on Activities:**

- ✓ **Plasmid isolation from recombinant clones.**
  - ✓ **Sequence verification using online tools (BLAST, SnapGene, Expasy).**
  - ✓ **Final Project:** Design a **cloning workflow** for a real-world research problem.
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### Expected Outcomes of the Workshop

- ✓ **Hands-on experience in TA cloning and gene manipulation.**
  - ✓ **Ability to design and troubleshoot molecular cloning experiments.**
  - ✓ **Proficiency in PCR, ligation, transformation, and plasmid isolation.**
  - ✓ **Knowledge of gene screening, sequencing, and functional analysis.**
  - ✓ **Preparation for research internships, biotech jobs, and academic projects.**
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### How to Register?

✉ Email: **[Your Email]**

☎ Phone: **[Your Contact Number]**

🌐 Website: **[Your Website]**