LAB: DNA Extraction

INPUT: Tree leaves

OUTPUT: Related tree DNA

KEY TERMS
- lysis buffer
- EDTA
- SDS
- GAPC
- silica gel chromatography
- cloning

KEY CONCEPTS
- GAPC = GAPDH
- ground up leaves in lysis buffer
- DNA + ethanol + silica gel = DNA bound to silica gel
- elution - warm H2O releases DNA

DNA Assignment
- Please try...
8, 15, 16

LAB: First Round PCR

Input: isolated gDNA

Output: Amplified GAPC-like sequence

**KEY TERMS**

- degenerate primers
- denaturation 95
- annealing 55
- extension 72
- thermocycler
- 40 cycles

**KEY CONCEPTS**

- Polymerase = non-specific, many times to increase chances of finding

- Control + outline PCR work
- Control + check contamination

1. Denature DNA
2. Anneal - add primer + DNA + dNTPs
3. Extension - add DNA polymerase buffer

Product of interest starts after cycle 1.
LAB: PCR Gel Electrophoresis

INPUT: GPE-like seqs

OUTPUT: info did PCR work?

KEY TERMS
- agarose gel
- ethidium bromide
- MW Ruler
- loading dye
- electrodes

MW Formula: 10^18

KEY CONCEPTS
- DNA has \( \theta \) charge (has positive lengths, negative ends)
- Separation by size:
  - Smaller DNA pieces run further
  - MW Ruler provides size reference
  - EtBr binds to DNA, when excited by UV light it fluoresces

DNA Assignment:
- Please

\[
\text{pH} \quad \left[ \frac{x}{e^{47}} \right] \quad \text{m} \, 20 (\text{A} + \text{T}) + 42 (\text{G} + \text{C})
\]
LAB: Nested PCR/PCR Purification

INPUT: GAPDH1 / GAPDH-1/2 + PCR mix

OUTPUT: unmodified GAPDH-1/2

KEY TERMS

- Nested
- Specific primer
- Annealing conditions
- Chromatography
- PCR

KEY CONCEPTS

- Nested M-HIV has specific primers
- Include GAPDH-1 2-2 only
- Chromatography digests 1st RD primers
- Heat inactivate HMwuclease or it acts nested primers
- Whiffle ball theory: "small" molecules get hung up in holes, big molecules pass through PCR product
LAB: Ligations

INPUT: spc-12

OUTPUT: ligated plasmid + spc insert

KEY TERMS:
- plat 1.2 vector
- ori
- Origin of Replication
- Eco47IR
- Mcs
- pLac DNA
- pLac

KEY CONCEPTS:
- blunt/sticky
- directionality
- Some sites are sticky, not blunt
- Many possible ligation products
- Unequal exchange, equal exchange
- Cuts off A overhang 1:1 ratio of
- homology

DNA and Protein Plea

\[ T_m = 20 \cdot (A+T) + 4 \cdot (C+G) \]
LAB: Transformation

INPUT: plasmid & donor (E)

OUTPUT: E. coli colony + plasmid (E)

KEY TERMS

TF buffer
CaCl2
DMSO

heated shock
electrotransfer
important cells
exponential growth phase

KEY CONCEPTS

\( n \text{ copies} \) vs. \( n \text{ copies} \)

CFU = number of viable cells that can form colonies

CONFIRM if the mutant contains a single mutation

E. coli and dependent regulatory pathway
**Key Terms**
- Liquid culture + inoculation
- Lipase
- Silica gel

**Key Concepts**
- High salt → precipitate the proteins
- Buffer + acidification to aggregate RNA
- Centrifuge:
- Silica gel column → isoelectric point

**Lab:**
- Planned purification

**Input:**
- Cell colony + institute

**Output:**
- Dried baker sheet

**Math:**
\[
\begin{align*}
47 & = 20(a - b) + 10 + 0.0102 \\
\end{align*}
\]