Brief Summary of the 7 Molecular Biology Projects (from lab manual)

Experiment # 1: Gene Cloning and Protein Expression
(using total RNA from mouse tissue in Experiment #3)

cDNA cloning of the mouse GAPDH gene in a plasmid expression vector (pMAL).
First and second strand cDNA synthesis and PCR to synthesize the GAPDH gene.
Ligation of the GAPDH cDNA into the plasmid expression vector pMAL.
Transformation of E. coli, selection of recombinant clones and DNA sequencing.
Expression and purification of the GAPDH fusion protein in E. Coli.
Measurement of protein concentration and running on PAGE protein gels.
Western blot to specifically detect the GAPDH fusion protein.

Experiment #2: Genome Analysis

Isolate and purify genomic mouse DNA from liver tissue.
Amplify the transthyretin (Ttr) gene using PCR.
Analysis of the methylation state of Ttr and Rvt genes in mouse genomic DNA.

Experiment #3: Gene Expression Analysis

Preparation of total RNA from mouse liver tissue.
Amplification of Ttr mRNA by reverse transcriptase PCR (RT-PCR).
Quantitative RT-PCR using real-time analysis (Sybr and TaqMan systems).

Experiment #4: Next-Generation Sequencing

Using total RNA from mouse tissue (Experiment #3) prepare a next-gen cDNA library.
Sequence the library on the next-gen Illumina MiSeq DNA Sequencer.
Bioinformatics to analyze millions of DNA sequence reads.

Experiment #5: CRISPR/cas9 Gene Editing in Yeast

Design a CRISPR/cas9 gene editing plasmid for yeast and amplify in E. coli.
Isolation and purification of the pCAS/ADE2 sgRNA plasmid.
Construct a barcode/editing DNA fragment using PCR.
Co-transform yeast with the pCAS plasmid and the barcode/editing DNA fragment.
Demonstrate successful genome editing by phenotype and genotype.

Experiment #6: RNA Interference (RNAi) in C. elegans

Grow C. elegans and isolate and purify eggs to produce a synchronous culture.
RNA interference by feeding C. elegans on E. coli containing Bli DNA.
Observe the RNA interference Bli phenotype in adult C. elegans.

Experiment #7: Human DNA/Genetic Analysis

Isolation of your own DNA from cheek cells from your mouth.
PCR amplification of your own DNA for DNA fingerprint analysis.
PCR amplificaiton of your taster gene: compare phenotype and genotype.