RT-LAMP Application Usage Examples

Quick Start Guide

1. Basic Primer Design

Command Line Usage (if applicable)

```
# Run the GUI application
./RT_LAMP_Designer

# For headless environments
QT_QPA_PLATFORM=offscreen ./RT_LAMP_Designer
```

GUI Workflow

1. **Launch Application**: Double-click `RT_LAMP_Designer` or run from terminal
2. **Load Sequence**:
   - Click "Load Sequence" or use File → Open
   - Select a FASTA file (e.g., `test_data/sars2_n.fasta`)
3. **Configure Parameters**:
   - Set target region (start/end positions)
   - Adjust primer length constraints
   - Set thermodynamic parameters
4. **Design Primers**: Click "Design Primers"
5. **Review Results**: Examine primer sets in the results panel
6. **Export Results**: Save results as Excel, CSV, or text format

2. Working with Sample Data

The package includes sample SARS-CoV-2 data for testing:

```
# Sample file location
test_data/sars2_n.fasta
```

Sample Sequence Information:
- **Target**: SARS-CoV-2 Nucleocapsid (N) gene
- **Length**: ~1,260 nucleotides
- **Use Case**: Ideal for testing RT-LAMP primer design

Step-by-Step with Sample Data

1. Launch RT-LAMP Designer
2. Load `test_data/sars2_n.fasta`
3. Use default parameters or try these settings:
   - **Target Region**: 200-800 (nucleotides)
   - **F3/B3 Length**: 18-22 bp
   - **FIP/BIP Length**: 40-60 bp
   - **Temperature**: 60-65°C

4. Click “Design Primers”
5. Review the generated primer sets

3. Advanced Features

Multiple Sequence Alignment

1. Load multiple FASTA sequences
2. Select “Advanced” → “Multiple Sequence Alignment”
3. Generate consensus sequence
4. Design primers based on consensus

Specificity Checking

1. After primer design, click “Check Specificity”
2. Configure BLAST parameters
3. Review specificity results
4. Filter primers based on specificity scores

Batch Processing

1. Select “Batch” → “Process Multiple Files”
2. Choose input directory with FASTA files
3. Configure output directory
4. Set processing parameters
5. Start batch processing

4. Parameter Optimization

Thermodynamic Parameters

```
Recommended Settings for RT-LAMP:
- Reaction Temperature: 60-65°C
- Salt Concentration: 50 mM
- Mg²⁺ Concentration: 8 mM
- dNTP Concentration: 1.4 mM
```

Primer Length Constraints

```
Standard LAMP Primer Lengths:
- F3/B3: 18-22 nucleotides
- F2/B2: 18-22 nucleotides
- F1c/B1c: 18-22 nucleotides
- FIP/BIP: 40-60 nucleotides (F1c+F2 or B1c+B2)
```

Loop Primers (Optional)

```
Loop Primer Settings:
- LF/LB Length: 15-25 nucleotides
- Position: Between F2-F1c or B2-B1c regions
- Tm: 5-10°C lower than main primers
```

5. Results Interpretation

Primer Set Quality Indicators

- **Tm Values**: Should be within 5°C of each other
- **GC Content**: 40-60% recommended
- **Secondary Structures**: Minimal hairpins and dimers
- **Specificity Score**: >90% for target specificity

Output Files

- **Excel Format**: Comprehensive results with multiple sheets
- **CSV Format**: Tabular data for further analysis
- **Text Format**: Human-readable summary
- **FASTA Format**: Primer sequences for synthesis

6. Troubleshooting Common Issues

No Primers Found

Possible Causes:
- Target region too short
- Stringent parameters
- Poor sequence quality

Solutions:
- Expand target region
- Relax length constraints
- Check sequence for ambiguous bases

Poor Primer Quality

Possible Causes:
- High GC content regions
- Repetitive sequences
- Secondary structures

Solutions:
- Try different target regions
- Adjust thermodynamic parameters
- Use consensus sequences for variable regions

Slow Performance

Possible Causes:
- Large sequences
- Complex secondary structures
- Limited system resources

Solutions:
- Process smaller regions
- Use batch processing for multiple sequences
- Close other applications

7. Export and Synthesis

Primer Ordering Format

The application can export primers in synthesis-ready format:

```
Primer Name: SARS2_N_F3
Sequence: 5'-ATGCTGCAATCGTGCTACAA-3'
Length: 20 bp
Tm: 58.2°C
GC%: 45.0%

Primer Name: SARS2_N_B3
Sequence: 5'-GACTGCCGCCTCTGCTC-3'
Length: 17 bp
Tm: 59.1°C
GC%: 70.6%
```

Quality Control Checklist

Before ordering primers:
- [ ] Verify sequence accuracy
- [ ] Check Tm calculations
- [ ] Confirm no cross-reactivity
- [ ] Validate primer concentrations
- [ ] Review synthesis modifications (if any)

8. Integration with Laboratory Workflow

Pre-Design Phase

1. **Target Selection**: Choose conserved regions
2. **Sequence Quality**: Verify sequence accuracy
3. **Literature Review**: Check existing primer sets

Design Phase

1. **Parameter Setting**: Use validated parameters
2. **Multiple Designs**: Generate several primer sets
3. **Quality Assessment**: Evaluate all metrics

Post-Design Phase

1. **In Silico Validation**: Check against databases
2. **Primer Synthesis**: Order from reliable suppliers
3. **Laboratory Testing**: Validate experimentally

9. Performance Benchmarks

Typical Processing Times

- **Single Sequence (1-5 kb)**: 10-30 seconds
- **Multiple Sequences (5-10)**: 1-5 minutes
- **Large Genome Regions (>10 kb)**: 2-10 minutes
- **Batch Processing (10+ files)**: 5-30 minutes

System Requirements for Optimal Performance

- **RAM**: 8GB+ recommended

- **CPU**: Multi-core processor
- **Storage**: SSD for faster file I/O
- **Display**: 1920x1080+ for GUI

10. Best Practices

Sequence Preparation

- Use high-quality, verified sequences
- Remove vector sequences and adapters
- Check for ambiguous nucleotides
- Validate sequence orientation

Parameter Selection

- Start with default parameters
- Adjust based on experimental requirements
- Consider target organism characteristics
- Account for reaction conditions

Result Validation

- Always validate computationally designed primers
- Test multiple primer sets when possible
- Consider experimental controls
- Document parameter choices and results

Support Resources

- **Documentation**: See README.md for detailed information
- **Source Code**: Available in src/ directory for customization
- **Sample Data**: Use test_data/ for learning and testing
- **Troubleshooting**: Refer to deployment guides for platform-specific issues

For additional support or questions, refer to the application documentation or rebuild from source code with modifications as needed.