

8-Molecular Biology Protocols

8.1- Characterization & DNA amplification

8.1.1-PCR

8.1.1.1-General PCR Methods & Guidelines

[8.1.1.1.5-Guidelines for Primer Design](#) [\[Updated by vg93 27Oct2016\]](#)

[8.1.1.1.5.1-Primer Design Checklist](#) [\[created by vg93 31Oct2016\]](#)

[8.1.1.1.7-Primer Ordering and Reconstitution](#) **SOP** [updated 2020-06-01 ram524]

[8.1.1.1.9-Ten Things That Can Kill Your PCR](#) **Information Sheet** [reviewed by mw16 07072016]

[8.1.1.1.10-Submitting sequences to GenBank](#) **SOP** [reviewed by mw16 07072016]

[8.1.1.1.11-Primer Database Search Protocol.pdf](#) **SOP** [updated 2020-06-01 ram524]

[8.1.1.1.12-rpoB sigB-AT Assignment](#) [\[MW\]](#)

[8.1.1.1.17-pGEM Ladder for Size Determination of PCR Products](#) **SOP** [updated by ser15 2020-05-18]

[8.1.1.1.18-Gel Electrophoresis of PCR Product](#) **SOP** [updated 2020-05-18 by ser15]

[8.1.1.1.19-PCR Product Purification and Sanger Sequencing Submission](#) **SOP** [updated 2020-05-18 by ser15]

[8.1.1.1.20-Sanger Sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit](#) **SOP** [created 2021-01-14 by sjr267]

[Sequencing Cost Comparison](#) **Supplemental File** [created 2020-12-25 by sjr267]

8.1.1.2-Listeria PCR

8.1.1.2.2-Listeria PCRs by Gene Name

[8.1.1.2.2.1-actA PCR](#) **SOP** [updated 2020-05-18 by ser15]

[8.1.1.2.2.3-Hly a b gene PCR for Listeria monocytogenes](#) **SOP** [updated 2020-05-18 by ser15]

[8.1.1.2.2.4-inlA ORF and Gene Fragment PCR and Sequencing](#) **SOP** [reviewed by mw16 07072016]

[8.1.1.2.2.5-sigB PCR](#) **SOP** [updated 2020-01-30 by js2568]

[8.1.1.2.2.17-Degenerate sigB PCR](#) **SOP** [updated 2020-09-05 by ser15]

[8.1.1.2.2.16-PCRs For Quadruple sigma factor Mutant.pdf](#) [\(Yichang and Veronica\)](#)

8.1.1.3-Salmonella PCR

[8.1.1.3.6-InvA colony PCR for Salmonella](#) **SOP** [updated 2020-04-27 amb629]

[8.1.1.3.7-PCR identification of common Salmonella serogroups](#) **SOP** [reviewed by mw16 07072016]

[8.1.1.3.8-PCR determination of H1 and H2-antigens for Salmonella](#) **SOP** [reviewed by mw16 07072016]

8.1.1.4-PCRs for Other Organisms

[8.1.1.4.1-E. coli 6-gene multiplex PCR.pdf](#)

[8.1.1.4.4-rpoB PCR Protocol](#) **SOP** [updated 2020-05-18 by ser15]

[8.1.1.4.5-PCR Amplification of Full Length 16s rDNA](#) **SOP** [updated 18Nov2016 by sjr267]

[8.1.1.4.5.1-PCR Amplification of 16s rDNA Internal Region](#) **SOP** [updated 2020-01-30 by js2568]

[8.1.1.4.6-ITS PCR for Yeast and Mold](#) **SOP** [updated 2020-06-26 by rle58]

8.1.2-Phenotypic Characterization

[\[see archive\]](#)

8.1.3-RFLP

[\[see archive\]](#)

8.1.4-Southern Blot

[see archive]

8.3-DNA preparation, Purification, & Quantification

[8.3.10-Quantification of Purified DNA by NanoDrop SOP](#) [updated 2020-11-20 sh988]

[8.3.13-Genomic_DNA_Extraction_Qiagen SOP](#) [updated by ajh22 4/28/16]

[8.3.14-HighThroughput_gDNA_Extraction_Qiagen_1_28_15.pdf](#)

[8.3.15-Take3_NucleicAcid_96_well_Quantification_01_20_15 Protocol](#) [reviewed by mw16 07072016]

[8.3.16-DNA Preparation for Whole Genome Sequencing SOP](#) [updated 27Oct2016 by sh988]

[8.3.16.1-Step-by-step checklist for Gram+ bacteria Supplemental file](#) [effective 2Sept2016 ajh22]

[8.3.16.2-Step-by-step checklist for Gram- bacteria Supplemental file](#) [effective 13Sept2016 ajh22]

[8.3.16.3-CDC MiSeq Nextera XT Library Preparation Reference](#) [reviewed 07Oct2016 by jk2739]

[8.3.16.4-Example Nextera Library Sample Preparation Spreadsheet Template](#) [reviewed 07Oct2016 by jk2739]

[8.3.18-Preparing Cell Lysates for PCR SOP](#) [updated 2020-05-18 by ser15]

8.4-DNA Sequencing Analysis

Sanger Sequencing

[8.4.1-Automated Sequence Editing SOP](#) [updated 2019-10-11 by js2568]

[8.4.5-Creating Consensus DNA Sequences using Sequencher SOP](#) [created 2020-04-28 ag67]

[8.4.6-rpoB Allelic Type \(AT\) Assignment SOP](#) [updated 2020-04-28 ag67]

[8.4.7-rpoB Database Management SOP](#) [updated 2021-04-01 by ag67]

Whole Genome Sequencing

[8.4.2-Quality Control and Assembly SOP](#) [reviewed by jk2739 12/19/2016]

[8.4.3-Short Read Submission \(SRA\) SOP](#) [reviewed by jk2739 12/19/2016]

[8.4.4-Assembly submission to NCBI SOP](#) [reviewed by rho2 11/25/2016]

8.5-DNA Recombination & Mutagenesis

[8.5.2-Cloning of DNA Fragments Protocol](#) [reviewed 8Aug2016 mw16]

[8.5.9-GUS promoter fusions in *Listeria monocytogenes* Protocol](#) [reviewed 08Aug2016 mw16]

[8.5.10-Integration of pPL2 derivatives into *L. monocytogenes* 10403S Protocol](#) [reviewed 08Aug2016 mw16]

[8.5.16-pMJG2 vector map.jpg Plasmid Map](#) [reviewed 08Aug2016 mw16]

[8.5.17-pMJG3 vector map.jpg Plasmid Map](#) [reviewed 08Aug2016 mw16]

[8.5.18-pMJG4 vector map.jpg Plasmid Map](#) [reviewed 08Aug2016 mw16]

[8.5.20-Whole Mutant-Soeing PCR for *L. mono* SOP](#) [reviewed by bmb29 4/6/2016]

[8.5.21-P22 Transduction of *Salmonella* Protocol](#) [reviewed by ram524 28Jul2017]

[8.5.22-Protocol for Lambda Red Recombinase in *Salmonella* SOP](#) [updated by ram524 2020-06-01]

[8.5.23-Gene Deletion Using I-SceI coupled with Lambda Red Recombination in *Salmonella* SOP](#) [updated by cq87 2021-07-18]

8.6-Microarray

[see archive]

8.7-Protein Methods

[8.7.1-Assay for beta-galactosidase activity of *B. subtilis* transformed with lacZ reporter plasmid Protocol](#) [mw16 checked07072016]

8.7.3-His tagged sigma factor protein purification protocol **Protocol** [mw16 checked07072016]

8.8.11-RNA Extraction Using TriReagent **SOP** [updated by arc326 2020-05-18]

8.8.13-Sybr Green qRT-PCR Protocol [updated by arc326 2020-05-18]

8.8.14-RNA Extraction from *Salmonella* using Tri-Reagent SOP [updated by arc326 2020-05-18]

8.8.15-RNA extraction from Salmonella using RNeasy Kit **SOP** [updated 2020-06-01 by ram524]

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