**Table S3. Scripts used to implement read healing pipelines.** All pipelines tested on isolates from Clusters 1 through 4. Ambiguous nucleotide content was evaluated using the script, countReadsWithAmbig.py, for all raw and healed reads. Custom scripts available for download from https://github.com/darlenewagner/NGS\_Multi\_Heal

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| --- | --- | --- |
| Healing Pipeline | Programmatic Dependencies† | Parameters |
| noNmin100 | Custom script, windowQualPrinseqLite\_R1andR2.py and prinseq v0.20.3 | minimum read length = 100bp, ambiguous nucleotides < 1 |
| fastxOnly-3pr | fastx-toolkit v0.0.13 or higher | R1 trimmed 3 bp from 3’,R2 trimmed 15 bp from 3’ |
| noNmin100-3pr | Custom scripts, windowQualPrinseqLite\_R1andR2.py and fastxQualAdaptTrimmer\_R1andR2.py, plus prinseq v0.20.3 and fastx-toolkit v0.0.13 | minimum read length = 100bp, ambiguous nucleotides < 1 R1 trimmed 3 bp from 3’,R2 trimmed 15 bp from 3’ |
| prinseq | Custom script, windowQualPrinseqLite\_R1andR2.py and prinseq v0.20.3 | trim quality from 3’ < 27 or 28trim quality window = 40 bpMinimum read length = 40 bp, ambiguous nucleotides < 2  |
| prinseq-3pr | Custom scripts, windowQualPrinseqLite\_R1andR2.py and fastxQualAdaptTrimmer\_R1andR2.py, plus prinseq v0.20.3 and fastx-toolkit v0.0.13 | trim quality from 3’ < 27 or 28trim quality window = 40 bpMinimum read length = 40 bp, ambiguous nucleotides < 2 R1 trimmed 3 bp from 3’,R2 trimmed 5 bp from 3’ |
| prinseq-5pr3pr | Custom scripts, windowQualPrinseqLite\_R1andR2.py and fastxQualAdaptTrimmer\_R1andR2.py, plus prinseq v0.20.3 and fastx-toolkit v0.0.13 | trim quality from 3’ < 27 or 28trim quality window = 40 bpMinimum read length = 40 bp, ambiguous nucleotides < 2 R1 trimmed 3 bp from 3’,R2 trimmed 5 bp from 3’R1 and R2 trimmed 1 bp from 5’ |
| bayesHammer | SPAdes 3.11 or higher | spades.py --only-error-correction |
| † wrappers for custom pipelines require Python 3.3 and Perl v5.12 or higher, SPAdes requires Python 2.7 or higher |