Supplemental Information for:

**Facilitating population genomics of non-model organisms through optimized experimental design for reduced representation sequencing**

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**Additional File 3. Comparisons of empirical and *in silico* restriction enzyme digestions.** Empirical Bioanalyzer results (left figure panels) with digested DNA are shown as concentration over fragment size and estimated loci numbers over locus size from *in silico* digestions (right figure panels) for all target taxa except fish (these are shown in Fig. 1).



**Figure S3.1.** Empirical Bioanalyzer results with digested DNA are shown as concentration over fragment size (a, c) and estimated loci numbers over locus size from *in silico* digestions (b, d). The tests were conducted with restrictions enzymes *EcoRI* (a, b) and *MspI* (c, d). Results for the ostracod species *Macropyxis hornei*, *Macrocyprina rocas* and *Macroscapha falcis* are shown next to *in silico* estimates using a related reference genome of *Cyprideis torosa* and two simulated genomes of 100 and 500 Mb size.

**Figure S3.2.** Empirical Bioanalyzer results with digested DNA are shown as concentration over fragment size (a, b, d, e) and estimated loci numbers over locus size from *in silico* digestions (c, f). The tests were conducted with restrictions enzymes *EcoRI* (a, b, c) and *MspI* (d, e, f). Results for the amphipods species *Eusirus pontomedon* (b, e) and *Charcotia obesa* (a, d) are shown next to *in silico* estimates using reference genomes of *Hyalella azteca*, *Parhyale hawaiensis* and one simulated genome of 1000 Mb size (note that this was the absolute size used for *in silico* computations, but resulting estimates were extrapolated to 10,000 Mb).

**Figure S3.3.** Empirical Bioanalyzer results with digested DNA are shown as concentration over fragment size (a, b, d, e, g, h) and estimated loci numbers over locus size from *in silico* digestions (c, f, i). The tests were conducted with restrictions enzymes *EcoRI* (a, b, c), *PstI* (d, e, f) and *MspI* (g, h, i). Results for the bivalve species *Aequiyoldia eightsii* (a, d, g) and *Laternula elliptica* (b, e, h) are shown next to *in silico* estimates using reference genomes of *Bathymodiolus platifrons, Crassostrea gigas* and *Pinctada imbricata*.

**Figure S3.4.** Empirical Bioanalyzer results with digested DNA are shown as concentration over fragment size (a, b, d, e, g, h) and estimated loci numbers over locus size from *in silico* digestions (c, f, i). The tests were conducted with restrictions enzymes *EcoRI* (a, b, c), *PstI* (d, e, f) and *MspI* (g, h, i). Results for the sea star species *Bathybiaster loripes* (a, d, g) and *Psilaster charcoti* (b, e, h) are shown next to *in silico* estimates using reference genomes of *Acanthaster planci*, *Patiria miniata*, and *Patiruella regularis*.

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**Figure S3.5.** Empirical Bioanalyzer results with digested DNA are shown as concentration over fragment size (a, c, e) and estimated loci numbers over locus size from *in silico* digestions (b, d, f). The tests were conducted with restrictions enzymes *EcoRI* (a, b), *PstI* (c, d) and *MspI* (e, f). Results for the bird species *Pagodroma nivea* are shown next to *in silico* estimates using a related reference genome of *Fulmarus glacialis* and two simulated genomes of 100 and 150 Mb size (note that this was the absolute size used for *in silico* computations, but resulting estimates were extrapolated to 1000 and 1500 Mb).