

Checklist of minimum metadata to be collected for mosquito microbiome samples and all controls (positive and negative) processed, along with examples following existing standards of recording and reporting arthropod and genomics data. A ready-to-use interactive and customizable template of these metadata records is freely available here: <https://mosquito-microbiome.org/resources/mmc-white-paper/>

Metadata fields	Example
<b>General</b>	
Study type	Field, semi-field, or laboratory
Sample name	Anopheles gambiae midgut, no template extraction/PCR control (negative control), ZymoBIOMICS microbial community standard or other known mock community (positive control), etc.
Sample ID	An_Gambiae_MG_01
Number per sample	Individual, pool of 3 individuals, etc.
Sample taxonomy	<i>Anopheles albimanus</i>
Developmental stage	egg, larval instar, pupa, or adult
Sex	Male, female, or both pooled
Age <sup>§</sup>	3 days post adult eclosion, 2-5 days post eclosion etc
Mating status	Virgin or mated/non-virgin
Gonotrophic status	Non gravid, fully gravid, or half-gravid
Blood fed	non blood-fed or blood-fed
Type of food provided <sup>‡</sup>	10% sucrose, human blood, 1:3 yeast & TetraMin, etc
Tissue processed	Whole mosquito, Midgut, Ovaries, Cuticle surface, etc.
Sample phenotype	virus/parasite infection status, insecticide resistance status, etc.
Collection date	YYYY, YYYY-MM, or YYYY-MM-DD
Collection time	hh, hh:mm, or hh:mm:ss
Biomolecule processed	DNA, RNA, protein, metabolites, etc.
Biomolecule isolation method	QIAGEN blood & tissue, Phenol-chloroform, etc.
Sequencing method	16S rRNA amplicon, Whole (meta)genome, metatranscriptomic, etc
Sequencing platform	Illumina, Oxford Nanopore, etc
Sequencing platform model	MiSeq (Illumina), MinIon (Oxford Nanopore), etc.
Sample storage preservative	None, Ethanol, RNALater®, etc
Sample storage temperature	-20 °C, -80 °C, etc
Sample storage duration	None, 6 months, 3 years, etc
<b>Specific to field studies</b>	
Collection country	Nigeria
Collection village or city	Iko Esai village
Location coordinates	00.000000, 00 00.0000, or 00°00'00.0"N 0°00'00.0"E
Climatic/environmental data	27 °C, 82% RH
Landcover	Savanna, Urban and built up, Tundra, etc. See Loveland et al. [1] for more examples
Collection method	Human landing catch, Mechanical aspirator, gravid trap, or larval dipping
Collection bait	CO2, Cattle
<b>Specific to laboratory studies</b>	
Name and location of laboratory (and/or facility)	The Short Lab, College of Food, Agricultural, and Environmental Sciences, The Ohio State University, Columbus, OH, USA
Strain	STECLA, KISUMU, ROCKEFELLER
Generation	F1, F6, F59, etc.
Maintenance temperature	27±2°C
Maintenance relative humidity	80±10%
Light-dark cycle	10-h light; 14-h dark
<b>Specific to semi field studies</b>	
Name and location of semi-field facility (if widely used/known)	MalariaSphere, Mbita Point Research & Training Center, International center of Insect Physiology and Ecology, Mbita, Kenya [2]
Type of semi-field structure	Glass house, mesh house, mesh cage, etc
Dimensions of semi-field structure	00.00 x 00.00 m

<sup>§</sup>May not apply to field-derived mosquitoes

<sup>‡</sup>Not applicable to field-derived mosquitoes

## References

- Loveland TR, Reed BC, Brown JF, Ohlen DO, Zhu Z, Yang L, et al. Development of a global land cover characteristics database and IGBP DISCover from 1 km AVHRR data. *International Journal of Remote Sensing*. 2000;21:1303–30.
- Knols BGJ, Njiru BN, Mathenge EM, Mukabana WR, Beier JC, Killeen GF. MalariaSphere: A greenhouse-enclosed simulation of a natural *Anopheles gambiae* (Diptera: Culicidae) ecosystem in western Kenya. *Malaria Journal*. 2002;1:19.