

S4 Table. Summary of sample preparation for *Ss-unc-22* whole-genome sequencing. *S. stercoralis* free-living adults were injected with RNP complexes targeting *Ss-unc-22* site #3, including an ssODN, and F₁ iL3s were collected. A subset of F₁ iL3s was screened for nicotine-twitching frequency to estimate the *Ss-unc-22* mutation rate and the remaining population was split in two for iL3 lysis, genomic DNA extraction, and Illumina library preparation. Wild-type iL3s were also screened for nicotine-twitching frequency and an Illumina library was prepared from a wild-type population in parallel with the *Ss-unc-22* libraries.

target	delivery	repair	# free-living adults injected (P ₀)	estimated # iL3s collected	# iL3s screened in 1% nicotine	% iL3s twitching in 1% nicotine	# WGS libraries prepared
3	RNP	ssODN	280	~9,240	182	40.7%	2 (<i>Ss-unc-22</i> A + B)
—	—	—	—	~5,000	154	0%	1 (wild type)