Oct 30, 2025

IDEEL- UNC Implementation of DBLa Var-Typing

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7n781lwz/v1

Infectious Disease Epidemiology and Ecology Lab[1]

[1]Infectious Disease Epidemiology and Ecology Lab, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

IDEEL

Infectious Disease Epidemiology and Ecology Lab
University of North Carolina

DOI: https://dx.doi.org/10.17504/protocols.io.q26g7n781lwz/v1

Protocol Citation: Infectious Disease Epidemiology and Ecology Lab 2025. IDEEL- UNC Implementation of DBLa Var-Typing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7n781lwz/v1

Protocol status: Working
We use this protocol and it's working

Created: October 27, 2025

Last Modified: October 30, 2025

Protocol Integer ID: 231055

Keywords: DBLa PCR, Amplification, Sequencing, Primers, unc implementation of dbla var, dbla var, continued plasmodium falciparum disease transmission, pcr assay, plasmodium falciparum disease transmission after an outbreak, use with the current dbla, molecular epidemiology, existing primer sequence, current dbla, pcr, primer sequence, sequencing approach, adapted primer, reverse primer, forward primer, standardized oligo segment, example publication of this protocol

Abstract

This protocol is designed for use with the current DBLa sequencing approach developed by the Day laboratory. An example publication of this protocol is: Ruybal-Pesántez S, Sáenz FE, Deed SL, Johnson EK, Larremore DB, Vera-Arias CA, Tiedje KE, Day KP. Molecular epidemiology of continued Plasmodium falciparum disease transmission after an outbreak in Ecuador. Front Trop Dis. 2023;4:1085862. doi: 10.3389/fitd.2023.1085862. Epub 2023 Mar 16. PMID: 39525803; PMCID: PMC11546077.

PCR assays are “Nextera-Adapted” by adding standardized oligo segments onto existing primer sequences.

For example:

- Non-Nextera-Adapted Primer:

1. Forward primer: CCATCAGGGAAATGTCCAGT
2. Reverse primer : TTTCCTGCATGTCTTGAACA
3. Forward primer addition: **TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**
4. Reverse primer addition: **GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**

Example:

- Nextera-Adapted Primer:

1. Forward: **TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CCATCAGGGAAATGTCCAGT
2. Reverse: **GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TTTCCTGCATGTCTTGAACA

PCR-based library preparation allows for a significant reduction in time to complete libraries, reduces the consumables required, and lessens the impact of library preparation on technicians. Pre-combined 96-well Unique Dual Index plates are utilized in combination with standardized barcoding worksheets to simplify the generation of final library indexing manifests.

Materials

Materials

Consumables:

- P200 pipettor and tips/ single or multichannel
- P20 pipettor and tips/ single or multichannel
- 25mL reagent reservoir
- 96-well PCR plates
- Nuclease-free PCR microtubes/strips/plates.
- Nuclease-free 1.5µL Eppendorf tubes (for master mixes).
- Cold blocks for microplates and 1.5µL tubes.

Reagents:

- 80% Ethanol
- Qubit or PicoGreen supplies for quantifying product
- Speedbeed mix (dx.doi.org/10.17504/protocols.io.x54v9p7b1g3e/v2)
- Roche Fast Start High Fidelity PCR System (Cat #: 03553400001)
- Molecular Grade Water (MGW)
- Pre-Combined UDI 96-well plate at 10µM each index (i7 and i5 Index primers)
- DBLa primers
- KAPA HiFi HotStart ReadyMix 2X (Cat #: 07958927001)

Materials: DBLa Primers

	A	B
	Primer	**Sequence**
	Nextera DBLaF	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCACMGT

	A	B
		TTYGC
	NexteraDBLaR	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCCATTCSTCGAACCA

Materials: I7 Index primers

	A	B	C	D
	Beginning	**Index**	**End**	**To order**
	CAAGCAGAAGACGGCATACGAGAT	CGCTCAGTTC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTCGCTCAGTTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACG	TATCTGACCT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACG

	A	B	C	D
	AGAT			AGATTATCTGACCTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TCGGATGTCG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTCGGATGTCGTCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CTTATGGAAT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCTTATGGAATGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TCCTATTGTG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTCCTATTGTGTCGTCTCGTGGGCTCGG

	A	B	C	D
				TGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GCGCGATGTT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGCGCGATGTTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	AGAGCACTAG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATAGAGCACTAGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TGCCTTGATC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTGCCTTGATCGTCTCGTGG

	A	B	C	D
				GCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CTACTCAGTC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCTACTCAGTCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TCGTCTGACT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTCGTCTGACTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GAACATACGG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGAACATACGGGTCTCGTGGGCTCGG

	A	B	C	D
	CAAGCAGAAGACGGCATACGAGAT	CCTATGACTC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCCTATGACTCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TAATGGCAAG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTAATGGCAAGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GTGCCGCTTC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGTGCCGCTTCTCGTCTCGTGGGCTCGG
	CAAGCAGAA	CGGCA	GTCTCGTGG	CAAGCAGAA

	A	B	C	D
	GACGGCATACGAGAT	ATGGA	GCTCGG	GACGGCATACGAGATCGGCATGAGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GCCGTAACCG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGCCGTAACGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	AACCATCTC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATAACCATTCTCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACG	GGTTGCCTCT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACG

	A	B	C	D
	AGAT			AGATGGTTGCCTCTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CTAATGATGG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCTAATGATGGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TCGGCCTATC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTCGGCCTATCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	AGTCAACCAT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATAGTCACC

	A	B	C	D
				ATG TCT CGT GG GCT CG G
	CAA GCA GAA GAC GG CAT ACG AGA T	GAG CGC AAT A	GTC TCG TGG GCT CG G	CAA GCA GAA GAC GG CAT ACG AGA TGA GC GCA ATA GTC TCG TGG GCT CG G
	CAA GCA GAA GAC GG CAT ACG AGA T	AAC AAG GC GT	GTC TCG TGG GCT CG G	CAA GCA GAA GAC GG CAT ACG AGA TAA CAA GG CGT GTC TCG TGG GCT CG G
	CAA GCA GAA GAC GG CAT ACG AGA T	GTA TGT AGA A	GTC TCG TGG GCT CG G	CAA GCA GAA GAC GG CAT ACG AGA TGT ATG TAG AAG TCT CGT GG

	A	B	C	D
				GCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TTCTATGGTT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTTCTATGGTTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CCTCGCAACC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCCTCGCAACCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TGGATGCTTA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGGATGCTTAGTCTCGTGGGCTCGG

	A	B	C	D
	CAAGCAGAAGACGGCATACGAGAT	ATGTCGTGGT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTATGTCGTGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	AGAGTGCGGC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTAGAGTGCGGCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TGCCTGGTGG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTTGCCTGGTGGGTCTCGTGGGCTCGG
	CAAGCAGAA	TGCGTG	GTCTCGTGG	CAAGCAGAA

	A	B	C	D
	GACGGCATACGAGAT	TCAC	GCTCGG	GACGGCATACGAGATTGCGTGTCACGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CATACACTGT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCATACTGTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CGTATAATCA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCGTATAATCAGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACG	TACGCGGCTG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACG

	A	B	C	D
	AGAT			AGATTACGCGGCTGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GCGAGTTACC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGCGAGTTACCGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TACGGCCGGT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTACGGCCGGTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GTCGATTACA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATGTCGATTA

	A	B	C	D
				CAGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CTGTCTGCAC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCTGTCTGCACGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	CAGCCGATTG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATCAGCCGATTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TGACTACATA	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGACTACATAGTCTCGTGG

	A	B	C	D
				GCTCGG
	CAAGCAGAAGACGGCATACGAGAT	ATTGCCGAGT	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTATTGCCGAGTGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GCCATTAGAC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGCCATTAGACTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	GGCGAGATGG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGGCGAGATGGGTCTCGTGGGCTCGG

	A	B	C	D
	CAAGCAGAAGACGGCATACGAGAT	TGGCTCGCAG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTGGCTCGCAGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TAGAATAACG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTAGAATAACGGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	TCCATGTTGC	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATTCCATGTTGCGTCTCGTGGGCTCGG
	CAAGCAGAA	TATCCA	GTCTCGTGG	CAAGCAGAA

	A	B	C	D
	GACGGCATACGAGAT	GGAC	GCTCGG	GACGGCATACGAGATTATCCAGGACGTCTCGTGGGCTCGG
	CAAGCAGAAGACGGCATACGAGAT	AGTGCCACTG	GTCTCGTGGGCTCGG	CAAGCAGAAGACGGCATACGAGATAGTGCCACTGTCTCGTGGGCTCGG

Materials: I5 Index Primers

	A	B	C	D
	Beginning	**Index**	**End**	**To order**
	AATGATACGGCGACCACCGAGATCTACAC	TCGTGGAGCG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACTCGTGGAGCGTCGTCGGCAGCGTC

	A	B	C	D
				GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	CTA CAA GAT A	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC CTA CAA GAT ATC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TAC GTT CAT T	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TAC GTT CAT TTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TGC CTG GTG G	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TGC CTG GTG GTC GTC

	A	B	C	D
				GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TCC ATC CGA G	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TCC ATC CGA GTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	GTC CAC TTG T	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC GTC CAC TTG TTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TGG AAC AGT A	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TGG AAC AGT ATC GTC GG

	A	B	C	D
				CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	CCT TGT TAA T	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC CCT TGT TAA TTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	GTT GAT AGT G	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC GTT GAT AGT GTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	ACC AGC GAC A	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC ACC AGC GAC ATC GTC GG CAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	CATACACTGT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCATACACTGTTTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GTGTGGCGCT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGTGTGGCGCTTTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	ATCACGAAGG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACATCACGAAGGGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	CGGCTCTACT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCGGCTCTACTTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GAATGCACGA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGAATGCACGATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	AAGACTATAG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACAAGACTATAGGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	TCGGCAGCAA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACTCGGCAGCAATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CTAATGATG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCTAATGATGGTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GGTTGCCTCT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGGTTGCCTCTTTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	CGCACATGGC	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCGCACATGGCTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GGCCTGTCCT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGGCCTGTCCTTCGTCGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CTGTGTTAGG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCTGTGTTAGGGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	TAAGGAACGT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACTAAGGAACGTTTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CTAACTGTAA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCTAACTGTAATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GGCGATGG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGGCGATGGTCGTCGCGCA

	A	B	C	D
				GC GTC
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	AAT AGA GCA A	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC AAT AGA GCA ATC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TCA ATC CAT T	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TCA ATC CAT TTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TCG TAT GC GG	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TCG TAT GC GGT CGT CG GCA

	A	B	C	D
				GC GTC
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	TCC GAC CTC G	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC TCC GAC CTC GTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	CTT ATG GAA T	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC CTT ATG GAA TTC GTC GG CAG CGT C
	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC	GCT TAC GGA C	TCG TCG GCA GC GTC	AAT GAT ACG GC GAC CAC CGA GAT CTA CAC GCT TAC GGA CTC GTC GG CAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	GAACATACGG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGAACATACGGTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GTCGATTACA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGTCGATTACATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	ACTAGCCGTG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACACTAGCCGTGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	AAGTTGGTGA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACAAGTTGGTGATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	TGGCAATATT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACTGGCAATATTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GATCACCGCG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGATCACCGCGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	TACCATCCGT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACTACCATCCGTTTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GCTGTAGGAA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGCTGTAGGAATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CGCACTAATG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCGCACTAATGTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	GACAACTGAA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGACAACTGAATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	AGTGGTCAGG	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACAGTGGTCAGGTCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CCATCTCGCCC	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCCATCTCGCCTCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	CTGCGAGCCA	TCGTCGGCAGC GTC	AATGATACGGCGACCACCGAGATCTACACCTGCGAGCCATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CGTTATTCTA	TCGTCGGCAGC GTC	AATGATACGGCGACCACCGAGATCTACACCGTTATTCTATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	GCAACATGGA	TCGTCGGCAGC GTC	AATGATACGGCGACCACCGAGATCTACACGCAACATGGATCGTCGGCAG

	A	B	C	D
				CGTC
	AATGATACGGCGACCACCGAGATCTACAC	GTCCTGGATA	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACGTCCTGGATATCGTCGGCAGCGTC
	AATGATACGGCGACCACCGAGATCTACAC	CAGTGGCACT	TCGTCGGCAGCGTC	AATGATACGGCGACCACCGAGATCTACACCAGTGGCACTTCGTCGGCAGCGTC

Step 1: General Lab measures

1 Ensure all work areas and pipettors are sterilized per laboratory protocol.

> Note
>
> - Clean pipettes and working bench tops with 70% ethanol.
> - UV-treat the working surface, all equipment, and tubes/strips.

2 DBLa PCR (PCR1) should be set up in a clean room, free of PCR amplification.

3 Indexing PCR (PCR2), clean-up, and quantification should be done in an area where PCR products can be worked with (i.e., a post-PCR space).

4 Thaw reagents and DNA samples On ice .

5 Briefly vortex and centrifuge each reagent before use, with the exception of enzymes.

> Note
>
> Gently mix enzymes by inverting the tube a few times, then centrifuge.

Step 2: Setting up Primers

6 Dilute the [M] 100 micromolar (µM) stock primers down to [M] 20 micromolar (µM) working solutions for DBLa forward and reverse primers.

7 Generation of UDI Plates

7.1 The final concentration of the i5/i7 primer mix should be [M] 10 micromolar (µM) . This can be done any number of ways, including diluting the [M] 100 micromolar (µM) stock

of each primer to [M] 10 micromolar (µM) and then mixing.

7.2 UDI Combinatorial Plates can be made ahead of time and stored at -20 °C for use.

7.3 Typically, we recommend making plates for a maximum of 10 indexing PCR reactions; thus, if contamination should occur, a new plate can be made without wasting reagents.

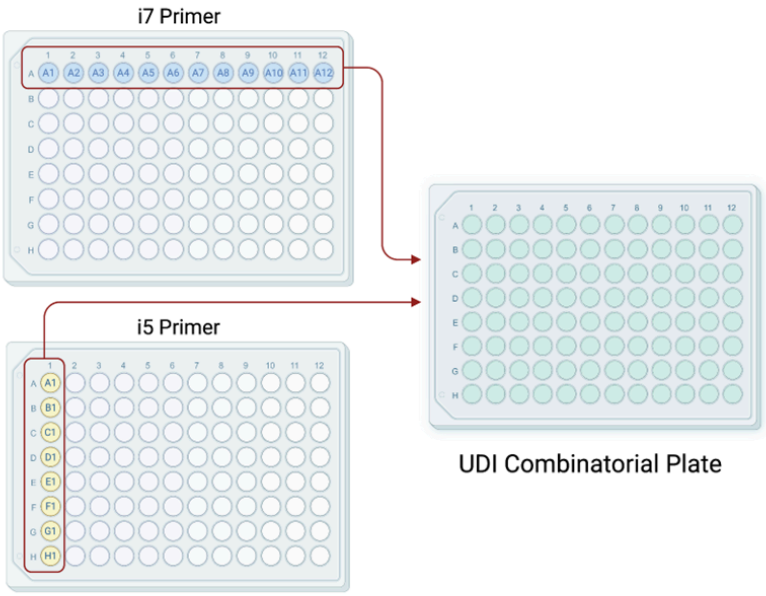

Figure legend: Generation of UDI (Unique Dual Index) plates. By ordering i5 (yellow) and i7 (blue) on plates in opposite orientation, UDI plates of combined i5 and i7 indexes (yellow + blue= green) can be made using a multichannel pipette to mix primers in a LoBind DNA plate. The column of i5 primers should be pipetted across all columns of the UDI plate and the i7 primer row pipetted across all rows of the plate.

Step 3: Initial DBLa PCR

8 Calculate the amount of reagents needed with a 10% excess to account for pipette loss.

9 Prepare the PCR master mix based on the table below.

	A	B
	Reagent	**Volume/rxn (µL)**
	Roche Fast Start High Fidelity PCR System Buffer (2X)	2.5
	Nucleotide Mix	0.5
	Fwd Primer (20uM)	2.5
	Rev Primer (20uM)	2.5
	Enzyme Blend	0.2
	Water	14.8

Note

- All reagents, PCR master mix, primer pools, and samples should be held in freezer blocks (tube or 96-well) unless stated otherwise.
- All assays are carried out with positive and negative controls (preferably 2 of each). For a positive control, we use a mocked dried blood spot containing an estimated parasite density of 100 Pf/µL. The positive control you use should be extracted the same way as the samples to be tested. For non-template control, we recommend using human DNA at a clinically relevant concentration (e.g. 16.77 ng/µL). If human DNA is not available, water can be used as a non-template control.

10 Combine the master mix and 2 µL sample DNA (25µl total reaction volume), seal caps/strips/plates, and place the reaction in a thermal cycler using the following conditions:

	A	B	C	D
	Step	**Duration**	**Cycles**	**Temperature**
	Activation	5 min	1 cycle	95° C
		1 min	1 cycle	42° C
		1 min	1 cycle	60° C
	Cycling	1 min	29 cycles	95° C
		1 min		42° C
		1 min		60° C

Step 4: PCR cleanup and library prep

45m

11 Bring Speedbeads to Room temperature for 00:30:00 . 30m

12 Mix fresh 80% Ethanol using 200 proof ethanol (100%, anhydrous) and molecular grade water.

13 Vortex Speed-Beads thoroughly, then add 50 µL to each well. Mix gently by pipetting.

14 Allow the mixture to incubate at Room temperature for 00:10:00 . 10m

> Note
>
> If there are droplets on the sides of the wells following incubation, cover the plate with a foil seal and briefly spin the plate to avoid losing product.

15 Place the plate on a 96-well magnetic block and wait 00:02:00 - 00:03:00 for the solution to clear. 3m

16 Aspirate the clear supernatant and discard.

17 Dispense 120 µL 80% Ethanol into each well, incubate 00:00:30 , then aspirate to wash. 30s

18 Repeat for a total of 2 washes.

19 Aspirate final drops of 80% Ethanol with P20 pipette tips. Allow bead pellets to dry momentarily for 00:00:30 to 00:01:30 . 1m 30s

20 Remove plate from the magnet.

21 Dispense 25 µL of Elution Buffer onto each bead pellet. Mix by pipetting to resuspend DNA.

22 Cover plate with foil seal and briefly centrifuge to collect product in the bottom of wells before storing or proceeding to library preparation. *The beads can remain in the eluent and may be added to the library preparation reaction. The eluent is not transferred to a new plate to limit cross-contamination of concentrated amplicon products.*

Step 5: Library Preparation (PCR2)

23 Thaw Pre-Combined UDI 96-well plate, purified PCR reaction, and KAPA Master Mix.

24 Calculate the amount of reagents needed with a 10% excess to account for pipette loss.

25 Mix master mix as described in the following table:

	A	B
	Reagent	**Volume/rxn**
	KAPA HiFi HotStart ReadyMix 2X	12.5 µL
	Molecular Grade Water	4.5 µL
	Total	**17.0 µL**

26 Combine KAPA HiFi HotStart ReadyMix 2X and water in a tube, then aliquot equal volumes into 0.2mL microtube strips. Pipette 17.0 µL Master Mix into each well with a multichannel pipettor.

27 Spin UDI plate to ensure there is no liquid on the plate sealing foil.

28 Remove the foil seal carefully to avoid contamination.

29 Add 2.5 µL of the appropriate UDI to each well. No need to mix yet.

30 Seal stock UDI plate before opening and adding samples to prevent contamination.

31 Add 5.0 µL of PCR1. Gently mix by pipetting.

- Sample and UDI plate should now have the following volumes in each well:

	A	B
	Reagent	**Volume/rxn**
	KAPA HiFi HotStart ReadyMix 2X	12.5 µL
	Molecular Grade Water	4.5 µL
	UDI Primer	2.5 µL
	Purified amplicons	5.0 µL
	Total	**24.5 µL**

32 Program the thermal cycler to the following conditions for 8-12 cycles.

	A	B	C	D
	Step	**Duration**	**Cycles**	**Temperature**
	Initial Activation Step	3 min	1	95° C
	Denaturation	30 sec	8-12	95° C
	Annealing	30 sec		55° C
	Extension	30 sec		72° C
	Final Extension	5 min	1	72° C
	Hold	Indefinite	1	4° C

Step 6: Contamination check

33 Prior to proceeding to sample pooling into a final library, the libraries generated from positive and non-template controls should be checked for contamination.

34 It is possible that negative controls will have a detectable band on gel below the bands consistent with amplification products. Non-template control bands typically represent primer dimer that should not yield data when processed through bioinformatic analysis.

Step 7: Pooling based on PicoGreen Concentration

54m 30s

35 Bring SpeedBeads to Room temperature ~ 00:30:00 . 30m

36 Mix fresh 80% Ethanol using 200 proof ethanol (100%, anhydrous) and molecular grade water.

37 Vortex Speed-Beads thoroughly before use.

38 Combine libraries with Speed-Beads at a 1:1 ratio.

39 Allow the mixture to incubate at Room temperature for 00:10:00 . 10m

40 Gently spin the plate to collect contents at the bottom of the wells.

41 Place the plate on a plate magnet and wait 00:02:00 - 00:03:00 for the solution to clear. 3m

42 Aspirate the clear supernatant and discard.

43 Keeping the plate on the magnet, dispense 80% Ethanol into the wells to wash. Ethanol wash volume should be higher than the combined library and Speed-Bead mixture.

44 Incubate 00:00:30 , then aspirate to wash. 30s

45 Repeat for a total of 2 washes.

46 Aspirate final drops of ethanol with P20 pipette tips. Allow bead pellets to dry on the magnet for 00:00:30 to 00:01:00 . 1m

Note

Dry beads to a satin-matte finish, but not to the point of cracking. This happens quickly if ethanol has been aspirated completely.

47 Remove the plate from the magnet and dispense 25 µL Elution Buffer onto each bead pellet. Mix by pipetting to resuspend DNA.

48 Allow the mixture to incubate at Room temperature for 00:05:00 . 5m

49 Gently spin the plate to collect product in the bottom of wells, then place on a plate magnet and wait 00:02:00 - 00:03:00 3m

50 Remove eluent to a new LoBind plate.

51 Quantify 2 µL of amplified libraries in 48 µL of 1X PicoGreen reagent in a black, flat bottom microplate appropriate for reading fluorescence.

Note

- If the reagent is not already provided at 1X, combine dye and buffer in a 1:200 ratio to create a working solution.
- If you are quantifying an entire 96-well plate of samples, a second plate will be necessary for assay standards.

52 Add 2 µL of each sample to a black, flat-bottomed polystyrene assay plate.

- The 90-degree angle where the well wall meets the flat bottom holds this droplet nicely.
- Add 2 µL of each standard to the appropriate wells.
- Standards 1-8 in duplicate = 16 wells required.

53 Fill each sample and standard wells with 48 µL 1X PicoGreen Reagent.

Note

Pipette to mix thoroughly, careful not to introduce bubbles.

54 Cover plate with optical film and incubate for 00:02:00 at Room temperature before assaying. Protect from light during incubation.

2m

55 Place the prepared quantitation plate into the multimode plate reader.

Note

The plate reader should be set up to analyze Fluorescence Intensity (FI) with an excitation wavelength around 480nm and an emission wavelength around 580nm.

56 Calculate each sample's concentration by creating a standard curve and plotting unknown fluorescence values. Divide the resulting value by two to report nanograms per microliter.

57 Negative controls can have detectable DNA by PicoGreen, typically in the form of dimers. To determine the concentration of the library in order to pool equimolar volumes of each sample, one should use a baseline (averaged NTC concentration) subtracted from the concentration for each sample to determine pooling volumes.

58 The library pool is ready to prepare for sequencing.