

## Supporting Information

**Title:** DuBA.flow – A low-cost, long-read amplicon sequencing workflow for the validation of synthetic DNA constructs

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**Table S1**

**Tab. S1 | Barcoding primer sequences used in this study.** M13 forward and reverse sequences are indicated bold.

<b>Forward barcoding primer</b>		<b>Reverse barcoding primer</b>	
<b>ID</b>	<b>Sequence (5' -&gt;3')</b>	<b>ID</b>	<b>Sequence (5' -&gt;3')</b>
SLo2420	AAGAAAGTTGTCCGGTGTCTTTGTGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2516	AAGAAAGTTGTCCGGTGTCTTTGTGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2421	TCGATTCCGTTTGTAGTCGTCTGTCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2517	TCGATTCCGTTTGTAGTCGTCTGTAGCGGATA <b>ACAATTTACACAGG</b>
SLo2422	GAGTCTTGTGTCCCAGTTACCAGGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2518	GAGTCTTGTGTCCCAGTTACCAGGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2423	TTCGGATTCTATCGTGTTCCTACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2519	TTCGGATTCTATCGTGTTCCTAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2424	CTTGTCCAGGGTTTGTGTAACCTTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2520	CTTGTCCAGGGTTTGTGTAACCTTAGCGGATA <b>ACAATTTACACAGG</b>
SLo2425	TTCTCGCAAAGGCAGAAAGTAGTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2521	TTCTCGCAAAGGCAGAAAGTAGCAGCGGATA <b>ACAATTTACACAGG</b>
SLo2426	GTGTTACCGTGGGAATGAATCCTTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2522	GTGTTACCGTGGGAATGAATCCTTAGCGGATA <b>ACAATTTACACAGG</b>
SLo2427	TTCAGGGAACAAACCAAGTTACGTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2523	TTCAGGGAACAAACCAAGTTACGTAGCGGATA <b>ACAATTTACACAGG</b>
SLo2428	AACTAGGCACAGCGAGTCTTGTTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2524	AACTAGGCACAGCGAGTCTTGTTAGCGGATA <b>ACAATTTACACAGG</b>
SLo2429	AAGCGTTGAAACCTTTGTCCCTCTCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2525	AAGCGTTGAAACCTTTGTCCCTCAGCGGATA <b>ACAATTTACACAGG</b>
SLo2430	GTTTCATCTATCGGAGGGAATGGACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2526	GTTTCATCTATCGGAGGGAATGGAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2431	CAGGTAGAAAGAAGCAGAATCGGACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2527	CAGGTAGAAAGAAGCAGAATCGGAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2432	AGAACGACTTCCATACTCGTGTGACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2528	AGAACGACTTCCATACTCGTGTGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2433	AACGAGTCTCTTGGGACCCATAGACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2529	AACGAGTCTCTTGGGACCCATAGAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2434	AGGTCTACCTCGCTAACACCACTGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2530	AGGTCTACCTCGCTAACACCACTGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2435	CGTCAACTGACAGTGGTTCGTACTIONCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2531	CGTCAACTGACAGTGGTTCGTACTIONAGCGGATA <b>ACAATTTACACAGG</b>
SLo2436	ACCCCTCAGGAAAGTACCTCTGATCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2532	ACCCCTCAGGAAAGTACCTCTGATAGCGGATA <b>ACAATTTACACAGG</b>
SLo2437	CCAAACCAACAACCTAGATAGGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2533	CCAAACCAACAACCTAGATAGGCAGCGGATA <b>ACAATTTACACAGG</b>
SLo2438	GTTCTCGTGCAGTGTCAAGAGATCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2534	GTTCTCGTGCAGTGTCAAGAGATAGCGGATA <b>ACAATTTACACAGG</b>
SLo2439	TTGCGTCTGTTACGAGAACTCATCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2535	TTGCGTCTGTTACGAGAACTCATAGCGGATA <b>ACAATTTACACAGG</b>
SLo2440	GAGCCTCTCATTGTCCGTTCTCTACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2536	GAGCCTCTCATTGTCCGTTCTCTAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2441	ACCACTGCCATGTATCAAAGTACGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2537	ACCACTGCCATGTATCAAAGTACGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2442	CTTACTACCCAGTGAACCTCCTCGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2538	CTTACTACCCAGTGAACCTCCTCGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2443	GCATAGTTCTGCATGATGGGTTAGCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2539	GCATAGTTCTGCATGATGGGTTAGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2444	GTAAGTTGGGTATGCAACGCAATGCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2540	GTAAGTTGGGTATGCAACGCAATGAGCGGATA <b>ACAATTTACACAGG</b>
SLo2445	CATACAGCGACTACGCATTCTCATCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2541	CATACAGCGACTACGCATTCTCATAGCGGATA <b>ACAATTTACACAGG</b>
SLo2446	CGACGTTAGATTACCTCTTACACCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2542	CGACGTTAGATTACCTCTTACAAGCGGATA <b>ACAATTTACACAGG</b>
SLo2447	TGAAACCTAAGAAGGCACCGTATCCCAGTCA <b>CGACGTTGTAAAACG</b>	SLo2543	TGAAACCTAAGAAGGCACCGTATAGCGGATA <b>ACAATTTACACAGG</b>

<b>Forward barcoding primer</b>		<b>Reverse barcoding primer</b>	
<b>ID</b>	<b>Sequence (5'→3')</b>	<b>ID</b>	<b>Sequence (5'→3')</b>
SLo2448	CTAGACACCTTGGGTTGACAGACCCCCAGTCA CGACGTTGTAAAACG	SLo2544	CTAGACACCTTGGGTTGACAGACCAGCGGATA ACAATTTACACAGG
SLo2449	TCAGTGAGGATCTACTTCGACCCACCCAGTCA CGACGTTGTAAAACG	SLo2545	TCAGTGAGGATCTACTTCGACCCAAGCGGATA ACAATTTACACAGG
SLo2450	TGCGTACAGCAATCAGTTACATTGCCAGTCA CGACGTTGTAAAACG	SLo2546	TGCGTACAGCAATCAGTTACATTGAGCGGATA ACAATTTACACAGG
SLo2451	CCAGTAGAAGTCCGACAACGTCATCCCAGTCA CGACGTTGTAAAACG	SLo2547	CCAGTAGAAGTCCGACAACGTCATAGCGGATA ACAATTTACACAGG
SLo2452	CAGACTTGGTACGGTTGGGTAACCTCCCAGTCA CGACGTTGTAAAACG	SLo2548	CAGACTTGGTACGGTTGGGTAACCTAGCGGATA ACAATTTACACAGG
SLo2453	GGACGAGAAGTCAAGTCAAAGGCCAGTCA CGACGTTGTAAAACG	SLo2549	GGACGAGAAGTCAAGTCAAAGGCCAGCGGATA ACAATTTACACAGG
SLo2454	CTACTTACGAAGCTGAGGGACTGCCAGTCA CGACGTTGTAAAACG	SLo2550	CTACTTACGAAGCTGAGGGACTGCAGCGGATA ACAATTTACACAGG
SLo2455	ATGTCCAGTTAGAGGAGGAAACACCCAGTCA CGACGTTGTAAAACG	SLo2551	ATGTCCAGTTAGAGGAGGAAACAAGCGGATA ACAATTTACACAGG
SLo2456	GCTTGCATTGATGCTTAGTATCACCCAGTCA CGACGTTGTAAAACG	SLo2552	GCTTGCATTGATGCTTAGTATCAAGCGGATA ACAATTTACACAGG
SLo2457	ACCACAGGAGGACGATACAGAGAACCAGTCA CGACGTTGTAAAACG	SLo2553	ACCACAGGAGGACGATACAGAGAAGCGGATA ACAATTTACACAGG
SLo2458	CCACAGTGTCAACTAGAGCCTCTCCCAGTCA CGACGTTGTAAAACG	SLo2554	CCACAGTGTCAACTAGAGCCTCTCAGCGGATA ACAATTTACACAGG
SLo2459	TAGTTTGGATGACCAAGGATAGCCCCAGTCA CGACGTTGTAAAACG	SLo2555	TAGTTTGGATGACCAAGGATAGCCAGCGGATA ACAATTTACACAGG
SLo2460	GGAGTTCGTCCAGAGAAGTACACGCCAGTCA CGACGTTGTAAAACG	SLo2556	GGAGTTCGTCCAGAGAAGTACACGAGCGGATA ACAATTTACACAGG
SLo2461	CTACGTGTAAGGCATACCTGCCAGCCAGTCA CGACGTTGTAAAACG	SLo2557	CTACGTGTAAGGCATACCTGCCAGAGCGGATA ACAATTTACACAGG
SLo2462	CTTTCGTTGTTGACTCGACGGTAGCCAGTCA CGACGTTGTAAAACG	SLo2558	CTTTCGTTGTTGACTCGACGGTAGAGCGGATA ACAATTTACACAGG
SLo2463	AGTAGAAAGGGTTCCTTCCCAGTCA CGACGTTGTAAAACG	SLo2559	AGTAGAAAGGGTTCCTTCCCAGCGGATA ACAATTTACACAGG
SLo2464	GATCCAACAGAGATGCCTTCAGTGCCAGTCA CGACGTTGTAAAACG	SLo2560	GATCCAACAGAGATGCCTTCAGTGAGCGGATA ACAATTTACACAGG
SLo2465	GCTGTGTTCCACTTCATTCTCCTGCCAGTCA CGACGTTGTAAAACG	SLo2561	GCTGTGTTCCACTTCATTCTCCTGAGCGGATA ACAATTTACACAGG
SLo2466	GTGCAACTTCCCACAGGTAGTTCCCCAGTCA CGACGTTGTAAAACG	SLo2562	GTGCAACTTCCCACAGGTAGTTCAGCGGATA ACAATTTACACAGG
SLo2467	CATCTGGAACGTGGTACACCTGTACCCAGTCA CGACGTTGTAAAACG	SLo2563	CATCTGGAACGTGGTACACCTGTAAGCGGATA ACAATTTACACAGG
SLo2468	ACTGGTGCAGCTTTGAACATCTAGCCAGTCA CGACGTTGTAAAACG	SLo2564	ACTGGTGCAGCTTTGAACATCTAGAGCGGATA ACAATTTACACAGG
SLo2469	ATGGACTTTGGTAACTTCTGCGTCCCAGTCA CGACGTTGTAAAACG	SLo2565	ATGGACTTTGGTAACTTCTGCGTAGCGGATA ACAATTTACACAGG
SLo2470	GTTGAATGAGCCTACTGGGTCTCCCAGTCA CGACGTTGTAAAACG	SLo2566	GTTGAATGAGCCTACTGGGTCTCAGCGGATA ACAATTTACACAGG
SLo2471	TGAGAGACAAGATTGTTGCGTGGACCCAGTCA CGACGTTGTAAAACG	SLo2567	TGAGAGACAAGATTGTTGCGTGGACAGCGGATA ACAATTTACACAGG
SLo2472	AGATTCAGACCGTCTCATGCAAAGCCAGTCA CGACGTTGTAAAACG	SLo2568	AGATTCAGACCGTCTCATGCAAAGAGCGGATA ACAATTTACACAGG
SLo2473	CAAGAGCTTTGACTAAGGAGCATGCCAGTCA CGACGTTGTAAAACG	SLo2569	CAAGAGCTTTGACTAAGGAGCATGAGCGGATA ACAATTTACACAGG
SLo2474	TGGAAGATGAGACCTGATCTACGCCAGTCA CGACGTTGTAAAACG	SLo2570	TGGAAGATGAGACCTGATCTACGAGCGGATA ACAATTTACACAGG
SLo2475	TCCTACTCAACAGGTGGCATGAAACCCAGTCA CGACGTTGTAAAACG	SLo2571	TCCTACTCAACAGGTGGCATGAAAGCGGATA ACAATTTACACAGG
SLo2476	GCTAGGTCAATCTCCTTCGGAAGTCCCAGTCA CGACGTTGTAAAACG	SLo2572	GCTAGGTCAATCTCCTTCGGAAGTAGCGGATA ACAATTTACACAGG

Forward barcoding primer		Reverse barcoding primer	
ID	Sequence (5'→3')	ID	Sequence (5'→3')
SLo2477	CAGGTTACTCCTCCGTGAGTCTGACCCAGTCA CGACGTTGTAAAACG	SLo2573	CAGGTTACTCCTCCGTGAGTCTGAAGCGGATA ACAATTTACACAGG
SLo2478	TCAATCAAGAAGGGAAAGCAAGGTCCCAGTCA CGACGTTGTAAAACG	SLo2574	TCAATCAAGAAGGGAAAGCAAGGTAGCGGATA ACAATTTACACAGG
SLo2479	CATGTTCAACCAAGGCTTCTATGGCCCAGTCA CGACGTTGTAAAACG	SLo2575	CATGTTCAACCAAGGCTTCTATGGAGCGGATA ACAATTTACACAGG
SLo2480	AGAGGGTACTATGTGCCTCAGCACCCAGTCA CGACGTTGTAAAACG	SLo2576	AGAGGGTACTATGTGCCTCAGCACAGCGGATA ACAATTTACACAGG
SLo2481	CACCCACACTTACTTCAGGACGTACCCAGTCA CGACGTTGTAAAACG	SLo2577	CACCCACACTTACTTCAGGACGTAAGCGGATA ACAATTTACACAGG
SLo2482	TTCTGAAGTTCCTGGGTCTTGAACCCAGTCA CGACGTTGTAAAACG	SLo2578	TTCTGAAGTTCCTGGGTCTTGAACAGCGGATA ACAATTTACACAGG
SLo2483	GACAGACACCGTTCATCGACTTTCGCCAGTCA CGACGTTGTAAAACG	SLo2579	GACAGACACCGTTCATCGACTTTCAGCGGATA ACAATTTACACAGG
SLo2484	TTCTCAGTCTTCCTCCAGACAAGGCCAGTCA CGACGTTGTAAAACG	SLo2580	TTCTCAGTCTTCCTCCAGACAAGGAGCGGATA ACAATTTACACAGG
SLo2485	CCGATCCTTGTGGCTTCTAACTTCCCAGTCA CGACGTTGTAAAACG	SLo2581	CCGATCCTTGTGGCTTCTAACTTACGCGGATA ACAATTTACACAGG
SLo2486	GTTTGTCACTACTCGTGTGCTCACCCAGTCA CGACGTTGTAAAACG	SLo2582	GTTTGTCACTACTCGTGTGCTCACAGCGGATA ACAATTTACACAGG
SLo2487	GAATCTAAGCAAACACGAAGGTGGCCAGTCA CGACGTTGTAAAACG	SLo2583	GAATCTAAGCAAACACGAAGGTGGAGCGGATA ACAATTTACACAGG
SLo2488	TACAGTCCGAGCCTCATGTGATCTCCAGTCA CGACGTTGTAAAACG	SLo2584	TACAGTCCGAGCCTCATGTGATCTAGCGGATA ACAATTTACACAGG
SLo2489	ACCGAGATCCTACGAATGGAGTGTCCCAGTCA CGACGTTGTAAAACG	SLo2585	ACCGAGATCCTACGAATGGAGTGTAGCGGATA ACAATTTACACAGG
SLo2490	CCTGGGAGCATCAGGTAGTAACAGCCAGTCA CGACGTTGTAAAACG	SLo2586	CCTGGGAGCATCAGGTAGTAACAGAGCGGATA ACAATTTACACAGG
SLo2491	TAGCTGACTGTCTTCCATACCGACCCAGTCA CGACGTTGTAAAACG	SLo2587	TAGCTGACTGTCTTCCATACCGACAGCGGATA ACAATTTACACAGG
SLo2492	AAGAAACAGGATGACAGAACCCTCCCAGTCA CGACGTTGTAAAACG	SLo2588	AAGAAACAGGATGACAGAACCCTCAGCGGATA ACAATTTACACAGG
SLo2493	TACAAGCATCCCAACACTTCCACTCCCAGTCA CGACGTTGTAAAACG	SLo2589	TACAAGCATCCCAACACTTCCACTAGCGGATA ACAATTTACACAGG
SLo2494	GACCATTGTGATGAACCTGTTGTCCCAGTCA CGACGTTGTAAAACG	SLo2590	GACCATTGTGATGAACCTGTTGTAGCGGATA ACAATTTACACAGG
SLo2495	ATGCTTGTACATCAACCCTGGACCCAGTCA CGACGTTGTAAAACG	SLo2591	ATGCTTGTACATCAACCCTGGACAGCGGATA ACAATTTACACAGG
SLo2496	CGACCTGTTTCTCAGGGATAACAACCCAGTCA CGACGTTGTAAAACG	SLo2592	CGACCTGTTTCTCAGGGATAACAACAGCGGATA ACAATTTACACAGG
SLo2497	AACAACCGAACCTTTGAATCAGAACCAGTCA CGACGTTGTAAAACG	SLo2593	AACAACCGAACCTTTGAATCAGAAAGCGGATA ACAATTTACACAGG
SLo2498	TCTCGGAGATAGTTCTCACTGCTGCCAGTCA CGACGTTGTAAAACG	SLo2594	TCTCGGAGATAGTTCTCACTGCTGAGCGGATA ACAATTTACACAGG
SLo2499	CGGATGAACATAGGATAGCGATTCCCAGTCA CGACGTTGTAAAACG	SLo2595	CGGATGAACATAGGATAGCGATTAGCGGATA ACAATTTACACAGG
SLo2500	CCTCATCTTGTGAAGTTGTTTCGGCCCAGTCA CGACGTTGTAAAACG	SLo2596	CCTCATCTTGTGAAGTTGTTTCGGAGCGGATA ACAATTTACACAGG
SLo2501	ACGGTATGTGCGAGTTCAGGACTACCCAGTCA CGACGTTGTAAAACG	SLo2597	ACGGTATGTGCGAGTTCAGGACTAAGCGGATA ACAATTTACACAGG
SLo2502	TGGCTTGATCTAGGTAAGGTGCAACCCAGTCA CGACGTTGTAAAACG	SLo2598	TGGCTTGATCTAGGTAAGGTGCAAGCGGATA ACAATTTACACAGG
SLo2503	GTAGTGGACCTAGAACCCTGTGCCACCCAGTCA CGACGTTGTAAAACG	SLo2599	GTAGTGGACCTAGAACCCTGTGCCAAGCGGATA ACAATTTACACAGG
SLo2504	AACGGAGGAGTTAGTTGGATGATCCCAGTCA CGACGTTGTAAAACG	SLo2600	AACGGAGGAGTTAGTTGGATGATAGCGGATA ACAATTTACACAGG
SLo2505	AGGTGATCCCAACAAGCGTAAGTACCCAGTCA CGACGTTGTAAAACG	SLo2601	AGGTGATCCCAACAAGCGTAAGTAAGCGGATA ACAATTTACACAGG

<b>Forward barcoding primer</b>		<b>Reverse barcoding primer</b>	
<b>ID</b>	<b>Sequence (5'→3')</b>	<b>ID</b>	<b>Sequence (5'→3')</b>
SLo2506	TACATGCTCCTGTTGTTAGGGAGGCCAGTCA CGACGTTGTAAAACG	SLo2602	TACATGCTCCTGTTGTTAGGGAGGAGCGGATA ACAATTCACACAGG
SLo2507	TCTTCTACTACCGATCCGAAGCAGCCCAGTCA CGACGTTGTAAAACG	SLo2603	TCTTCTACTACCGATCCGAAGCAGAGCGGATA ACAATTCACACAGG
SLo2508	ACAGCATCAATGTTTGGCTAGTTGCCAGTCA CGACGTTGTAAAACG	SLo2604	ACAGCATCAATGTTTGGCTAGTTGAGCGGATA ACAATTCACACAGG
SLo2509	GATGTAGAGGGTACGGTTTGAGGCCAGTCA CGACGTTGTAAAACG	SLo2605	GATGTAGAGGGTACGGTTTGAGGCAGCGGATA ACAATTCACACAGG
SLo2510	GGCTCCATAGGAACTCACGCTACTCCCAGTCA CGACGTTGTAAAACG	SLo2606	GGCTCCATAGGAACTCACGCTACTAGCGGATA ACAATTCACACAGG
SLo2511	TTGTGAGTGGAAAGATACAGGACCCCAGTCA CGACGTTGTAAAACG	SLo2607	TTGTGAGTGGAAAGATACAGGACAGCGGATA ACAATTCACACAGG
SLo2512	AGTTTCCATCACTTCAGACTTGGGCCAGTCA CGACGTTGTAAAACG	SLo2608	AGTTTCCATCACTTCAGACTTGGGAGCGGATA ACAATTCACACAGG
SLo2513	GATTGTCTCAAACGCCACCTACCCCAGTCA CGACGTTGTAAAACG	SLo2609	GATTGTCTCAAACGCCACCTACAGCGGATA ACAATTCACACAGG
SLo2514	CCTGTCTGGAAGAAGAATGGACTTCCCAGTCA CGACGTTGTAAAACG	SLo2610	CCTGTCTGGAAGAAGAATGGACTTAGCGGATA ACAATTCACACAGG
SLo2515	CTGAACGGTCATAGAGTCCACCATCCCAGTCA CGACGTTGTAAAACG	SLo2611	CTGAACGGTCATAGAGTCCACCATAGCGGATA ACAATTCACACAGG