SINGULAR GENOMICS

REFERENCE GUIDE

Adapters and Indices for the G4 Sequencing Platform

Singular adapters and unique dual indices maximize flexibility for the independently addressable lanes of the G4 Sequencing Platform.

The G4™ Sequencing Platform by Singular Genomics™ is the most powerful benchtop sequencer to date. The G4 Sequencing Platform and Singular sequencing chemistry have been designed from the ground up, resulting in increased speed and flexibility, and support a wide range of library preparation workflows. Singular Genomics has developed an adapter strategy with 96 unique dual indices that enable efficient use of each independently addressable lane and flow cell, and allows for flexibility in the index sequence lengths. This reference guide describes the adapters and indices, explains how to integrate them in library preparation workflows, and provides guidelines for using custom libraries on the G4 Sequencing Platform.

SINGULAR ADAPTERS FOR LIBRARY PREP

Singular Adapter Sequences

Singular adapters are used during library preparation to add several functional nucleotide tags to each end of the inserts to be sequenced (Fig 1). At the 5′ ends, Singular proprietary platform sequences S1 and S2 are attached as anchors for the formation of clusters on the flow cell. The SP1 and SP2 tags, which are identical to the SP1 and SP2 sequencing primers used in many existing applications, are positioned directly adjacent to the insert. When constructing libraries for multiplexed reads, index 1 and index 2 sequences are placed in between S1 and SP1, and S2 and SP2 respectively. The inserts and indices are sequenced using the primers described in Table 1.

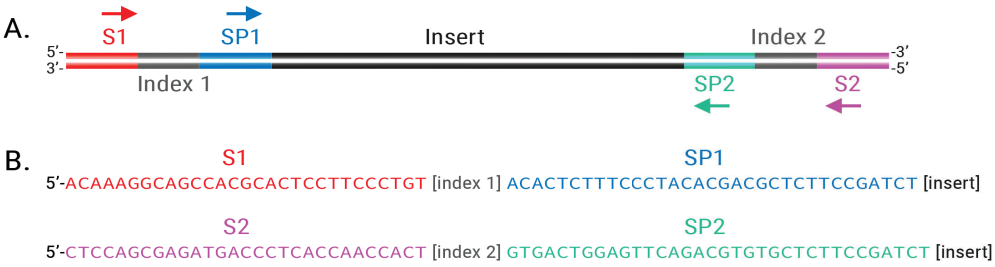

Figure 1: Singular Library with Dual Indices. **A**. Structure of Singular library, arrows denote direction of sequencing. **B**. Final sequence of adapters on a library with dual indices.

Table 1: Sequencing Primers.

Primer	Sequence Order	Sequenced Segment	Sequence of Primer
S1	1	Index 1	5′-ACAAAGGCAGCCACGCACTCCTTCCCTGT
SP1	2	Insert Read 1	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT
S2	3	Index 2	5′-CTCCAGCGAGATGACCCTCACCAACCACT
SP2	4	Insert Read 2	5′-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Universal Stem-Loop Adapter

Singular Genomics uses a universal stem-loop adapter during library preparation as first step to introduce the nucleotide sequences necessary for cluster generation and sequencing. The stem-loop adapter consists of short complementary sequences at the 5′ and 3′ ends, a non-complementary segment in the center with uracil in the middle, and a T-overhang at the 3′ end (Figure 2). The 3′ end contains the SP1 sequencing

Figure 2: Sequence of the Singular Universal Stem-Loop Adapter.

primer sequence, while the 5′ end is complementary to the standard SP2 sequencing primer (SP2′), without the A at the 5′ end. SP1 and SP2 are sequencing primers widely used in existing sequencing protocols.

Standard Library Preparation

Adding the necessary sequences for cluster generation and sequencing to your library is a straight-forward procedure. After you process and fragment a DNA sample, add an A-tail to the 3′ ends (Fig. 3, step 1). This A-tail provides an efficient target to ligate with the 3′ T-tail of the stem-loop adapter (Fig. 3, step 2). Next, the cleavable site in the middle of the loop is excised (Fig.3, step 3) using a Uracil-DNA Glycosylase[1] (UDG) in appropriate buffer conditions or a suitable alternative. Cleaving the backbone opens the loops and results in a linear fragment with the SP1 sequence at the 5′ end, and the SP2′ sequence at the 3′ end. These sequences are then used to add the Singular S1 cluster sequence and index 1 at the SP1 end, and Singular S2 cluster sequence and index 2 at the SP2 end through a limited amplification step (Fig. 3, step 4-6). Primers without indices are used if no multiplexing is required (Table 2).

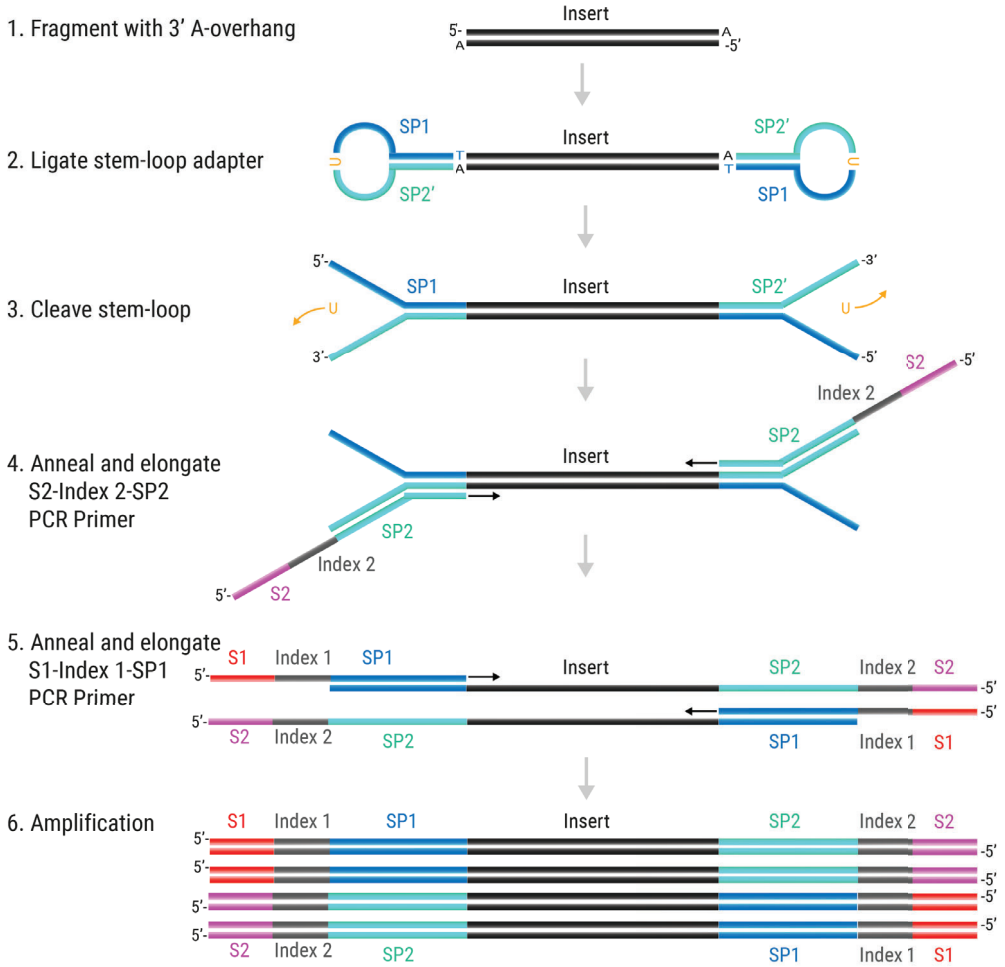

Figure 3: Singular Standard Library Preparation.

Primer	Sequence of Primer
S1-Index-SP1	S1 SP1 5′-ACAAAGGCAGCCACGCACTCCTTCCCTGT[index 1]ACACTCTTTCCCTACACGACGCTCTTCCGATCT
S2-Index-SP2	S2 SP2 5′-CTCCAGCGAGATGACCCTCACCAACCACT[index 2]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
S1-SP1	S1 SP1 5′-ACAAAGGCAGCCACGCACTCCTTCCCTGTACACTCTTTCCCTACACGACGCTCTTCCGATCT
S2-SP2	S2 SP2 5′-CTCCAGCGAGATGACCCTCACCAACCACTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Table 2: PCR primers for introducing cluster sequences (S1 and S2) with or without indices.

The steps described above result in a library that is ready for clustering and sequencing on the G4 Sequencing Platform (Fig.1). The S1 and S2 sequences are used to form clusters on the flow cell. Then, depending on the application, the inserts and indices are sequenced using the primers described in Table 1.

PCR-Free Library Preparation

PCR-free library preparation has certain advantages, including eliminating PCR bias and a simplified workflow, though with lower yield. For PCR-free sequencing, Singular uses a different stem-loop adapter. In addition to the uracil and SP1 and SP2′ sequences, the PCR-free stem-loop adapter also contains the S1 and the complementary sequence to S2 (Fig.4A). For indexed applications, Singular has embedded dual indices within the adapter design (Fig.4B). This means no amplification is required to add the index sequences, which simplifies the workflow (Fig. 4C).

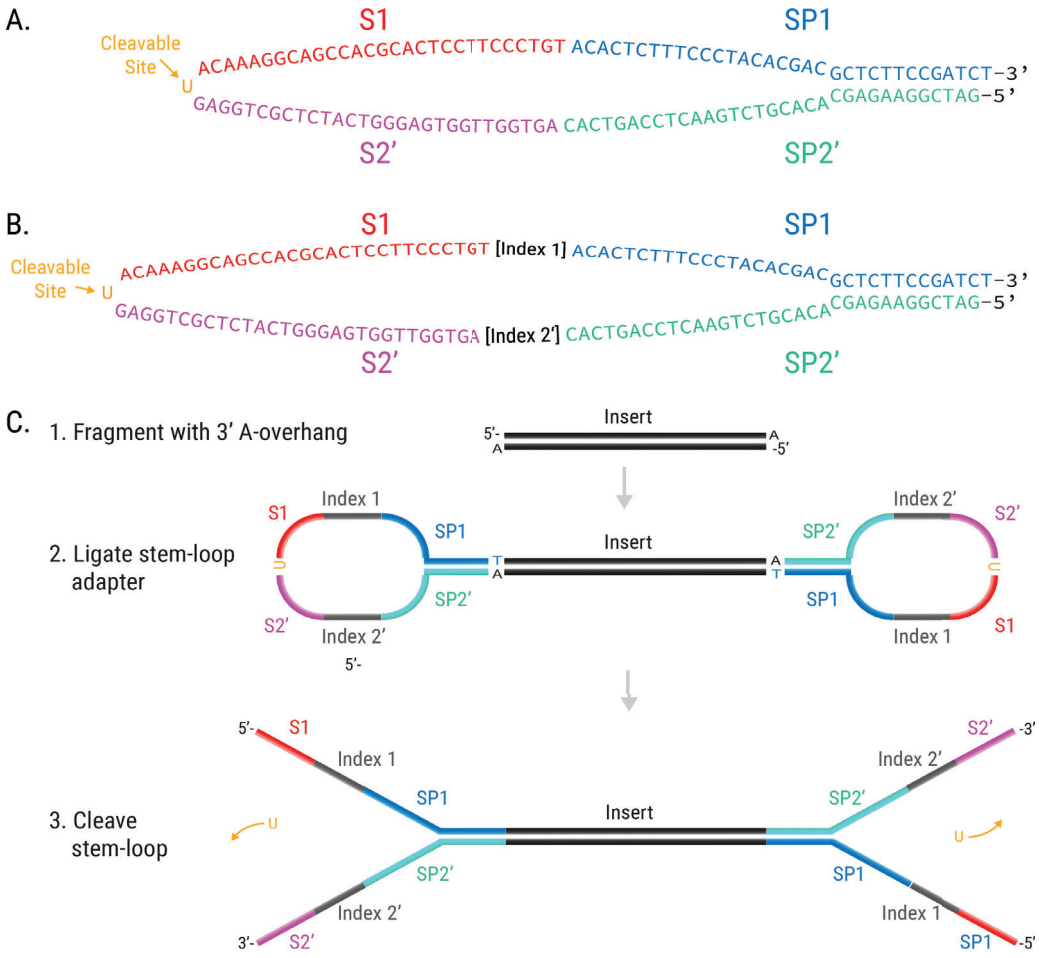

Figure 4: Singular PCR-free Library Preparation. **A**. Sequence of Singular stem-loop adapter for PCR-free library preparation. **B**. Like A, but with dual indices. **C**. Singular PCR-free library preparation steps.

SINGULAR INDEXING STRATEGY

Indices are short DNA barcodes added during library prep that allow you to correctly assign reads to their corresponding sample via demultiplexing. When pooling multiple libraries within a single lane, you should always assign a unique combination of indices to each library. An ideal index design avoids misassociation of reads, and the assignment process is resistant to errors, including those introduced during index synthesis. The index sequences should be as divergent as possible while having similar GC content and avoiding extended homopolymer stretches. Indices that are many changes (insertions, deletions, and/or substitutions) away from other index sequences minimize the possibility of misassociation.

Singular dual indices have been designed with these principles in mind, and have the following features:

- Singular Genomics provides 96 unique dual indices (UDIs) of 12 bases for sample indexing.
- Robust UDI design allows for efficient demultiplexing with as few as 8 cycles, which enables efficient demultiplexing of 96 libraries. Extending reads to 12 cycles assures maximal differentiation between samples.
- The indices have been empirically tested to ensure even library representation in multiplex experiments.
- Quality control analysis of indices ensures high purity oligomer synthesis.
- Rapid demultiplexing can be performed on or off instrument via the Singular Genomics Demultiplex Tool, an accelerated version of fgbio's DemuxFastqs.

The complete set of Singular UDIs is listed in Appendix 1: Singular Dual Indices.

It is best practice to use dual indices, which enable identification and removal of mis-associated indices that can arise during library prep and index hopping. While index hopping in cluster amplification is rare (< 0.1% or reads), levels close to 2% and up to 10% have been reported for other platforms[2,3,4]. We have measured index hopping on the G4 Sequencing Platform, and have observed very low levels of index hopping in the order of 0.07% (for details see Appendix 2: Index Hopping), indicating index hopping is not a substantial source of error for the G4 Sequencing Platform.

ADAPT LIBRARY FORMAT TO SINGULAR SEQUENCING

Considerations When Using Custom Sequencing Primers

If you design your own custom adapters and primers, the adapters need to contain S1 and S2 sequences for clustering on the G4. The S1 and S2 sequences need to be located on the ends of the fragment, so your custom sequences and insert will be amplified during cluster generation (Fig. 5). Note that you must validate custom primers yourself; Singular Genomics cannot guarantee performance or compatibility of custom primers.

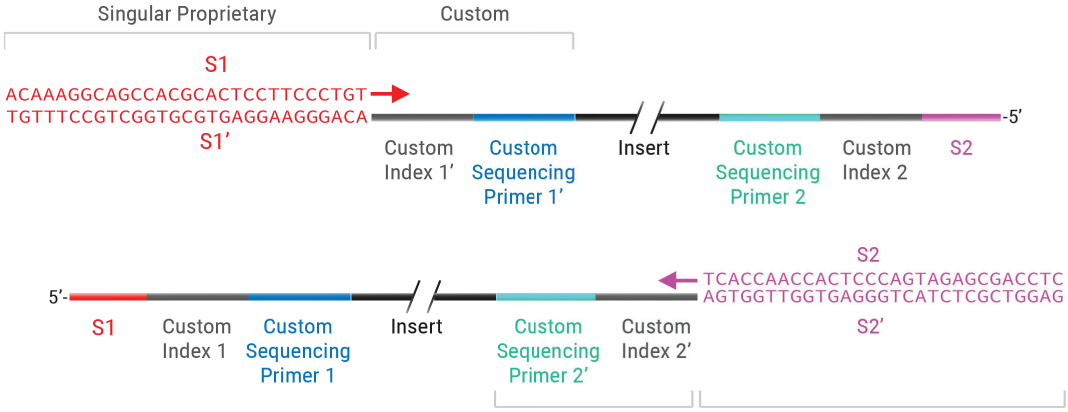

Figure 5: Outline of Custom Library during Cluster Generation. Indicated are the custom parts of the adapter, as well as the Singular proprietary sequences needed for cluster generation with Singular S1 and S2 oligos.

Your custom sequencing primers need to account for the melting temperature (Tm) relative to sequencing temperature. Also be aware of the salt concentration of the buffer for the sequencing primers, as it impacts hybridization efficiency and may impact amplification and sequencing efficiency. Custom sequencing primers need to be able to bind at 66°C (while assuming 50 mM Na^+). Ideally, your custom primers are very similar in GC content and length to SP1 and SP2 sequencing primers (52% and 33 bp). Also avoid short GC-rich sequencing primers. Contact the Singular Genomics Customer Care team for additional technical guidance.

Existing Library Conversion

Converting existing libraries to Singular libraries is usually straightforward. Many libraries already contain SP1 and SP2 sequences adjacent to the insert. You can do a simple PCR using either the S1-index1-SP1 and S2-index2-SP2 primers, or, if no index is required, use the S1-SP1 and S2-SP2 primers (Table 2 and Fig. 6). After this, use a PCR purification kit to clean up your product and follow the regular workflow for clustering on the G4.

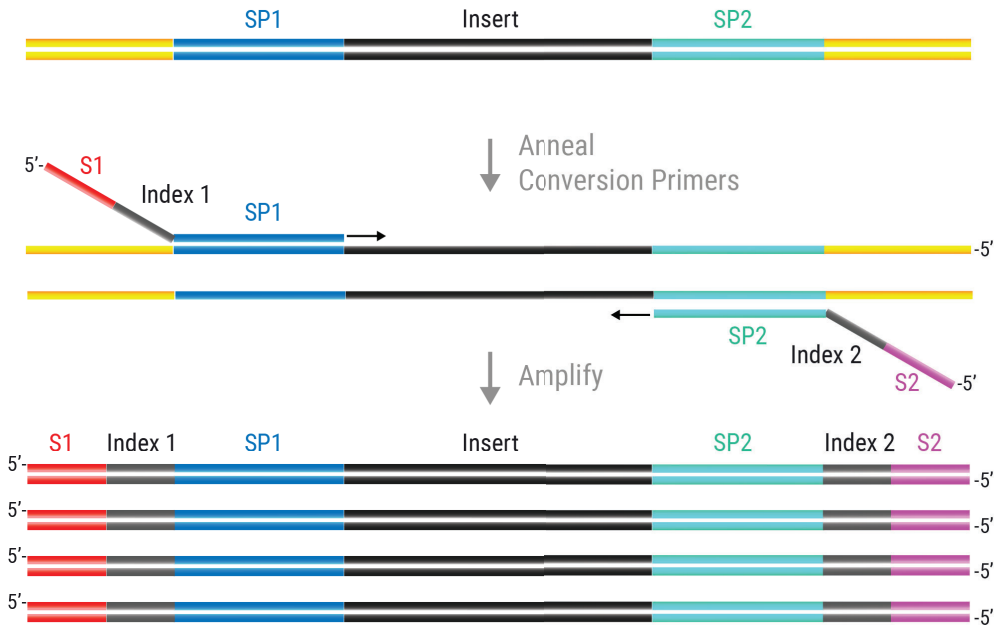

Figure 6: Strategy for Adapting Existing Libraries to Singular Sequencing.

Note that the SP1 and SP2 sequencing primer sequences the G4 uses are identical to the ones used in many legacy platforms, while the indices and S1 and S2 are unique for Singular Genomics.

If you have an existing library without SP1 and SP2 sequencing primers, you will have to design your own conversion primers. They should minimally consist of the S1 and S2 sequences at the 5′ end for clustering on the G4, and your custom sequencing primer sequences for anchoring on your library fragment (see Design Custom Libraries for additional guidelines, and contact Singular Genomics Customer Care for help).

Adapter Trimming

Trimming adapters from Singular Genomics data can be done using common NGS bioinformatic tools such as cutadapt[5] or BBDuk[6]. We recommend using at least 12 bp of the adapter sequence to trim. For standard runs using the SP1 and SP2 sequencing primers, the sequences that can be used to trim R1 and R2 are listed in Table 3.

Table 3: Trimming Sequences.

Sequencing Primer	Sequenced Segment	Recommended Trimming Sequence
SP1	Read 1	AGATCGGAAGAGCACA
SP2	Read 2	AGATCGGAAGAGCGTC

RESOURCES

Customer Care

For any questions regarding this document, or for further assistance, contact Singular Genomics Customer Care:

Email: **care@singulargenomics.com**
Call: +1-442-SG-CARES (1-442-742-2737)
Website: **www.singulargenomics.com**

Ordering Products

To order the products described in this reference guide from Singular Genomics, use the following part numbers:

Part Number	Product
700110	SG UDI - Set of 96
700111	SG UDI - Set of 24 [Set A]
700112	SG UDI - Set of 24 [Set B]
700115	G4 Non-Indexed PCR-Free Library Prep Adapters (24 Reactions)
700116	G4 Indexed PCR-Free Library Prep Adapters (24 Reactions)
700117	G4 Universal Library Prep Adapters (96 Reactions)
700118	G4 Universal Library Prep Adapters (24 Reactions)
700119	G4 Non-Indexed Library Prep Primers (24 reactions)

REFERENCES

1. Pearl, LH. Structure and function in the uracil-DNA glycosylase superfamily. Mutat Res. 2000; 460(3-4):165-81. doi: 10.1016/s0921-8777(00)00025-2
2. Costello M, Fleharty M, Abreu J, Farjoun Y, Ferriera S, Holmes L, Granger B, Green L, Howd T, Mason T, Vicente G, Dasilva M, Brodeur W, DeSmet T, Dodge S, Lennon NJ, Gabriel S. Characterization and remediation of sample index swaps by non-redundant dual indexing on massively parallel sequencing platforms. BMC Genomics. 2018; 19:332. doi: 10.1186/s12864-018-4703-0
3. Effects of Index Misassignment on Multiplexing and Downstream Analysis. index-hopping-white-paper-770-2017-004.pdf
4. Sinha R, Stanley G, Gulati GS, Ezran C, Travaglini KJ, Wei E, Chan CKF, Nabhan AN, Su T, Morganti RM, Conley SD, Chaib H, Red-Horse K, Longaker MT, Snyder MP, Krasnow MA, Weissman IL. Index switching causes "spreading-of-signal" among multiplexed samples in Illumina HiSeq 4000 DNA sequencing. bioRxiv. 2017; 125724. doi: 10.1101/125724
5. https://cutadapt.readthedocs.io/en/stable/
6. https://jgi.doe.gov/data-and-tools/software-tools/bbtools/bb-tools-user-guide/bbduk-guide/
7. Schwendinger J. Sequence with confidence: understand index hopping and how to resolve it. https://www.10xgenomics.com/blog/sequence-with-confidence-understand-index-hopping-and-how-to-resolve-it
8. Farouni R, Djambazian H, Ferri LE, Ragoussis J, Najafabadi HS. Model-based analysis of sample index hopping reveals its widespread artifacts in multiplexed single-cell RNA-sequencing. Nature Communications. 2020; 11: 2704
9. Van der Valk T, Vezzi F, Ormestad M, Dalén L, Guschanski K. Index hopping on the Illumina HiseqX platform and its consequences for ancient DNA studies. Mol Ecol Resour. 2020; 20(5):1171-1181. doi: 10.1111/1755-0998.13009
10. Ros-Freixedes R, Battagin M, Johnsson M, Gorjanc G, Mileham AJ, Rounsley SD, Hickey JM. Impact of index hopping and bias towards the reference allele on accuracy of genotype calls from low-coverage sequencing.Genet Sel Evol. 2018; 50(1):64. doi: 10.1186/s12711-018-0436-4.

APPENDIX 1: SINGULAR DUAL INDEX SEQUENCES

Indicated are the Singular dual index sequences in each set. S1 and S2 in the index name indicate the index can be sequenced using the S1 primer and S2 primer, respectively. After each 12-base index sequence, there is a single nucleotide spacer before SP1 or SP2 sequences start. See also Table 2.

Set A

Dual Index 1		Dual Index 2	
S1-Index1	TAAGACCCTACT T	S2-Index1	GGGACATATTGA C
S1-Index2	CGAAGTACATCC C	S2-Index2	TAGGACGTAACG G
S1-Index3	TAGCCTTCCAAA C	S2-Index3	AGTATGGCAAGA C
S1-Index4	GCCTTTCAAGTC C	S2-Index4	TAGAGTCGTCGT G
S1-Index5	CAACGGTTCCGG C	S2-Index5	ACGTTTCGCTCG T
S1-Index6	GTTGCATGGCCC T	S2-Index6	TAGGGAACGATG G
S1-Index7	ATCGTTGCTATC G	S2-Index7	ATGACTCCGCAT T
S1-Index8	CCTCGAATTCAT C	S2-Index8	GGTTGCTACCGG T
S1-Index9	TGAACGTCCGCC C	S2-Index9	TCCTCGATTGAA G
S1-Index10	CATCTAGCAAGC T	S2-Index10	ATGTAGCGTCTC A
S1-Index11	TATCGAGGCAAC C	S2-Index11	CATCATGCGTAC C
S1-Index12	GAGACGTAGCAA G	S2-Index12	ACCTTGACCGGG G
S1-Index13	ATCATGCGCCCG T	S2-Index13	TTGACGAGATCT A
S1-Index14	AGGAGCTAGGGA G	S2-Index14	GGGCTAATGTCA T
S1-Index15	ATCGACCATGCT C	S2-Index15	TTAGGAGCGAAC T
S1-Index16	TGCGAATCGACA C	S2-Index16	GTACATCGAGTA G
S1-Index17	ATGTTCCCCTCT T	S2-Index17	AGGCTTTGTCAT T
S1-Index18	TCGCTCATCTAG A	S2-Index18	CGACGATATTTG G
S1-Index19	CCTAAGGTAAAC C	S2-Index19	AAACTCCGTTGT T
S1-Index20	GAATAGCGCTTA T	S2-Index20	ACGTACCAAGAC C
S1-Index21	CGATGTACATCC T	S2-Index21	TCCGATGTCGGC G
S1-Index22	CAAGTCGAAACC T	S2-Index22	AGGTTACCGCGT A
S1-Index23	GTAACGGATAGC T	S2-Index23	ATGCCGAAACGT T
S1-Index24	GAAGCTTGGTCA G	S2-Index24	ACATACGCGGGG G

Set B

Dual Index 1		Dual Index 2	
S1-Index25	AACCCGTAACCA T	S2-Index25	GGATCTAGGACG G
S1-Index26	AATGCTCCCCTA C	S2-Index26	TCGACTCTCCGT T
S1-Index27	GTATGACGGATG G	S2-Index27	TACGCTAGACAA A
S1-Index28	GCAAAGCTTGGA C	S2-Index28	ATTTCGGGTAAG T
S1-Index29	TCTAACCGGCTA G	S2-Index29	CTAGCCAACGCC G
S1-Index30	ATTGGAGCCCGC C	S2-Index30	TGATAGCCGGTT C
S1-Index31	TGTCCGATCTAT A	S2-Index31	GCATGGTTCCTA T
S1-Index32	ACATCGCATGTT C	S2-Index32	GACGGTAATGAG T
S1-Index33	ACTTCCGACAAT A	S2-Index33	CGAAGTCATCAA C
S1-Index34	GCTCCTATGCCT T	S2-Index34	TGGACTACAAAC T
S1-Index35	CATAACGCGAAT G	S2-Index35	ATACGCGACATT G
S1-Index36	CGGACAATGATT G	S2-Index36	TCCCTATGATAC C
S1-Index37	GCTACTTGGAAA A	S2-Index37	GAGCTCTACGCA T
S1-Index38	TAAGCGAGTAGT C	S2-Index38	CTAGAGGTTACC T
S1-Index39	TGCTTGACTCCG G	S2-Index39	AACCCTTGTTCG T
S1-Index40	GACATCGTCGGG C	S2-Index40	GTCGTAAGGGGT T
S1-Index41	AGCTATGGGACG A	S2-Index41	TATACCCGGCCC A
S1-Index42	ACGACTAGGCTC T	S2-Index42	TGCTAAGCGAGC C
S1-Index43	TACCGTACGATA A	S2-Index43	CGTCCTAAAACT T
S1-Index44	TAGAAGGCGCGT C	S2-Index44	ATATCGGGTAAG A
S1-Index45	CGTCATCAAGGA G	S2-Index45	GAGACGTTCTTA A
S1-Index46	CCGTAAGATAGA C	S2-Index46	GCTTAACGATCA T
S1-Index47	TAGACTCGTTTC C	S2-Index47	CGCTAGTACTAT G
S1-Index48	TATCGGCTTGGT C	S2-Index48	ACGGGTTATTAG A

Set C

Dual Index 1		Dual Index 2	
S1-Index49	TCAAGAGCGGAG G	S2-Index49	CGGACTTTTGTA G
S1-Index50	TTACCCGTAGAA A	S2-Index50	TCCATTGCTTCT A
S1-Index51	GCTCTCAATCGG G	S2-Index51	GTCTCATGGCGG A
S1-Index52	GTCTACGTTTAC C	S2-Index52	TGACTTGGAGAA A
S1-Index53	TCCGTATGAGAC C	S2-Index53	ACCGTATCCGAT C
S1-Index54	CGCCAATACGTC A	S2-Index54	CTATGGGACGGT T
S1-Index55	GATGGTCTAGCA C	S2-Index55	TAGTTCCCATTC C
S1-Index56	CTCGCTTAAGGC G	S2-Index56	CTCCAAGACATC A
S1-Index57	GGCAACATGGGT C	S2-Index57	ATTACCGCGGTA C
S1-Index58	AGACTCTCATCA A	S2-Index58	CTAAGCTCCTAA C
S1-Index59	TGACAAGGTCAA A	S2-Index59	TGAACGTCCTTC T
S1-Index60	CGGTATGTCATC G	S2-Index60	CCATGCAATCTA C
S1-Index61	GACTCATGAATG A	S2-Index61	TAGCGTTCATTG G
S1-Index62	CGTAGACATTGA T	S2-Index62	AGCATTCGAGCC T
S1-Index63	CATTCGCTCCCT G	S2-Index63	AACCTCGAACAT A
S1-Index64	ACAATCGGGGAC T	S2-Index64	CAAATCGCGGAA A
S1-Index65	GGACTTAGAGCG C	S2-Index65	GTCAAGAGGTTA A
S1-Index66	GACCGATTCTCG A	S2-Index66	TTAAGGCCGGGA G
S1-Index67	TGGAAACCCGAG T	S2-Index67	TCGAAAGGGAAA C
S1-Index68	GGCCTAATGGAA G	S2-Index68	GGAGTCAAATAG T
S1-Index69	TTGTACGCGTAC A	S2-Index69	CCGTTCTATACA A
S1-Index70	ATGTCGAGTTGC A	S2-Index70	TGCGAATGCGAA C
S1-Index71	CTTCGTACCTCC A	S2-Index71	GCGATCATGACT C
S1-Index72	TTAGGTCCGAGA A	S2-Index72	CCGAAATCCAAC C

Set D

Dual Index 1		Dual Index 2	
S1-Index73	CTAGCTCTTCGT T	S2-Index73	TCGGAGTTTTAC C
S1-Index74	CTTGTCCAACTT G	S2-Index74	CTATCCGTCCCG C
S1-Index75	CTTAGCGACCCA T	S2-Index75	TAACGCGTACCC C
S1-Index76	CGTAGGTTAACA A	S2-Index76	CTCGTACTTAGC A
S1-Index77	AGCATTCCATGT G	S2-Index77	GACTCGAAATGA A
S1-Index78	TCGTTACCAACG C	S2-Index78	TTAACCGGCCGA C
S1-Index79	TTGCTAGGACAT G	S2-Index79	TTCCTAGCAACC C
S1-Index80	CGAGACTTCTAC A	S2-Index80	GAATAGCGTCCC T
S1-Index81	GGTCTATGTTTG T	S2-Index81	AATGACGGATGT G
S1-Index82	GATGCCATAGTA G	S2-Index82	AGTAGCTCGTCC C
S1-Index83	GTACGAGTTCCT C	S2-Index83	TGGCCATTCTCC T
S1-Index84	TTCCATCGGTAG A	S2-Index84	ACGCCATAACCG T
S1-Index85	ACGCTATCATCT T	S2-Index85	CGTTAACCCGCT T
S1-Index86	GTCCAAGAGTTC C	S2-Index86	TTGAGCCTTGCT A
S1-Index87	CGAATGGTAAGA T	S2-Index87	ACCCGTAACAAG G
S1-Index88	AGGTTTCGGTAT C	S2-Index88	AAAGGTCGCCCC A
S1-Index89	GGTTCAAGATAC T	S2-Index89	TACGAGCTCCTC A
S1-Index90	GTTAAGCGGGCG A	S2-Index90	GGCATAGAGCGA T
S1-Index91	AAGCTACGCGAA T	S2-Index91	GGTAGCATTCGG T
S1-Index92	TTGAGCGTCCGA C	S2-Index92	CGACAAGTTCGA G
S1-Index93	CCTATGCAATTG T	S2-Index93	GGCAATGTACTT C
S1-Index94	CTATTGCCTACG A	S2-Index94	CCGGTAAACCCA C
S1-Index95	CCTCTTGAAGAG A	S2-Index95	GCCCATTAGATT G
S1-Index96	TGGGTTACGGGC G	S2-Index96	CAAGGTTCTCTT C

APPENDIX 2: INDEX HOPPING

Indices are widely used for multiplexing to identify which reads belong to which samples, but sometimes errors happen. There are several causes for index misassociation. Some of them, like poor oligo synthesis or well-to-well contamination, can be eliminated through good practices. Others are caused by the chemistry employed during cluster formation (for example, index hopping).

A common cause of index hopping is contamination with free adapters and primers during cluster generation. These free adapters can hybridize with the complementary sequence of an adapter from a different sample and extend. This free hybrid molecule can seed a cluster with an incorrect index for that insert in a different well. There are other causes of index hopping, like low diversity in sequence that allows two fragments to bind to each other. These fragments can then form hybrids that extend and seed clusters with mismatched indices.[2,7]

While index hopping in bridge amplification usually stays well below 1% of the reads, levels close to 2%, with levels as high as 10% have been reported on other SBS platforms.[2,3,4] These levels can lead to serious problems in downstream analysis in a variety of applications, like somatic variant detection and differential expression.[8,9,10] We therefore wanted to characterize the potential for index hopping on the patterned flow cells of the G4 Sequencing Platform.

We ran 4 lanes each with 24 indexed Salmonella whole-genome samples using different unique dual indices for each lane. The data were analyzed to detect any unexpected dual index pairs between the 24 dual indices, which are indicative of index hopping events. As shown in Fig. 7, in all lanes, index hopping events were rare, with on average only 0.07% of reads having aberrant dual index combinations.

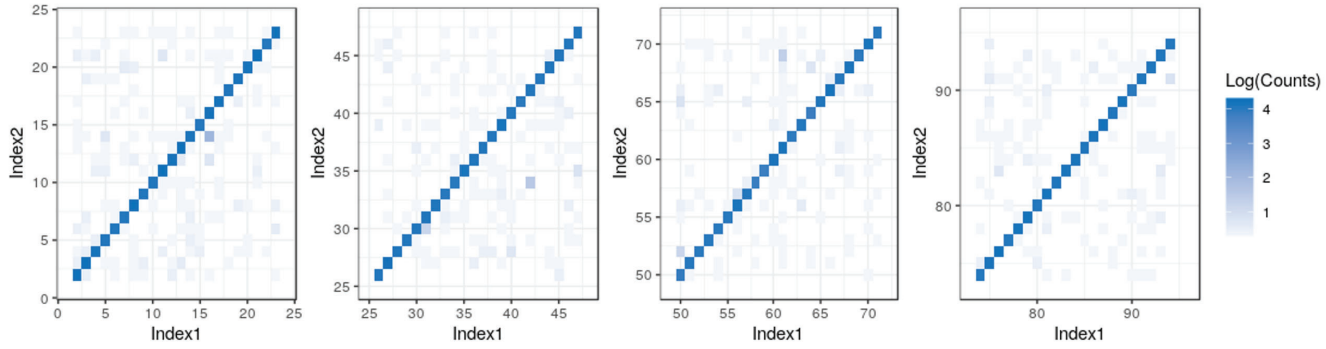

Figure 7: Counts of dual index pairs between 24 dual indices on each lane. Expected combinations are located on the diagonals.

Even though index hopping on the G4 Sequencing Platform occurs at low frequency, its impact on downstream analysis can still be minimized further. Singular Genomics uses unique dual indices and filters out unexpected dual index pairs during demultiplexing. In addition, there are laboratory best practices to prevent index misassignment:

- Make sure to completely remove free adapters after the ligation step.
- Store prepared libraries at the recommended temperature of -20° C.
- Employ good practices to prevent cross-contamination of oligos during library preparation.
- Singular Genomics provides adapters of high purity. If you plan to use custom adapters, make sure they are of the highest quality.

In conclusion, a low level of inherent index hopping, dual indices, and best practices combine to ensure that index hopping is not a substantial source of artifacts for the G4 Sequencing Platform.

SINGULAR GENOMICS

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