
DuplexSeq Assay Performance Report



2021-09-28

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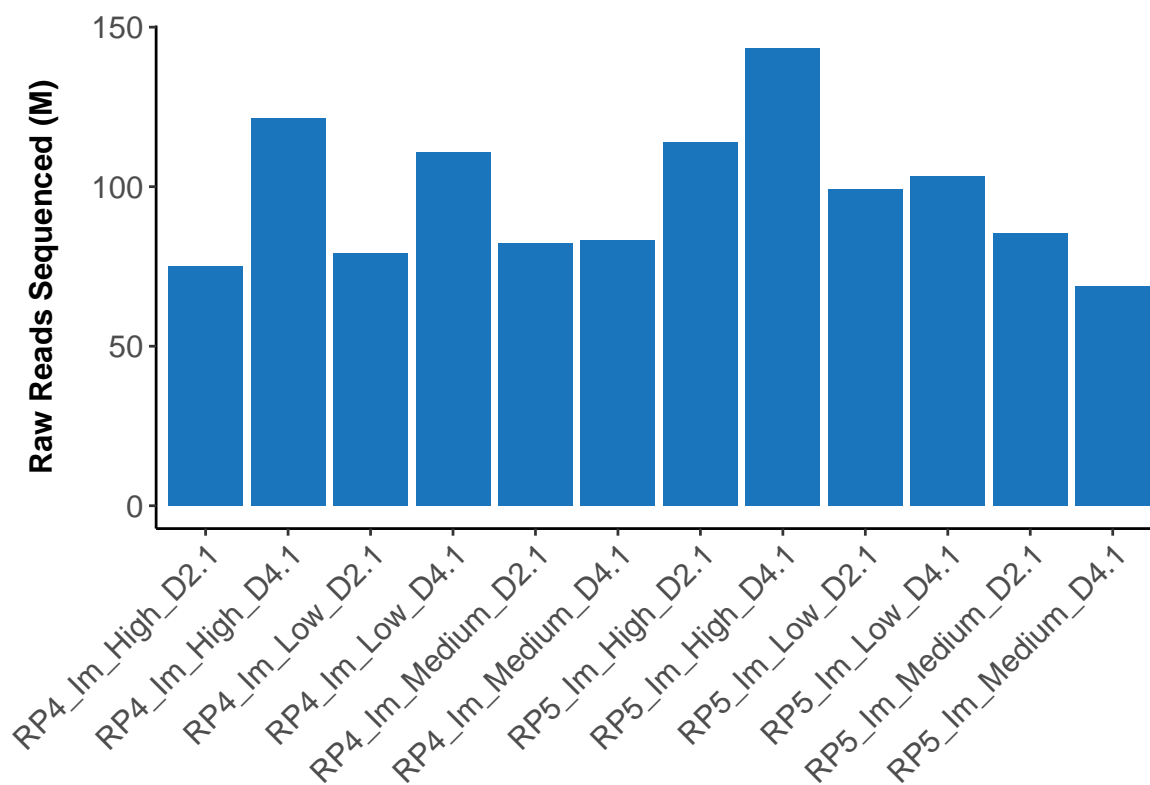
Report Overview

This PDF report is intended to provide metrics on the overall technical performance of the analyzed DuplexSeq libraries. The metrics table provides summary metrics for each analyzed library. Each library should be assessed based on the metric values observed relative to the metric values expected. Any samples deviating significantly from the group should be scrutinized to evaluate if the deviation is biological in origin or a technical outlier requiring further investigation.

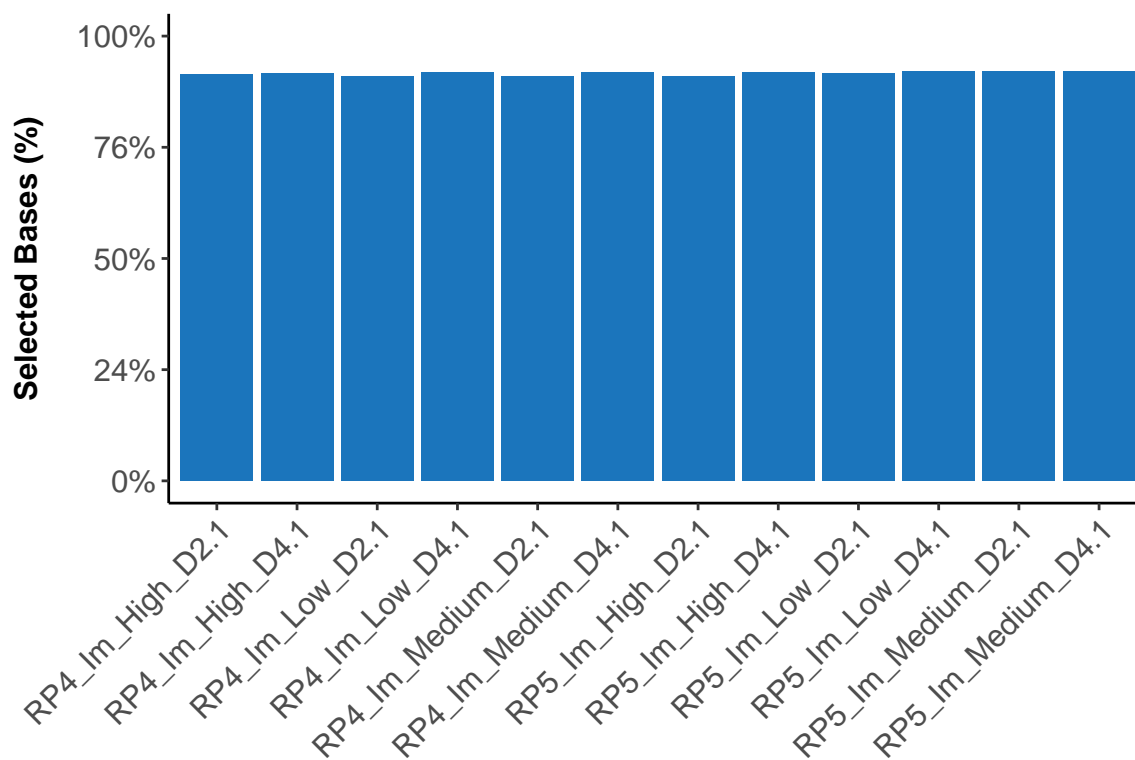
Assay Performance Metrics Table

| Library Name | Passing Filter Raw Reads | Percent Selected Bases from Raw Reads | Insert Size | | On Target Duplex Depth | | Peak Tag Family Size On-Target | Informative Duplex Bases (non-N) | Percent Ns | Average G.E. To Duplex Conversion | TwinStrand Software Version |
|--------------------|--------------------------------|--|-----------------------|--------------------------------------|--|---|--------------------------------------|---|------------|--|-----------------------------------|
| | | | Median Insert Size | Standard Deviation Insert Size | Mean On- Target Duplex Depth | Maximum On- Target Duplex Depth | | | | | |
| RP4.Im.High.D2.1 | 75,090,442 | 91.45% | 1,997 | 3,857 | 16,400 | 20,966 | 25 | 24,294,121 | 0.93% | NA | 3.11.0 |
| RP4.Im.High.D4.1 | 121,333,360 | 91.60% | 911 | 2,783 | 12,209 | 15,694 | 60 | 18,856,685 | 0.80% | NA | 3.11.0 |
| RP4.Im.Low.D2.1 | 79,266,624 | 90.95% | 2,068 | 4,018 | 6,219 | 8,132 | 2 | 9,063,661 | 0.94% | NA | 3.11.0 |
| RP4.Im.Low.D4.1 | 110,681,438 | 91.78% | 1,958 | 3,849 | 21,517 | 28,088 | 33 | 31,260,561 | 0.92% | NA | 3.11.0 |
| RP4.Im.Medium.D2.1 | 82,334,384 | 90.95% | 2,076 | 4,022 | 15,117 | 19,895 | 27 | 22,263,209 | 0.99% | NA | 3.11.0 |
| RP4.Im.Medium.D4.1 | 83,137,646 | 91.86% | 1,671 | 3,752 | 15,873 | 20,701 | 42 | 23,588,570 | 0.87% | NA | 3.11.0 |
| RP5.Im.High.D2.1 | 113,777,940 | 90.89% | 2,084 | 4,070 | 10,750 | 14,009 | 53 | 15,631,934 | 0.94% | NA | 3.11.0 |
| RP5.Im.High.D4.1 | 143,339,020 | 91.85% | 2,054 | 3,955 | 7,369 | 9,575 | 2 | 10,955,333 | 0.89% | NA | 3.11.0 |
| RP5.Im.Low.D2.1 | 99,136,880 | 91.56% | 2,022 | 3,926 | 14,464 | 18,624 | 50 | 21,642,087 | 0.78% | NA | 3.11.0 |
| RP5.Im.Low.D4.1 | 103,181,908 | 92.10% | 1,962 | 3,851 | 6,434 | 8,298 | 97 | 9,633,835 | 0.73% | NA | 3.11.0 |
| RP5.Im.Medium.D2.1 | 85,419,668 | 92.17% | 1,712 | 3,820 | 9,884 | 12,727 | 53 | 14,786,632 | 0.86% | NA | 3.11.0 |
| RP5.Im.Medium.D4.1 | 68,852,554 | 92.16% | 1,720 | 3,743 | 5,811 | 7,446 | 62 | 8,686,418 | 0.99% | NA | 3.11.0 |

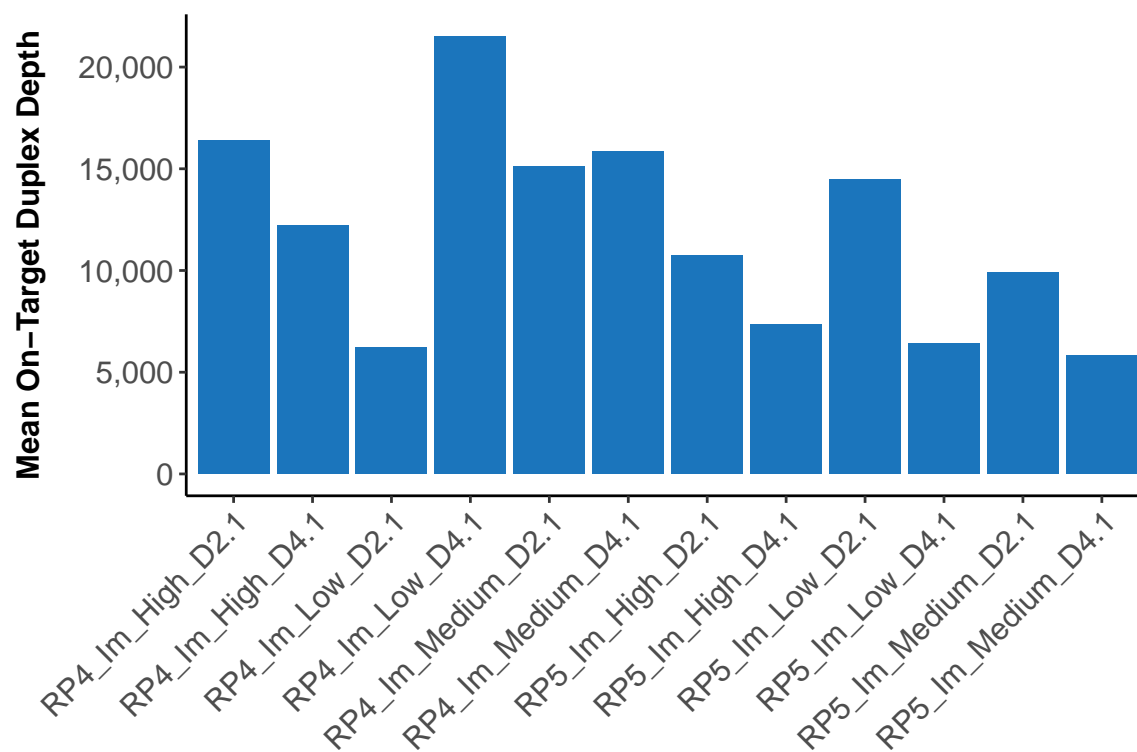
Passing Filter Raw Reads Plot



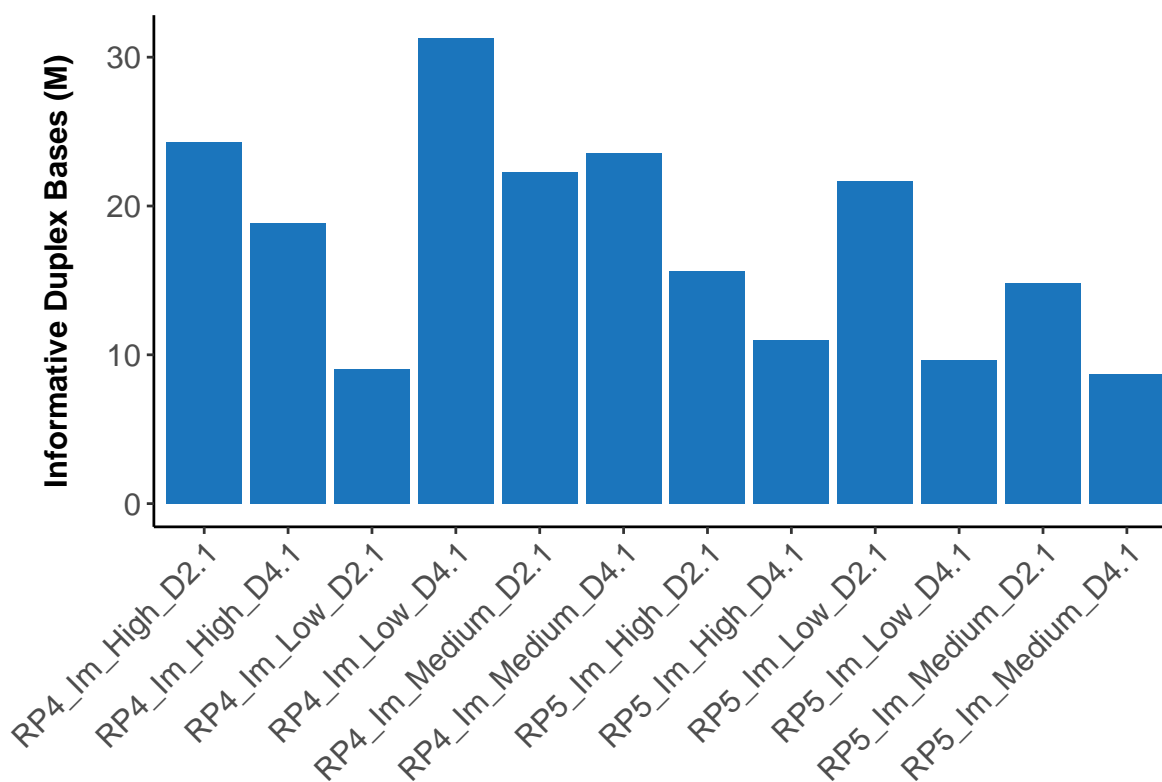
Percent Selected Bases Plot



Mean On-Target Duplex Depth Plot



Informative Duplex Bases Plot



SSCS Family Size Plots

RP4_Im_High_D2.1

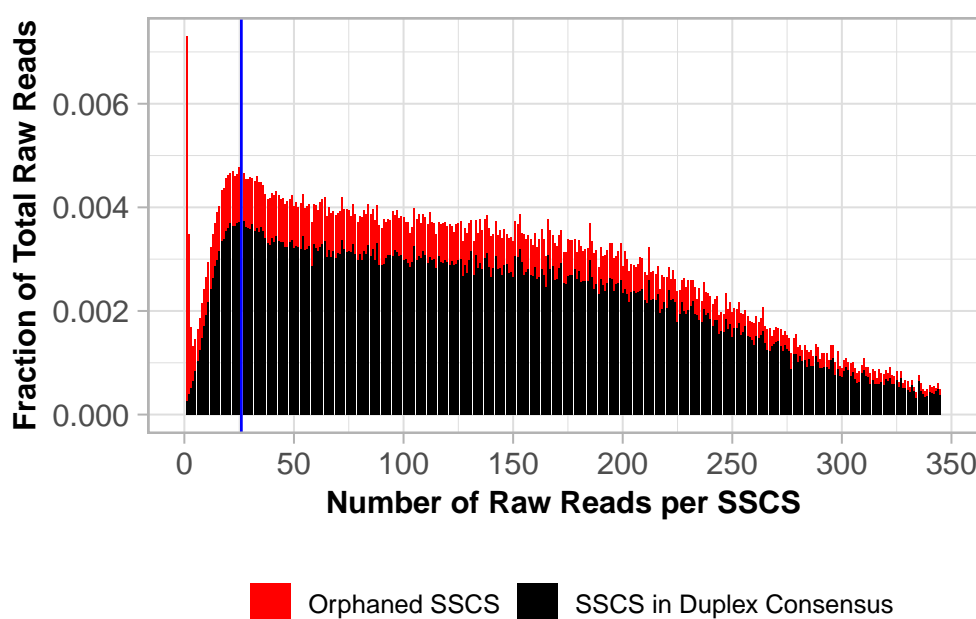
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_High_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 26 at 0.38%

Peak Orphaned SSCS Family Size: 1 at 0.70%

Omitting family sizes above 345 (2.3% of raw reads in SSCS from duplex co.



RP4_Im_High_D4.1

All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak

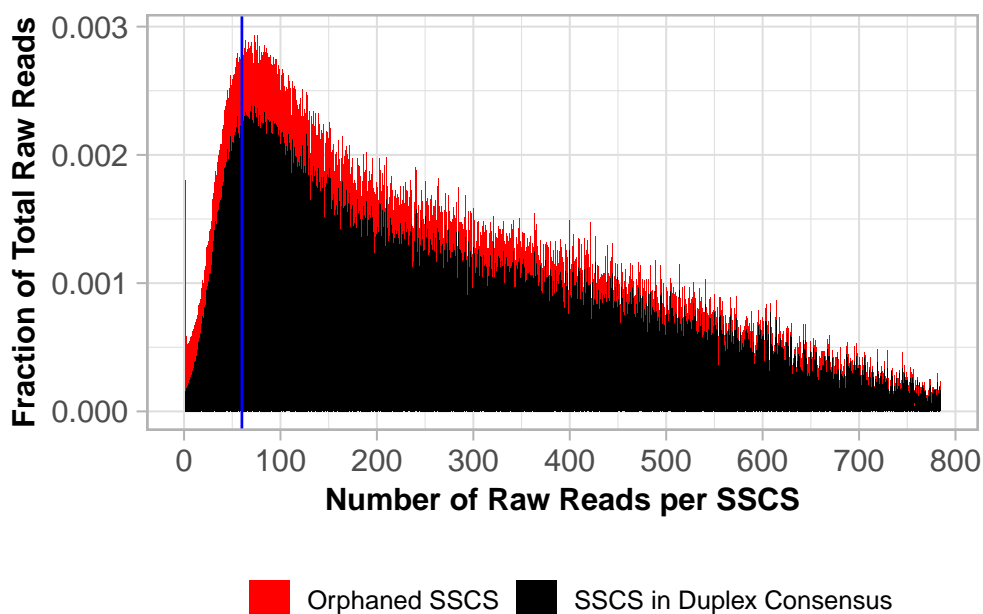
Tag Family Size On-Target”, these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_High_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 60 at 0.24%

Peak Orphaned SSCS Family Size: 1 at 0.16%

Omitting family sizes above 784 (2.3% of raw reads in SSCS from duplex co.



RP4_Im_Low_D2.1

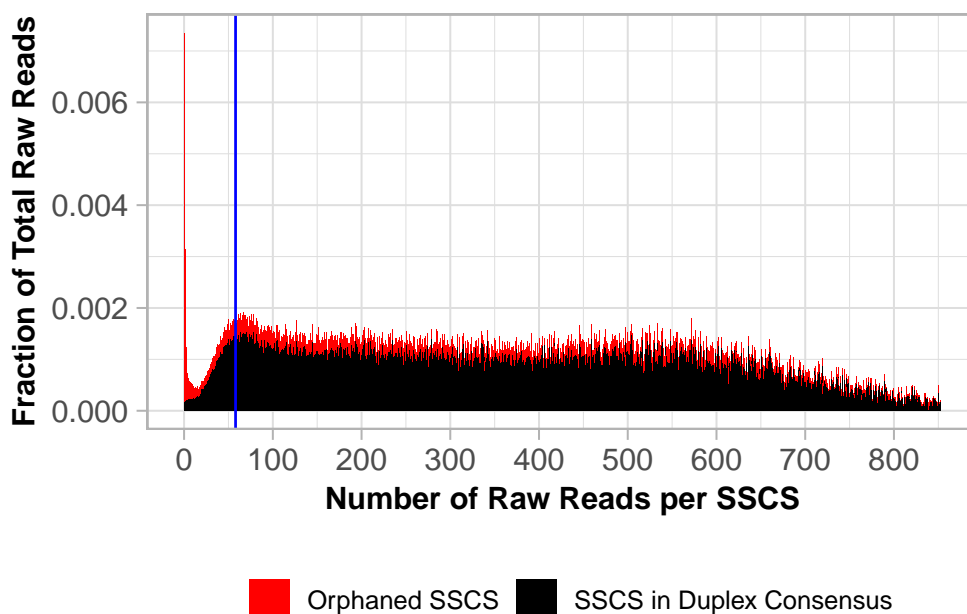
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric “Peak Tag Family Size On-Target”, these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_Low_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 58 at 0.16%

Peak Orphaned SSCS Family Size: 1 at 0.72%

Omitting family sizes above 852 (2.3% of raw reads in SSCS from duplex co.

**RP4_Im_Low_D4.1**

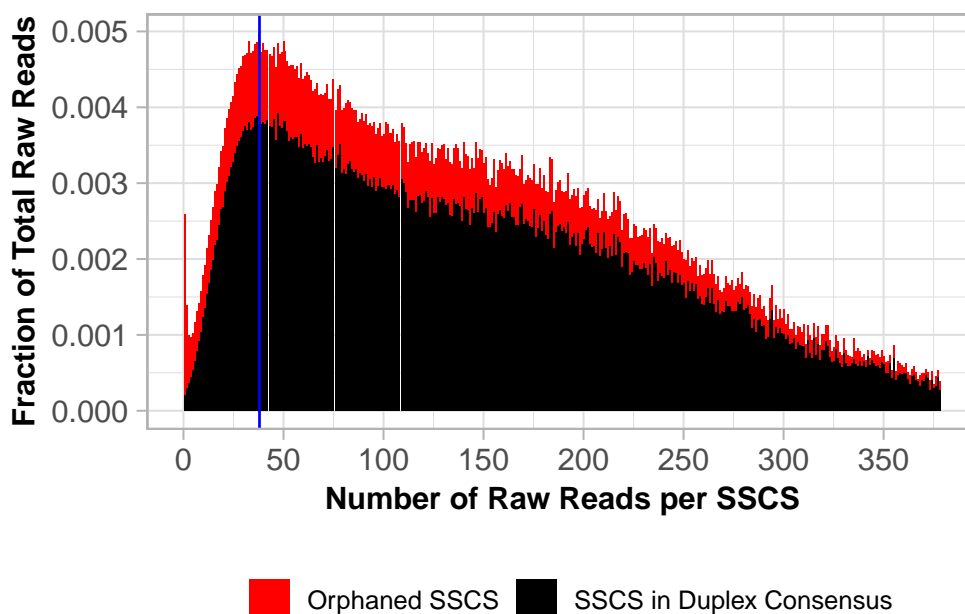
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_Low_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 38 at 0.40%

Peak Orphaned SSCS Family Size: 1 at 0.24%

Omitting family sizes above 378 (2.3% of raw reads in SSCS from duplex co.

**RP4_Im_Medium_D2.1**

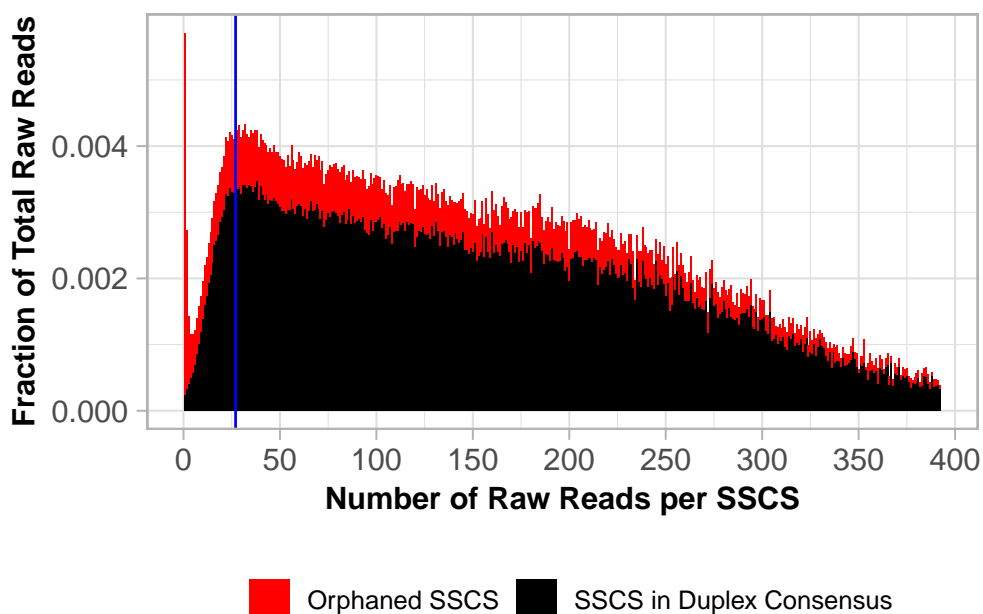
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_Medium_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 27 at 0.35%

Peak Orphaned SSCS Family Size: 1 at 0.55%

Omitting family sizes above 392 (2.3% of raw reads in SSCS from duplex co.

**RP4_Im_Medium_D4.1**

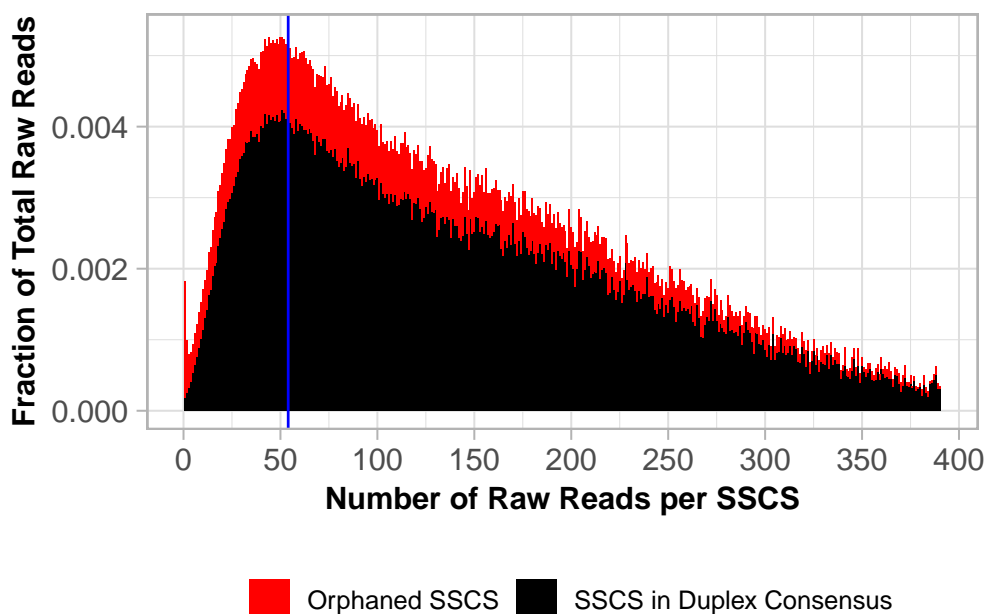
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP4_Im_Medium_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 54 at 0.43%

Peak Orphaned SSCS Family Size: 1 at 0.16%

Omitting family sizes above 390 (2.3% of raw reads in SSCS from duplex co.

**RP5_Im_High_D2.1**

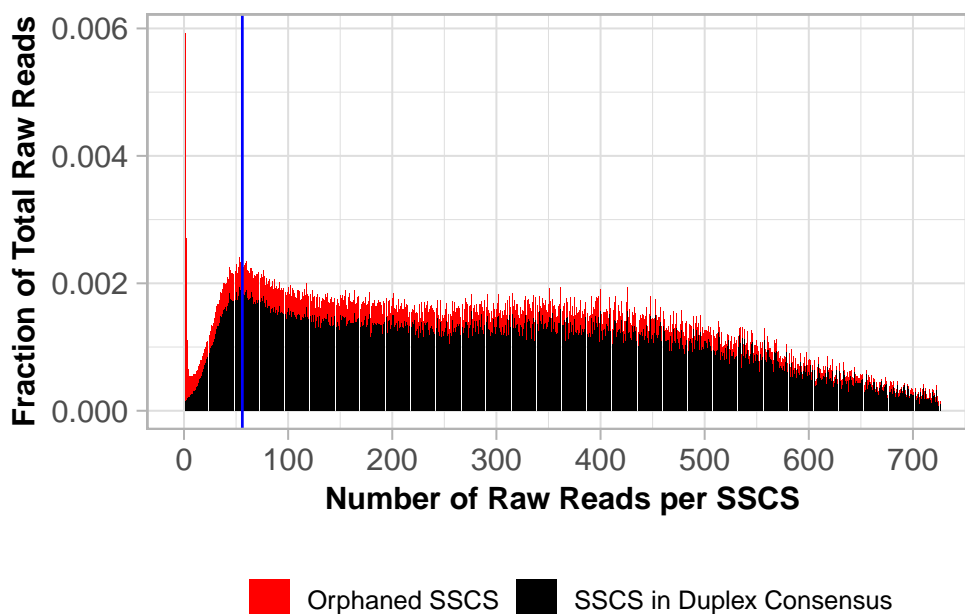
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_High_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 56 at 0.20%

Peak Orphaned SSCS Family Size: 1 at 0.58%

Omitting family sizes above 726 (2.3% of raw reads in SSCS from duplex co.

**RP5_Im_High_D4.1**

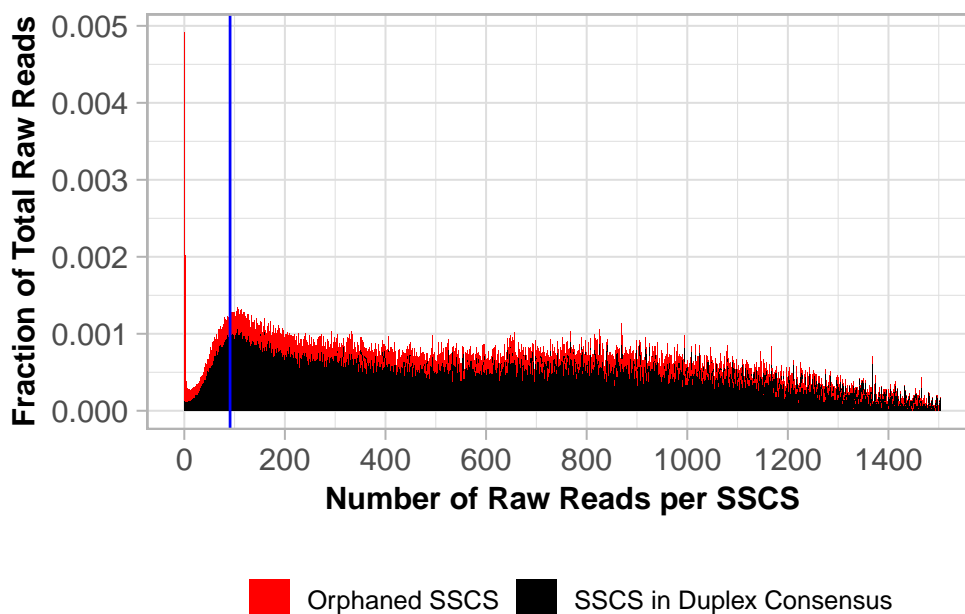
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_High_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 91 at 0.11%

Peak Orphaned SSCS Family Size: 1 at 0.48%

Omitting family sizes above 1503 (2.3% of raw reads in SSCS from duplex c

**RP5_Im_Low_D2.1**

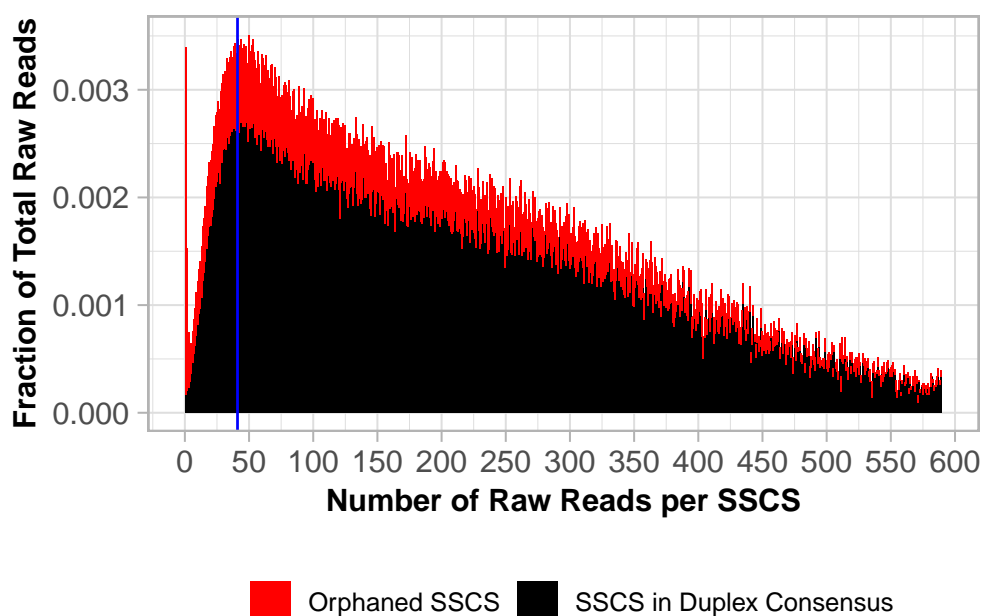
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_Low_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 41 at 0.28%

Peak Orphaned SSCS Family Size: 1 at 0.32%

Omitting family sizes above 589 (2.3% of raw reads in SSCS from duplex co.



RP5_Im_Low_D4.1

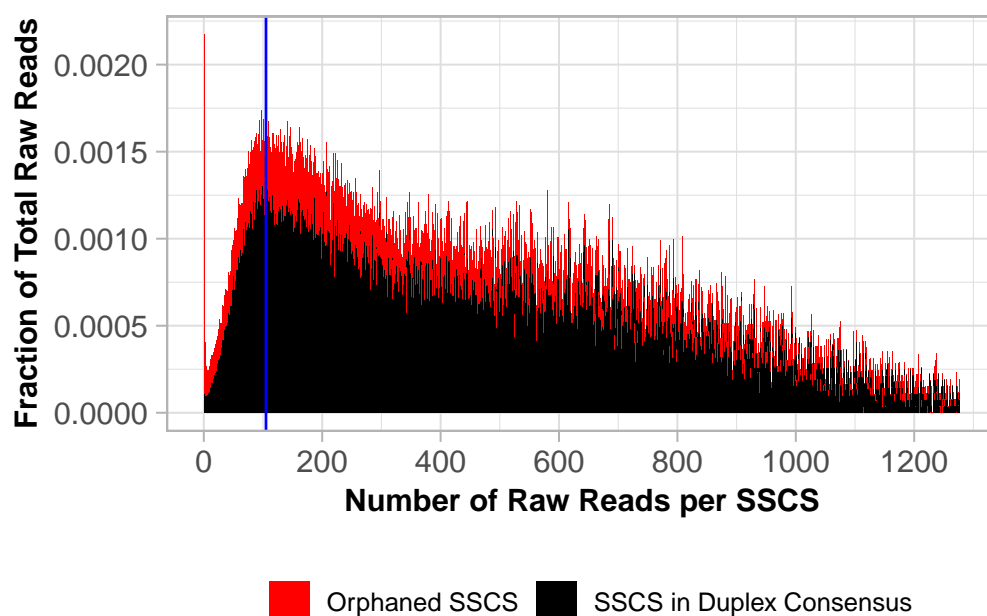
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_Low_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 105 at 0.14%

Peak Orphaned SSCS Family Size: 1 at 0.21%

Omitting family sizes above 1277 (2.3% of raw reads in SSCS from duplex c



RP5_Im_Medium_D2.1

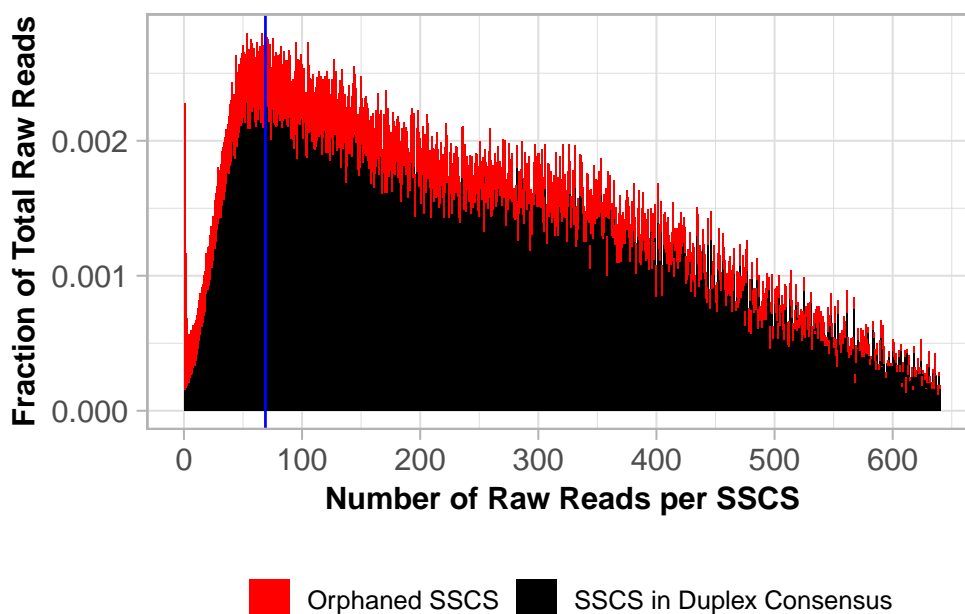
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_Medium_D2.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 69 at 0.23%

Peak Orphaned SSCS Family Size: 1 at 0.21%

Omitting family sizes above 640 (2.3% of raw reads in SSCS from duplex co.

**RP5_Im_Medium_D4.1**

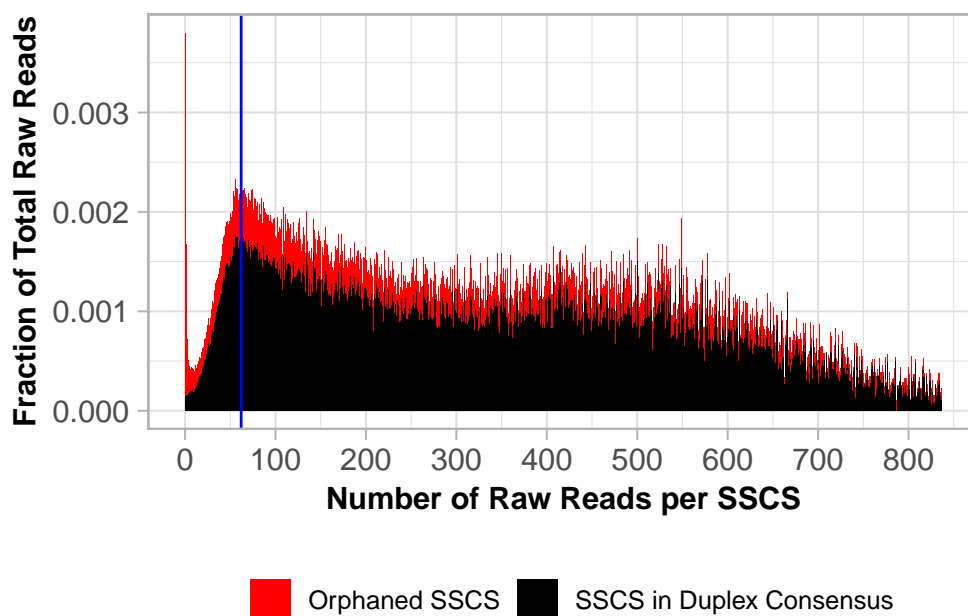
All single strand consensus sequences (SSCS) are tabulated for every library in the plots that follow. The proportion of raw reads that contribute to SSCS of various sizes is visualized for both SSCS's that are orphaned and for SSCS's that are members of a complete duplex consensus. The peak of both distributions is explained in the plot subtitles. Unlike the assay performance summary metric "Peak Tag Family Size On-Target", these plots are derived from all reads from the library regardless of their mapping coordinates.

RP5_Im_Medium_D4.1 SSCS Family Sizes

Peak SSCS in Duplex Consensus Family Size: 62 at 0.18%

Peak Orphaned SSCS Family Size: 1 at 0.36%

Omitting family sizes above 836 (2.3% of raw reads in SSCS from duplex co.



Metrics Definitions

| | Definition |
|---------------------------------------|--|
| Library Name | Name of the sample, inferred from the file name. |
| Passing Filter Raw Reads | The total number of passing-filter sequencing reads that were assigned to the sample index. |
| Percent Selected Bases from Raw Reads | The fraction of passing-filter aligned bases that are located on or near the baited region. |
| Median Insert Size | The median length of all inserts that are sequenced based on the alignment of the paired-end reads. |
| Standard Deviation Insert Size | The standard deviation of all inserts that are sequenced based on the alignment of the paired-end reads. |
| Mean On-Target Duplex Depth | The mean coverage of the target region in the Duplex Consensus alignment file. |
| Maximum On-Target Duplex Depth | The maximum coverage of the target region in the Duplex Consensus alignment file |
| Peak Tag Family Size On-Target | The most frequent raw read family size across all single strand consensus that is greater than one. If there are multiple "most frequent" family sizes then the smallest family is reported. |
| Informative Duplex Bases (non-N) | The number of Duplex Consensus bases examined which is exclusive of any No-calls (ambiguous bases). |
| Percent Ns | The percent of No-calls (ambiguous bases) as determined from the total pool of filtered Duplex Consensus bases. |
| Average G.E. To Duplex Conversion | The percentage genome equivalents that are successfully converted into Duplex Consensus. |
| TwinStrand Software Version | The version of the TwinStrand DuplexSeq Software that was used to generate variant calls. |

Contact Information

TwinStrand Biosciences, Inc.

twinstrandbio.com

support@twinstrandbio.com

3131 Elliott Ave, Suite 750 • Seattle, WA 98121 • USA

(206) 202-TWIN

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