# NucBreak: Location of structural errors in a genome assembly by using paired-end Illumina reads

# *Supplementary materials*

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## **Supplementary methods and results**

#### **1. Fragment size estimation**

Only read pairs satisfying the following conditions are used for fragment size estimation:

- 1. Each read in a pair is uniquely aligned
- 2. Both reads are mapped to the same genome sequence
- 3. The reads have different orientations relative to the genome sequence
- 4. The read with the reverse orientation is located at the same position or further down on the sequence compared to the mapping locations of the forward-oriented read
- 5. The forward- and reverse-oriented reads are not soft-clipped at both sides. However, the alignments of properly mapped reads may contain short substitutions, insertions and deletions.

The fragment size is calculated by the formula:

 $frag\_size = ref\_end_2 - ref\_st_1 + 1$ , where

 $ref\_end_2$  - the location of reverse-oriented read end at the genome chromosome  $ref\_st_1$  - the location of forward-oriented read start at the genome chromosome

The fragment sizes are sorted in ascending order, and for each fragment size the number of read pairs  $(HP)$  having the given fragment size is calculated. Then  $min$  frag size and max\_frag\_size are found:

$$
min\_frag\_size = \{frag\_size_i : #P(frag\_size_i) \ge 10 \text{ and } \forall j < i \text{ #P}(frag\_size_j) < 10
$$
\n
$$
and \exists k = i + 1 \dots i + 10 \text{ #P}(frag\_size_k) \ge 10 \text{ and } \exists k \ge 3 \}
$$

$$
max\_frag\_size = \{frag\_size_i : #P(frag\_size_i) \ge 10 \text{ and } \forall j > i \#P(frag\_size_j) < 10
$$
\n
$$
and \exists k = i - 11 \dots i - 1 \#P(frag\_size_k) \ge 10 \text{ and } \exists k \ge 3\}
$$

If the number of corresponding read pairs is less than 10 for any fragment size, then:

 $min\_frag\_size = \{max(0, frag\_size_i - 50): \forall k \neq i \#P(frag\_size_i) \geq \#P(frag\_size_k)\}$  $max\_frag\_size = {frag\_size_i + 50: \forall k \neq i #P(frag\_size_i) \geq #P(frag\_size_k)}$ 

#### **2. Fragment size detection between properly mapped read pairs**

Since the reads from properly mapped reads pairs may be soft-clipped in the start or at the end of the read depending on the read orientation, a fragment size inside properly mapped reads is calculated by the extended formula:

 $frag\_size = ref\_end_2 + end\_clipped\_dist_2 - ref\_st_1 - start\_clipped\_dist_1 + 1$ , where

 $ref\_end_2$  - the location of the reverse-oriented read end at the genome chromosome  $end\_clipped\_dist_2$  - the number of soft-clipped bases at the end of the reverse-oriented read  $ref\_st_1$  - the location of the forward-oriented read start at the genome chromosome start clipped dist<sub>1</sub>- the number of soft-clipped bases in the beginning of the reverse-oriented read

#### **3. The Velvet, ABySS and SPAdes parameter settings used to obtain assemblies**

SPAdes was run with the "-t 2 -k 33 --cov-cutoff 2" parameter settings.

ABySS was run with "k=64" parameter setting.

Velveth was run with k-mer length equal to 31.

Velvetg was run with "-ins length 180 -scaffolding yes -min\_contig\_lgth 250 -cov\_cutoff 5" parameter settings.

## **4. The NucBreak, REAPR and FRCbam parameter settings used to detect assembly errors**

In the Sections 3.1 and 3.2, we used the following parameter settings for the tools:

- NucBreak was run with "--min frag\_size 620 --max\_frag\_size 790" parameter settings
- In case of REAPR, perfectmap was run with 700 bp average insert size
- FRCbam was run with "--pe-max-insert 776" and the value for "--genome-size" parameter was detected automatically by using python script for each modification case.

In the Section 3.3, we used the following parameter settings for the tools:

- in case of REAPR, perfectmap was run with 300 bp average insert size
- FRCbam was run with "--pe-max-insert 776 --genome-size 112000000" parameter settings

In the Section 3.4, we used the following parameter settings for the tools:

- In case of REAPR, perfectmap was run with the following average insert sizes depending on the genome dataset used:
	- Salmonella dataset 500 bp
	- Staphylococcus dataset 400 bp
	- Escherichia dataset 300 bp
	- Pseudomonas dataset 180 bp
	- Bordetella dataset 450 bp
	- Brucella dataset 500 bp
	- Klebsiella dataset 200 bp
	- Enterobacter dataset 300 bp
- FRCbam was run with the following parameter settings depending on the genome dataset used:
	- Salmonella dataset "--pe-max-insert 1060 --genome-size 4810000"
	- Staphylococcus dataset "--pe-max-insert 1040 --genome-size 2860000"
	- Escherichia dataset "--pe-max-insert 1110 --genome-size 5480000"
	- Pseudomonas dataset "--pe-max-insert 844 --genome-size 6820000"
	- Bordetella dataset "--pe-max-insert 890 --genome-size 4110000"
	- Brucella dataset "--pe-max-insert 1120 --genome-size 3300000"
	- Klebsiella dataset "--pe-max-insert 950 --genome-size 5720000"
	- Enterobacter dataset "--pe-max-insert 819 --genome-size 5040000"

#### **5. Result evaluation**

The ground truth entries may be represented as dots (e.g. in case of deletions, simple relocations or translocations) or as intervals (e.g. in case of insertion, duplications, relocations with overlap). If a ground truth entry is an interval, it may be fully covered with reads mapped back to the query sequences (e.g. in case of inversions) or remain uncovered (e.g. in case of inserted regions that are not present in the reference genome). In the first case, a tested tool is expected to mark the regions corresponding to the start- and/or end-points of the ground truth entry as breakpoints, while in the second case the whole entry is expected to be predicted as a breakpoint.

We say that if a ground truth entry coincides with an obtained breakpoint or the ground truth entry start- and/or end-points coincide with obtained breakpoints, then we have a true positive (TP). If a ground truth entry does not coincide with any of obtained breakpoints, then we have a false negative (FN). To get TPs and FNs, we have run BEDTools with the pairtopair -both' option. With this option, BEDTool reports an overlap between two intervals A and B if both ends of A overlap B. If BEDTool reports an overlap for a whole ground truth entry or for its start- and/or end-points, then we get a TP, otherwise a FN. Having obtained the number of TPs and FNs, we calculate sensitivity by the formula:

$$
Sensitivity = \frac{\#TP}{\#TP + \#FN}
$$

Unlike ground truth entries, an obtained result can correspond only to one interval: either to a whole ground truth entry or to its start- or end-point. If an obtained breakpoint does not coincide with any of the ground truth entries and with any of the ground truth entry start- and end-points, then the given obtained breakpoint is a false positive (FP). To get FP, we have run BEDTools with the ''pairtopair -notboth' option. With this option, BEDTool reports an overlap between two intervals A and B, if one or neither of A's ends overlap B. If BEDTool reports an overlap for an obtained breakpoint with a whole ground truth entry or with its ends, then we get a FP. Having obtained the number of FPs, we calculate FDR by the formula:

$$
FDR = \frac{FP}{FP + TP}
$$

## **Supplementary figures**



**Figure S2** Properly mapped read pair formation. The black line represents an assembly. The arrows represent all possible read mapping locations. The cases a) and b) correspond to the situations when no read pairs are formed or just one read pair is formed, respectively. The cases c) and d) show examples when several read pairs are formed from two given reads. The case d) is an example of the situation when reads are mapped to a tandem repeat.



**Figure S3** Properly mapped read pair categorization. The black line represents an assembly. The assembly regions marked by red colour correspond to repeated regions. The repeated regions are identical or near-identical copies of the same repeat. The arrows represent all possible read mapping locations.



**Figure S4** Read paths and path gaps. The black line represents an assembly. The assembly regions marked by red colour correspond to repeated regions. The repeated regions are identical or near-identical copies of the same repeat or copies of different repeats. The arrows represent read paths. The arrows of the same colour correspond to the read paths of the same type. The rectangles between the read paths indicate path gaps. The example demonstrates the correct order of the read paths in the absence of assembly errors.

## Single path



## Multiple path



## Single\_Multiple path



## Multiple\_Single path



**Figure S5** Possible type order and locations of read paths in the absence of breakpoints.



**Figure S6** Sensitivity results for the insertion, duplication and tandem duplication groups, obtained using the simulated datasets.



**Figure S7** Sensitivity results for the deletion, deletion repeat and deletion tandem groups, obtained using the simulated datasets. The deletion\_repeat group contains deletions of interspersed repeats or their parts. The deletion tandem group contains deletions of tandem repeats or their parts.



**Figure S8** Sensitivity results for the inversion, relocation and relocation overlap groups, obtained using the simulated datasets. The relocation group consists of relocations with either inserted regions between misjoined regions (size varied between 10 and 1000) or without them (size is equal to 0). The relocation\_overlap group consists of relocations with overlapped misjoined regions.



**Figure S9** Sensitivity results for the insertion, duplication and tandem duplication groups, obtained using the simulated datasets.



Figure S10 Sensitivity results for the deletion, deletion\_repeat and deletion\_tandem groups, obtained using the simulated datasets. The deletion repeat group contains deletions of interspersed repeats or their parts. The deletion tandem group contains deletions of tandem repeats or their parts.



Figure S11 Sensitivity results for the inversion, relocation and relocation overlap groups, obtained using the simulated datasets. The relocation group consists of relocations with either inserted regions between misjoined regions (size varied between 10 and 1000) or without them (size is equal to 0). The relocation\_overlap group consists of relocations with overlapped misjoined regions.



**Figure S12** Sensitivity results for the insertion, duplication and tandem duplication groups, obtained using the datasets from the Assemblathon 1 project.



Figure S13 Sensitivity results for the deletion, deletion\_repeat and deletion\_tandem groups, obtained using the datasets from the Assemblathon 1 project. The deletion repeat group contains deletions of interspersed repeats or their parts. The deletion\_tandem group contains deletions of tandem repeats or their parts.



Figure S14 Sensitivity results for the inversion, rearrangement and rearrangement\_overlap groups, obtained using the datasets from the Assemblathon 1 project.The rearrangement group consists of relocations and translocations with either inserted regions between misjoined regions (size varied between 1 and 1000) or without them (size is equal to 0). The rearrangement\_overlap group consists of relocations and translocations with overlapped misjoined regions.



**Figure S15** Sensitivity results for the reshuffling and substitution groups, obtained using the datasets from the Assemblathon 1 project.



**Figure S16** Sensitivity results for the insertion, duplication and tandem duplication groups obtained using the bacterial genome datasets.



Figure S17 Sensitivity results for the deletion, deletion\_repeat and deletion\_tandem groups, obtained using the bacterial genome datasets. The deletion\_repeat group contains deletions of interspersed repeats or their parts. The deletion\_tandem group contains deletions of tandem repeats or their parts.



**Figure S18** Sensitivity results for the inversion, rearrangement and rearrangement\_overlap groups, obtained using the bacterial genome datasets. The rearrangement group consists of relocations and translocations with either inserted regions between misjoined regions (size varied between 1 and 1000) or without them (size is equal to 0). The rearrangement overlap group consists of relocations and translocations with overlapped misjoined regions.



**Figure S19** Sensitivity results for the reshuffling and substitution groups, obtained using the bacterial genome datasets.

## **Supplementary tables**

**Table S1** Genome modifications implemented during the simulation process. G and A denote a reference genome and assembly, respectively. All other letters denote reference genome and assembly sequence regions. Diff means difference. C' is the reverse complement of C.



- 13. G: CxCxC A: CxCCxC Diff: tandem\_duplication
- 14. G: RxRxRxTKTKTKTK A: RxTRxRxTKTKTKTK Diff: duplication
- 15. G: RxRxRxTKTKTKTK A: RxKRxRxTKTKTKTK Diff: duplication
- 16. G: RxRxRxTKTKTKTK A: RxTKRxRxTKTKTKTK Diff: duplication
- 17. G: RxRxRxTKTKTKTK A: RxKTRxRxTKTKTKTK Diff: duplication
- 18. G: RxRxRxCCCC A: RxCCCCRxRxCCCC Diff: duplication
- 19. G: RxRxRxTKxTKxTK A: RxTRxRxTKxTKxTK Diff: duplication
- 20. G: RxRxRxTKxTKxTK A: RxKRxRxTKxTKxTK Diff: duplication
- 21. G: RxRxRxCxCxC A: RxCRxRxCxCxC Diff: duplication
- 22. G: RxRxR A: RxRCxR Diff: insertion
- 23. G: RxRxRxC A: RxRCxRxC Diff: duplication
- 24. G: TKxTKxTK A: TKxTKTxTK Diff: duplication
- 25. G: TKxTKxTK A: TKxTKKxTK Diff: tandem\_duplication
- 26. G: RxRxRxTKTKTKTK A: RxRTxRxTKTKTKTK Diff: duplication
- 27. G: RxRxRxTKTKTKTK A: RxRKxRxTKTKTKTK Diff: duplication
- 58. G: DPPPPxCCCC A: DCCCCPPPPxCCCC Diff: duplication
- 59. G: LLLLD A: LLLLCD Diff: insertion
- 60. G: LLLLDxC A: LLLLCDxC Diff: duplication
- 61. G: LLLLDxTKxTKxTK A: LLLLTDxTKxTKxTK Diff: duplication
- 62. G: LLLLDxTKxTKxTK A: LLLLKDxTKxTKxTK Diff: duplication
- 63. G: LLLLDxCxCxC A: LLLLCDxCxCxC Diff: duplication
- 64. G: TKTKTKTKD A: TKTKTKTKTD Diff: duplication
- 65. G: TKTKTKTKD A: TKTKTKTKKD Diff: tandem\_duplication
- 66. G: TKTKTKTKD A: TKTKTKTKKTD Diff: duplication
- 67. G: PPPPDxTKTKTKTK A: PPPPTDxTKTKTKTK Diff: duplication
- 68. G: PPPPDxTKTKTKTK A: PPPPKDxTKTKTKTK Diff: duplication
- 69. G: PPPPDxCCCC A: PPPPCDxCCCC Diff: duplication
- 70. G: PPPPDxTKTKTKTK A: PPPPKTDxTKTKTKTK Diff: duplication
- 71. G: PPPPDxCCCC A: PPPPCCCCDxCCCC Diff: duplication
- 72. G: PPPP A: PPCPP Diff: insertion
- 28. G: RxRxRxTKTKTKTK A: RxRTKxRxTKTKTKTK Diff: duplication
- 29. G: RxRxRxTKTKTKTK A: RxRDTxRxTKTKTKTK Diff: duplication
- 30. G: RxRxRxCCCC A: RxRCCCCxRxCCCC Diff: duplication
- 31. G: RxRxRxTKxTKxTK A: RxRTxRxTKxTKxTK Diff: duplication
- 32. G: RxRxRxTKxTKxTK A: RxRKxRxTKxTKxTK Diff: duplication
- 33. G: RxRxRxCxCxC A: RxRCxRxCxCxC Diff: duplication
- 34. G: RDxRDxRD A: RDxRCDxRD Diff: insertion
- 35. G: RDxRDxRDxC A: RDxRCDxRDxC Diff: duplication
- 36. G: TKxTKxTK A: TKxTKKxTK Diff: tandem\_duplication
- 37. G: RDxRDxRDxTKTKTKTK A: RDxRTDxRDxTKTKTKTK Diff: duplication
- 38. G: RDxRDxRDxTKTKTKTK A: RDxRKDxRDxTKTKTKTK Diff: duplication
- 39. G: RDxRDxRDxCCCC A: RDxRCDxRDxCCCC Diff: duplication
- 40. G: RDxRDxRDxTKTKTKTK A: RDxRKTDxRDxTKTKTKTK Diff: duplication
- 41. G: RDxRDxRDxCCCC A: RDxRCCCCDxRDxCCCC Diff: duplication
- 42. G: RDxRDxRDxTKxTKxTK A: RDxRTDxRDxTKxTKxTK Diff: duplication
- 73. G: PPPPxC A: PPCPPxC Diff: duplication
- 74. G: PPPPxTKxTKxTK A: PPTPPxTKxTKxTK Diff: duplication
- 75. G: PPPPxTKxTKxTK A: PPKPPxTKxTKxTK Diff: duplication
- 76. G: PPPPxCxCxC A: PPCPPxCxCxC Diff: duplication
- 77. G: TKTKTKTK A: TKTKKTKTK Diff: tandem\_duplication
- 78. G: PPPPxTKTKTK A: PPTPPxTKTKTK Diff: duplication
- 79. G: PPPPxTKTKTK A: PPKPPxTKTKTK Diff: duplication
- 80. G: PPPPxCCC A: PPCPPxCCC Diff: duplication
- 81. G: PPPPxTKTKTK A: PPKTPPxTKTKTK Diff: duplication
- 82. G: PPPPxCCC A: PPCCCPPxCCC Diff: duplication
- 83. G: DKL A: DKTKL Diff: insertion
- 84. G: DKLxT A: DKTKLxT Diff: insertion
- 85. G: LxKLxLxT A: LxKTKLxLxT Diff: insertion
- 86. G: KLxKLxKLxT A: KLxKTKLxKLxT Diff: insertion
- 87. G: LKxLKxLKxT A: LKxLKTKxLKxT Diff: insertion





In the simulated modifications, the following lengths of regions were used:

- 1. Distance between each manipulation case =2500 bp
- 2. len(H)=800 bp
- 3. len(B)=800 bp
- 4. len(x)=800 bp
- 5. len(C)={17,30,100,250,800} bp

6. len(TK)={[50,70],[100,150],[250,250],[600,600]} bp, where first number in a pair is len(T) and second number in a pair is len(K)

- 7. len(R)=600 bp
- 8. len(D)=600 bp
- 9. len(L)=200 bp
- 10. len(P)=400 bp
- 11. len(F)=len(T) in len(TK)
- 12. len(S)=1500 bp
- 13. len(V)=1500 bp
- 14. len(Z)=15000 bp



**Table S2** List of bacterial genomes.

**Table S3** Number of ground truth errors in each group.





