Supplementary file 1.The detail information for 25 aligners collected from different websites and published articles

Software	OS	Language	Reference	Links
BOAT	Linux/Unix 32/64bit	C	PMID: 19958483	http://boat.cbi.pku.edu.cn
Bowtie	Linux/Unix 32/64bit Windows Mac OS X/Solaris	C++	PMID:19261174	http://bowtie-bio.sourceforge.net/index.shtml
BS Seeker	Linux/Unix 32/64bit Mac OS X 32/64bit	Python	PMID:20416082	http://sourceforge.net/projects/bsseeker/files/BS%20Seeker2004-23-2010/examples.tgz/download
BWA	Linux/Unix 32/64bit	C++	PMID:19451168	http://bio-bwa.sourceforge.net/
GASSST	Linux/Unix 32/64bit	C++	PMID: 2073910	http://www.irisa.fr/symbiose/projects/gassst/
GenomeMapper	Linux/Unix 32/64bit	C	PMID:19761611	http://1001genomes.org/downloads/genomemapper.html
GSNAP	Linux/Unix 32/64bit	C	PMID: 20147302	http://research-pub.gene.com/gmap/
GNUMAP	Linux/Unix 32/64bit	C	PMID:19861355	http://dna.cs.byu.edu/gnumap/download.cgi

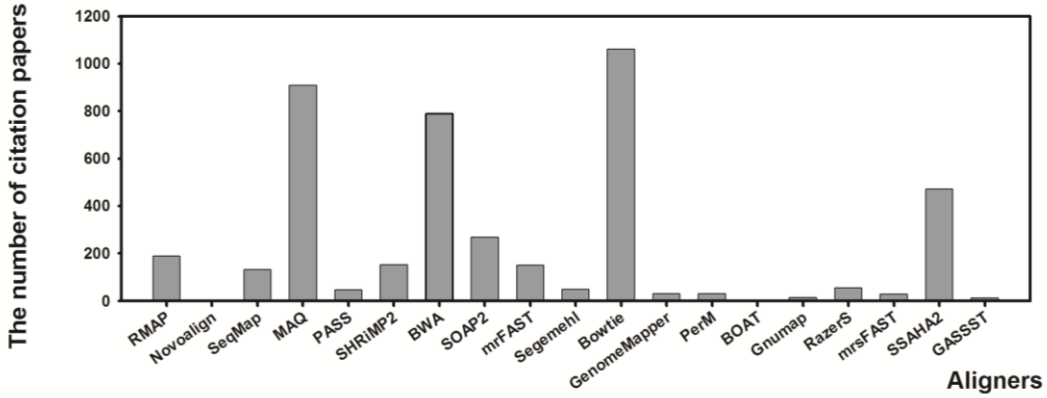
Supplementary file 2. The version list of the aligners involved in the evaluation process

Software	Version	websites
Bowtie	bowtie-v0.12.7	http://bowtie-bio.sourceforge.net/index.shtml
SOAPv2	SOAPaligner-v2.21	http://soap.genomics.org.cn/
BWA	bwa-0.5.8a	http://bio-bwa.sourceforge.net/
PerM	PerM-v0.3.6	http://code.google.com/p/perm/
mrsFAST	mrsfast-v2.3.0.2	http://sourceforge.net/projects/mrfast/files/mrsfast/2.3.0.2/
GASSST	Gassst-v1.28	http://www.irisa.fr/symbiose/projects/gassst/
PASS	pass-v1.62	http://pass.cribi.unipd.it/cgi-bin/pass.pl
BOAT	boat-v1.0	http://www.seqan.de/downloads/projects.html#c13
RazerS	RazerS-20100618	http://bowtie-bio.sourceforge.net/index.shtml
RMAP	rmap-v2.05	http://rulai.cshl.edu/rmap/
MAQ	maq-0.7.1	http://maq.sourceforge.net/
Novoalign	Novocraft-v2.05	http://www.novocraft.com/
mrFAST	mrFast-v2.1.0.1	http://mrfast.sourceforge.net/
SeqMap	SeqMap-v 1.0.12	http://biogibbs.stanford.edu/~jiangh/seqmap/
Segemehl	Segemehl-v0.1.2	http://www.bioinf.uni-leipzig.de/Software/segemehl/
SHRiMAP	SHRiMP-v2.2.1	http://compbio.cs.toronto.edu/shrimp/
Gnumap	Gnumap-v3.0.2	http://dna.cs.byu.edu/gnumap/
GenomeMapper	genomemapper0.4.3	http://1001genomes.org/downloads/genomemapper.html
SSAHA2	ssaha2_v2.5.3	http://www.sanger.ac.uk/resources/software/ssaha2/

MAQ	Linux/Unix 32/64bit	C++	PMID:18714091	http://maq.sourceforge.net/
MOM	Windows 32/64bit Linux/Unix 32/64bit	Java	PMID: 19228804	http://mom.csbc.vcu.edu/
Mosaik	Windows 32/64bit Linux/Unix 32/64bit	C++	N/A	http://bioinformatics.bc.edu/marthlab/Mosaik
mrFAST & mrsFAST	Windows 32/64bit Linux/Unix 32/64bit	C	PMID: 19718026 PMID: 19447966 PMID: 19483690	http://mrfast.sourceforge.net/
MUMmer	Linux/Unix 32/64bit	C++	PMID: 14759262	http://mummer.sourceforge.net/
Novoalign	Linux/Unix 32/64bit	C++	N/A	http://www.novocraft.com/
PASS	Windows 32/64bit Linux/Unix 32/64bit	C++	PMID:19218350	http://pass.cribi.unipd.it/cgi-bin/pass.pl
PerM	Windows 32/64bit Linux/Unix 32/64bit	C++	PMID: 19675096	http://code.google.com/p/perm/
RazerS	Windows 32/64bit Linux/Unix 32/64bit Mac X OS	C++	PMID: 19592482	http://www.seqan.de/projects/razers.html

	32/64bit			
RMAP	Linux/Unix 32/64bit	C++	PMID:19736251	http://rulai.cshl.edu/rmap/
Segemehl	Linux/Unix 32/64bit	C	PMID:19750212	http://www.bioinf.uni-leipzig.de/Software/segemehl/
SeqMap	Windows 32/64bit Linux/Unix 32/64bit Mac X OS 32/64bit	C++	PMID: 18697769	http://biogibbs.stanford.edu/~jiangh/seqmap/
SHRiMP	Linux/Unix 32/64bit	Python	PMID: 19461883	http://compbio.cs.toronto.edu/shrimp/
SliderII	Windows 32/64bit Linux/Unix 32/64bit Mac X OS 32/64bit	Java	PMID: 18974170	http://www.bcgsc.ca/platform/bioinfo/software/SliderII
SOAP2	Linux/Unix 64bit	C++	PMID: 18227114 PMID: 19497933	http://soap.genomics.org.cn/
SSAHA2	Linux/Unix 32/64bit Mac X OS 32/64bit	N/A	PMID: 11591649	http://www.sanger.ac.uk/resources/software/ssaha2.html
BFAST	Linux/Unix 32/64bit	Python	PMID: 19508732 PMID: 19907642	https://sourceforge.net/apps/mediawiki/bfast/index.php?title=Main_Page

Supplementary file 3. The numbers of citation papers for multiple aligners



Supplementary file 4. A python script was mainly used as memory usage monitor

```
#!/usr/bin/env python



import os

import time



max_mem = 0;

PID = raw_input("Please input your target PID:")

print '      The max_RAM used during this stage is:'

while True:

    fd = os.popen('ps aux')

    fd.readline()



    for line in fd:

        tmp_list = line.split()

        if tmp_list[1] == PID:

        if max_mem < float(tmp_list[3]):

                    max_mem = float(tmp_list[3])

                    print '        ',max_mem



    time.sleep(1)
```

Supplementary file 5. A perl script was implemented for computational simulation for *in silico* data

```
#!/usr/bin/perl -w



=pod

This Perl script generate simulated single-end short reads,

written by Yifei Tang,

from Center for Systems Biology, Soochow University,

with no warranty.

We used part of thought by Juliane Dohm and Claudio Lottaz.

=cut



$Usage = <<EOF;

Users can simply run this script followed by 5 arguments:

    1. Genome sequence file in Fasta format which you will generate reads from.

    2. Number of reads to be generated.

    3. Length of each read.

    4. Assumed sequencing error rate (%).

    5. Assumed sequencing indels rate (%).

    6. Indels length.

    7. Base name of output files. (<basename>_SE.fa)

Eg. perl simulation_SE.pl hg.fa 50000 36 0.8 0.0001 4 simread

This command will generates 50000 36-length short reads from "hg.fa

with sequencing error rate 0.8%, output to simread_SE.fa.

EOF



#Check if a leagal command line is given.

die "$Usage\n" if (@ARGV != 7);
```

```
#Define variables.
my $in = $ARGV[0];
my $totalreads = $ARGV[1];
my $readlen = $ARGV[2];
my $errorrate = $ARGV[3];
my $insertionrate = $ARGV[4];
my $deletionrate = $ARGV[4];
my $indellength = $ARGV[5];
my $basename = $ARGV[6];
my (@seq, @rev_seq, @chro, @chro_len, @chro_reads);
my $chro_index = 0, $total_len = 0, $errors = 0, $read_index = 0;

#Open input and output files.
open (IN, $in) || die "Genome sequence file not found!\n";
open (OUT, ">$basename\_SE.fa");
open (INDEL, ">indels.txt");

#Read the genome sequence file line by line.
print "Reading genome sequence...\n";
while (<IN>) {
	chomp;
	$_ =~ s/[\n\r]//;
	if ($_ =~ /\>/) {
		if ($chro_index != 0) {
			my $rev_seq = reverse ($seq[$chro_index]);
			$rev_seq =~ tr/ATCGU/TAGCA/;
```

```
            $rev_seq =~ tr/atcgu/tagca/;
            $rev_seq[$chro_index] = $rev_seq;
            $chro_len[$chro_index] = length ($seq[$chro_index]);
            $total_len += $chro_len[$chro_index];
            print "Length of chromsome $chro_index... \"$chro[$chro_index]\" is $chro_len[$chro_index]\n";
        }
        my $chro = substr ($_, 1, length ($_));
        $chro[++$chro_index] = $chro;
        print "Processing chromsome $chro_index... \"$chro\"\n";
    }elsif ($_ =~ /[ATCGUatcguNn]/) {
        $seq[$chro_index] .= $_;
    }
    if (eof) {
        my $rev_seq = reverse ($seq[$chro_index]);
        $rev_seq =~ tr/ATCGU/TAGCA/;
        $rev_seq =~ tr/atcgu/tagca/;
        $rev_seq[$chro_index] = $rev_seq;
        $chro_len[$chro_index] = length ($seq[$chro_index]);
        $total_len += $chro_len[$chro_index];
        print "Length of chromsome $chro_index... \"$chro[$chro_index]\" is $chro_len[$chro_index]\n";
    }
}
print "Complete with reading genome sequence with the total length of $total_len.\n";
#Determine reads to be generated of each chromsome.
print "Determining reads to be generated of each chromsome...\n";
for (my $n = 1; $n <= $chro_index; $n++) {
    $chro_reads[$n] = int (($chro_len[$n] / $total_len) * $totalreads);
```

```
	print "Chromsome $chro[$n] will generate $chro_reads[$n] reads.\n";
}
print "Complete with determing reads to be generated of each chromsome.\n";
```

```
#Main part simulating short reads.
print "Generating short read pairs...\n";
for (my $n = 1; $n <= $chro_index; $n++) {
	for (my $m = 0; $m < $chro_reads[$n]; $m++) {
		$read_index++;
		my $start = int (rand ($chro_len[$n] - $readlen));
		my ($ifrev, $errorIndex, $errorBase);
		my ($read, $errorread, $indelread);
		my ($adjustedOriSeq, $adjustedPostSeq);
		if (rand (1) >= 0.5) {
			$read = substr ($seq[$n], $start, $readlen);
```

```
            $ifrev = "+";
        }else {
            $read = substr ($rev_seq[$n], $start, $readlen);
            $ifrev = "-";
        }
        #Generating sequencing error
        ($errorread, $errorIndex, $errorBase) = &seq_error ($read);
        #Generating indels
        ($indelread, $adjustedOriSeq, $adjustedPostSeq) = &indels ($errorread);
        #Checking if uncertain bases reach the number of 10 (N)
        my $uncertainbases = 0;
        foreach my $base (split (//,$indelread)){
            $uncertainbases++ if ("\U$base" eq "N");
        }
        if ($uncertainbases >= 10) {$read_index--; redo;}
        if ($errorread ne $indelread) {
            print INDEL ">$read_index\_$chro[$n]\_$start\n$adjustedOriSeq\n$adjustedPostSeq\n";
        }
        if ($read ne $errorread) {
            ++$errors;
            chop ($errorIndex), chop ($errorBase);
            print OUT ">read_$read_index\_$chro[$n]\_$start\_$errorIndex\_$errorBase\n$indelread\n";
        }else {
            print OUT ">read_$read_index\_$chro[$n]\_$start\n$indelread\n";
        }
    }
    print "Complete with generating short reads from $chro[$n].\n";
```

```
}

print "Complete with generating all short reads.\n";

print "You get $errors errors out of $read_index of all generated reads.\a\n";



#Close files opened.

close IN;

close OUT;

close INDEL;
```

#Subroutine processing sequencing error.

```
sub seq_error {

	my $tmpread = $_[0];

	my @base = split (//, $tmpread);

	$tmpread = "";

	my $errorIndex = "";

	my $errorBase = "";

	for (my $n = 0; $n < @base; $n++) {

		if (rand (100) > $errorrate) {

			$tmpread .= $base[$n];

		}else {

			my %fourbase = ("A", 0, "T", 0, "C", 0, "G", 0);

			delete $fourbase{"\U$base[$n]"};

			my @fourbase = keys (%fourbase);

			my $index = $n + 1;

			my $i = rand (1);
```

```
                if ($i < 0.33) {
                        $tmpread .= $fourbase[0];
                        $errorIndex .= "$index,";
                        $errorBase .= $base[$n] . "->" . $fourbase[0] . ",";
                }elsif ($i <0.66) {
                        $tmpread .= $fourbase[1];
                        $errorIndex .= "$index,";
                        $errorBase .= $base[$n] . "->" . $fourbase[1] . ",";
                }else {
                        $tmpread .= $fourbase[2];
                        $errorIndex .= "$index,";
                        $errorBase .= $base[$n] . "->" . $fourbase[2] . ",";
                }
        }
    }
    return ($tmpread, $errorIndex, $errorBase);
}


                #Deleting deletion sequence
                for ($n = 0; $n < $indellength; $n++) {
                        $adjustedOriSeq .= $base[$i+$n];
                        $adjustedPostSeq .= "-";
                }
                $i = $i + $indellength - 1;
        }else {
                $postSeq .= $base[$i];
                $adjustedOriSeq .= $base[$i];
```

```
                $adjustedPostSeq .= $base[$i];
            }
        }
        return ($postSeq, $adjustedOriSeq, $adjustedPostSeq);
}
```

```
#Subroutine processing indels.
sub indels {
        my $oriSeq = $_[0];
        my $postSeq = "";
        my $adjustedOriSeq = "";
        my $adjustedPostSeq = "";
        my @base = split (//, $oriSeq);
        my @fourbase = ("A", "T", "C", "G");
        for(my $i = 0; $i < @base; $i++) {
            if (rand (100) <= $insertionrate) {
                print "Insertion happens.\n";
                #Generating random insersion sequence
                for ($n = 0; $n < $indellength; $n++) {
                    my $randIndice = rand (1);
                    if ($randIndice < 0.25) {
                        $postSeq .= $fourbase[0];
                    }elsif ($randIndice < 0.5) {
                        $postSeq .= $fourbase[1];
                    }elsif ($randIndice < 0.75) {
                        $postSeq .= $fourbase[2];
                    }else {
```

```
                    $postSeq .= $fourbase[3];

                }

                $adjustedOriSeq .= " ";

                $adjustedPostSeq .= "+";

            }

        }

        if (rand (100) <= $deletionrate) {

            print "Deletion happens.\n";
```