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RESEARCH ARTICLE

Genome-wide *in-silico* identification and characterization of simple sequence repeats in onion (*Allium cepa* L.)

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Abstract

Onion (*Allium cepa* L.) is a globally significant vegetable crop with a large genome that presents challenges for genetic study and breeding. A study was undertaken to identify and characterize simple sequence repeats (SSRs) in the onion genome using *in-silico* method to develop new molecular markers for breeding and genetic diversity analysis. Using the KRAIT tool, 470,700 SSRs were identified from the onion genome, with dinucleotide repeats being the most abundant. Primers were designed for 171 SSR loci and tested on a subset of onion genotypes, resulting in 18 primers displaying clear amplification and polymorphism. These primers were further validated on 22 onion genotypes and *Allium fistulosum*, revealing a total of 49 alleles with an average polymorphic information content (PIC) value of 0.456. The study demonstrated the potential of these SSR markers in genetic diversity analysis and breeding programs for onion. The newly developed SSR markers enhance the genomic resources available for *Allium cepa* and provide valuable tools for future molecular breeding efforts.

Keywords: Genetic diversity, Microsatellites, Characterization, Onion, Male sterile.

Introduction

Onion (*Allium cepa* L.) is the third most important vegetable crop produced worldwide, after tomatoes and potatoes, and is frequently consumed as a functional and staple vegetable in many countries. With an annual world production of 106.5 million tonnes, harvested over an area of 5.7 million hectares, India contributes as the highest producer with 26.6 million tonnes (FAOSTAT 2021). Onion derives its economic value not only for its culinary use but also for compounds like flavonoid, sulfur-containing S-alk(en)yl cysteine sulphoxides and fructo-olisaccharides that have been found to be beneficial for human health (Grifiths et al. 2002; Mogren et al. 2007). Onions, although they can self-pollinate, are considered an outcrossing crop displaying the characteristic of protandry, whereby the stamens release pollen before the pistils mature. This pollination behavior, characterized by its high heterozygous genetic background, self-incompatibility, high inbreeding depression, along its biennial life cycle, has been a challenge for the breeding and characterization of improved traits (McCallum 2007; Jayaswall et al. 2019).

Since the discovery of cytoplasmic male sterility (CMS) in 1943 by Jones and Clarke, major headways have been made in developing F_1 hybrids in conjunction with other breeding approaches such as inbred line development and mass selection, in order to produce populations with improved qualities. Integrated use of genomic tools such as molecular markers, mapping populations, and linkage maps can greatly enhance the breeding program of a crop by allowing a deep understanding of traits at a genetic level. High-throughput genome sequencing, such as next-generation sequencing (NGS) and RNA transcriptome sequencing, has opened up opportunities for the advancement of marker development. In the case of onions, linkage maps

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and molecular markers have played a major part in the molecular breeding strategies. King et al. (1988) pioneered the construction of the first genetic map using amplified fragment length polymorphism (AFLP) and randomly amplified polymorphic DNA (RAPD). Another linkage map comprising 14 linkage groups was developed by integrating rice expressed sequence tags (EST) that showed significant similarities into the previously constructed onion genetic map (Martin et al. 2005). Genetic maps based on single-nucleotide polymorphisms (SNPs) and high-resolution melting markers (HRM) (Duangjit et al. 2013; Jo et al. 2017; Choi et al. 2020) and PCR-based assay targeting indel and SNP markers identified from the sequenced data (Baldwin et al. 2012b) have been developed. Reference transcripts of the large genome of onion have also been constructed through RNA sequencing (Kim et al. 2015; Sohn et al. 2016). Molecular markers have been extensively employed for the construction of genetic maps (McCallum et al. 2008; Damon and Havey 2014; Chand et al. 2018), genetic fingerprinting (Almontero and Espino 2016) and analyzing genetic diversity in onion using RFLPs (McCallum et al. 2001), RAPDs (Bradeen and Havey 1995), AFLPs (Van Heusden et al. 2000) and simple sequence repeats (SSRs) (Fischer and Bachmann 2000; Jakse et al. 2005; Khar et al. 2011; Mallor et al. 2014). Up until recently, a reference genome for onion was not available, but that changed when Finkers et al. (2021) unveiled the first *de novo* genome sequence with 91% coverage (14.9 Gb) of the expected genome size (16.3 Gb). This genome data will accentuate the breadth and depth of our understanding of the distribution of the genes and repeats in this crop. With this development, more SSR-containing region and their distribution can be explored to identify genome-wide polymorphic SSR markers, identify quantitative trait loci that control traits of economic importance.

Genomic SSR markers have been extensively used for genetic mapping and population diversity studies as they are co-dominant, highly polymorphic and allow it to be possible to detect of variation even between closely related accessions. In *A. cepa,* SSR markers have been developed for cultivar discrimination, genetic diversity and mapping studies. SSR has been used for diversity analysis and for distinguishing the accessions of Spanish onion landraces, Turkish onion germplasm, and Indian onion accessions (Mallor et al. 2014; Rivera et al. 2016; Hanci and Gokce 2016; Khar et al. 2011; Lyngkhoi et al. 2021). Baldwin et al. (2012a) developed SSR markers for quantitative estimation of diversity within and among onion populations. A similar study of intra- and inter-variation among local onion accessions from arid region of Tunisia was evaluated using microsatellites markers whereby great variability was observed for all the traits studied (Chalbi et al. 2023). But the number of SSR markers available for genetic and other molecular studies is significantly less. Owing to the large genome size and low estimated gene density of the onion, large regions of the onion genome are still left to be investigated. Therefore, the present study was undertaken to develop new SSR markers from genomic sequence produced through RNA sequencing and the most robust markers were selected and polymorphism validated in different onion accessions and the phylogenetic relationship examined between them to evaluate the usefulness of the newly designed SSR markers for their subsequent application in breeding research.

Materials and methods

Genome-wide survey for SSR motifs and primer design

The assembled genome of onion accession number GCA_905187595.1 was retrieved from NCBI (https://www.ncbi.nlm.nih.gov/search/all/?term=GCA_905187595.1). KRAIT tool (Du et al. 2018) was used to detect various types of SSRs and screening was done with the criteria of mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeat motifs with occurrences of 12, 7, 5, 4, 4 and 4, respectively. The identified SSRs were classified into two groups: Group I comprised hypervariable SSRs with a motif length of ≥50 nt, while Group II consisted of potentially variable SSRs with motif lengths ranging from 20 to 50 nt. To design primers for the predicted SSRs, Primer3 (Untergasser et al. 2012) was employed with default parameters, which is integrated into the KRAIT tool (Fig. 1).

Plant materials

To assess the utility of the newly synthesized SSR markers, a set of 22 onion genotypes, along with *Allium fistulosum* as an out-group species, was used (Supplementary Fig. 1). Amongst the genotypes, eight were open-pollinated varieties, 7 were male-sterile lines, 7 were maintainer lines and 2 were *kharif* onion breeding lines. These genotypes were maintained in the experimental field of the Division of Vegetable Science, ICAR-Indian Agriculture Research Institute, Pusa, New Delhi, India.

Experimental validation of SSR primer pairs

For primer validation, genomic DNA from the onion genotypes was extracted from the young leaf samples following the modified CTAB method (Murray and Thompson 1980). A total of 171 primer pairs (ACKI novel primer) were initially screened against a subset of eight onion genotypes, i.e., PRO6, Punjab Naroya, POSOP02A, POS02B, Pusa Madhavi, Bhima Super, POS03B, Bhima Dark Red, following normal PCR conditions for different primers with Mastercycler Nexus Thermal Cycler (Eppendorf) (Supplementary Table S1). Subsequently, 18 ACKI primers showing clear amplifications were selected for validation of the amplification of the SSR primer set identified in the present work. For every PCR

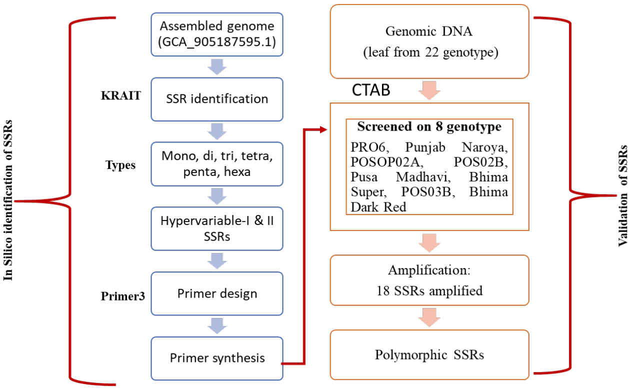

Fig. 1. Outline of the stepwise procedure employed to mine SSR loci and identify polymorphic loci and genetic characterization

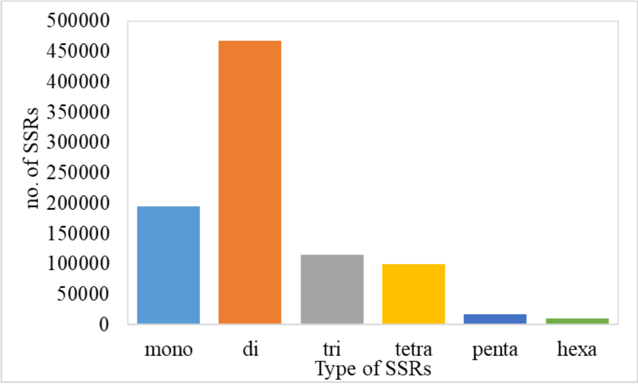

Fig. 2. Distribution of various types of SSRs

amplification, the reaction was carried out in a 10 µL reaction volume containing 5.0 µL of 2 × PCR buffer (Dream Taq PCR Master Mix -Thermo Scientific), 0.5 µL each of forward and reverse primers (10 pmol) and 1.0 µL (20 ng) of template DNA. PCR was performed with the following conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, annealing temperature for 45 seconds, 72°C for 1-minute, and a final extension at 72°C for 7 minutes. PCR products were separated on 3% agarose gels, visualized, and photographed in a gel documentation system (Cell Bioscience Alphaimager HP). Along with these newly developed primers, other previously developed markers, namely ACM primers (5) and gACK primers (3) were also used to study the diversity of the onion genotypes.

Marker data analysis

For each marker system, standard diversity indices like the number of alleles, expected heterozygosity (H), polymorphic information content (PIC) and discriminating power (D) were calculated using the iMEC software program (Amiryousefi et al. 2018). For the genetic diversity study, the amplified loci showing two or more scorable bands among the onion genotypes were used for genetic characterization. A matrix of similarity coefficients was prepared with the SIMQUAL module using NTSYS-pc version 2.00 (Rohlf 2000) to analyse the phylogenetic relationship among the genotypes and the clustering map was constructed based on the genetic distances and the unweighted pair group method with arithmetic mean (UPGMA).

Results

SSR marker identification and development

A total of 470700 SSRs were identified from the onion genome. The frequencies of mono, di, tri, tetra, penta and hexa repeats were observed as 194620 (21.5%), 467180 (51.6%), 114545 (12.6%), 99051 (10.9%), 17598 (1.9%) and 10674 (1.1%), respectively (Fig. 2). The dinucleotide repeats were identified as the most abundant and contributed to about 51.6% of total repeats on onion genome. Additionally, the hyper-variable motifs were identified with parameters: motif length >=50 bp, considered as hypervariable group I. Among hypervariable group I, 17603 motifs (1.94%) were found distributed as one mono-, three tri and three hexa- repeats. A total of 326007 (36%) SSRs were identified under hypervariable group II, ranging between 20 to 50 bp (Supplementary Table S2, for data, contact author on e-mail). The distribution of mono, di, tri, tetra, penta and hexa repeats in hypervariable group I and II is shown in Table 1.

Distribution of SSRs on chromosomes

One hundred and seventy-one SSR primer pairs were selected from the set of *in-silico* identified SSR loci. The location of SSRs was assigned on the chromosomes on the basis of the assembled genome (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_905187595.1/). The distribution of 171 SSRs on genomic localization is given in Figure 3. Around 30 SSR markers were assigned to different chromosomes whereas 141 were assigned to unplaced scaffolds. Out of the 17 polymorphic markers, ACKI030 was assigned to Chromosome 2, ACKI125 and ACKI145 to chromosome 4, ACKI021 and ACKI103 were assigned to Chromosome 5, whereas primer ACKI014, ACKI029, ACKI037, ACKI038, ACKI085, ACKI091, ACKI092, ACKI093, ACKI101, ACKI121, 128, ACKI140, ACKI141 were aligned to unplaced scaffolds (Table 2).

Table 1. List of SSRs falling in the categories Hypervariable I and II

Type of SSRs	Hyper-variable I	Hyper-variable II
Mono	977	22598
Di	12116	224770
Tri	717	17934
Tetra	2970	33256
Penta	70	17528
Hexa	753	9921

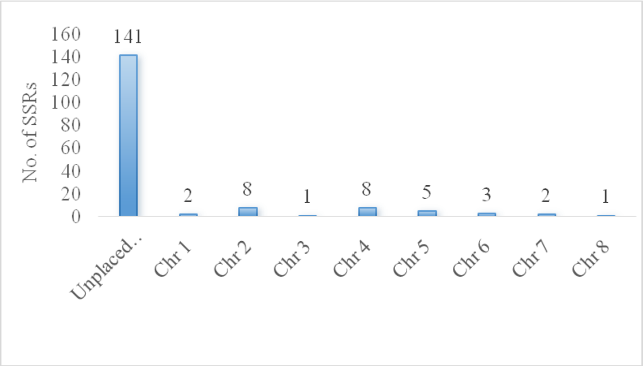

Fig. 3. Assignment of identified SSRs on onion chromosomes

Primer validation and characterization

One hundred and seventy-one SSR primer pairs were selected from the set of *in-silico* identified SSR loci and genotyped on a subset of eight genotypes to confirm and validate the polymorphic potential of these SSR loci. A total of eighteen primer pairs that displayed clear amplification and polymorphism were selected and then genotyped on a panel of 22 onion accessions and *Allium fistuosum* for further validation. In total, 49 alleles were amplified from the eighteen SSR primer pairs with an average of 2.7 alleles at each locus ranging from 2 to 4 alleles (Fig. 4). Additionally, the average PIC value was 0.456 that ranged from 0.119 (ACKI029) to 0.649 (ACKI092)with an average expected heterozygosity (H) value of 0.538 and discriminatory power of 0.450. Eight primers had a PIC value of 0.5 and above (Table 2).

The performance of the additional primers, viz., ACM series and gACK series, was also analyzed and the number of alleles averaged 2.1 alleles, with PIC ranging from 0.156 (gACK044) to 0.549 (ACM221), with an average of 0.336. The expected heterozygosity ranged from 0.162 (gACK044) to 0.628 (ACM221) with an average of 0.422 per locus. The primer pairs showed discriminatory power ranging from 0.166 (gACK044) to 0.826 (gACK007) with an average of 0.449 (Table 3).

The genotype data analyzed using NTYSYS-2.00 software divided the examined genotypes into four major clusters with similarity coefficients ranging from 0.54 to 0.88 (Fig. 5). Cluster I included 7 genotypes, Cluster II was the largest cluster containing 60% (14 genotypes), 1 genotype (Bhima Super) in Cluster III. *Allium fistulosum,* which was used as an out-group was clustered out separately in Cluster IV from all the rest of the genotypes as expected. In Cluster I, POSOP2A and POS03A presented a high similarity with POSOP2B and POS03B, respectively. POSOP2A and POSOP2B, which are male sterile and maintainer lines generated from the background of Pusa Sona (yellow onion), were grouped closely with Pusa Madhavi (red onion) and PRO6 (red onion) was grouped in the same sub-cluster with them. POS3A (male sterile) and POS3B (maintainer) were developed from the background of Pusa Red. Punjab Naroya (red onion) was grouped furthest away from the rest. Most of the genotypes were grouped together in Cluster II. POS08A was highly similarity to POS08B, which belongs to the same background of Arka Pragati. So do POS07A and POS07B, which have originated from the genetic background of Pusa Red and share high similarity. In this same sub-cluster, POS17A and POS17B were also grouped together. POS01A and POS01B were in high similarity, and POS12A and POS12B showed high similarity. Of the two elite lines, POS24K was in high similarity

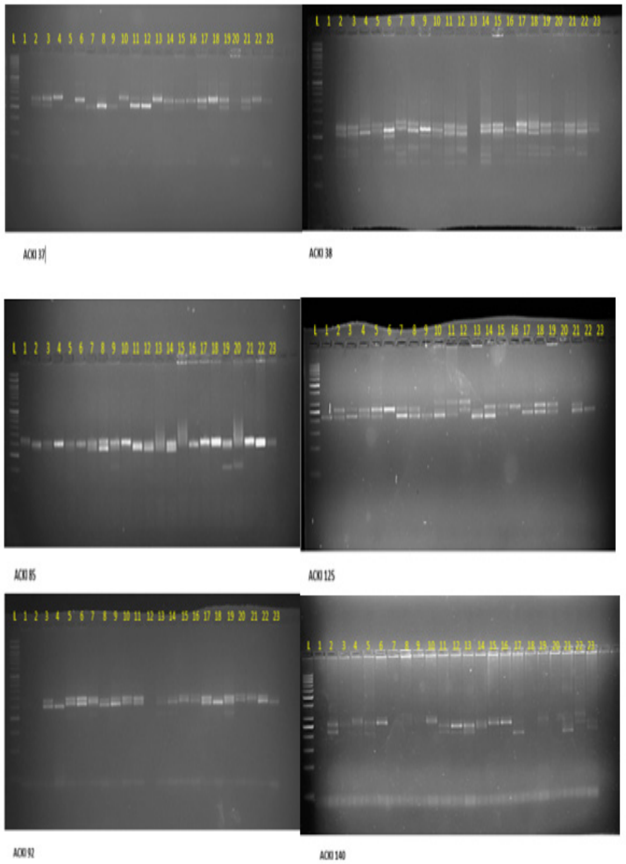

Fig. 4. Amplification of 23 genotypes with the SSR primers ACKI37, ACKI38, ACKI85, ACKI125, ACKI92, ACKII140

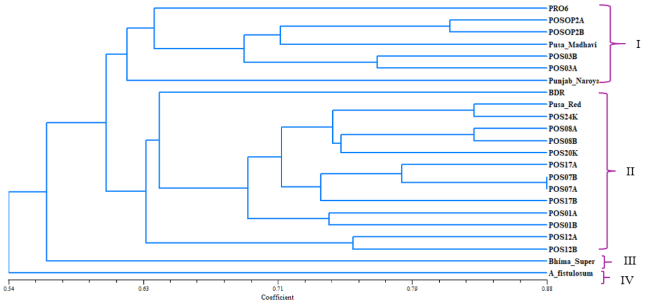

Fig. 5. Genetic diversity analysis of 22 onion genotypes and *A. fistulosum* with SSR using genetic diversity data from 26 polymorphic markers

Table 2. Summary of SSR motifs and polymorphism of novel ACKI primer pairs tested in onion genotypes

S. No.	Primer	Motif	Repeat	Chromosome Position	Primer sequence (5′-3′)	Tm (°C)	Allele size (bp)	Allele Number	H	PIC	D
1	ACKI014	GTAT	38	Unplaced scaffolds	CCTCTGTGCGTGAATCTTATCC AGGACCAATGTTGTCTTTGGG	60	162-216	3	0.499	0.374	0.774
2	ACKI021	ATACAT	24	Chromosome 5	GACTTAGGGTCCCTGTTCCC ACAACAATTCAATGCCAAGCC	58	250-300	3	0.476	0.362	0.850
3	ACKI029	TATATG	23	Unplaced scaffolds	ACTAGGTTCAAAGTTGCTGTCC CCTACATATGTGTCTTCGTTGTCC	58	250-277	2	0.123	0.119	0.128
4	ACKI030	ATATAC	23	Chromosome 2	TCGTTACCTCTTCCTTTAGCCC TCTCGATCCGACGATTTTAATGC	58	212-282	3	0.572	0.480	0.371
5	ACKI037	CATATA	22	Unplaced scaffolds	GCAGAAAGCATATAGATGGAGACG AGACCTAGGAGTCCAGAGAGG	60	227-283	4	0.522	0.449	0.496
6	ACKI038	TATATG	22	Unplaced scaffolds	TGATCTGCCTATTTGTGTGAATGC AGCTGATACATTTTGTTAGCAAGC	60	220-240	2	0.590	0.509	0.181
7	ACKI085	TAG	40	Unplaced scaffolds	GTTTGGATCAACGGACGACC ACCGTTGAGTGCTTAGAAATGC	60	227-260	3	0.642	0.573	0.318
8	ACKI091	TATG	29	Unplaced scaffolds	GTTGACTGCAGCATCATCGG AGCACTTAACCAAAAGGTAAGATGC	58	169-197	2	0.539	0.433	0.075
9	ACKI092	TATG	29	Unplaced scaffolds	TGCACTTAAGGGCATTAGTTTAGG CCTATAGGAATCCCACCCGC	58	194-220	3	0.703	0.649	0.376
10	ACKI093	TATG	29	Unplaced scaffolds	ACGACTTGTTGGTGACACCG ATGTAACACAGACCAGGGGC	60	250-255	2	0.624	0.554	0.021
11	ACKI101	TATATG	19	Unplaced scaffolds	AGCGAGGATAGTATCATGGTCC CGTTGATTCCCTTACATATACATATGC	60	274-340	3	0.603	0.519	0.566
12	ACKI103	ATA	38	Chromosome 5	TCACCCAGAACAACTGCTCC AACACTTCAGGGTTGCAGGC	60	168-288	4	0.446	0.347	0.888

13	ACKI121	CATATA	18	Unplaced scaffolds	CAAGGACTGCACAAGATAGGC TCTTAATTTTGGCCTCAACCCC	60	273-283	2	0.540	0.434	0.529
14	ACKI125	TATATG	18	Chromosome 4	GGCGGCGTTCCTTGATAGG TGAGATGGTTGCATGTGGGG	60	230-314	3	0.525	0.425	0.351
15	ACKI128	ATATAC	18	Unplaced scaffolds	TCCGAAAGACAAGTCACTGGG TCAGTATGTAGGGACCATGTCC	60	280-320	3	0.586	0.521	0.578
16	ACKI140	AG	2	Unplaced scaffolds	AAGTCTCGGCCAAAACCTCG AGTCACCGTGTTTTTGTCGC	60	200-278	3	0.578	0.502	0.577
17	ACKI141	ACAAT	21	Unplaced scaffolds	AGAGAACACAGAGTCAAAATCATTGG TCAGGAACAATGAGGCCAGC	60	292-323	2	0.612	0.543	0.498
18	ACKI145	TACA	26	Chromosome 4	GAACTAAATATTTCTGTCACCTGAACC ATGTTTCCTTCTCAGATAGACACC	60	250-270	2	0.517	0.421	0.531
	Mean							2.7	0.538	0.456	0.450

H = Expected heterozygosity; PIC = Polymorphic information content; D = Discriminating power

with Pusa Red, and POS2OK shared similarity with POS08A and POS08B. Our results indicate that the SSR markers had the ability to assess molecular diversity.

Discussion

Identification of SSR markers on the basis of NGS is a highly effective and low-cost method and owing to the high specificity and highly conserved nature, SSR markers are suitable for conducting genetic mapping and diversity analyses in crops (Yang et al. 2015). All of the newly designed SSR primer pairs were able to amplify in all the onion genotypes used in this study and all the markers were polymorphic. PIC values of 0.5 and above were recorded in 44% of the newly developed markers and ranged within the whole set from 0.1 to 0.6, which is comparable to previous related studies. With a collection of 34 Indian onion accessions along with some wild species and with a set of similar 19 SSR primers, Khar et al. (2011) observed PIC from 0.0 to 0.7. In another genetic diversity study, Baldwin et al. (2012) observed that the 20 SSR markers that they employed showed polymorphism with a PIC value of 0.2 to 0.8. Mallor et al. (2014) in a larger collection of Spanish onion genotypes recorded a PIC of 0.0 to 0.7 with 12 SSR markers. Marker ACKI092 exhibited the highest PIC value, indicating its usefulness in detecting polymorphism in the onion accessions. In comparison, the highest PIC value recorded by ACM and gACK primer pairs was 0.549 (ACM221), which suggests that the new ACKI primers may be better markers for distinguishing between the onion lines in the study.

The cluster analysis revealed that the male sterile lines and maintainer lines, which were selected from the genetic background of the open-pollinated varieties, were grouped together in a cluster. Cluster II had a collection of almost all the derived lines except for POSOP02A, POSOP02B and POS03A, POS03B, which were classified in cluster I along with the open-pollinated varieties. The two male sterile and maintainer lines derived from Sukhsagar, i.e., POS17A, POS17B and POS01A and POS01B were grouped closely together in Cluster II. Also in Cluster II, POS07A and POS07B selected from Pusa Red, POS17A and POS17B selected from Sukhsagar, POS01A and POS01B also selected from Sukhsagar were all red. Clusters of analysed accessions did not reveal any specific features based on bulb colour as yellow bulbed POSOP02A and POS02B, which were lines selected from Pusa Sona, and red colour onions i.e., PRO6, Pusa Madhavi, Pusa Red and Punjab Naroya, and POS03A and POS03B, which were lines selected from the background of Pusa Red, all grouped in Cluster I. Other studies have also indicated a lack of correlation between clustering patterns and bulb skin colour or shape as well (Hanci and Gökçe 2016; Mallor et al. 2014; Mitrova et al. 2015). A similar observation in Indian onion accessions has been reported in which cluster formations had no bearing

Table 3. Polymorphism of additional SSR markers

S. No.	Primer	Tm (°C)	Allele size (bp)	Allele Number	H	PIC	D	Reference
1	ACM004	55	207-230	2	0.199	0.189	0.199	Jakše et al. 2015
2	ACM018	60	271-279	2	0.603	0.519	0.464	Jakše et al. 2015
3	ACM124	55	269-300	2	0.314	0.265	0.356	Jakše et al. 2015
4	ACM221	55	175-180	2	0.628	0.549	0.371	McCallum et al. 2008
5	ACM235	55	300-310	2	0.499	0.374	0.733	McCallum et al. 2008
6	gACK007	60	200-240	3	0.487	0.368	0.826	Singh et al. 2022
7	gACK044	60	180-200	2	0.162	0.156	0.166	Singh et al. 2022
8	gACK105	60	222-236	2	0.486	0.432	0.484	Singh et al. 2022
	Mean			2.1	0.422	0.356	0.449	

on the colour, growing season, or geographical origin of the accessions (Khar et al. 2011). Our cluster data also revealed similar findings whereby accessions maintained over a wide geographical locations (Delhi, Punjab, Maharashtra and West Bengal) were grouped together under the same clusters supporting the previous observations made. This might be attributed to the exchange of germplasm among farmers from different geographical regions. Although in Cluster I, some accessions from similar geographical regions were grouped together. For instance, PRO6 and Punjab Naroya, both from Punjab and Pusa Madhavi and lines selected from Pusa Sona (POSOP2A and POSOP2B), both from Delhi, were clustered together. The two elite lines POS20K and POS24K, were categorized under the sub-cluster with Pusa Red. *A. fistulosum* was in a separate cluster all by itself in Cluster IV, which is unambiguous as it is a different species from the rest, which belonged to *A. cepa*. Bhima Super was also placed as the sole individual in Cluster III, signifying its distance from the rest. Nevertheless, this degree of genetic distance displayed by Bhima Super suggests its usability as a viable parent in the onion breeding program. Considering that the markers produced in this study were able to generate good polymorphism and also exhibited a degree of transferability in *A. fistulosum*, they are conducive to their usage in mapping and diversity analyses. Hence these newly developed SSR markers have considerable potential in molecular breeding of onion.

New bioinformatics tools, made possible by the advent of next-generation DNA sequencing techniques, now offer more efficient methods as the output from NGS can be used as input for *in-silico* analyses, which have proven to be an effective approach for the de novo identification and characterization of repetitive DNAs (Dias et al. 2021). This study highlights the efficacy and cost-effectiveness of using SSR markers identified through NGS for genetic mapping and diversity analyses in onion. Approximately 44% of the markers exhibited PIC values of 0.5 and above, indicating their robustness in detecting polymorphism. Along with this, Cluster analysis revealed that male sterile and maintainer lines derived from open-pollinated varieties grouped together, while derived lines mostly clustered separately. The ability of the markers to produce good polymorphism and their transferability to *A. fistulosum* underscores their suitability for mapping and diversity analyses, highlighting their considerable potential in the molecular breeding of onions. The findings contribute to the broader understanding of onion genetics and support the development of improved onion varieties through molecular breeding strategies.

Supplementary material

Supplementary Table S1 is provided, which can be accessed at www.isgpb.org

Author's contribution

Conceptualization of research (AK, MM, NS); Designing of the experiments (AK, SS); Contribution of experimental materials (AK); Execution of field/lab experiments and data collection (MZ); Analysis of data and interpretation (MZ, AK, SS); Preparation of the manuscript (MZ, AK, SS).

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Supplementary Table S1. Details of sequence chromosome number and motif etc.

Id	Sequence	Chromosome position	Standard	Motif	Type	Repeat	Start	End	Length	Product
17720	LR994629.1	1	ATAC	TATG	4	27	264830958	264831065	108	288
5953	LR994629.1	1	ATATAC	TATATG	6	17	89794891	89794992	102	252
42348	LR994630.1	2	ATATAC	TATATG	6	30	311247037	311247216	180	278
25400	LR994630.1	2	ATATAC	ATATAC	6	23	62821673	62821810	138	263
29801	LR994630.1	2	ATAC	TATG	4	34	126551516	126551651	136	265
45568	LR994630.1	2	ATATAC	ATATGT	6	20	360485887	360486006	120	287
31554	LR994630.1	2	AG	AG	2	58	151598873	151598988	116	281
37012	LR994630.1	2	ATAC	TATG	4	28	233608185	233608296	112	253
29798	LR994630.1	2	ATAC	TATG	4	26	126514696	126514799	104	257
22080	LR994630.1	2	ATATAC	ATACAT	6	17	14162765	14162866	102	292
63363	LR994631.1	3	ATATAC	TGTATA	6	17	225813248	225813349	102	278
81571	LR994632.1	4	ATATAC	TATGTA	6	25	174108958	174109107	150	276
70557	LR994632.1	4	ATATAC	TATATG	6	20	7344479	7344598	120	294
87869	LR994632.1	4	ATATAC	TATACA	6	20	269025525	269025644	120	290
77627	LR994632.1	4	ATATAC	ATATAC	6	20	112465395	112465514	120	251
71629	LR994632.1	4	ATATAC	TATATG	6	18	23183263	23183370	108	260
84921	LR994632.1	4	ATAC	ACAT	4	27	223310785	223310892	108	283
70707	LR994632.1	4	ATAC	TACA	4	26	9540156	9540259	104	275
75798	LR994632.1	4	ATAC	TGTA	4	26	84016949	84017052	104	254
91076	LR994633.1	5	ATATAC	ATACAT	6	24	27204621	27204764	144	284
93461	LR994633.1	5	ATATAC	ATATAC	6	21	63292787	63292912	126	275
101121	LR994633.1	5	ATATAC	TATGTA	6	21	179081667	179081792	126	269
93830	LR994633.1	5	AAT	ATA	3	41	69433657	69433779	123	268
93828	LR994633.1	5	AAT	ATA	3	38	69405393	69405506	114	259
114827	LR994634.1	6	ATAC	TATG	4	33	192306425	192306556	132	293
120276	LR994634.1	6	ATAC	TATG	4	33	274735343	274735474	132	290
106197	LR994634.1	6	ATAC	TGTA	4	26	65878206	65878309	104	281
135899	LR994635.1	7	ATAC	TATG	4	35	231046723	231046862	140	279
132208	LR994635.1	7	ATATAC	TATGTA	6	19	176724558	176724671	114	252
146984	LR994636.1	8	ATAC	ATAC	4	29	166008431	166008546	116	290
658036	CAJJJL010064964.1	unplaced scaffolds	ATATAC	ATATAC	6	34	223322	223525	204	300
337189	CAJJJL010039057.1	unplaced scaffolds	AG	CT	2	100	888977	889176	200	287
772330	CAJJJL010073824.1	unplaced scaffolds	ATAC	ATAC	4	44	409856	410031	176	283
772333	CAJJJL010073824.1	unplaced scaffolds	ATAC	ATAC	4	44	420293	420468	176	283
827354	CAJJJL010077887.1	unplaced scaffolds	ATAC	ATAC	4	44	256757	256932	176	283
841002	CAJJJL010078829.1	unplaced scaffolds	ATATAC	GTATAT	6	29	179448	179621	174	296
522583	CAJJJL010053900.1	unplaced scaffolds	ATATAC	TATGTA	6	28	1131216	1131383	168	284
401007	CAJJJL010044055.1	unplaced scaffolds	ATATAC	ATACAT	6	28	360800	360967	168	272
186785	CAJJJL010011899.1	unplaced scaffolds	ATAC	ATAC	4	40	77933	78092	160	264

Forward	Tm1	Gc1	Stability1	Reverse	Tm2	Gc2		Primer name
CTAGACCAACATGAACACGGC	59.27	52.38	5.68	GCTTTGCGCTCTGCTTCC	59.82	61.11	3.46	ACKI130
CGTTGGTTCAGATGCAATTCCC	60.42	50	3.97	CAAGGGGAATCAATTCGATCCC	59.11	50	3.85	ACKI164
TCCTTTATTTCAACAACTAGTCCCG	59.06	40	5.14	CGCCATTAAGTCGCCATTCG	60.04	55	3.34	ACKI003
TCGTTACCTCTTCCTTTAGCCC	59.5	50	5.19	TCTCGATCCGACGATTTTAATGC	59.27	43.48	3.56	ACKI030
ACTGGTCCTGGTTACATGCG	60.04	55	4.73	CCTTATCAAGTGCAGGGAAGC	58.98	52.38	3.86	ACKI036
CAGGTGAAGATGGGTTTGGC	59.11	55	4.52	AGTTGATCAGGTTTAAAACGGTCG	59.79	41.67	4.79	ACKI067
TCTCTAAGCCTTTAAACTCTCAACC	58.53	40	3.77	TACATGCATCTCAGCCACCC	59.82	55	4.61	ACKI094
GCTTTAGAGCTACGTGGTTTGC	60.16	50	3.68	AGAGCTCTTATGTACAACCAAATCC	58.88	40	3.01	ACKI113
ACTGGTCCTGGTTACATGCG	60.04	55	4.73	CCTTATCAAGTGCAGGGAAGC	58.98	52.38	3.86	ACKI147
ACACAGAAAGTCAGAATAGAATATCCG	59.08	37.04	4.18	GAAGTCAGGTGGTTCTGGGG	59.96	60	4.96	ACKI151
TGGAATATGATAGAGAGAGGAGGC	58.73	45.83	4.7	GGGGGCGGATTGGATATAGG	59.45	60	2.57	ACKI153
CAAACAATAAAGTAAGGACTTATGGGC	59.03	37.04	5.36	GTTCACACCCGTCATAAGCG	59.28	55	4.68	ACKI017
TGTGTTCTTGTGTGCTTGTGC	60.14	47.62	4.57	GGGGGTCCATAAGTTATAACTCTCG	60.28	48	4.04	ACKI063
GGGACCGACCAATATTGATAGACC	60.56	50	3.85	TCATCGTGAAGTTGGTGTTTCG	59.46	45.45	3.46	ACKI064
TGGTATCATGGTATGGAGCCC	59.01	52.38	5.19	AACCAACCTTTATCCGGGGC	60.32	55	5.8	ACKI077
GGCGGCGTTCCTTGATAGG	60.89	63.16	2.57	TGAGATGGTTGCATGTGGGG	60.32	55	4.96	ACKI125
TCATTGAAGTGCAGCGAGG	58.15	52.63	4.63	TGCCTTGCTCGTATACTATTTTATGG	59.35	38.46	2.74	ACKI133
GAACTAAATATTTCTGTCACCTGAACC	58.55	37.04	3.62	ATGTTTCCTTCTCAGATAGACACC	58.02	41.67	4.16	ACKI145
GTTAGGATACCAGCGAGGGG	59.32	60	4.79	AGCACTCGAGAGTTTAACGGG	60.07	52.38	5.28	ACKI149
GACTTAGGGTCCCTGTTCCC	59.09	60	3.97	ACAACAATTCAATGCCAAGCC	58.23	42.86	4.35	ACKI021
TGAGTGGAAGACATCTACCCC	58.53	52.38	4.95	AGGGTAGAGAAGCAAGTGCC	59.38	55	5.01	ACKI049
AGACTTTGTATGTGCGATTTGGG	59.56	43.48	4.12	TCCCTCTTACGAGCCTATGC	58.67	55	3.14	ACKI051
TCACCCAGAACAACTGCTCC	59.89	55	4.7	AACACTTCAGGGTTGCAGGC	61.11	55	4.85	ACKI060
TCACCCAGAACAACTGCTCC	59.89	55	4.7	AACACTTCAGGGTTGCAGGC	61.11	55	4.85	ACKI103
TCATTACGAAGTTGATGTTTTCGG	58.08	37.5	4.3	TCGACAACTACTTTATGCTTTGAGC	59.88	40	4.26	ACKI039
TGGTTTGTTTTGATGGGTACGC	59.97	45.45	4.42	ACATCCAAAGTACCAAATATCAAACCC	60.09	37.04	4.11	ACKI040
TCTTGTAATGTTCTTTGAGCCTGG	59.24	41.67	4.45	TGGTTGTCATCATCAAAAATGGGG	60.02	41.67	4.96	ACKI142
GTTCACCATAAACCGGCTGG	59.19	55	4.85	AATTACGTGACCAAATTCCAACG	58.21	39.13	4.1	ACKI023
AGCCATTTTAAAGTTAGTCCGATCG	59.71	40	3.69	ACAACCAATACTTGATGATCAAAGC	58.38	36	3.51	ACKI100
AAGAAATATGTTCCTAGATGAGTACCC	58.35	37.04	3.69	CTTAGAGTCCCTATCCCTTTGGG	59.35	52.17	4.12	ACKI086
AAAACCCGAACAGGTAACCC	58.01	50	4.11	GGAATGGTTTCTATCTTGAGAGAGC	59.24	44	4.09	ACKI001
CCACTTAAACTCCTCCTCCGC	60.41	57.14	5.54	TTGTCAACCATGTCATCACCG	58.85	47.62	4.94	ACKI002
GTTTCGATGACAACAACTATACAATGG	59.31	37.04	3.16	TGTAGTGTCATTTGAGCCTCG	58.04	47.62	4.63	ACKI004
GTTTCGATGACAACAACTATACAATGG	59.31	37.04	3.16	TGTAGTGTCATTTGAGCCTCG	58.04	47.62	4.63	ACKI005
TCGGTTGAGAGAATACCTAGGC	59.04	50	3.93	GAAAACCCGAACAGGTAACCC	59.12	52.38	4.11	ACKI006
TTGCACTTTGATATGGATGTTGC	58.25	39.13	4.17	GCAACCAATGTGTATTCAATCATCC	59.25	40	3.51	ACKI007
TCGCTAACTTTAATAATTCCCACCC	59.18	40	4.61	CAGTCGAACAAGGGGGACG	60.37	63.16	4.79	ACKI008
TTAACCGTTGTGGATGCAGG	58.47	50	4.85	CACGCCGCTTATCAGAATAGG	58.94	52.38	2.57	ACKI009
GCTTGAATTATGATTTGGGATGTGC	59.54	40	4.57	GTTATAAATGCCTGTAAATTGACATGC	58.24	33.33	4.06	ACKI010

722809	CAJJJL010070036.1	unplaced scaffolds	ATAC	GTAT	4	39	35614	35769	156	284
872634	CAJJJL010080870.1	unplaced scaffolds	ATAC	TATG	4	39	305359	305514	156	269
774876	CAJJJL010074026.1	unplaced scaffolds	ATAC	ATAC	4	38	51344	51495	152	281
553583	CAJJJL010056390.1	unplaced scaffolds	ATAC	GTAT	4	38	342041	342192	152	270
678308	CAJJJL010066604.1	unplaced scaffolds	ATATAC	ATATAC	6	25	867424	867573	150	297
592110	CAJJJL010059542.1	unplaced scaffolds	ATATAC	TGTATA	6	25	61244	61393	150	280
195006	CAJJJL010014429.1	unplaced scaffolds	ATATAC	TACATA	6	24	297982	298125	144	299
326256	CAJJJL010038215.1	unplaced scaffolds	ATATAC	ATATGT	6	24	243157	243300	144	297
899842	CAJJJL010082861.1	unplaced scaffolds	ATACAC	TGTATG	6	24	81642	81785	144	285
761279	CAJJJL010072975.1	unplaced scaffolds	ATAC	ATAC	4	35	217888	218027	140	300
633220	CAJJJL010062935.1	unplaced scaffolds	ATAC	TGTA	4	35	74328	74467	140	272
667076	CAJJJL010065724.1	unplaced scaffolds	ATAC	TACA	4	35	26262	26401	140	265
301598	CAJJJL010036212.1	unplaced scaffolds	ATATAC	TATGTA	6	23	57550	57687	138	300
282452	CAJJJL010034079.1	unplaced scaffolds	ATATAC	TATATG	6	23	219967	220104	138	291
703939	CAJJJL010068577.1	unplaced scaffolds	ATATAC	ACATAT	6	23	308986	309123	138	286
687880	CAJJJL010067359.1	unplaced scaffolds	ATATAC	TATATG	6	23	130679	130816	138	271
733948	CAJJJL010070917.1	unplaced scaffolds	ATAC	TGTA	4	34	152661	152796	136	282
751348	CAJJJL010072223.1	unplaced scaffolds	ATAC	TACA	4	34	660549	660684	136	278
263665	CAJJJL010031670.1	unplaced scaffolds	ATAC	ATAC	4	34	346848	346983	136	277
556420	CAJJJL010056647.1	unplaced scaffolds	ATAC	ACAT	4	34	319662	319797	136	270
772362	CAJJJL010073824.1	unplaced scaffolds	ATAC	ATAC	4	34	827983	828118	136	270
658220	CAJJJL010064983.1	unplaced scaffolds	ATATAC	CATATA	6	22	410780	410911	132	285
420620	CAJJJL010045665.1	unplaced scaffolds	ATATAC	TATATG	6	22	225555	225686	132	267
853281	CAJJJL010079596.1	unplaced scaffolds	ATAC	GTAT	4	33	553967	554098	132	273
730334	CAJJJL010070667.1	unplaced scaffolds	ACCCG	CGACC	5	26	1026744	1026873	130	257
890038	CAJJJL010082169.1	unplaced scaffolds	ATAC	GTAT	4	32	72148	72275	128	298
474046	CAJJJL010050104.1	unplaced scaffolds	ATAC	TATG	4	32	270521	270648	128	265
802445	CAJJJL010076041.1	unplaced scaffolds	ATAC	ATAC	4	32	599501	599628	128	262

AAAAGGGAAAGAAAGCGCCG	59.69	50	6.46	ACGATATGTTAGGCCTAAAACCG	58.63	43.48	4.44	ACKI011
ATCGATACAATGTACATCAGTTTGC	58.05	36	3.68	TGTGGTGCGGTTACATTTGC	59.69	50	3.68	ACKI012
TGCTTTACTCAAAACCATATCACCG	59.88	40	4.94	GGTTTTCACTTAGTTTTGGAAAGAGG	59.02	38.46	3.69	ACKI013
CCTCTGTGCGTGAATCTTATCC	58.87	50	2.59	AGGACCAATGTTGTCTTTGGG	58.4	47.62	4.12	ACKI014
AGGCGACATTCCTCGATTGG	60.18	55	3.16	GTGGGTGGTACAGGGTATGG	59.46	60	2.74	ACKI015
TTCAAACCTCGGGGTGTTGG	60.47	55	3.77	TCAGCCTATCCTATCTACACGC	58.92	50	5.34	ACKI016
GTGAACAATTAGGAATCCATGTTTCC	58.96	38.46	3.13	AGGTATTACTTGGTGTGTTCAAATAGG	59.39	37.04	2.57	ACKI018
TTCGAATCTTGAGTCTTGAGTCC	58.19	43.48	3.85	TGTTGGTTTTGATGACAACAACC	58.5	39.13	3.77	ACKI019
CTGGTGTAGATATGGTTCTTGCC	58.87	47.83	4.52	CAAAAGCAAACTAGACTATACATGACC	58.61	37.04	4.02	ACKI020
AGATGTTGGTTTTTGATGATAACAACC	59.13	33.33	3.77	AGTCTTGAGTCTTTTTACTGATCGG	58.84	40	4.18	ACKI022
GAATACCTAGGCTCTGGAGTGG	59.37	54.55	4	CCTGAATAGGTAACCCAACAGATAGG	60.46	46.15	2.57	ACKI024
TCCATTGTGCTAGCTGCTCC	60.11	55	4.7	GTACAGGCACAAGTCAAGGC	59.12	55	4.35	ACKI025
TCTACTTCCTTGCTGTTGTTGC	59.12	45.45	4.17	CCACACAGCAAACCTAGACC	58.48	55	3.85	ACKI026
GAATGGATCCTGACAAGGTGC	58.98	52.38	5.01	GTCTTCTTGACTCCGCATATGC	59.46	50	3.14	ACKI027
TCAACACTTCTTCTCCACATGG	58.26	45.45	3.66	CAAGCAAAGACATTGACTCTGC	58.44	45.45	4.26	ACKI028
ACTAGGTTCAAAGTTGCTGTCC	58.52	45.45	4.02	CCTACATATGTGTCTTCGTTGTCC	59.2	45.83	4.02	ACKI029
AATGGCTACCATCCAAGGGC	60.11	55	5.19	TGATGAACTTGGTTGGACTATCC	58.16	43.48	2.59	ACKI031
CACGGCTGCTGATAATTCCC	59.05	55	3.97	GAAGGGCACAAACAAGCAGG	59.97	55	4.85	ACKI032
AGTCGGGTCGTCACATTTGG	60.32	55	3.28	GAAAACCCGAACAGGTAACCC	59.12	52.38	4.11	ACKI033
TGGAAAACCCGGATAGGTAACC	59.76	50	2.85	TGACAGTTGGTATCAAAGCGC	59.2	47.62	5.92	ACKI034
AGTGATAGGGTTAGTGATGCCC	59.29	50	5.36	AGGTACTCCTAAGGGAAAAGGG	58.54	50	3.95	ACKI035
GCAGAAAGCATATAGATGGAGACG	59.38	45.83	4.18	AGACCTAGGAGTCCAGAGAGG	59.15	57.14	3.69	ACKI037
TGATCTGCCTATTTGTGTGAATGC	59.9	41.67	3.56	AGCTGATACATTTTGTTAGCAAGC	58.36	37.5	4.01	ACKI038
AGATTATCGCATTGATGGATTGGG	59.3	41.67	4.12	CCATCACCTACTCTTGGACTGG	59.83	54.55	4	ACKI041
TTCCGACCCATTTACGACCC	59.75	55	4.46	CTACCCAACCCGTGTAACCC	60.04	60	4.11	ACKI042
CTACTCCTTCCCAGCATCGG	59.61	60	4.18	CACACACCCCCAAGTATAAAGC	59.25	50	3.51	ACKI043
CCTTCCTAAGGGGGAATCAACG	60.42	54.55	4.1	AGAGCCTATGAGCCACATGC	59.89	55	4.06	ACKI044
TTTCCTCTTCCGTTTCCTGC	58.11	50	4.85	GATTGGGGGTTGGGTTAATGC	59.52	52.38	3.56	ACKI045

454437	CAJJJL010048510.1	unplaced scaffolds	ATAC	TATG	4	32	3166	3293	128	261
693871	CAJJJL010067839.1	unplaced scaffolds	ATATAC	ATATAC	6	21	71939	72064	126	293
874965	CAJJJL010081048.1	unplaced scaffolds	ATATAC	ATATAC	6	21	186543	186668	126	289
425236	CAJJJL010046045.1	unplaced scaffolds	ATATAC	ATACAT	6	21	881996	882121	126	271
842549	CAJJJL010078940.1	unplaced scaffolds	ATATAC	TACATA	6	21	25178	25303	126	266
748038	CAJJJL010071957.1	unplaced scaffolds	ATATAC	ATATAC	6	21	392423	392548	126	265
760649	CAJJJL010072924.1	unplaced scaffolds	ATATAC	ATATAC	6	21	613317	613442	126	262
387719	CAJJJL010042945.1	unplaced scaffolds	ATATAC	ATACAT	6	21	239189	239314	126	261
708540	CAJJJL010068895.1	unplaced scaffolds	ATATAC	ATGTAT	6	21	327567	327692	126	261
837302	CAJJJL010078556.1	unplaced scaffolds	ATATAC	TATGTA	6	21	98761	98886	126	250
428243	CAJJJL010046262.1	unplaced scaffolds	ATAC	TATG	4	31	165871	165994	124	275
810408	CAJJJL010076648.1	unplaced scaffolds	ATAC	GTAT	4	31	189521	189644	124	264
712735	CAJJJL010069244.1	unplaced scaffolds	AT	AT	2	61	129129	129250	122	294
466854	CAJJJL010049542.1	unplaced scaffolds	ATATAC	TACATA	6	20	51639	51758	120	295
363537	CAJJJL010041178.1	unplaced scaffolds	ATATAC	ATGTAT	6	20	16082	16201	120	289
806949	CAJJJL010076383.1	unplaced scaffolds	ATATAC	TATATG	6	20	633853	633972	120	288
284119	CAJJJL010034254.1	unplaced scaffolds	ATATAC	TATATG	6	20	458273	458392	120	284
272291	CAJJJL010033180.1	unplaced scaffolds	ATATAC	ATATAC	6	20	101278	101397	120	274
490329	CAJJJL010051335.1	unplaced scaffolds	ATATAC	ATACAT	6	20	677656	677775	120	272
691588	CAJJJL010067668.1	unplaced scaffolds	ATATAC	ATACAT	6	20	490204	490323	120	270
507323	CAJJJL010052684.1	unplaced scaffolds	ATATAC	TATACA	6	20	69421	69540	120	268
160614	CAJJJL010003722.1	unplaced scaffolds	ATATAC	TATATG	6	20	14167	14286	120	258
648174	CAJJJL010064146.1	unplaced scaffolds	ATATAC	ATATAC	6	20	38600	38719	120	258
890979	CAJJJL010082237.1	unplaced scaffolds	ATATAC	TATATG	6	20	200069	200188	120	256
506181	CAJJJL010052603.1	unplaced scaffolds	ATATAC	ATATGT	6	20	6435	6554	120	255
478743	CAJJJL010050505.1	unplaced scaffolds	ATAC	ACAT	4	30	365589	365708	120	289
156803	CAJJJL010002591.1	unplaced scaffolds	ATAC	ATAC	4	30	296969	297088	120	282
721259	CAJJJL010069930.1	unplaced scaffolds	ATAC	GTAT	4	30	23647	23766	120	281

GTGATCCAAAGATTATTTCCAAGTTGG	59.24	37.04	3.77	TCAAAAGCCTTTCCATAATCCATAGG	59.45	38.46	2.57	ACKI046
AGGCAATCAAAGTGATCAGGC	58.9	47.62	4.85	TGGTAGTTGCATACTTTCTGTGC	59.25	43.48	4.57	ACKI047
AAGTCTAAATTAATTTCGATCCAGCC	58.07	34.62	4.85	TGCACATTTATATGTTGCACTTGC	59.14	37.5	4.01	ACKI048
GAAGGTGCTGCTGAAGATATGG	59.12	50	2.74	CGAGTTTCCCTAGTGTTTCTCC	58.41	50	3.71	ACKI050
GGGTTTGTTTAGCACCCTTAAGG	59.74	47.83	2.69	TCATGCTACTACAACCTTGATGG	58.23	43.48	3.51	ACKI052
GAGTTATGAAGGCGTTCGTCG	59.42	52.38	5.12	TTCTTATCTCGATACACCTTGAGC	58.17	41.67	4.26	ACKI053
TCTTGGTTGATCAGAGTTTACTGC	59	41.67	4.4	TTCCGGAGACATGCATGAGG	59.82	55	3.86	ACKI054
GCGGTGGTTCTTTTCAAAACC	58.8	47.62	3.27	AACCTTCTGATACAGTCGAAAGG	58.18	43.48	3.11	ACKI055
AGGAAGTGCTTTGCTGTTGC	59.61	50	4.17	AACAGAACTGCACTGGCTCG	60.88	55	5.03	ACKI056
TTTTTAGTTAGGGGACAGTTCTAGC	58.53	40	3.42	GCATCGTGAGCATTAATGGTAGG	59.81	47.83	3.18	ACKI057
ACGTGGTCATACATACATAAGTACG	58.57	40	3.67	ACCTCACATCTTAGAGTGTATATGGC	60.02	42.31	4.4	ACKI058
AGACATATAAAATGAGACAGCAAGCG	60.02	38.46	4.68	AAGCTTGGAGTTGCCTCTGG	60.25	55	3.86	ACKI059
AGAAGCAAACAAATGAACACAACC	59.13	37.5	3.77	TAGGCCACCTGGTTGTTAGC	59.67	55	3.09	ACKI061
AAATTTATAAATTGCAGTCTAGGGTCC	58.09	33.33	4.46	TGGCTATGTTACACATGTGCG	58.99	47.62	5.34	ACKI062
CACTATTGGCTTGGAGATAATGGG	59.24	45.83	4	TAAATCGTGCGAAAAGGCGG	59.56	50	6.13	ACKI065
ATGTACACGTTTACATATTGCACG	58.21	37.5	5.34	AATGGCGTGTTGTAGAGTGC	58.84	50	4.4	ACKI066
CACCCATCTTTGTAGCCACG	58.91	55	4.94	ATGAGGGCGACTCTTTTGGC	60.68	55	4.52	ACKI068
ATCTTTGTACGAACCGTTCCC	58.31	47.62	3.97	ACAAGTGGAGATAGTATCTTGGTCC	59.64	44	4.46	ACKI069
TCCCACCACTTAGACCATAAGC	59.5	50	3.09	AGGAATGAGATACTTGTGTGTTGC	59.3	41.67	4.17	ACKI070
CCAATGTTCCAAATCAACTGTAAAACC	60.04	37.04	3.27	AAAAGGCTGATTTCAAAAATCAAACC	58.29	30.77	3.27	ACKI071
GCCTAACCTACTCGAGTATAAAATAGC	59.25	40.74	2.97	TGTTTCCTTGTCCATGGGGG	59.89	55	5.4	ACKI072
GAATTGGCTTGGGGATAATGGG	59.37	50	4	TGTCATCAAAACCATCAATCTCAAGG	60.07	38.46	3.61	ACKI073
TGCTAGGAGTTCGAGATTTTTCG	58.83	43.48	3.46	TGGGTTGAATTATAAGAGAATGGGC	59.17	40	5.36	ACKI074
CAGATTGATTGGCAGATTGTTGC	59.14	43.48	4.17	ATCTCACAAACGTAGCAGAGC	58.39	47.62	4.09	ACKI075
TGCATGCGACTTATACACTTTAGG	59.19	41.67	2.69	ATGGATCGAGAGTGCACACG	60.18	55	4.49	ACKI076
TGAGTGAGAGAGAGCCAAAGC	59.73	52.38	3.51	TTGTGGAAGCAAGTGCAAGG	59.25	50	3.61	ACKI078
GGGGATATGGTTAATACCTTAACGC	59.35	44	4.84	AGAGGATGCTTGGAATTCGC	58.33	50	4.7	ACKI079
TTTGTTACTGCATTTCCTCAATCG	58.3	37.5	3.34	AAATCATGAATATGGAGCAAAAGGG	58.18	36	3.95	ACKI080

167785	CAJJJL010005949.1	unplaced scaffolds	ATAC	ACAT	4	30	134001	134120	120	265
284755	CAJJJL010034307.1	unplaced scaffolds	ATAC	ATAC	4	30	876952	877071	120	263
255180	CAJJJL010030243.1	unplaced scaffolds	ATAC	ATAC	4	30	714450	714569	120	260
208180	CAJJJL010017977.1	unplaced scaffolds	ATAC	ATAC	4	30	860709	860828	120	256
623554	CAJJJL010062209.1	unplaced scaffolds	ATG	TAG	3	40	152993	153112	120	250
353916	CAJJJL010040373.1	unplaced scaffolds	ATAC	GTAT	4	29	43278	43393	116	283
606859	CAJJJL010060751.1	unplaced scaffolds	ATAC	CATA	4	29	99171	99286	116	283
534535	CAJJJL010054901.1	unplaced scaffolds	ATAC	GTAT	4	29	192609	192724	116	282
852057	CAJJJL010079531.1	unplaced scaffolds	ATAC	TATG	4	29	72311	72426	116	264
642284	CAJJJL010063688.1	unplaced scaffolds	ATAC	TATG	4	29	84259	84374	116	258
410235	CAJJJL010044826.1	unplaced scaffolds	ATAC	TATG	4	29	559366	559481	116	255
475361	CAJJJL010050227.1	unplaced scaffolds	ATAC	TATG	4	29	246297	246412	116	250
818093	CAJJJL010077198.1	unplaced scaffolds	AC	CA	2	58	97974	98089	116	255
790416	CAJJJL010075182.1	unplaced scaffolds	ATATAC	TGTATA	6	19	193934	194047	114	275
731101	CAJJJL010070719.1	unplaced scaffolds	ATATAC	ATATAC	6	19	151965	152078	114	266
211161	CAJJJL010018851.1	unplaced scaffolds	ATATAC	ATACAT	6	19	297796	297909	114	262
765081	CAJJJL010073252.1	unplaced scaffolds	ATATAC	TATGTA	6	19	718921	719034	114	261
834713	CAJJJL010078371.1	unplaced scaffolds	ATATAC	TATATG	6	19	825882	825995	114	252
324671	CAJJJL010038096.1	unplaced scaffolds	ATATAC	ACATAT	6	19	22888	23001	114	250
161806	CAJJJL010004067.1	unplaced scaffolds	ATAC	TATG	4	28	21664	21775	112	300
587907	CAJJJL010059181.1	unplaced scaffolds	ATAC	ATAC	4	28	343070	343181	112	290
650389	CAJJJL010064329.1	unplaced scaffolds	ATAC	CATA	4	28	272902	273013	112	284
788609	CAJJJL010075053.1	unplaced scaffolds	ATAC	GTAT	4	28	56713	56824	112	282
731655	CAJJJL010070756.1	unplaced scaffolds	ATAC	TATG	4	28	141884	141995	112	280
356711	CAJJJL010040608.1	unplaced scaffolds	ATAC	TATG	4	28	192117	192228	112	268
363726	CAJJJL010041190.1	unplaced scaffolds	ATAC	ACAT	4	28	364797	364908	112	262
647456	CAJJJL010064102.1	unplaced scaffolds	ATAC	TATG	4	28	66449	66560	112	261
534728	CAJJJL010054917.1	unplaced scaffolds	ATAC	ATAC	4	28	69076	69187	112	257

TGGTATCATGGTATGGAGCCC	59.01	52.38	5.19	ATCCCCCGTTGTAACCAACC	59.96	55	3.77	ACKI081
AAACAGAGTGAAAACTGACATGC	58.07	39.13	4.06	TCTAAGTTAAATGATGCTTGTTTGTGG	58.92	33.33	4.17	ACKI082
ACGATATATCCAACCTTCTCTGAGG	59.52	44	3.86	TTGTCATTGGGTCTGCCTCC	59.96	55	4.3	ACKI083
ACATTTGGTATCAGAGCACTTGG	58.99	43.48	3.61	AGCTGATCTAACAACCAAGGAGG	60.05	47.83	4.3	ACKI084
GTTTGGATCAACGGACGACC	59.21	55	4.79	ACCGTTGAGTGCTTAGAAATGC	59.52	45.45	3.56	ACKI085
AGGCTCGAGTCAGCTAATGG	59.25	55	3.16	CTATATCATGCAACCAAAACTGGC	58.72	41.67	4.85	ACKI087
CTCATTCATGCAGCGTTCCC	59.62	55	3.97	CATTCATATACCGACCCCCTCC	59.76	54.55	4.3	ACKI088
CTCAAGAACTGAACACCGGC	59.13	55	6.13	GGGAGAGGCCACTTCAATAGC	60.48	57.14	2.97	ACKI089
TGATTTGTACCTGATGTATCTTGTTGC	60.15	37.04	4.17	AATACAGAGTATTTAATAATGCAGCGG	58.28	33.33	5.52	ACKI090
GTTGACTGCAGCATCATCGG	59.62	55	4.18	AGCACTTAACCAAAAGGTAAGATGC	60.05	40	3.91	ACKI091
TGCACTTAAGGGCATTAGTTTAGG	59.05	41.67	2.69	CCTATAGGAATCCCACCCGC	59.39	60	6.13	ACKI092
ACGACTTGTTGGTGACACCG	60.81	55	4.94	ATGTAACACAGACCAGGGGC	59.67	55	5.8	ACKI093
GCTATCAAGAAGAATTTGAGTTGTAGC	59.09	37.04	3.58	AATCGCAAATATGACGCCGG	59.41	50	6.13	ACKI095
AAGCGAATCAACACTACGGG	58.28	50	5.28	TTATAGACTTGAGATGAGATTTGGGG	58.26	38.46	4.96	ACKI096
GATCAACACATGCGCTCGC	60.3	57.89	5.03	CTTTTCGTGGCTATAGATTATTGGC	58.4	40	4.52	ACKI097
ACCATGAATCAAGGACCCGC	60.39	55	6.13	CGACTTTAGATTGAGTAGTTGGCG	59.73	45.83	5.69	ACKI098
AAATGCCATGGTAGGGGTCG	60.11	55	4.79	CACATGAATTCCCGATCCATGC	60.03	50	4.06	ACKI099
AGCGAGGATAGTATCATGGTCC	58.91	50	4.46	CGTTGATTCCCTTACATATACATATGC	58.4	37.04	3.14	ACKI101
ATGGATCGAGAGTGCACACG	60.18	55	4.49	TGCATGCGACTTATACACTTTAGG	59.19	41.67	2.69	ACKI102
CCAATTTAAAACGAACAAGATGTGC	58.42	36	4.57	AGCTCTGTAGGTTGTGGTTGG	59.93	52.38	3.77	ACKI104
CGTTTCCTGCCTTCATATCTTCC	59.44	47.83	3.46	ATCCAATGAATCAGAAAATAAGATCGG	58.21	33.33	4.18	ACKI105
CATCTTGCGTGAAGAAATTGCC	59.08	45.45	4.52	AGCAGAATGTCTCGATAGGAGG	59.11	50	4.3	ACKI106
TCCCAAAAATCAAAAGCCTTTTCC	58.87	37.5	3.13	CTCCCCTTGTTGAATCTTAGCC	58.72	50	3.93	ACKI107
AGTCTAAAATTCCAAGTGTGTAGGG	58.76	40	3.53	CTCTTCTGGATGGTCCTCCC	58.87	60	4.3	ACKI108
CGGCATGTGCATTGATTTTGC	59.94	47.62	3.68	TGTAACACACACATACACTCCC	58	45.45	4.3	ACKI109
GCTACAAACATCGAGAGTTTTTGC	59.39	41.67	3.68	TCATTTAAATTGAAATCAACGCCCC	59.13	36	5.8	ACKI110
TTGTACAAGTGGCGTACCGG	60.32	55	5.28	CCCATAGTCGCCTCATTCATCC	60.61	54.55	3.51	ACKI111
TGGTTGTTTGGTTGAGTTTCCG	59.84	45.45	4.3	TGGTGAAAGACCCCTTAGCG	59.68	55	4.26	ACKI112

287074	CAJJJL010034565.1	unplaced scaffolds	ATATAC	TATATG	6	18	208740	208847	108	300
792006	CAJJJL010075281.1	unplaced scaffolds	AATCGT	AATGCT	6	18	94467	94574	108	295
373382	CAJJJL010041880.1	unplaced scaffolds	ATATAC	TATGTA	6	18	6217	6324	108	290
241296	CAJJJL010027900.1	unplaced scaffolds	ATATAC	GTATAT	6	18	48196	48303	108	287
303147	CAJJJL010036345.1	unplaced scaffolds	ATATAC	TATATG	6	18	314603	314710	108	285
541899	CAJJJL010055444.1	unplaced scaffolds	ATACAC	ATACAC	6	18	251485	251592	108	280
342712	CAJJJL010039506.1	unplaced scaffolds	AATAGC	CTATTG	6	18	170199	170306	108	276
292487	CAJJJL010035277.1	unplaced scaffolds	ATATAC	CATATA	6	18	46623	46730	108	275
254838	CAJJJL010030176.1	unplaced scaffolds	ATATAC	ATACAT	6	18	201937	202044	108	272
812596	CAJJJL010076819.1	unplaced scaffolds	ATATAC	ACATAT	6	18	586585	586692	108	270
815246	CAJJJL010076994.1	unplaced scaffolds	ATATAC	TATACA	6	18	37776	37883	108	268
478380	CAJJJL010050482.1	unplaced scaffolds	ATATAC	TATGTA	6	18	876524	876631	108	259
479362	CAJJJL010050534.1	unplaced scaffolds	AAATAC	TGTATT	6	18	406739	406846	108	255
601292	CAJJJL010060290.1	unplaced scaffolds	ATATAC	ATATAC	6	18	55592	55699	108	253
415438	CAJJJL010045205.1	unplaced scaffolds	ATAC	GTAT	4	27	258716	258823	108	291
545881	CAJJJL010055745.1	unplaced scaffolds	ATAC	ATAC	4	27	86654	86761	108	288
552904	CAJJJL010056319.1	unplaced scaffolds	ATAC	ATAC	4	27	241951	242058	108	287
767162	CAJJJL010073419.1	unplaced scaffolds	ATAC	ATAC	4	27	398767	398874	108	281
714403	CAJJJL010069378.1	unplaced scaffolds	ATAC	TATG	4	27	608355	608462	108	273
435301	CAJJJL010046821.1	unplaced scaffolds	ACGC	GTGC	4	27	125141	125248	108	261
610735	CAJJJL010061088.1	unplaced scaffolds	ATAC	ACAT	4	27	291906	292013	108	253
688563	CAJJJL010067432.1	unplaced scaffolds	ATAC	ATAC	4	27	94445	94552	108	252
541664	CAJJJL010055426.1	unplaced scaffolds	AT	TA	2	54	545805	545912	108	255
813921	CAJJJL010076915.1	unplaced scaffolds	AG	AG	2	53	164384	164489	106	286
210114	CAJJJL010018525.1	unplaced scaffolds	AATAC	ACAAT	5	21	45785	45889	105	271
591988	CAJJJL010059523.1	unplaced scaffolds	ATAC	ATAC	4	26	485110	485213	104	281
802433	CAJJJL010076041.1	unplaced scaffolds	ATAC	TGTA	4	26	440124	440227	104	278
640886	CAJJJL010063569.1	unplaced scaffolds	ATAC	ATAC	4	26	485283	485386	104	271

ACAACAATTCAATGCCAAGCC	58.23	42.86	4.35	TCCACCTACATATGTTTAACACCC	58.25	41.67	4.61	ACKI114
TTGTTGAGATAGGTTATAGAGCACC	58.18	40	5.01	GTGTGTGCCAGATTTCCTATGC	59.9	50	3.14	ACKI115
GCTATGGGTTTCACTGTGATTGC	60.43	47.83	3.56	TGAAGGCCATAAAATCAAGGAGC	59.3	43.48	4.7	ACKI116
GACCCTAATGCTGGTCGACC	60.18	60	4.79	GGTCCGTGGTAATTTTCTGAGC	59.58	50	4.26	ACKI117
TCGAAAAAGTGGGTCGTTGC	59.34	50	4.17	GTTGTTTCAATGACCTCGATTTCC	58.85	41.67	3.13	ACKI118
TGGGTCAAAGCGAATGTTGC	59.69	50	4.17	GGGGAAGTTAGGAAGGCATGG	60.41	57.14	3.66	ACKI119
TGTATCTCGTGTATTTTGTAGCGG	58.96	41.67	5.52	CGTAGAATCTTATAACACGATGGAGC	59.69	42.31	4.7	ACKI120
CAAGGACTGCACAAGATAGGC	58.99	52.38	3.93	TCTTAATTTTGGCCTCAACCCC	58.83	45.45	4.95	ACKI121
AACTACTTCACTGTCATTTCATTGC	58.33	36	3.56	CTAGCATAGTGACAAACATGTGC	58.1	43.48	4.57	ACKI122
TCATCATTCTTCTCCTAACCAAAGC	59.12	40	3.51	TGACCATCATCGATCATATACATAGC	58.57	38.46	2.97	ACKI123
AGCGAACACTTAGCCTTTTGG	59.12	47.62	3.28	GGACGAGATAAATCTTGACAGAAGG	59.25	44	3.46	ACKI124
AGGATCGATGCGGTCTTTGG	60.18	55	3.28	GCCTTTATGCGTGAATCGTACC	60.03	50	3.34	ACKI126
GAATGGATTGGATGGCTTGGG	59.31	52.38	4.12	TGCTAACTCATCATGTGATAGGTCC	60.22	44	4.46	ACKI127
TCCGAAAGACAAGTCACTGGG	59.93	52.38	4.45	TCAGTATGTAGGGACCATGTCC	58.69	50	4.02	ACKI128
TCTCTTCTTTCATTGCCTTGACC	58.93	43.48	4.02	AGTAAAAGTGCCAGATACCCCC	59.76	50	5.4	ACKI129
ACAACCAAAATACCAAACCCACC	59.87	43.48	4.61	GTCAATGCCCGGGGAAGG	60.44	66.67	3.46	ACKI131
TGTTTTTGCGTTTTGAATCGACC	59.45	39.13	4.79	TCTCCACCATTTTCTCTCTCTTCC	59.78	45.83	3.46	ACKI132
TCTTAAACCATTTTGAGCCGAACC	60.02	41.67	3.62	AGCTCAAAGCAATTCAACAATTGG	59.25	37.5	3.16	ACKI134
TCTGGAGGAAGAACAACTACAGC	59.99	47.83	4.4	CGAGAAAAGAATCAGCACACCG	60.16	50	4.94	ACKI135
TCATATGTGTTACGATAACAGATGCC	59.35	38.46	4.4	GTCCGGATCTTTTGTGTCACC	59.2	52.38	4.02	ACKI136
TCATTCTTCAGCAATCAATACCTGC	59.93	40	4.85	ATATACACTAACACTGTTGGCTAGG	58.18	40	3.02	ACKI137
AAACAAATTAAAGTGACCTGGGC	58.04	39.13	5.36	AATAGTCATACTGCGTGCGC	58.79	50	6.09	ACKI138
CTCCGCTAGCAGTCGTAACC	60.25	60	2.85	ACGAATGGCAGACTGGATCG	60.18	55	3.69	ACKI139
AAGTCTCGGCCAAAACCTCG	60.6	55	4.63	AGTCACCGTGTTTTTGTCGC	59.62	50	5.19	ACKI140
AGAGAACACAGAGTCAAAATCATTGG	59.79	38.46	3.16	TCAGGAACAATGAGGCCAGC	60.32	55	4.85	ACKI141
TGATGGCTAATCCGTTCAATCC	58.52	45.45	3.01	TCATCGCTTTGGGCTACTAGC	60.2	52.38	3.42	ACKI143
TGATCCACAAAAGTGAAGAGTGC	59.43	43.48	4.4	CACACACACACACACACACC	59.83	55	4.16	ACKI144
TGTGGAGCTAGTACTTTCCTTCC	59.49	47.83	3.46	CGGGAGGTGAACACAATTGG	59.12	55	3.16	ACKI146

758912	CAJJJL010072804.1	unplaced scaffolds	ATAC	CATA	4	26	59297	59400	104	256
348037	CAJJJL010039908.1	unplaced scaffolds	ATAC	ATAC	4	26	192179	192282	104	252
826631	CAJJJL010077861.1	unplaced scaffolds	ATATAC	TATACA	6	17	67138	67239	102	285
202756	CAJJJL010016580.1	unplaced scaffolds	ATATAC	ACATAT	6	17	952134	952235	102	275
241661	CAJJJL010027974.1	unplaced scaffolds	ATATAC	ACATAT	6	17	191262	191363	102	269
189371	CAJJJL010012674.1	unplaced scaffolds	ATATAC	TGTATA	6	17	11016	11117	102	266
324300	CAJJJL010038068.1	unplaced scaffolds	ATATAC	TATACA	6	17	498943	499044	102	264
367627	CAJJJL010041476.1	unplaced scaffolds	ATATAC	TATGTA	6	17	757148	757249	102	264
508483	CAJJJL010052756.1	unplaced scaffolds	ATATAC	TATACA	6	17	880402	880503	102	262
695476	CAJJJL010067977.1	unplaced scaffolds	AATACT	ATACTA	6	17	50675	50776	102	255
554045	CAJJJL010056421.1	unplaced scaffolds	ATATAC	ATACAT	6	17	994174	994275	102	254
405289	CAJJJL010044416.1	unplaced scaffolds	ATATAC	CATATA	6	17	507414	507515	102	253
487777	CAJJJL010051138.1	unplaced scaffolds	ATATAC	ATATAC	6	17	51438	51539	102	253
475409	CAJJJL010050231.1	unplaced scaffolds	ATATAC	ATATGT	6	17	351797	351898	102	252
670744	CAJJJL010066042.1	unplaced scaffolds	ATAC	TGTA	4	25	87210	87309	100	285
330469	CAJJJL010038528.1	unplaced scaffolds	ATAC	ACAT	4	25	42216	42315	100	267
731656	CAJJJL010070756.1	unplaced scaffolds	ATAC	TATG	4	25	158058	158157	100	265
377554	CAJJJL010042179.1	unplaced scaffolds	ATAC	TATG	4	25	290575	290674	100	259
758964	CAJJJL010072804.1	unplaced scaffolds	ATAC	TATG	4	25	801201	801300	100	257
866716	CAJJJL010080475.1	unplaced scaffolds	ATAC	ATAC	4	25	387043	387142	100	256

TGTGTCTGTCGGAAGTGAACC	60.2	52.38	3.62	TGGCCTAGGTGACATAGAAGC	59.24	52.38	3.86	ACKI148
GTCGCACTTCATTTCGATTGC	58.83	47.62	3.56	ATCACAACAAAGGCAACGGC	59.97	50	5.68	ACKI150
TGCTGCGTTTTATTCTTCTCGC	60.16	45.45	5.03	GCAACGTCAAGATAAGCGGC	60.25	55	6.53	ACKI152
ATGTGCTTTCAACTGAACTTGC	58.29	40.91	4.01	TGGAAGTTAATCCATGAATAACGGC	59.7	40	5.68	ACKI154
TGTCCATAATGCTCACGTGC	58.63	50	5.34	TGATACCATGATCCAAAAGAGATGG	58.7	40	3.51	ACKI155
TGGCTAGGGTATTCTTTCGGG	59.23	52.38	5.14	CCATGTGATTCCATCTACGCG	59.21	52.38	6.01	ACKI156
ACGCCTGACTCGAACTATGC	60.18	55	3.14	TGTGTGTGTGTGTATGTATGTATGG	59.13	40	2.74	ACKI157
AGCGGAGATGAAAACTTGTGG	58.85	47.62	4.17	TCCCAAAGCACAATATCTCAGG	58.11	45.45	3.86	ACKI158
TGTCCCAAACATTTTATAGGTTTGC	58.54	36	3.68	GTAGATCTATTCATTGGAGTTGTAAGC	58.02	37.04	3.09	ACKI159
TGGATTTATGCACGTGAATGCC	59.9	45.45	4.4	ACATTGATTAAAAGTTACCGGCCG	60.14	41.67	6.13	ACKI160
CTGGCCTAGGTGACACATGG	60.11	60	3.66	GCCCCAGATCCATGACACC	60.15	63.16	4.16	ACKI161
CGCTTACTCCAAACAAGCATGG	60.41	50	3.66	GGTCGATTGATTTCTTTTCTTCAAAGC	60.15	37.04	3.51	ACKI162
ACATATAAGTGTGCGTGTGGG	58.37	47.62	4.61	TGTATATGTGCTAGGATTTACATCGC	59.19	38.46	4.58	ACKI163
TGCTACTGGATGACATAAATATGTGG	59.06	38.46	4.17	TGCTTTTCGACGTGTATTACCG	59.34	45.45	4.02	ACKI165
GCTCAAAGGCACCAGAGACG	61.29	60	4.18	CTGGTGCACTTATCGTTGCG	59.9	55	4.85	ACKI166
ATTTACGCTGTTTCTCAATATACATCC	58.23	33.33	3.51	GGTCTATTAAAAAGCACATGATATGGC	59.19	37.04	4.4	ACKI167
AGTCTAAAATTCCAGTGTGTAGGG	58.19	41.67	3.53	CTCTTCTGGATGGTCCGTCC	59.54	60	4.79	ACKI168
ACCAAACGAGAACTGATGCC	58.48	50	4.4	TGCCTATGTTATTTCAGTCACTGC	59.36	41.67	4.4	ACKI169
TGTGTATAGTGTACTCACCGGC	59.58	50	6.13	GGGTCGTTACATACTTCTCCCC	59.9	54.55	4.81	ACKI170
AGATAAGGAAGGAGTTACTGCCG	59.61	47.83	5.69	ACAAGCTATAACAAGAATGATCAGCC	59.74	38.46	4.85	ACKI171