Genetics and population analysis

KASPspoon: an *in vitro* and *in silico* PCR analysis tool for high-throughput SNP genotyping

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Associate Editor: Alfonso Valencia

Received on March 22, 2018; revised on December 15, 2018; editorial decision on December 29, 2018; accepted on January 4, 2019

Abstract

**Motivation:** Fine mapping becomes a routine trial following quantitative trait loci (QTL) mapping studies to shrink the size of genomic segments underlying causal variants. The availability of whole genome sequences can facilitate the development of high marker density and predict gene content in genomic segments of interest. Correlations between genetic and physical positions of these loci require handling of different experimental genetic data types, and ultimately converting them into positioning markers using a routine and efficient tool.

**Results:** To convert classical QTL markers into KASP assay primers, KASPspoon simulates a PCR by running an approximate-match searching analysis on user-entered primer pairs against the provided sequences, and then comparing *in vitro* and *in silico* PCR results. KASPspoon reports amplimers close to or adjoining genes/SNPs/simple sequence repeats and those that are shared between *in vitro* and *in silico* PCR results to select the most appropriate amplimers for gene discovery. KASPspoon compares physical and genetic maps, and reports the primer set genome coverage for PCR-walking. KASPspoon could be used to design KASP assay primers to convert QTL acquired by classical molecular markers into high-throughput genotyping assays and to provide major SNP resource for the dissection of genotypic and phenotypic variation. In addition to human-readable output files, KASPspoon creates Circos configurations that illustrate different *in silico* and *in vitro* results.

**Availability and implementation:** Code available under GNU GPL at (http://www.ageri.sci.eg/index.php/facilities-services/ageri-softwares/kasp spoon).

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**Supplementary information:** Supplementary data are available at *Bioinformatics* online.

1 Introduction

Classically acquired quantitative trait loci (QTL) have been introduced using numerous field trials and laboratory experiments with significant correlations with economical useful traits. The expansion in genome sequencing avenues has provided public genome repositories with massive amounts of biological information regarding different genome sequences and single nucleotide variations (Doddamani et al., 2015). Simultaneously, advanced molecular marker technologies provide extremely high levels of assay robustness and accuracy with significant cost savings. An example of these technologies, the KASP assay (He et al., 2014), has been efficiently
used to detect and validate nucleotide variations, single nucleotide polymorphisms (SNPs)/InDels, related to important traits across different organisms (Kolmer et al., 2018).

As a result, harvesting more gain from published QTL across different organisms requires correlations between genetic and physical positions of these loci, handling different experimental genetic data types, and ultimately converting them into positioning markers using a routine and fast computational tool. In silico PCR is one of the most widely used techniques to determine the physical position of these loci. In silico PCR is a computational procedure that estimates PCR results theoretically using a given set of primers to amplify DNA sequences from a sequenced genome or transcriptome (Lexa et al., 2001).

In recent years, a plethora of software programs have been developed to aid in silico PCR analysis, including Primer-BLAST (Ye et al., 2012), SNPCheck (http://ngrl.man.ac.uk), FastPCR (Kalendar et al., 2011), Primerssearch-EMBOSS (Rice et al., 2000) and PUNS (Boutros and Okey, 2004). Nevertheless, researchers have a long journey when decreasing and correlating prior or subsequent information regarding the genetic data of these markers to obtain conclusive results they can employ.

This study presents a PCR primer test application, called ‘KASPspoon,’ for routine manipulation and analysis of PCR primers. The final and main goal of KASPspoon is converting classically acquired QTL information into more comprehensive, routine and accurate molecular marker technologies such as a KASP assay. To reach this goal, KASPspoon can be used efficiently to compare in vitro (laboratory observed) and in silico (predicted) PCR results, and reports SNPs that are close to or adjoined by PCR amplifiers by integrating a database for known SNPs. KASPspoon uses this information to design KASP assay primers to convert QTL markers into PCR-amplified chromosomal regions. Report files that contain all SSRs that lie between the PCR-amplified regions.

When genetic linkage map is provided, KASPspoon can compare between the physical (bp) and genetic (cM) positions for PCR markers, where information provided by in silico PCR analysis will be used to assign genetic linkage group(s) to physical chromosome(s).

If a list of SNP variations is provided, KASPspoon can generate KASP assay primers that can be used for SNP genotyping. These KASP primers are designed to target all SNPs that are nearby PCR-amplified chromosomal regions. Report files that contain all KASP-targeted genes and marker loci are generated. The KASP sequences are designed according to a KASP primer design manual published by LGC (www.lgcgroup.com). Primer3 tool (Untergasser et al., 2012) was used to design two allele-specific forward primers, and a common reverse primer for allele-specific assays such as KASP assay. These primers designed by KASPspoon use a user-provided SNP database to create degenerate PCR primers to provide primers with minimal mismatches, where the target nucleotide is marked by ‘[]’ and untargeted nucleotides are masked according to IUPAC codes.

KASPspoon will generate different Circos configurations for in silico PCR statistical results, comparison between in silico and in vitro PCR data and linkages, and in silico (physical) maps.

The search returns a sequence output file in FASTA format, containing all sequences in the database that lie between, and include, the primer pair. FASTA header describes the region in the database and primer(s) names. Comma-separated output files generated by KASPspoon include:

1. in silico PCR-generated amplimer information
2. in silico amplimers near/adjoining genes
3. SNPs near in silico amplimers in VCF
4. in silico amplimers adjoining SSRs
5. in vitro and in silico amplimers acquired by the same PCR primer that share the same approximate band size
6. location of genes adjoining or close to PCR primer regions
7. genomic areas that are covered using this primer set
8. primer set coverage statistics with both base-pair and percentage scales (compared to length of total genome sequence covered and chromosomal sequence length)
9. chromosomal assignment for linkage genetic groups
10. KASP primers’ sequence and information
11. final report files containing different information about this run in an abbreviated form.

2 Design and implementation

KASPspoon handles a different experimental genetic data type, correlates between the genetic and physical position of loci, and generates ultimate positioning markers for KASP assay. Snapshots of KASPspoon outputs are shown in Supplementary File S1.

KASPspoon was developed as a stand-alone package for PCR primer analysis using both C and Perl programming languages. The Boyer–Moore–Horspool (Horspool, 1980) and Baeza-Yates–Perleberg (Baeza-Yates and Perleberg, 1992) string approximate-matching algorithms were used through C to search genomic sequences provided PCR primer-pair sequences were used as queries. For primer genome coverage statistics and PCR-walking procedure, the overlap layout consensus algorithm with a user-defined gap between amplifiers is used to report primer(s’) covered area (Supplementary File S1).

KASPspoon uses common biological data formats as an input and only amplifiers that do not exceed user-defined maximum primer mismatch or maximum total mismatch (forward primer mismatch + reverse primer mismatch), or those that do not have a mismatch in the first user-defined 3’ nucleotides are reported (Supplementary File S1). KASPspoon can compare in silico (predict PCR product size) and in vitro PCR results (observed PCR product size) by defining the maximum molecular weight mismatch between the in silico and in vitro amplifiers if the in vitro PCR product length (in base pairs) is provided (approximately). For comparing in silico and in vitro PCR amplifiers, KASPspoon generates a text file containing amplimers that exist in both and that contain (or do not) genes. In addition, MISA Perl script (pgrc.ipk-gatersleben.de/misa/) is integrated inside KASPspoon tool to report all SSRs that lie between the PCR-amplified regions.

As a result, harvesting more gain from published QTL across different organisms requires correlations between genetic and physical positions of these loci, handling different experimental genetic data types, and ultimately converting them into positioning markers using a routine and fast computational tool. In silico PCR is one of the most widely used techniques to determine the physical position of these loci. In silico PCR is a computational procedure that estimates PCR results theoretically using a given set of primers to amplify DNA sequences from a sequenced genome or transcriptome (Lexa et al., 2001).

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9. chromosomal assignment for linkage genetic groups
10. KASP primers’ sequence and information
11. final report files containing different information about this run in an abbreviated form.
Supplementary File S2) produced 892 bands, covering 168,082 bp (0.048%) of the chromosomal genome, in which 89.6% were chromosome-specific and 24% had genes nearby (Supplementary File S4) and investigate SNP effects using SnpEff was used to detect SNPs nearby and to design KASP primers (Doddamani et al., 2010) was used to compare the genetic and in silico map position for 241 primers. Most of these markers were successfully assigned to corresponding chromosomes as assumed by Nayak et al. (2010), and others had a high number of markers belonging to other chromosomes. Chickpea SNP database (Doddamani et al., 2015) was used to detect SNPs nearby and to design KASP primers (Supplementary File S4) and investigate SNP effects using SnpEff tool (Cingolani et al., 2012). About 99.41% of the additional SNPs were ‘MODIFIER.’ Two had a ‘HIGH’ impact on two chickpea uncharacterized proteins.

### 3 Results

Although KASPspoon showed a medium processing speed compared to tools uses BLAST as a search engine such as Primer-BLAST and SNPCheck, KASPspoon provides several additional advantages that are lacking in some or all published tools, such as being free, source code availability, primer coverage, in silico and in vitro PCR comparison, SNP or SSR or anchor primer reporting, physical and linkage map comparison, output graphical illustration, KASP primer design and human readable and easy manipulated outputs (Table 1). The 462 previously published chickpea SSR markers (Supplementary File S2) produced 892 bands, covering 168,082 bp (0.048%) of the chromosomal genome, in which 89.6% were chromosome-specific and 24% had genes nearby (Supplementary Files S1 and S3). Published chickpea linkage map (Nayak et al., 2010) was used to compare the genetic and in silico map position for 241 primers. Most of these markers were successfully assigned to corresponding chromosomes as assumed by Nayak et al. (2010), and others had a high number of markers belonging to other chromosomes. Chickpea SNP database (Doddamani et al., 2015) was used to detect SNPs nearby and to design KASP primers (Supplementary File S4) and investigate SNP effects using SnpEff tool (Cingolani et al., 2012). About 99.41% of the additional SNPs were ‘MODIFIER.’ Two had a ‘HIGH’ impact on two chickpea uncharacterized proteins.

### 4 Conclusion

KASPspoon could be successfully integrated in different genomics procedures such as primer design, genome mapping, QTL fine mapping, genome wide association analysis, PCR-walking and SNP genotyping. Combining KASPspoon with SNP selection programs such as SnpEff could decrease the number of SNPs used for KASP assay primer design. Adding more than one genome in one basket for in silico PCR could help to select potential polymorphic markers through genomes. Circos configurations will help to give a grand overview regarding QTL chromosomal position and closeness to genes or SNPs.

### 4.1 Availability

Source code (Linux installer), Microsoft Windows installer, manual, sample data, and sample output are available for non-commercial purposes and can be downloaded from http://www.ageri.sci.eg/index.php/facilities-services/ageri-softwares/kaspspoon.

### Acknowledgements

The authors would like to dedicate this work to the soul of Prof. Dr Sami Adawy. We also thank Mr Morad Mokhtar (Molecular Genetics and Genome Mapping Lab., Agricultural Genetic Engineering Research Institute, ARC, Egypt) and Abdulqader Jighly (Department of Economic Development, Jobs, Transport and Resources, Australia) for their valuable support during this study.

Conflict of Interest: none declared.

### Funding

This work was funded by the Grain Legume and Dryland Cereals (GLDC) and Grain Research and Development Cooperation (GRDC).

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