QuantPrime

- a flexible tool for reliable high-throughput primer design for quantitative PCR

http://www.quantprime.de

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Samuel Arvidsson, March 11th 2009, qPCR 2009 Meeting Freising, Weihenstephan

Reference:
Samuel Arvidsson, Mirosław Kwaśniewski, Diego Mauricio Riaño-Pachón and Bernd Mueller-Roeber: QuantPrime - a flexible tool for reliable high-throughput primer design for quantitative PCR. *BMC Bioinformatics* 2008, **9**:465

Background

- Projects going on in our group
  - Leaf growth and senescence (mainly *Arabidopsis*)
  - Salt stress in rice
  - Systems biology of biomass accumulation (*Chlamydomonas*)
- Working with transcriptomics in plants and algae
  - Transcription factors (TFs)
  - Membrane channels, pumps and transporters
    - Expressed on low levels → microarrays not feasible
- qPCR "platforms" for different gene groups
  - Covering many transcripts (hundreds to thousands)
  - Keep costs down:
    - SYBR Green I based assays
    - Small reaction volumes

Why develop QuantPrime?

- Need of software for creation of qPCR platforms for high-throughput transcriptomics
- Loads of primer design software available, but…
  - there are important features missing or not suited for high-throughput design (most free software)
  - they are very expensive and not customizable for our own demands (most commercial software)
- Companies are offering such services, but…
  - on a fundamental research budget quite expensive and the service often a “black box“

What we need…

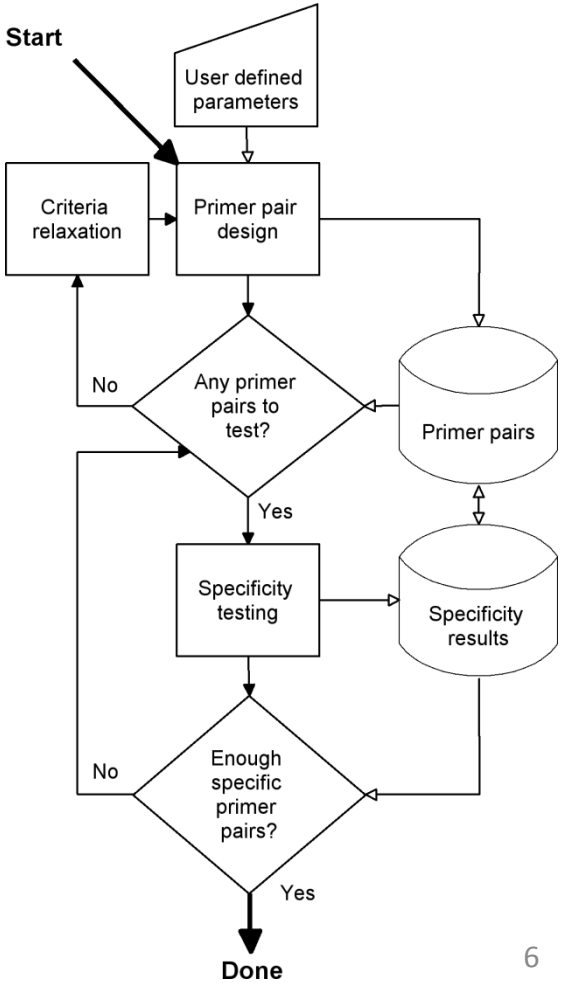
- Automated design which can…
  - directly use common annotations of sequence sets
  - have extensive basic primer parameters (melting temperature, GC content etc.) with ranking of primers
  - organize sequences, transcript identifiers and primer pairs well
- Automated specificity checking which should…
  - do checking against transcriptomes and genomes
  - score the specificity of the primer pairs
  - handle splice variants of transcripts
- And of course, it should be reliable and fast…

So…

- I needed about 200 primer pairs for my project
- Instead of …
  - paying a company for the primer design (too easy)
  - spending the time checking and testing the primer pairs designed by simple tools (too much manual work for an engineer)
- … I decided to invest time in making a tool that does just what I needed (more fun)
- After many discussions, much programming, more GUI design and some extensive testing that tool ended up becoming “QuantPrime“

What can QuantPrime do?

- Designs primer pairs and hybridization probes for expression measurements with qPCR
- Organizes…
  - parameters for design and specificity testing
  - sequences and annotations
  - primer design projects
  - primer pairs
- Predicts specificity of primer pairs



Automated primer design

- Primer3 with extra parameters
  - Primer 3‘-end G/C-content limitations
  - Transcript 3‘-biased design, when using oligo-dT cDNA synthesis
  - Filtering of primers against similar transcripts
- Genomic DNA amplicon avoidance
  - Exon-junction spanning primers
  - Large-intron-spanning
  - No manual annotation necessary

```
GGTGGACATTCTCTGTCTCCTCGAGTCAACAAGG^ACGAGTGGGTGGTGTGTCGCATTTTTCAAAAGAATTCTGGAGGGA
AGGGAAGTTTTTTGTTCTCGGATTCCTGCATGCGCAGCCATGCGGTGATGTACCAGATTGAGGACACAAGGTCGTCTTTG
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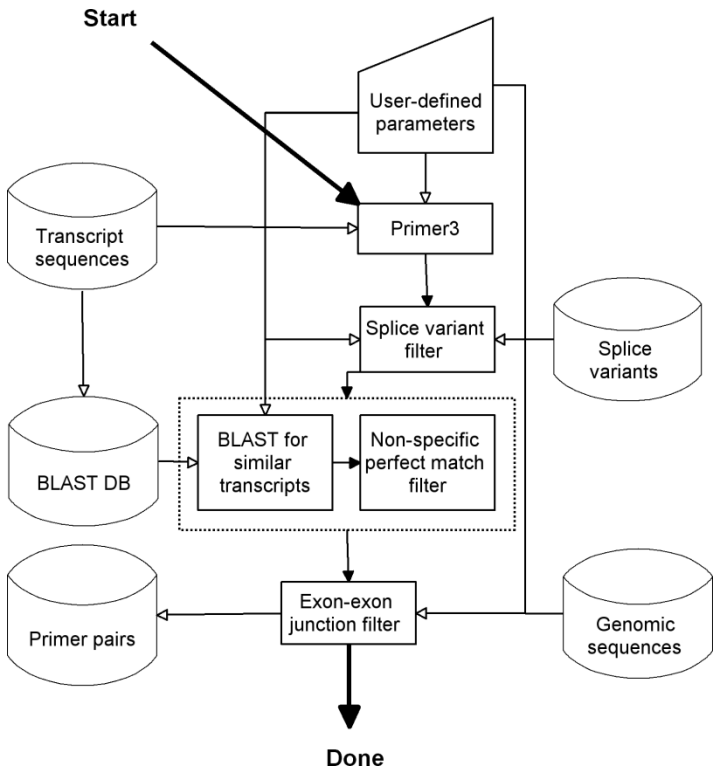
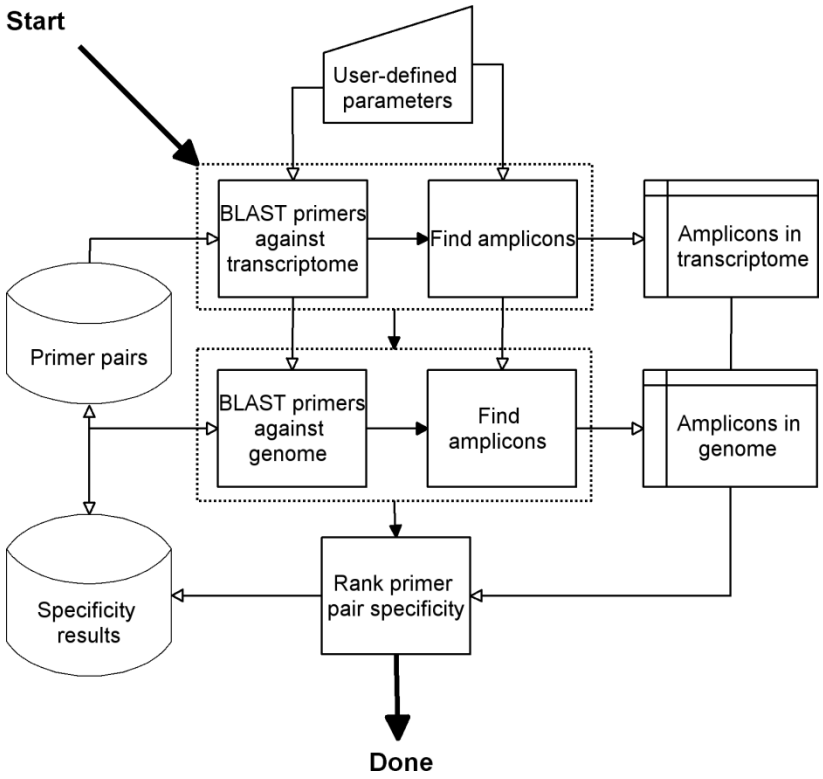
- Hybridization probes
  - Together with primer pairs or alone (e.g. for QISH)



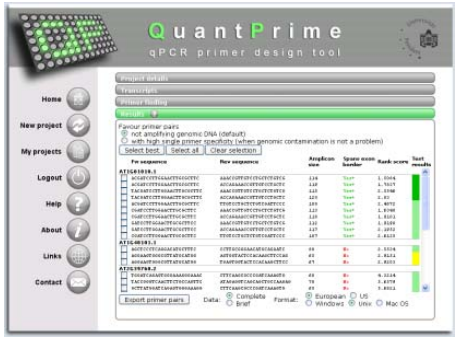
Specificity testing

- Amplicon finder based on BLAST used on
  - transcript sequences
  - gene model sequences
  - unannotated genome regions
- Ranking of primer pair specificity

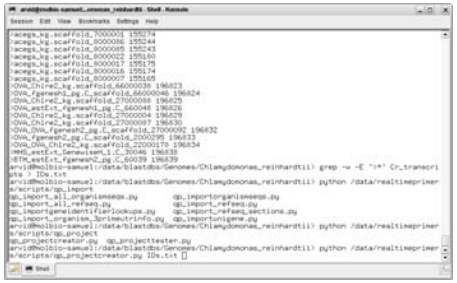


User interfaces

- Public web server
  - http://www.quantprime.de
  - Free to use for non-profit research, free to try for commercial research

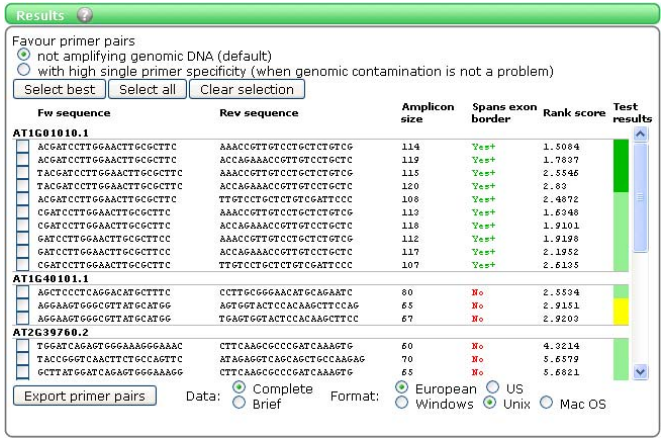


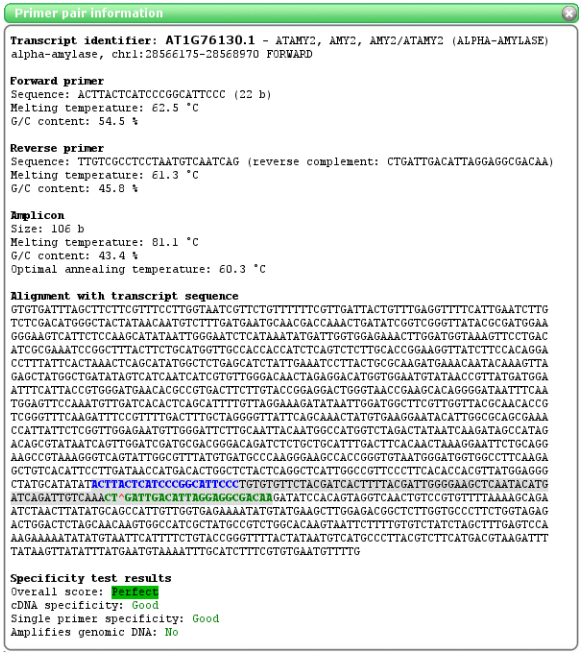
- Local installation
  - Command-line user interface and/or local web server user interface
  - Free to use for non-profit research



Public web server

- Integrated graphical user interface





- Currently 345 annotations of 295 species available
- Standard protocols for SYBR Green qPCR predefined, custom protocols possible

Tutorial…

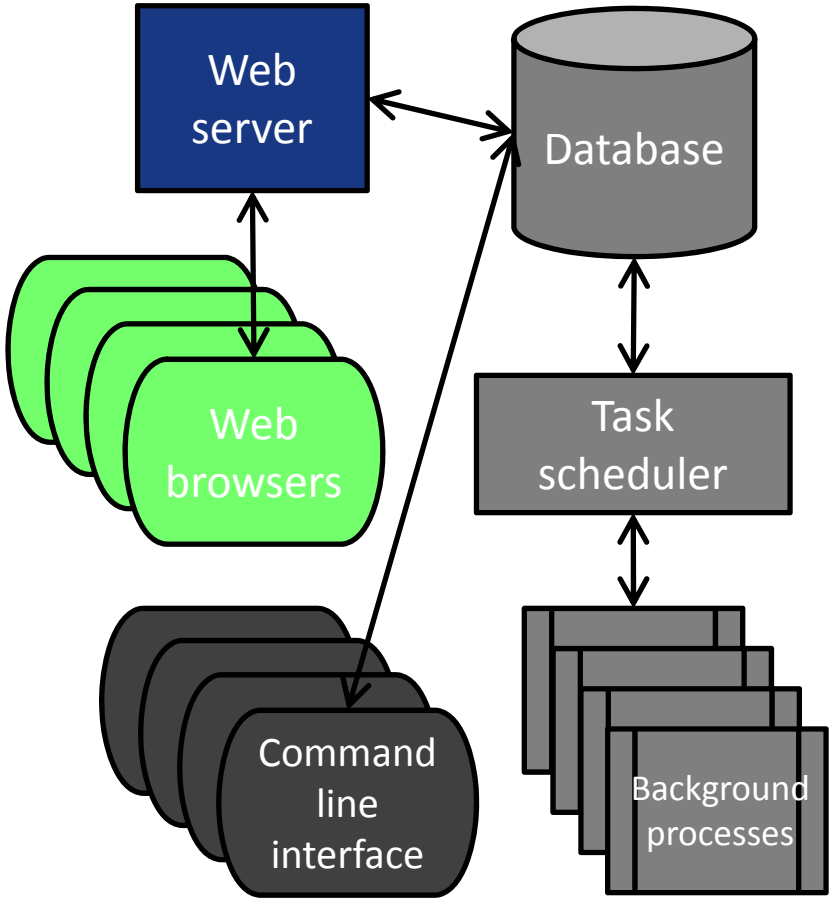
- http://www.quantprime.de/

Local installation

- Recommended for true high-throughput applications
- Calculation and database nodes can easily be installed on clusters
- Web interface for intranet applications
- Command line interface for integration in design pipelines

System architecture

- Easily extendable with new features and interfaces
- Possible to integrate in a design pipeline
- Suitable for parallelization

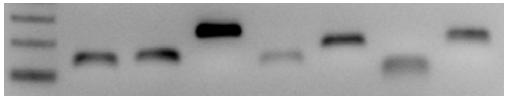
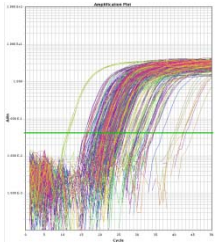
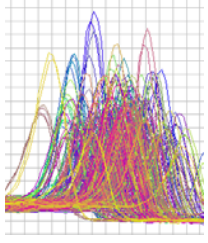
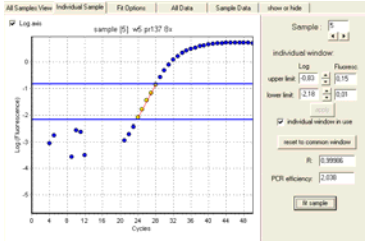


Experimental results

- Primer pairs designed by QuantPrime tested in real experiments

Organism	Total transcripts	Detectable	QC passed of detectable	Predicted gDNA-safe
Arabidopsis thaliana	175	165 (94 %)	160 (97 %)	149 (85 %)
Chlamydomonas reinhardtii	33	29 (88 %)	28 (97 %)	24 (73 %)
Hordeum vulgare	30	28 (93 %)	27 (96 %)	-
Physcomitrella patens	32	32 (100 %)	32 (100 %)	21 (66 %)
Total	270	254 (94 %)	247 (97 %)	194 (81 %)

- Quality control: amplicon size, melting curve, efficiency

Performance results

Results of *in silico* benchmarking of QuantPrime

Species	Transcripts	Total search time	Average search time	Primer pair specificity ranking[1]		
				Acceptable[2]	Good[3]	Very good[4]
Arabidopsis thaliana	5000	20:22:06	15 s	4916 (98%)	4323 (86%)	2534 (50%)
Vitis vinifera	5000	50:45:33	37 s	4765 (95%)	3927 (78%)	2315 (46%)
Drosophila melanogaster	5000	13:48:45	9.9 s	4894 (97%)	4075 (81%)	3096 (61%)
Chlamydomonas reinhardtii	5000	12:11:07	8.8 s	4568 (91%)	3999 (79%)	2349 (46%)
Oryza sativa ssp japonica	5000	83:31:12	60 s	4658 (93%)	3821 (76%)	1984 (39%)
Hordeum vulgare	23078	22:56:59	3.6 s	22145 (95%)	21564 (93%)	-

Primer pairs designed for hypothetical high-throughput experiments, for random transcripts of each species. The numbers of successfully designed primer pairs for the different specificity ranks and the search times are reported for each species (percentages refer to the total number of transcripts tested).

[1] Percentages indicate for how many of the transcripts primer pairs of at least the rank given were identified. [2] 'Acceptable' amplifies only the specific sequence, but one primer has a high similarity with a non-target sequence and the primer pair might amplify genomic DNA. [3] 'Good' amplifies only the target sequence, but one primer has a high similarity with a non-target sequence or the pair might amplify genomic DNA. This is the highest possible rank for primer pairs designed for species without a genome annotation. [4] 'Very good' amplifies only the target sequence, both primers are highly specific to this sequence and will not amplify genomic DNA.

Arvidsson *et al. BMC Bioinformatics* 2008 **9**:465 doi:10.1186/1471-2105-9-465

Public server usage

- Since Nov 1st 2008: 200 occassional users; more than 100 frequent users
  - From all over the world
  - Working with all kinds of organisms, e.g. yeast, tomato, water buffalo and human
- Sequence databases added on request

Summary

- QuantPrime is an easy-to-use yet flexible primer design tool for low- to high-throughput qPCR
- Robust; primer pairs have > 96 % success in experimental tests
- The public server, which is free to use for non-profit researchers, has many sequence sets installed, even for uncommon species not covered by commerical assays and software
- The link: http://www.quantprime.de/
  - Check out the paper for more details on algorithms and parameters (link on website)
- Coming up:
  - TF platform for tomato designed with QuantPrime, in testing
  - TF platform for *Physcomitrella* (moss) planned
  - RAM and CPU boost for the public server to better cope with big genomes
  - RDML export
  - More features…

Thanks to…

- The QuantPrime co-developers:


Dr. Mirosław Kwaśniewski


Dr. Diego M. Riaño-Pachón


Prof. Dr. Bernd Müller-Röber

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  - DMRP: BMBF, GABI-FUTURE grant 0315046
  - BMR: BMBF, GoFORSYS, FKZ 0313924

- … you for your attention!

VaTEP
Vacuolar Transport Equipment for Growth Regulation in Plants

DAAD Deutscher Akademischer Austausch Dienst
German Academic Exchange Service

Bundesministerium für Bildung und Forschung
FORSYS
GoFORSYS