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rdml: A Mathematica package for parsing and importing Real-Time qPCR data

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Abstract

Objective: The purpose and objective of the research presented is to provide a package for easy importing of Real-Time PCR data markup language (RDML) data to Mathematica.

Results: Real-Time qPCR is the most widely used experimental method for the accurate quantification of gene expression. To enable the straightforward archiving and sharing of qPCR data and its associated experimental information, an XML-based data standard was developed—the Real-Time PCR data markup language (RDML)—devised by the RDML consortium. Here, we present *rdml*, a package to parse and import RDML data into Mathematica, allowing the quick loading and extraction of relevant data, thus promoting the re-analysis, meta-analysis or experimental re-validation of gene expression data deposited in RDML format.

Keywords: Mathematica, Wolfram, RDML, PCR, qPCR, DNA, RNA

Background

Real-Time quantitative polymerase chain reaction (qPCR) is a sensitive and powerful experimental method routinely used in molecular biology labs for quantifying DNA and RNA. qPCR instruments collect a large set of data per run, providing the basis for the quantification and validation of PCR amplification products. An instrument-independent format to store and exchange these data collections was published in 2009 as the first version of the Real-Time qPCR data markup language (RDML) [1]. This is an XML-based data standard, containing not only the measurements from the thermocycler, but also the metadata necessary to fully characterize the experimental conditions used to obtain those results. However, it is not trivial to automatically extract all the relevant information from such a comprehensive and technique-specific XML-based format.

Main text

Accordingly, we have developed the `rdml.m` package, which extends Wolfram's language functionality by allowing RDML version 1.2 files to be readily imported and loaded into Mathematica™ . The data from all mandatory and optional elements are validated first, and subsequently returned as a `Dataset` object; allowing the user to take full advantage of the recently introduced database-like operations for `Dataset` objects in Mathematica.

Making RDML data easily accessible is an important step toward promoting the peer-validation of published datasets managed by the RDML consortium (http://www.rdml.org/). There are a few software applications compatible with RDML, namely the *RDML Ninja* [2], *qbase+* [3], *RDML R package* (https://github.com/kablag/RDML/), and *LinRegPCR* [4], of which only the first and the last fully support RDML version 1.2. Our package brings these data to Mathematica users wishing to take advantage of the symbolic character and new data query potential of the Wolfram Language, hence broadening the spectrum of software able to access, analyse and manipulate RDML data.

Two of the main advantages of this *rdml* package are: (1) the fact that it does not require the user to become familiarized with the sizeable RDML schema, and (2) it does not require an extensive learning of new package-specific functions. Instead, we have expanded the built-in `Import` function from Mathematica, making it directly capable of importing RDML files. For this, we registered

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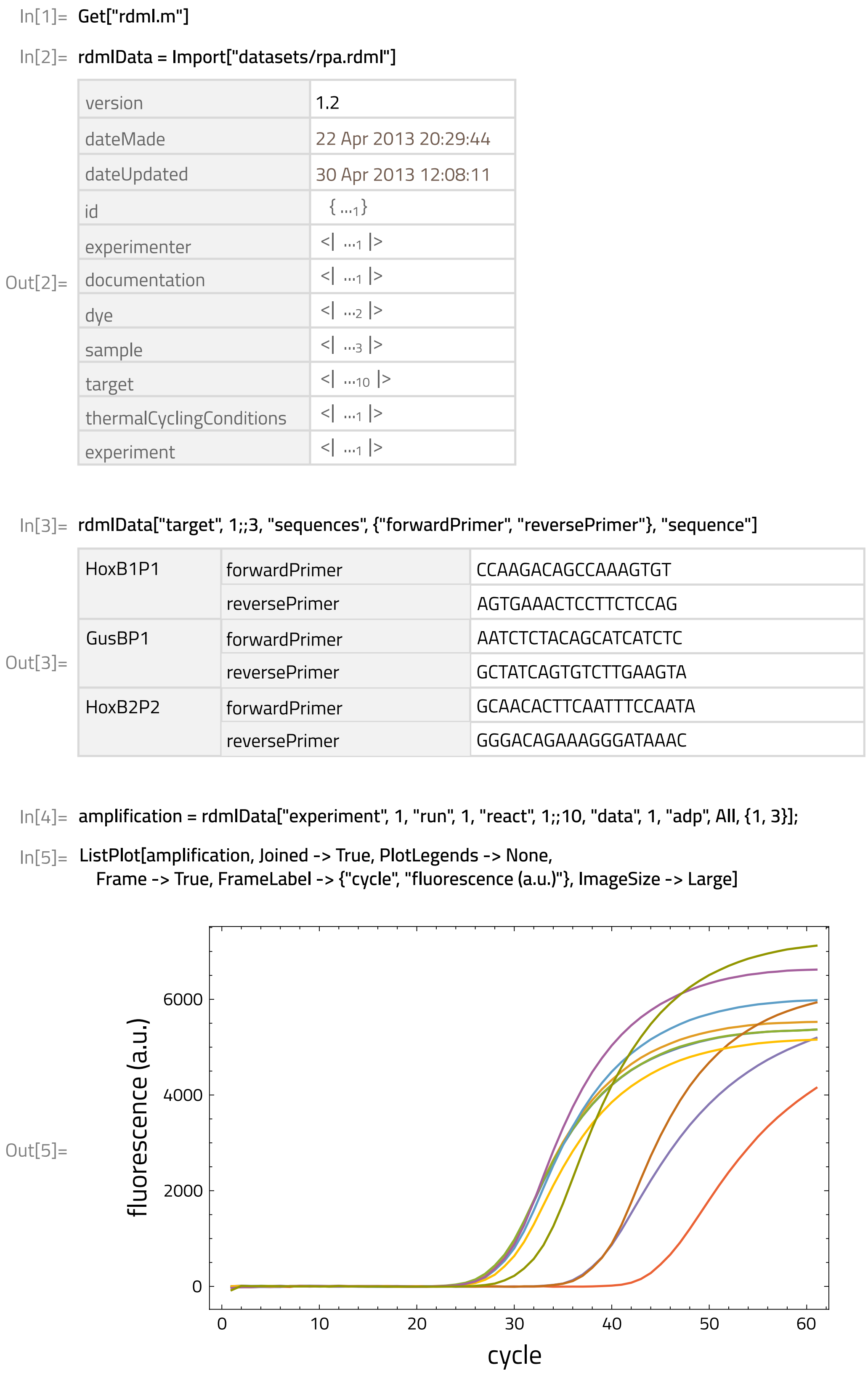

```
In[1]=  Get["rdml.m"]

In[2]=  rdmlData = Import["datasets/rpa.rdml"]
```

Out[2]=

version	1.2
dateMade	22 Apr 2013 20:29:44
dateUpdated	30 Apr 2013 12:08:11
id	{ …1 }
experimenter	<| …1 |>
documentation	<| …1 |>
dye	<| …2 |>
sample	<| …3 |>
target	<| …10 |>
thermalCyclingConditions	<| …1 |>
experiment	<| …1 |>

```
In[3]=  rdmlData["target", 1;;3, "sequences", {"forwardPrimer", "reversePrimer"}, "sequence"]
```

Out[3]=

HoxB1P1	forwardPrimer	CCAAGACAGCCAAAGTGT
	reversePrimer	AGTGAAACTCCTTCTCCAG
GusBP1	forwardPrimer	AATCTCTACAGCATCATCTC
	reversePrimer	GCTATCAGTGTCTTGAAGTA
HoxB2P2	forwardPrimer	GCAACACTTCAATTTCCAATA
	reversePrimer	GGGACAGAAAGGGATAAAC

```
In[4]=  amplification = rdmlData["experiment", 1, "run", 1, "react", 1;;10, "data", 1, "adp", All, {1, 3}];

In[5]=  ListPlot[amplification, Joined -> True, PlotLegends -> None,
          Frame -> True, FrameLabel -> {"cycle", "fluorescence (a.u.)"}, ImageSize -> Large]
```

Out[5]=

Fig. 1 Screenshot of a typical rdml usage session in Mathematica

specific import functions for the various RDML master elements: dateMade, dateUpdated, id, experimenter, documentation, dye, sample, target, thermalCyclingConditions and experiment. This registration is achieved by using the function `ImportExport`RegisterImport` as indicated in (https://reference.wolfram.com/language/tutorial/DevelopingAnImportConverter.html). Additionally, we overload `Import` to allow it to automatically recognize the file extensions `.rdml` and `.rdm`.

The returned `Dataset` expression is a nested structure of lists and associations. The hierarchy in the returned object preserves the original hierarchy defined in the RDML schema v1.2. Each XML element that is not a leaf element, is either represented by an `Association` or a `List`. We use `Associations` for lists of RDML elements that have an `id` XML-attribute, using the `id` value as `Key`; XML elements with no `id` are represented as `Lists`. Leaf elements are converted to either `Symbol` (`True` or `False`), `Integer`, `Real`, `String` or `DateObject`. Special numeric codes such as `INF` are appropriately dealt with, being converted to Mathematica equivalent expressions, i.e., `Infinity`. Missing or non-schema-compliant elements are converted to `Missing[NotAvailable]`.

RDML validation

Another important feature of this package is the validation of the input file against the RDML schema version 1.2. This step is optional by setting `ValidateAgainstXSD` to `True` when importing. For this, we leverage on Java's support for XML schema by using J/Link in Wolfram (http://reference.wolfram.com/language/JLink/tutorial/CallingJavaFromTheWolframLanguage.html). Additionally, *rdml* is partially compatible with versions 1.0 and 1.1, only to the extent of the overlap between the schemas.

Package overview

Once loaded, the *rdml* package extends the `Import` function from Mathematica, enabling it to read and parse RDML files. The importer returns a `Dataset` object containing all valid data, which is then easily browsable using Mathematica's newly implemented query language for `Datasets`.

Loading the `rdml.m` package

First, the package file must be made available to Mathematica. For this, make sure that the current working directory is where the `rdml.m` file is located, and simply load the package with: `Get ["rdml.m"];` once loaded, the user can access the documentation notebook via the function: `HelpPageRDML[]`.

Importing RDML data

To import an RDML file use the built-in Mathematica `Import` function. For example, to import one of the sample dataset files included with the package, the `rpa.rdml` file (which can also be found in the `datasets` folder in https://github.com/ramiromagno/rdml), simply run: `Import["datasets/rpa.rdml"]`.

Next, the user can browse and freely explore the data within Mathematica. In Fig. 1, `Out[2]` shows the returned summarized `Dataset` object for the `rpa.rdml`. The main RDML elements are all present, as well as the schema `version` of the input file. Values for most elements are long expressions, hence shown in abbreviated form. Short expression values, such as those for `dateMade` and `dateUpdated`, can be fully displayed and are automatically returned as `DateObjects`. Once returned, the `Dataset` object structure can be easily traversed by using `Dataset` queries or `Part` operations, as illustrated in `In[3]`. This example shows how to access the nucleotide sequences of the primers used to amplify three of the PCR targets.

Discussion

The *rdml* importer is an open source Wolfram package that validates and automatically loads RDML data into Mathematica. Standard RDML files are generated by most of the widely used qPCR equipments. This package makes archived qPCR data, and all of its experimental settings, readily available to Mathematica users without the additional burden of becoming familiarized with the RDML schema and having to learn new package-specific functions.

Limitations

Although the package here presented is free and open source software, Mathematica itself is not open source software.

Abbreviations

RDML: Real-Time PCR data markup language; qPCR: Real-Time quantitative PCR; PCR: polymerase chain reaction; XML: extensible markup language.

Authors' contributions

RM and ID designed and implemented the package. RPA contributed with the main test dataset. RM, ID, RPA and IP wrote the paper. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The *rdml* package, as well as input example files, are freely available at https://github.com/ramiromagno/rdml.

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