The B-55’s

# Abstract

*Human adenovirus B-55* (HAdV-B55) is a re-emergent pathogen that threatens dense populations.

# Introduction

The *Adenoviridae* family includes genera that infect a wide range of hosts and cell types. The *Mastadenovirus* genus includes species that infect mammalian hosts. They are globally distributed and are responsible for sporadic outbreaks in densely populated regions and close-living quarters. Symptoms range from acute respiratory disease to organ failure, depending on the viral species and host immune strength. Accordingly, individuals with developing or weakened immune systems account for most outbreak deaths.

# Methods

## Data

A Bash script coordinated the retrieval of nucleotide sequences and metadata in order to generate a local BLAST database. The Entrez Direct E-utilities scripts provided a command-line interface to query and retrieve data from the federated set of National Center for Biotechnology Information (NCBI) databases. The script generated the following query to download the sequences from the Nucleotide database.

```sql

(txid714978[PORG] OR txid343463[PORG]) AND biomol\_genomic[PROP] NOT gbdiv\_pat[PROP] NOT gbdiv\_syn[PROP]

```

Each square bracket occurs immediately after a search value and encloses a keyword that specifies the search field. The PORG and PROP fields correspond to primary organism and property respectively. The first two PORG terms use NCBI Taxonomy identifiers to limit the search to HAdV-55 and HAdV-11a respectively. The first PROP term sets the molecule type while the next two exclude GenBank divisions (Romiti and Cooper, 2011). Specifically, these terms limit the search to genomic DNA while excluding patents and synthetic constructs respectively. The \*\*esearch\*\* and \*\*efetch\* programs executed the query and retrieved the FASTA-formatted sequence data respectively. The script piped the results directly into the \*\*makeblastdb\*\* program to generate a BLAST database.

## Metadata

The same Bash script also coordinated the retrieval and normalization of sequence metadata to create a database of collection dates for subsequent Bayesian analysis. The script retrieved the set of accessions via \*\*blastdbcmd\*\* and piped them into the \*\*esummary\*\* to download the JSON-formatted metadata. The \*\*jq\*\* program processed the records from standard input using the following query.

```jq

.result | del(.uids) | map([.accessionversion, .subtype, .subname] | @tsv) | .[]

```

The query ran a series of filters to transform the JSON into a tab-separated file. The \*subtype\* and \*subname\* properties are pipe-delimited strings that correspond to keys and values respectively. An R script processed the data from standard input, using tidyverse functions to split the key-value pairs into new columns (Wickham, 2017). Another R script relied on the lubridate package to automatically convert the collection\_date field values into a consistent ISO-8601 format (Spinu et al., 2018). This series of commands generated a sorted tab-separated file mapping accessions to collection dates.

## Sequence Extraction

The full genome extraction method involved a series of piped commands. The \*\*blastdbcmd\*\* program dumped a space-separated list of accession-length pairs. Next, \*\*awk\*\* selected sequences with lengths greater than or equal to 34 kbp.

The gene extraction method required a query sequence. The \*\*blastdbmcd\*\* program generated the query for each gene based on a reference accession and sequence coordinates…

# Alignment

The MAFFT program performed multiple sequence alignments (Katoh, 2002). MAFFT achieves performance gains via multithreading while maintaining accuracy via application of the Fast Fourier Transform on the sequence data to quickly identify homologous regions. Parameters included the --auto and --adjustdirection flags. The former automatically sets algorithm heuristics based on the sequence data and the latter automatically adjusts sequence direction for each entry if the reverse complement is optimal. The calling script redirected standard error into a log file to record alignment progress and heuristic selection.

# Phylogeny

The IQ-TREE program inferred phylogenies (Nguyen et al., 2015). The program performed a series of likelihood tests to select the optimal number of threads and sequence evolution model based on the input data. The former compared the effect of adding additional threads on efficiency and the latter exploited the ModelFinder algorithm to estimate the optimal substitution model (Kalyaanamoorthy et al., 2017). Parameters included the -alrt and -bb flags to set the number of bootstrap replicates to 1,000 for the approximate likelihood ratio test of branches (Anisimova et al., 2011) and branch support (Hoang et al., 2018). The -bnni parameter also reduced the risk of model violations associated with ultrafast bootstrap testing via nearest neighbor interchange. The program automatically created a log file and exported the maximum likelihood tree in a variety of formats, including Newick.

## Molecular Clock

Testing the strict molecular clock hypothesis on the genome, fiber, and hexon nucleotide sequences of each species required the TempEst (Rambaut et al., 2016) and BEAST (Drummond and Bouckaert, 2015). The programs evaluated the clock signal and estimated model parameters respectively. A filtering step removed sequences lacking the collection\_date metadata.

The TempEst program tests the strength of the strict molecular clock hypothesis for a given phylogeny. It plots the taxon date against the root-to-tip patristic distance and fits a regression line with an objective function that optimizes the correlation coefficient, R-squared value, or mean-squared residuals. This is an interactive tool that facilitates the identification of outliers that may result from incorrect collection dates, vaccine strains, or contaminated sequence data. Model parameters are only useful for data exploration since the variables are dependent. Development of an R script automated this process by plotting the model for the cross product of root-to-tip distance metrics and model objective functions. The script invoked the rtt and distRoot function of the ape (Paradis et al., 2019) and adephylo (Jombart et al., 2017) packages.

The BEAUti program is a graphical tool that outputs BEAST model parameters as XML files.

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