Pubmlst.org – the new home for the *Borrelia* MLSA database

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Abstract

For *Borrelia*, a multilocus sequence typing (MLST) scheme was developed based on sequence fragments of eight housekeeping loci (i.e. *clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, *uvrA*). The MLST/MLSA database accompanying the scheme has been moved to <http://pubmlst.org/borrelia> and now includes relapsing fever spirochetes. The new database is introduced here.

Multilocus sequence typing (MLST) and multilocus sequence analyses (MLSA) have been developed for epidemiological purposes, i.e. to aid understanding the relationship of bacterial pathogens at an intermediate time scale (Enright and Spratt, 1999; Urwin and Maiden, 2003). While MLST is used to resolve intraspecific relationships, MLSA has been recognized as suitable tools to address taxonomic questions, i.e. it is used at the genus level (Bishop *et al.*, 2009). MLST and MLSA are based on the sequence information of internal fragments of about 500 bp of housekeeping genes; often seven loci are chosen (Urwin and Maiden, 2003). The use of sequence information renders it unambiguous and portable. Even today traditional MLST and Sanger sequencing remains a fast and economical alternative to whole genome sequencing, in particular for bacteria that are difficult to culture (Jungnick *et al.*, 2015; Mukhacheva and Kovalev, 2013).

In 2008, a Multilocus sequence typing (MLST) scheme was published for *Borrelia burgdorferi* sensu stricto that was based on eight housekeeping loci located on the main linear chromosome (Hoen *et al.*, 2009; Margos *et al.*, 2008). Because of the way the primers were designed, the scheme was extendable to other species of the *Borrelia burgdorferi* sensu lato species complex and used to study the populations within *Borrelia* genospecies and for taxonomic purposes (Hanincova *et al.*, 2013; Jungnick *et al.*, 2015; Margos *et al.*, 2014; Margos *et al.*, 2012; Margos *et al.*, 2013; Mechai *et al.*,

2014; Mukhacheva and Kovalev, 2013; Ogden *et al.*, 2011; Takano *et al.*, 2011; Vitorino *et al.*, 2008; Vollmer *et al.*, 2013).

For all individual MLST and MLSA schemes specific databases maintain sequences, isolate specific information and tools for analyses (Aanensen and Spratt, 2005; Jolley *et al.*, 2004; Perez-Losada *et al.*, 2013). These databases are specifically designed to host MLST/MLSA data and serve to make these data available to the scientific community (Jolley, 2009). Since its development and introduction the *Borrelia* MLSA database was hosted and maintained at Imperial College London under <http://borrelia.mlst.net>. In February 2015 the database was moved to the Pubmlst.org website (<http://pubmlst.org/databases>) and extended to be used also for relapsing fever spirochetes. Here some features of the new interface are shown and described.

The new face of the *Borrelia* MLSA database is shown in Figure 1A. The website is divided into two parts: one that holds allele profile and sequence definitions and one that holds isolate data. Within each of the subdivisions, it is possible to browse, search and download data (Figure 1B). The database also offers the option to break down data according to their country of origin, species, year of collection and others (Figure 1C).

The move of the MLST/MLSA database for *Borrelia* was accompanied with an extension of the database to relapsing fever spirochetes. Primer for relapsing fever spirochetes were designed to conserved regions of the eight housekeeping loci, i.e. *clpA*, *clpX*, *nifS*, *pepX*, *pyrG*, *recG*, *rplB*, *uvrA*, using multiple alignments of *B. duttonii* strain Ly (GenBank accession no. NC_011229.1), *B. hermsii* strain DAH (GenBank accession no. CP000048.1), and *B. turicatae* strain 91E135 (GenBank accession no. CP000049.1). For primer design the program OligoExplorer v. 1.5 (<http://www.genelink.com>) was used. Primers were designed in such a way that the same fragments used for the Lyme borreliosis group of spirochetes could be sequenced and used in data analyses. The recently published detailed protocol for PCR conditions (Wang *et al.*, 2014) is still valid and can also be found in the database.

Relapsing fever spirochetes are more diverse than LB spirochetes and therefore, degenerate primers had to be designed for the eight genes to allow amplification of the same region that was used for analyses of LB spirochetes. These primers were tested on a number of relapsing fevers spirochetes. DNA samples including *B. recurrentis*, *B. duttonii*, *B. miyamotoi*, and *B. persica*. Table 1 shows the primer sequences and results of successful amplification.

For relapsing fever species, the *nifS* primers showed such a high degree of degeneracy that they did not work for PCR amplification. For *B. miyamotoi*, specific primers for *nifS* amplification were designed using *nifS* sequences extracted from two fully sequenced genomes available in GenBank (*B. miyamotoi* strain LB2001, GenBank accession no. NZ_CP006647.1/CP006647.2 and strain FR64, GenBank accession no. NZ_CP004217.1/CP004217.1). In addition, the other degenerate primers showed little utility for sequencing PCR amplicons; therefore, specific sequencing primers were designed. For this, *B. miyamotoi* products amplified using degenerate primer were cloned into a pGEM-Vector (Promega, Mannheim, Germany) and sequenced using vector specific primers (SP6, T7). These sequences were used in multiple alignments in combination with *B. miyamotoi* sequences from GenBank to design sequencing primers that were specific for *B. miyamotoi* (Table 2). Multiple alignments were conducted in MEGA5 (Kumar *et al.*, 2004).

Since *B. miyamotoi* occurs sympatrically with *B. burgdorferi* s.l., efforts were made to design primers that would not cross-react with *B. burgdorferi* s.l.. This was, however, not possible for all genes and

primers that cross-react to *B. burgdorferi* s.l. are designated with * in Table 2. The application of MLSA to *B. miyamotoi* strains from Asia, USA and Europe showed that (i) loci can be amplified directly from ticks as the material from Germany constituted tick DNA extracts and (ii) the two *B. miyamotoi* strains from USA (LB2001 and M1029) did not differ in these loci supporting previous claims that local *B. miyamotoi* may be genetically very homogeneous (Bunikis *et al.*, 2004; Richter *et al.*, 2003).

For other relapsing fever spirochetes, only seven genes were amplified (*clpA*, *clpX*, *pepX*, *pyrG*, *recG*, *rplB* and *uvrA*). The Pubmlst.org database, however, provides the option to submit incomplete allelic profiles. These incomplete profiles can be saved and connected to isolate data. Hence, for relapsing fever spirochetes, data for seven loci can be used to build phylogenetic trees and for other statistical analyses (e.g. genetic distance analyses). We suggest that the MLSA scheme described here will be a useful tool for population analyses of relapsing fever spirochetes which may be difficult to achieve using single locus approaches (Elbir *et al.*, 2013). It may also be suitable for taxonomy in relapsing fever spirochetes.

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Figure legends

Figure 1A The new face of the *Borrelia* MLST database. Upon double click on “*Borrelia* spp” on the web site <http://pubmlst.org/databases>, the *Borrelia* MLST database opens. The database is divided into a “profile and sequence definitions” and “isolates”. In profiles and sequence definitions, allele sequences, allelic profiles and sequence type information can be obtained as well as concatenated sequences of sequence types. The database “isolates” contains isolate specific information including strain name, year of isolation, geographic and biological origin, collector and submitter.

Figure 1B Browse, search and download functions are available inside the sequence or isolate specific databases. The figure shows the option to query the sequence database with an unknown gene sequence.

Figure 1C In addition to search, browse and download sequences or isolate specific information, the database offers options to breakdown information contained in the database. The example provided shows a breakdown of isolates according to country of origin.

Table 2 *Borrelia miyamotoi* MLSA primer

PCR primers				Sequencing primer		
Gen	direction	Primer name	Sequence/ Degeneracy, 5' to 3'	direction	Primer name	5' to 3'
clpA	forward	BmclpAF1268	TTGATCTCTTAGATGATCTTGG	forward	BmclpAF1268	TTGATCTCTTAGATGATCTTGG
	reverse	BmclpA2051R	CAAACATAAACCTTTTCAGCCTTTAATA	reverse	BmclpA2051R	CAAACATAAACCTTTTCAGCCTTTAATA
clpX	forward	clpX104F	CTGTTGCYATTTGTTTTGAATGYTC	forward	BmclpF268	TTATCTGTTGCTGTTTATAATC
	reverse	clpX1277R	TAAAGTTCTTTTGCCCAAGG	reverse	BmclpX1155R	TTCAAACATAACATCTTTAAGTAATTCTTC
nifS	forward	BmnifF31	GAAAAAGTAAACTCCCTCAGAAAGG	forward	BmnifF31	GAAAAAGTAAACTCCCTCAGAAAGG
	reverse	BmnifS892R	CAATGATGCCTGCAATATTTGGTG-3	reverse	Bmnif892R	CAATGATGCCTGCAATATTTGGTG
pepX	forward	pep361F	5'-AGAGAYTTAAGYTTAKCAGG-3'	forward	BmpepF358	GATAGAGACTTAAATTTAGCAGGAGTTG
	reverse	pep1202R	5'-GTTTCTCTTAAAGAYTGCATTCC-3'	reverse	Bmpep1187R	TGCATTCCCCACATTGGAGTTC
pyrG	forward	pyrF415	5'-CTTYTAGTWATTGARATTGGTGGT	forward	BmpyrF417	TTTAGTAATTGAGATTGGTGGTC
	reverse	pyr1261R	5'-CAGCATCAAYTATRCCACAAAC-3	reverse	Bmpyr1252R	TATTCCACAAACATTACGAGC
recG	forward	rec908F*	5'-CTAGYATTCCTYTAATTGAGGC-3	forward	BmrecF909	TAGCATTCCCTTTAGTTGAGGC
	reverse	rec1779R*	5'-TTCRGTTAAAGGTTCTTATAAAG-3	reverse	Bmrec1671R	CTCAGCATGCTCAACTACC
rplB	forward	rpl18F*	5'-ATTAAGACTTATARGCCAAAAAC-3	forward	BmrplF23	GACTTATAGGCCAAAAACTTC
	reverse	rplR761*	5'-GGCTGNCCCCAAGGWWGAT	reverse	Bmrpl759R	GATACAGGATGACGACCACC
uvrA	forward	uvr1170F*	5'-GAGGCGTTATCTTKCAACTG-3			
	reverse	uvr2181R*	5'-AGACTCTGGAAGCTTWGC-3			
	forward	uvrF1435	GCTKAAATTTTTRATTGATGTTGGA	forward	BmuvrF1437	5'-TTAAATTTTAAATTGATGTTGGACT
	reverse	uvrR2306	5'-CARGGAACAAAAACATCRGGC	reverse	Bmuvr2147R	5'-TCTGTAAAAAACCCAACATAAGTTGC-3

*cross reactivity with *B. burgdorferi*

Table 1 **MLSA Primer relapsing fever spirochetes**

gene	approximate 5' position	primer name	Sequence/degeneracy 5' to 3'	Remarks
clpAF	1258	clpAF1258	GATAAAGCTTTTGA YY TATTAGATGG	worked only for <i>B. recurrentis</i>
	1262	clpAF1262	AAGCTTTTGA YY TATTAGATGGTC	
clpAR	2098	clpAR2098	CAAAAAAAAA S ATC HARR TTTTTCATTTTTTAG	<i>B. recurrentis</i>
	2276	clpAR2276	TCATATTT D AT R GT D TCGTC	
clpXF	104	clpXF104	CTGTTGC Y ATTTGTTTTGAATGYTC	sometimes non-specific bands
	109	clpXF109	GC Y ATTTGTTTTGAATGTTCTAAAATATG	
	268	clpXF268	TT R TC Y GTTGCTGTTTATAATC	
clpXR	1183	clpXR1183	CTTTTTTAATTTGCTTA STW GAAAGG	
	1277	clpXR1277	TAAAGTTCTTTTGCCCAAGG	
nifS			nd	
pepXF	361	pepF361	AGAGAYTTAAG Y TTA K CAGG	non-specific bands in combination with pepR1187
	364	pepF364	GAYTTAAG Y TTA K CAGGAR T TTG	
pepXR	1187	pepR1187	TGCATTCCCCACATTGG	
	1202	pepR1202	GTTTCTCTTAAAGAY T GCAATTCC	
	1207	pepR1207	C Y ATAGTTTCTCTTAAAGAY T GTC	
pyrGF	372	pyrF372	TGG R AA R TATTTAGG K AGAACTG	may not work with pyrR1261 may work with pyrR1252
	379	pyrF379	TATTTAGG K AGAACTGTACAGC	
pyrGR	1252	pyrR1252	TAT R CCACAAACATTACG K GC	may not work with pyrF379 may not work with pyrF372
	1261	pyrR1261	CAGCATCAAYTAT R CCACAAAC	
	1375	pyrR1375	CAAGTCGCATTGT W GCAC	
recGF	898	recF898	GCKTTTCT M TCTAG Y ATTCC	
	908	recF908	CTAG Y ATTCCCT Y TAATTGA R GC	
recGR	1779	recR1779	TT R GTTAAGGTTCTTATAAAG	
rplBF	3	rplF3	GGAGAAAAATATGGG K ATTAAGAC	
	18	rplF18	ATTAAGACTTATA R GCCAAAAAC	
	24	rplF24	GACTTATA R GCCAAAAACTTC	
rplBR	759	rplR759	GATACAGGATG W CGACC	
	765	rplR765	CCAAGG W GATACAGGATG	
	769	rplR769	G R CCCCAAGG W GATAC	

uvrAF	1170	uvrF1170	GAGGCGTTATCTT W CAAC	sometimes non-specific bands with uvrR2147
	1173	uvrF1173	GCGTTATCTT W CAACTGAATC	
uvrAR	2147	uvrR2147	TC D GTAAAAAATCCAACATAAGTTGC	
	2153	uvrR2153	CTAATCTC D GTAAAAAATCCAACATAAG	
	2178	uvrR2178	TCTAGACTCTGGAAGCTT	