B. burgdorferi plasmid profile

1. Grow *B. burgdorferi* to a density $\geq 10^8$/mL in BSKII

2. Extract total gDNA using the Promega Wizard prep kit, or your extraction method of choice (for a chloroform/methanol DNA extraction procedure refer to the Transposon Mutagenesis protocol)

3. Using either the Purser/Norris or Akins primer sets, PCR for plasmid content using the following mixture per reaction:

   1 μL gDNA (approx. 50 ng)
   2 μL 10X PCR buffer
   3.2 μL dNTP mix (working stock containing 1.25 mM/each dNTP)
   1 μL forward primer (10-20 pmols)
   1 μL reverse primer (10-20 pmols)
   0.2 μL Taq polymerase (1 U)
   0.2 μL 150 mM $MgCl_2$
   11.4 μL ddH_2O
   20 μL total

Our method for simplifying this procedure is as follows:

4. Make a working stock of the Akins or Pursor/Norris primers sets in a 96 well PCR plate. Dilute the forward and reverse primers for each plasmid in the same well to 20 pmol/μL. To make interpretation of the plasmid profiles easier, prepare the working stocks in the 96 well plate in the same order as the published data sheet.

5. When setting up your plasmid screening, pipet 2 μL of the primer dilutions into the corresponding wells of a 96 well PCR reaction plate.

6. Make a master mix containing enough Taq polymerase, PCR buffer, dNTPs, $MgCl_2$, and ddH_2O for the entire primer set plus 10% extra (29 reaction volumes/genomic DNA when using the Akins primer set, or 21 reaction volumes/genomic DNA when using the Purser/Norris primer set).

Example: Akins PCR master mix for one set of gDNA (~32 rxns):

15 μL gDNA (approx. 1-2 μg)
64 μL 10X PCR buffer
102.4 μL dNTP mix (working stock containing 1.25 mM/each dNTP)
6.4 μL Taq polymerase (5 U/μL)
6.4 μL 150 mM $MgCl_2$
460.8 μL ddH_2O
655 μL total

7. Pipet 20 μL of the PCR master mix into each well of the PCR plate containing the 2 μL of primers for each plasmid.

8. Run PCR reaction under the following conditions:

1. 94°C for 5 min.
2. 94°C for 30 seconds
3. 55°C fro 30 seconds
4. 72°C for 1 min.
5. Cycle to step 2 for 29 more times
6. 72°C for 7 min.

9. Run reactions on agarose gel.

Akins' Primer Set:

Plasmid	Primer	Sequence	Tm	Location
lp54	BBA60-5'	ATG AGC AAA AAA GTA ATT TTA ATA T	55.3	40957-40981
	BBA60-3'	CAC TAA TTC TTT TTG AAT TAC TAA T	53.7	40151-40175
cp26	BBB03-5'	ATG CCT CCA AAA GTG AAG ATA AAA A	64.4	2162-2186
	BBB03-3'	TAG CTT ATA ATT AAA AAT TAT TGA T	51.7	840-864
cp9	BBC10-5'	ATG CAA AAA ATA AAC ATA GCT AAA T	58.0	6784-6808
	BBC10-3'	ATC TTC TTC AAG ATA TTT TAT TAT A	51.3	6284-6308
lp17	BBD11-5'	GTG TAT ACT GAC CCA AGG TCA ATT A	61.7	6681-6705
	BBD11-3'	CAA TAA TGT GAT ATT TTT AAG AAA T	54.2	7607-7631
lp25	BBE17-5'	ATG AAA GTA ATA ATA TTG TTA ATT T	51.2	10685-10709
	BBE17-3'	CTT ATG AAA AAT CAT ATC AAA TGC A	60.6	10203-10227
lp28-1	BBF18-5'	ATG GAA GAA CGA AGA AAA AAA GTG G	65.9	9561-9585
	BBF18-3'	ATA GAG ATA AGT CTT AAG GTT TAG A	52.7	10025-10049
lp28-2	BBG13-5'	ATG GCG CTG ATT ACA TTA ATT GTC G	68.1	12331-12355
	BBG13-3'	AAT CTT GAA GAA CCT TGC ATC TTT A	62.8	11504-11528
lp28-3	BBH18-5'	CTG AAA ATG AAG GAG AAG CGG GTG G	72.8	12105-12129
	BBH18-3'	TAG GCT AAT ACC AAT TCG TAC AAA T	60.5	13193-13217
lp28-4	BBI28-5'	ATG AAA TGC CAT ATA ATT GCA ACT A	62.0	17850-17874
	BBI28-3'	AAT CCG ACA GAT CTG GTT TGT CCA G	69.9	17305-17329

Plasmid	Primer	Sequence	Tm	Location
lp38	BBJ23-5’	TTG AAT GGG GTA ATT ATG AGA GAA A	63.2	16505-16529
	BBJ23-3’	ATT TTT TAT AGG AAA ATC CAT AAA C	56.6	17302-17326
lp36	BBK23-5’	ATG AAA GCC GTT ATA CCT AGT TAT A	57.9	15736-15760
	BBK23-3’	CTC AAA TTT CAA TCC CTT TGA CAA A	65.6	14837-14861
cp32-8	BBL39-5’	ATG GAG AAA TTT ATG AAT AAG AAA A	57.8	26370-26394
	BBL39-3’	TTT TAA ATT TCT TTT AAG CTC TTC T	57.0	26876-26900
	BBL40-5’	ATG AAT AAA AAA ACA ATT ATT ATT T	53.3	26931-26955
	BBL40-3’	ATC TTC TTC ATC ATA ATT ATC CTC A	58.6	28040-28064
cp32-6	BBM32-5’	AAA ACC TAA AAT AAT AAC AAT AGC G	57.6	20732-20756
	BBM32-3’	AAT CCA TTT GAA AAT CAA AAG AAG A	62.5	21393-21417
	BBM38-5’	TTT TTA GAT ACA AAA AAA GAA GAG T	55.5	26380-26404
	BBM38-3’	AAA TAT TTT TAA AGC CCA AAC CCC G	67.6	26863-26887
cp32-9	BBN28-5’	ATG AAA ATC ATC AAC ATA TTA TTT T	56.3	17302-17326
	BBN28-3’	CAC TCT TTA TAT GAT TAA GTG CAC C	59.5	17572-17596
	BBN32-5’	AAA ACC TAA AAT AAT AAC AAT AGC G	57.6	20828-20852
	BBN32-3’	TAT TAG ATT TTC TTT ATA TCT ATT C	49.1	21425-21449
cp32-7	BBO32-5’	AAA ACC TAA AAT AAT AAC AAT AGC G	57.6	20777-20801
	BBO32-3’	AAA AAA TTC TTA TTG TCT TTA AGC G	59.6	21358-21382
	BBO40-5’	TGA TCC TAA TAA CAG AGC AAT AGC A	62.5	27123-27127
	BBO40-3’	TTT ACC ATT TAT ACC ATC AAT ATC C	58.5	27591-27615

Plasmid	Primer	Sequence	Tm	Location
cp32-1	BBP32-5’	AAA ACC TAA AAT AAT AAC AAT AGC G	57.6	20777-20801
	BBP32-3’	AAA TCT ATC ATT TTT TGC TAT TCT C	57.6	21416-21440
	BBP38-5’	ATG GAG AAA TTT ATG AAT AAG AAA A	57.8	26235-26259
	BBP38-3’	TTT TAA ATT TCT TTT AAG CTC TTC T	57.0	26741-26765
lp56	BBQ05-5’	ATG AAA TAC TAT ATA TGT GTG TGT G	54.0	2744-2768
	BBQ05-3’	AAG GTT ACT TAT TGA AAA TAT CTG A	55.9	3469-3493
	BBQ08-5’	CAA ACA TTA TAA CAA TTG CAA GCC C	65.7	5790-5814
	BBQ08-3’	GAA TTT TTC CCT TTA TGT AAA AAG A	59.4	5185-5209
cp32-4	BBR41-5’	TAA TAA GAA TTC TAA GGG GTA CGA G	59.5	26109-26133
	BBR41-3’	TGT TTT TGA TTT TAT AAT CTT CTC C	58.2	26683-26707
	BBR42-5’	GTA ACT AGT AAA GAT TTA GAA GGG G	56.5	26922-26946
	BBR42-3’	AAT CCA ACA CCA CCT TGT CTT TGG A	70.1	27346-27370
cp32-3	BBS35-5’	AAA ACC TAA AAT AAT AAC AAT AGC G	57.6	21268-21292
	BBS35-3’	TTA TTA TCA AAA ATA TAG GTA AAA A	52.3	21782-21806
	BBS41-5’	AGT TTT TGT TTT GAT AAT TTC TTG C	60.3	26743-26767
	BBS41-3’	AGT ATT AGT ACC ATC ATT AAC AGA A	54.5	27175-27199
lp5	BBT03-5’	ATG AAT GGA ATA ATT AAC GAT ACA C	58.7	1208-1232
	BBT03-3’	AAT ATT AGG ATG AAG ATT ATA AAT T	52.4	1549-1573
lp21	BBU04-5’	TTT CAT TCG TTA AGG AGA GTT TGC C	66.8	1493-1517
	BBU04-3’	CAT TTA TTA TTT CCA ATA ATT CGG A	60.6	2554-2578