Isolation of Spirochete DNA Using the Wizard® Genomic DNA Purification Kit

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Purified genomic DNA can be isolated rapidly from the spirochetes Treponema pallidum *(syphilis agent) and* T. denticola *(periodontal disease agent) using the Wizard® Genomic DNA Purification System (1-3). The method previously used in our laboratory (4) involved organic (phenol/chloroform/isoamyl alcohol) extraction and required approximately three days to complete. The method described here requires 1-2 hours and consistently achieves higher DNA yields. Higher genomic DNA yields are particularly valuable for our work with* T. pallidum*, because this species must be cultured* in vivo*, producing only limited amounts of starting material. Using the Wizard® Genomic Kit for* Treponema *species, we have dramatically reduced input cell numbers from 1-2 x 10^{10} to 2-4 x 10^{9}. Yields are 0.8-1µg/µl in 100µl. Additional advantages to this method are its greater simplicity and the elimination of hazardous chemicals and concerns of accompanying waste disposal.*

Methodology

1. Cells were grown and harvested.

   T. denticola cells were grown under anaerobic conditions.

   T. pallidum cells were isolated from rabbit testicles using hydroxypropylmethylcellulose according to the method of Baseman, Nichols and Mogerly (5).

2. Cells were collected by centrifugation and washed three times with PBS.

3. For both treponemal species, 2-4 x 10^{9} cells were processed according to the Gram negative bacteria protocol provided in the *Wizard® Genomic DNA Purification Kit Technical Manual* #TM050 (6). No other modifications were made. Resulting DNA, following restriction enzyme digestion, is shown in Figure 1.

Conclusions

In our laboratory, the Wizard® Genomic DNA Purification Kit is a valuable tool for rapidly extracting highly pure DNA from treponemes. DNA extracted using this system has been routinely used for amplification, cloning and restriction analyses. This method has the additional benefit of eliminating the disposal costs and exposure hazards associated with organic phenol and chloroform in other extraction kits and protocols, including that used in the recent report on the complete genome sequence of *T. pallidum* (7).

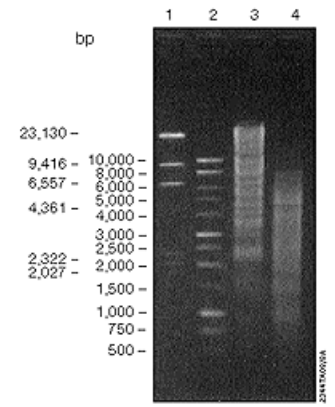

Figure 1. Extraction and digestion of genomic DNA from *Treponema pallidum* and *T. denticola*. *Treponema* sp. DNA was extracted according to the described procedure, digested (2µg) and resolved in a 0.8% agarose gel. Lane 1, Promega Lambda DNA/*Hin*d III Markers (Cat. G1711); lane 2, Promega 1kb DNA Ladder (Cat.# G5711); lane 3, *T. pallidum* genomic DNA digested with *Bam*H I; lane 4, *T. denticola* genomic DNA digested with *Hin*d III.

REFERENCES

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4. Saito, Y. and Miura, S. (1963) *Biochim. Biophys. Acta* **72**, 619.
5. Baseman, J.B., Nichols, J.C. and Mogerly, S. (1979) *Infect. Immun.* **23,** 392.
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Ordering Information

Product	Size	Cat.#
Wizard® Genomic DNA Purification Kit	100 x 300µl	A1120
	50 x 3ml	A1125
Lambda DNA/*Hin*d III Markers	100µg	G1711
1kb DNA Ladder	500µl	G5711
