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(54) **METHOD FOR PREPARING
SINGLE-STRANDED DNA LIBRARIES**

(76) Inventors: **Gina L. Costa**, Beverly, MA (US);
John H. Leamon, Guilford, CT (US);
Jonathan M. Rothberg, Guilford, CT (US); **Michael P. Weiner**, Guilford, CT (US)

Correspondence Address:
MINTZ LEVIN COHN FERRIS GLOVSKY & POPEO
666 THIRD AVENUE
NEW YORK, NY 10017 (US)

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(57) **ABSTRACT**

This invention relates to methods of generating single stranded DNA libraries for use in amplification and sequencing reactions. In various aspects, the disclosed methods include: fragmenting DNA; polishing the fragments' ends; ligating the fragments to universal adaptors; performing strand displacement and extension of the nicked fragments; purifying the double-stranded ligation products; capturing the double-stranded ligation products onto a solid support; and isolating single stranded DNA library fragments, and binding these fragments to another solid support.

Figure 1

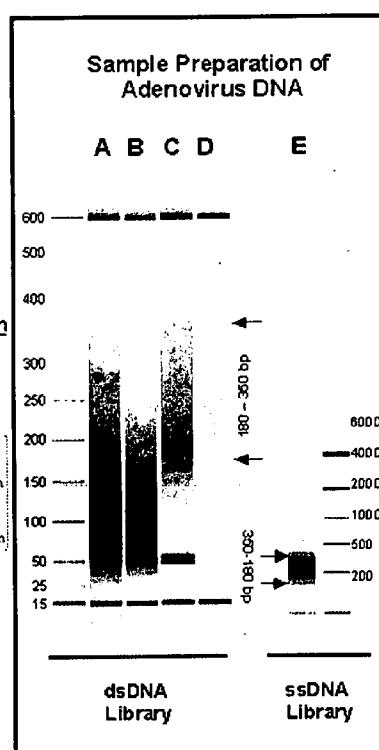
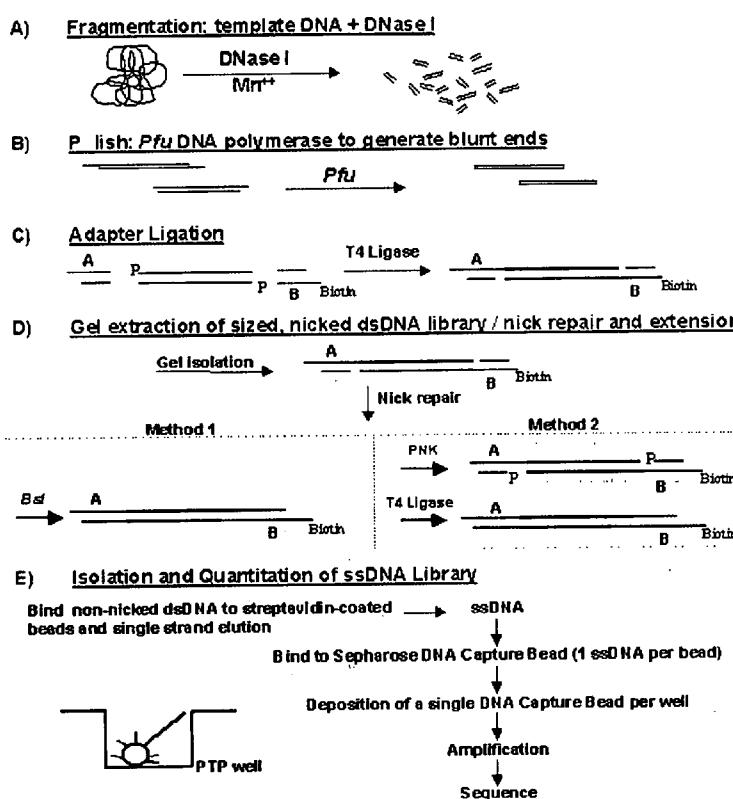


Figure 2A

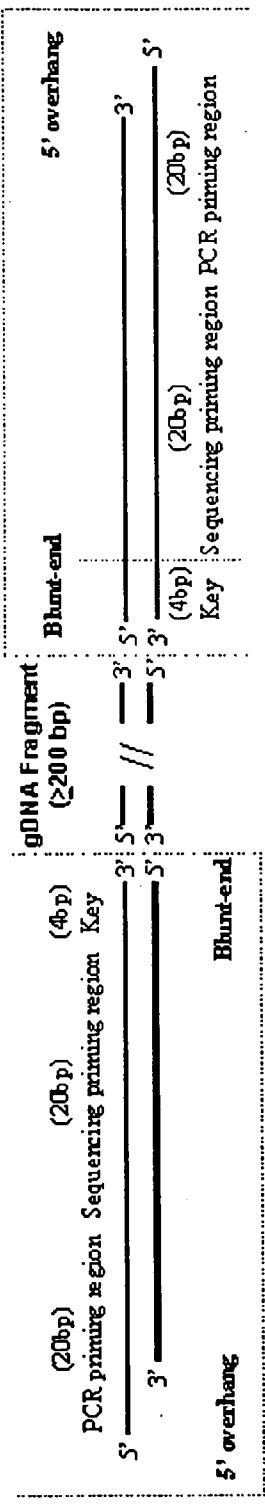
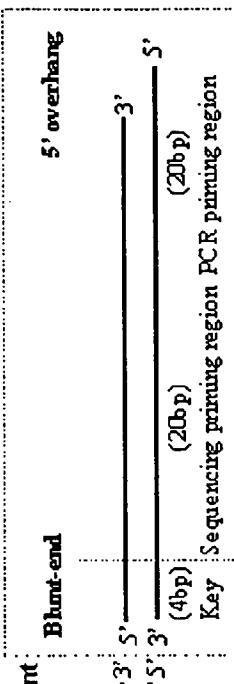
Universal Adaptor A**Universal Adaptor B**

Figure 2B

Adaptor A

MMP1A MMP7A Key DNA fragment

5' cgttttccctgtgtgccttg-ccatctgttccctccctgtc-atgc-
3' --- 5' -gcat-gacacgcaacaggatagg-gacacgacaaacag
5' --- 3' -cgta-ctgtgcgttgtccctatcc-ctgtgcgtgcgttacc-Biotin 5'
Key MMP1B
Key MMP2B

Figure 2C

(4 base 5' overhang) Adaptor ADNA fragmentAdaptor B (4 base 5' overhang)

Sense Strand 5' [PCR primer (20 bp)-----Seq Primer (20 bp)-Key (4 bp)] DNA Fragment [Key (4 bp)-Seq Primer (20 bp)] 3'
Anti Strand 3' [PCR primer (20 bp)-Seq Primer (20 bp)-Key (4 bp)] DNA Fragment [Key (4 bp)-Seq Primer (20 bp)] 3' (**BEAD**)

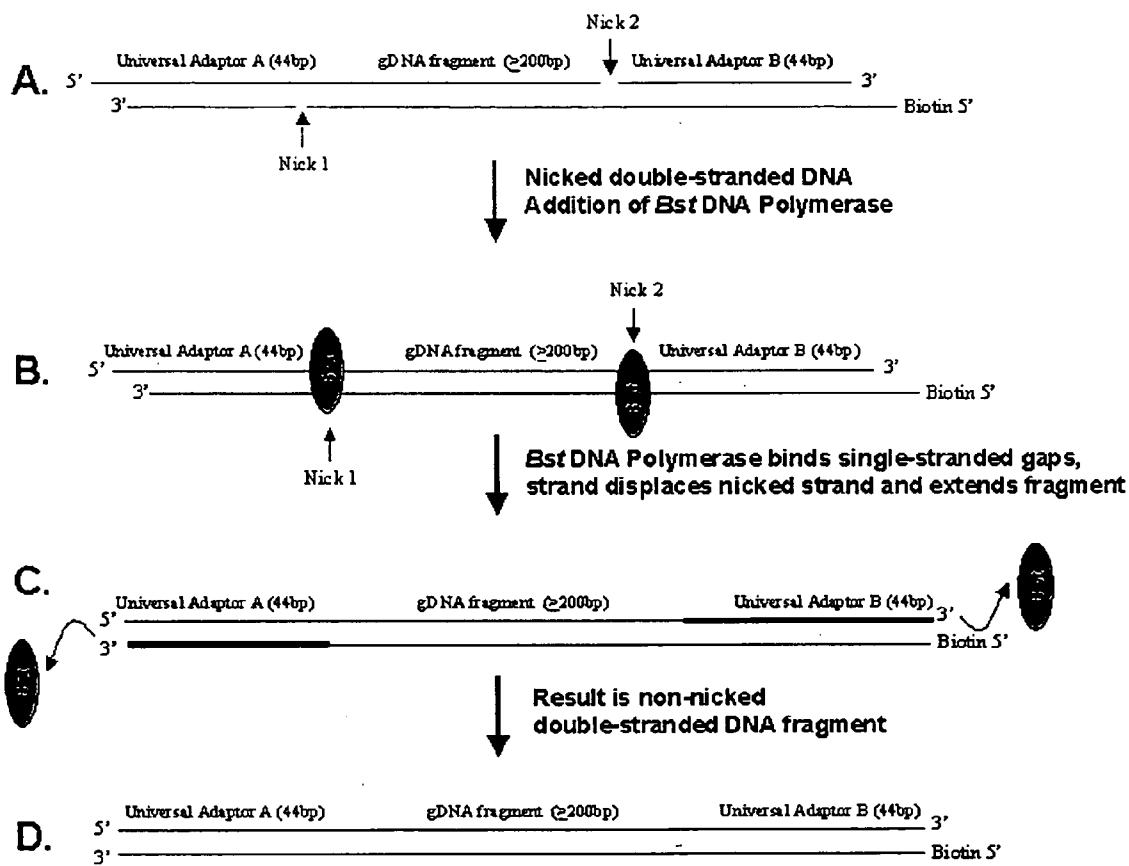
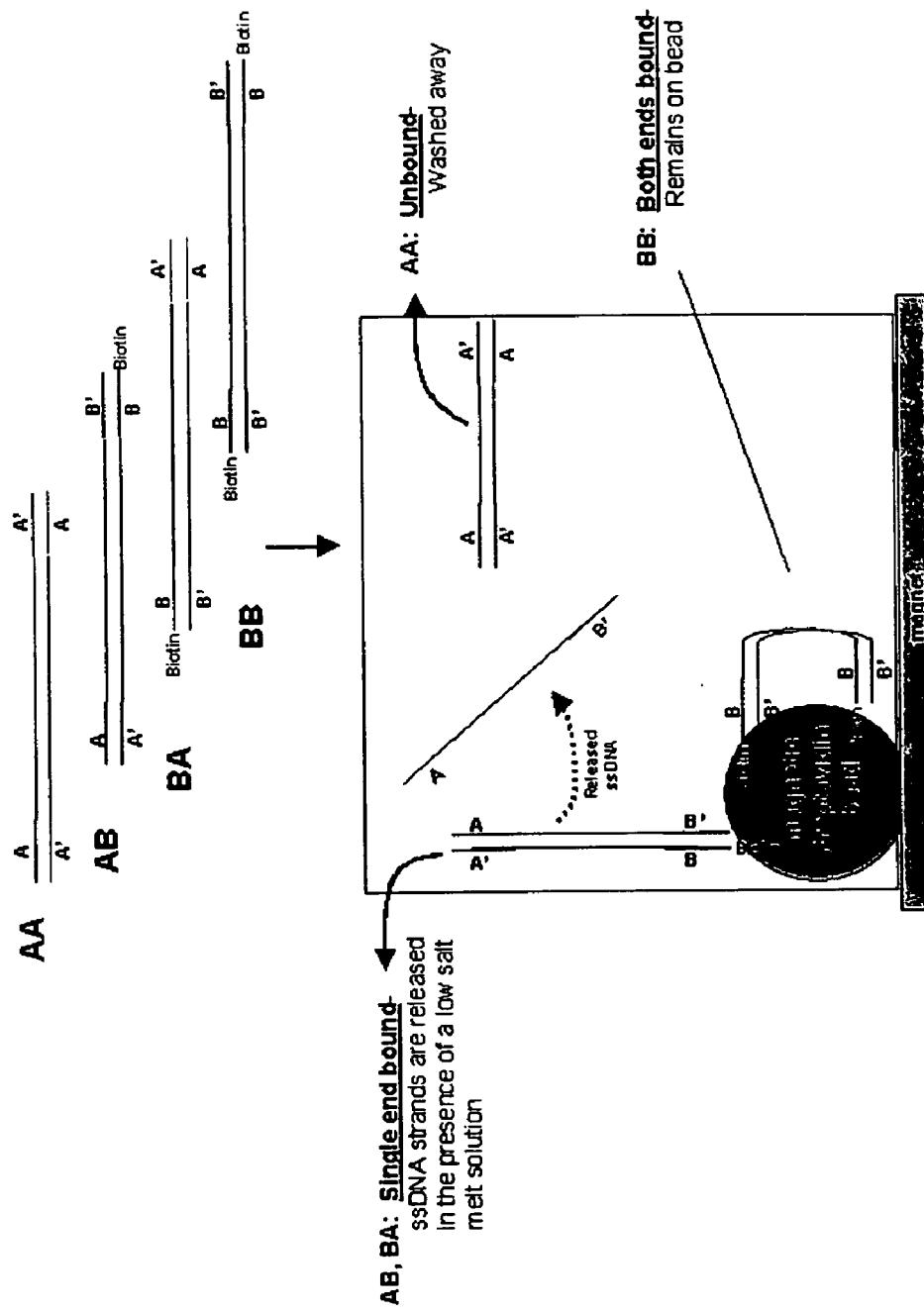
Figure 3

Figure 4

Possible double-stranded Genomic DNA Library Species:

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