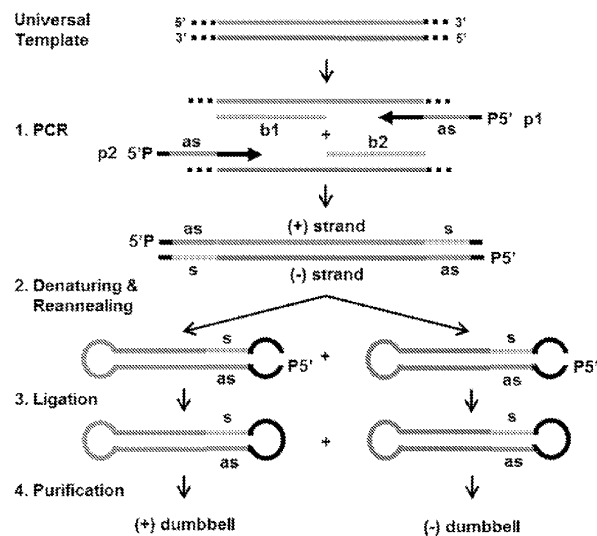




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- (71) Applicant: NATIONAL UNIVERSITY OF SINGAPORE [SG/SG]; 21 Lower Kent Ridge Road, Singapore 119077 (SG).
- (72) Inventors: PATZEL, Volker; c/o National University of Singapore, Yong Loo Lin School of Medicine / Department of Microbiology and Immunology, 21 Lower Kent Ridge Road, Singapore 119077 (SG). CYRILL, Samantha Leeanne; 109-20 Queen's Blvd., Apt. 3B Forest Hills, New York, New York 11375 (US).
- (74) Agent: DAVIES COLLISON CAVE ASIA PTE. LTD.; 10 Collyer Quay #07-01, Ocean Financial Centre, Singapore 049315 (SG).
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(54) Title: METHOD FOR THE GENERATION OF DUMBBELL-SHAPED DNA VECTORS

Figure 1



(57) **Abstract:** Dumbbell-shaped DNA minimal vectors represent genetic vectors solely composed of the gene expression cassette of interest and terminal closing loop structures. Dumbbell vectors for small hairpin RNA or microRNA expression are extremely small-sized which is advantageous with regard to cellular delivery and nuclear diffusion. Conventional strategies for the generation of small RNA-expressing dumbbell vectors require cloning of a respective plasmid vector which is subsequently used for dumbbell protection. Here, we present a novel cloning-free method for the generation of small RNA-expressing dumbbell vectors which also does not require any restriction endonucleases. The method comprises the PCR amplification of a universal DNA template using primers containing the sense or antisense strand of the sequence of interest, the denaturing and refolding of the amplified product to form stem-loop structures, and the structures are covalently closed using DNA ligases to obtain dumbbell structures.

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