Charon BS (+) and (−), versatile λ phage vectors for constructing directional cDNA libraries and their efficient transfer to plasmids

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We describe a modified lambda vector for high efficiency directional cDNA cloning. The library (or individual clones) in this vector can be transferred to plasmids in one step for further analysis. Bluescript KS H13(+) or KS H13(−) plasmid DNA (from Stratagene cloning system, CA) was digested with NotI, and ligated to a mixture of NotI adapters which have HindIII or EcoRI ends but do not regenerate these sites. The resulting product was then cloned between the Charon 15 (1) lambda arms generated by HindIII and EcoRI digestion. The vector was termed Charon BS (+) or (−) based on the plasmid KS H13(+) or KS H13(−) it contained. A map of the vector is shown in the figure.

cDNA libraries were prepared in Charon BS vector as described (2,3). To evaluate the fidelity of a library, clones for HLA class I genes were isolated by screening a cDNA library from a human lymphoblastoid cell line with the HLA-B7 probe DNA (4). All the eight purified cDNA clones had poly A and five were full length when compared with the genomic sequences (Swaroop and Shukla, unpublished data).

To transfer phage libraries or individual clones to Bluescript plasmid, the phage DNA was digested with NotI. After incubation at 65°C for 30 min, ligation of DNA under dilute concentrations (10–20 µg/ml) produced circular plasmid molecules, which were used to transform competent cells.

High efficiency of cloning in the phage vector and the easy conversion of clones to plasmids (>10^6 ampicillin-resistant colonies/µg of phage DNA) without passage through a single-stranded DNA intermediate has allowed us to construct deep cDNA libraries in Bluecript plasmid. Presence of an Fl origin and T3/T7 RNA polymerase promoters can be exploited to prepare single-stranded circular DNA or large amounts of strand-specific RNA for subtractive cDNA cloning. Knowledge of the direction of cDNA insert is valuable in improving the efficiency of subtraction procedure.

![Diagram of Charon BS vector](image)

Fig: Charon BS - The restriction enzyme symbols are as follows: B-BamHI; D-DraI; B-HindIII; K-KpnI; B-EcoRI; S-SalI; S-SacI; D-DdeI; B-HindIII. N sites in the Charon 15 phage have been removed. The sites in plasmid, however, are available for directional cDNA cloning. T3 and T7 indicate respective RNA polymerase promoters in Bluecript plasmid. Both pBR and HJ origin of replication are present. Map is in the 8-lambda gens.

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