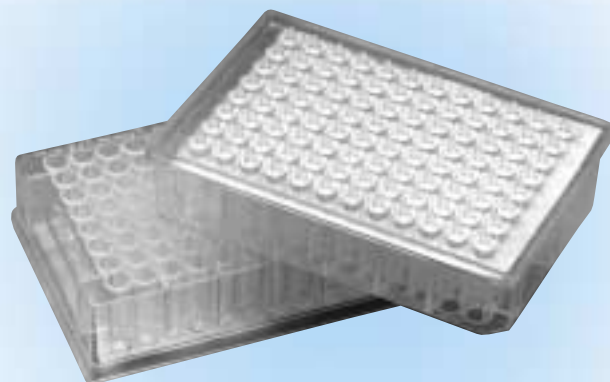


High Throughput Genomics Microplate: BAC, PAC, Fosmid and Cosmid DNA Isolation



The rise in whole genome sequencing projects has necessitated the development and use of vectors able to carry and propagate large DNA inserts. Physical maps of contiguous clones can only be prepared by cloning very long fragments of genomic DNA. Whole genes including remote control regions can encompass multiple tens of kb. Functional genomics studies require vectors capable of carrying and expressing large genes and related gene families in cultured cells.

A number of large-insert cloning vectors are currently in use. Cosmids and their more stable companions, fosmids, can accommodate DNA fragments 35 - 45 kb in length. P1 bacteriophage can propagate larger DNA fragments up to 95 kb but have lower transformation efficiencies. Bacterial artificial chromosomes (BACs) and P1-derived BACs, (PACs) can routinely carry much larger DNA fragments (150 - 250 kb) and are easily manipulated.

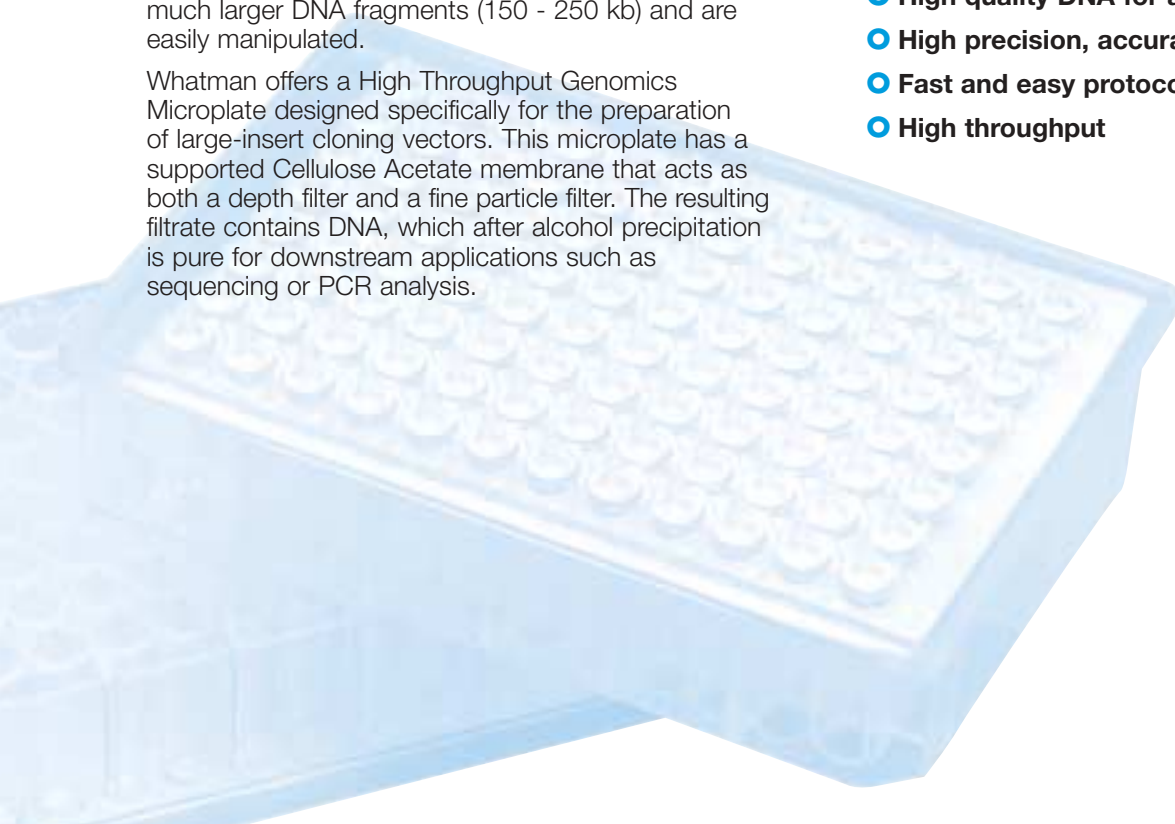
Whatman offers a High Throughput Genomics Microplate designed specifically for the preparation of large-insert cloning vectors. This microplate has a supported Cellulose Acetate membrane that acts as both a depth filter and a fine particle filter. The resulting filtrate contains DNA, which after alcohol precipitation is pure for downstream applications such as sequencing or PCR analysis.

Features

- 96 well, 800µL per well, 8 x 12 format clear polystyrene plate
- Individual discrete disks of Whatman Cellulose Acetate 0.45µm at the bottom of each well
- Nonclogging membrane filter
- Patented long drip director design for the non-crosstalk collection of filtrate
- Compatible with many automated platforms and liquid handlers

Benefits

- High quality DNA for analysis
- High precision, accuracy and reproducibility
- Fast and easy protocol
- High throughput



Protocol

This is a general procedure for isolating vector DNA from bacterial cultures using the Whatman High Throughput Genomics Microplate.¹

- Inoculate 1.2mL of culture media containing the appropriate antibiotic with a single colony from a plate and grow at 37°C overnight with agitation (~325rpm) in a 96-well High Throughput Bacterial Growth Plate.**
- Pellet cells by centrifugation at 1800xg for 5 minutes and decant the supernatant. Leave the culture plate inverted to drain residual media and blot gently on a clean paper towel. Place the culture plate containing the pelleted cells on ice while performing steps 3, 4 & 5.**
- Resuspend pellets by adding 150µL of chilled GET/RNase Buffer (50mM Glucose, 25mM Tris-HCl pH 8.0, 10mM EDTA, 0.12mg/mL RNase A) and mix by agitation or pipette tip aspiration.**
- Lyse cells by adding 150µL of Lysis Buffer (0.2N NaOH, 1% SDS). Mix thoroughly by agitation or by inversion if plate is sealed with a clear adhesive seal.**
- Neutralize the cell lysate by addition of 150µL of chilled Neutralization Buffer (3M Potassium Acetate, pH 5.5)*. Mix thoroughly by agitation or inversion if plate is sealed with a clear adhesive seal. Incubate the plate on ice for 10 minutes.**
- Spin the plate 25 minutes at 4000xg.**
- Using a multichannel pipette, transfer 400µL of the supernatant to the 0.45µm Cellulose Acetate filterplate stacked on top of an 800µL collection plate containing 330µL of 100% ethanol.**
- Centrifuge the plate stack at 3000xg for 15 minutes. Separate the plates and decant the ethanol from the collection plate leaving the DNA pellet behind (pellet may be invisible). Leave the plate inverted on paper towels to drain for 2 minutes.**
- Wash the pellet by adding 250µL of 80% ethanol to each well and centrifuge at 3000xg for 15 minutes. Decant the supernatant.**
- Dry the DNA pellet in a Speed-Vac or by inverting on a paper towel until dry.**
- Resuspend the DNA pellet from fosmid, BAC, PAC and P1 clones in 30µL of TE (10mM Tris, 0.1mM EDTA, pH 8.0) or 150µL of TE for cosmid clones.**
- The purified DNA is ready for downstream processing.**

*Decreasing the pH of the 3M Potassium Acetate to pH 4.9 is reported to give cleaner fosmid DNA¹.

Reference: ¹Marra et al., Genome Research 7: 1072-1084 1997.

Products Required

Cat No.	Description	Units/Case
7700-2808	High Throughput Genomics Microplate, 0.45µm Cellulose Acetate	25
7701-5800	UNIPLATE™ Collection Plate, 800µL natural polypropylene	25
7701-5205	High Throughput Bacterial Growth Plate	25
7704-0001	Clear Polyester Thin Cold Sealing Film, adhesive backing	100

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