

# Whole Genome Shotgun Library Approach For Microbial Sequencing Projects at the JGI

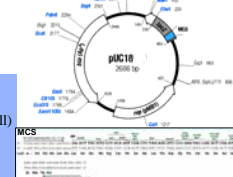
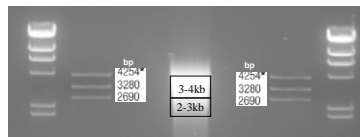
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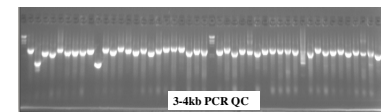
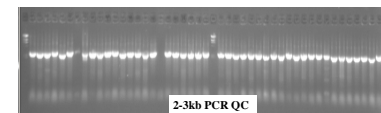
The US Department of Energy's Joint Genome Institute is a high-throughput sequencing center and user facility that has sequenced a large number of microbial genomes. The strategy for most projects calls for construction of whole genome shotgun libraries from high-molecular weight DNA isolated from an axenic culture. In general, the JGI produces 3 insert size-selected libraries for all whole genome shotgun projects. We generate a 3kb high-copy pUC18 library, an 8kb low-copy pMCL200 library, and a 40kb pCC1FOS fosmid library. The DNA is randomly sheared, fragments are end-repaired for blunt-end cloning, and then size selected on an agarose gel, extracted and purified. 3 & 8kb inserts are cloned into the appropriate vector and transformed into *E. coli*. 40kb inserts are cloned, packaged and infected by phage into *E. coli*. PCR using primers flanking the inserts are used to determine the percentage of clones with inserts for both the 3 and 8kb libraries, before proceeding to production sequencing. Clones (10-384-well plates) from each of the 3 & 8kb libraries are initially sequenced and library quality is assessed at this stage before full sequencing is completed. Both 3 & 8kb libraries are sequenced to 4x sequencing coverage and the 40kb library is sequenced to 30x clone coverage. The 3 library approach generally results in more complete genome coverage at the draft stage, and pairing information allows for contig mapping and repeat resolution during the genome assembly/finishing step.

## Small Insert: 3kb Library Construction

- Randomly shear 3-5ug of genomic DNA
- Blunt-end Repair
- Size select on gel
- Extract 2 band sizes of 2-3 & 3-4kb
- Gel purify
- Blunt-end ligate into pUC18
- Transform into electromax DH10B cells
- PCR QC 24 clones of each library

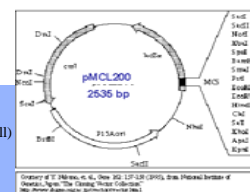
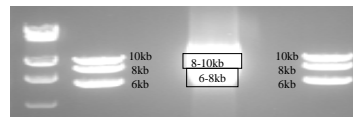


- Name: pUC18 (2.7kb)
- Replicon: pMB1
- Purpose: High Copy (~500/cell)
- Selectable Marker: Amp
- Color Selection: lacZ
- Cloning Site: SmaI

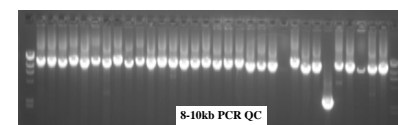
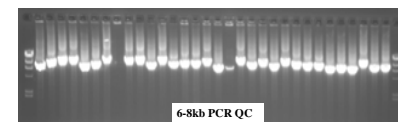


## Mid-size Insert: 8kb Library Construction

- Randomly shear 10-20ug of genomic DNA
- Size select on gel
- Extract 6-10kb fragment
- Gel purify
- Blunt-end Repair
- Size select on gel
- Extract 6-8, & 8-10kb fragments
- Gel purify
- Blunt-end ligate into pMCL200
- Transform into electromax DH10B cells
- PCR QC 24 clones of each library

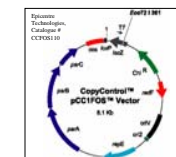
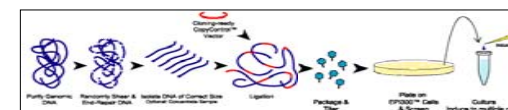
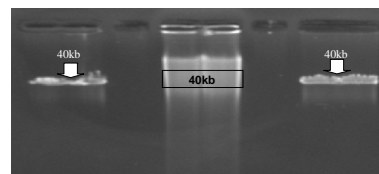


- Name: pMCL200 (2.5kb)
- Replicon: p15A
- Purpose: Low Copy (10 copies/cell)
- Selectable Marker: cml
- Color Selection: lacZ
- Cloning Site: EcoRV



## Large Insert: 40kb Library Construction

- Randomly shear 20ug of genomic DNA
- Size select on a pulse-field gel
- Extract 40kb fragment
- Gel purify
- Blunt-end Repair
- DNA Cleanup
- Blunt-end ligate into pCC1FOS
- Package into phage
- Infect *E. coli*



## Microbial Assembly/Finishing

LBNL-60350 Poster

	old 3kb lib.		plus 8kb and 40kb		QD/prefinishing	
	Major Contigs	Genome size (MB)	Major Contigs	Genome size (MB)	Major Contigs	Genome size (MB)
<b>Novosphingobium aromaticivorans</b>	197	4.17	13	4.21	9	4.215
<b>Cytophaga hutchinsonii</b>	118	4.36	23	4.41	22	4.41
<b>Methanosarcina barkeri</b>	478	3.88	77	4.83	67	4.84
<b>Ralstonia metallidurans</b>	432	NA	165	6.83	45	6.83

