Bigelow | Single Cell Genomics Center

Updated December 5, 2014

Description of Services

Service	Cat. #	Unit	Bigelow	Other nonprofit	Corporate
SERVICE GROUP: PRE-WGS					
SAG generation	S-101	384-well plate	$2,700	$3,200	$5,400
Bacteria SAG identification	S-102	384-well plate	$2,200	$2,600	$4,400
Archaea SAG identification	S-103	384-well plate	$2,200	$2,600	$4,400
Eukarya SAG identification	S-104	384-well plate	$2,200	$2,600	$4,400
PCR-sequencing, customer primers	S-106	384-well plate	$2,800	$3,400	$5,600
SAG re-arraying	S-105	96-well plate	$400	$450	$800
SAG re-MDA	S-007	1 SAG	$400	$450	$800
SERVICE GROUP: SAG WHOLE GENOME SEQUENCING (WGS)					
Prokaryote SAG WGS express	S-014	1 SAG	$1,900	$2,300	$3,800
Prokaryote SAG WGS, small batch	S-015	10 SAGs	$8,900	$9,900	$17,800
Prokaryote SAG WGS, medium batch	S-016	48 SAGs	$25,900	$29,900	$51,800
Prokaryote SAG WGS, large batch	S-017	384 SAGs	$149,900	$179.900	$299,800
SERVICE GROUP: MISCELLANEOUS					
Consultation	S-011	1 hour	free	$260	$450
Customized Services	S-100		Req. a quote	Req. a quote	Req. a quote

All prices are in US dollars (USD). 15% discount is applied if more than 10 of the same items (catalog number) are ordered simultaneously. A 30% discount is applied for services S-102, S-103, and S-104, when fewer than 10 SAGs are identified per 384-well plate. Service fees do not include shipping costs.

Overview

Single cell genomics consists of a series of integrated processes, starting with appropriate collection and preservation of environmental samples, followed by physical separation, lysis, and whole genome amplification of individual cells, then proceeding to either targeted loci or whole genome sequencing and sequence interpretation (1). SCGC offers a comprehensive suite of single cell genomics services, from single cell separation to genome sequencing and bioinformatics. SCGC also provides advice on environmental sample collection and storage protocols (please see Preparation and Shipment on SCGC website) and post-sequencing analyses (customized services). The general methods that are currently employed by SCGC have been described previously (2-7). However, please keep in mind that continued method improvement is a significant component of SCGC activities, and many details of our protocols regularly evolve.

SERVICE GROUP: PRE-WGS

SAG generation (S-101)

Single amplified genomes (SAGs) are whole genome amplification products obtained from individual cells. The SCGC SAG generation service includes individual cell separation into wells of a 384-well plate FACS, cell lysis, and single cell multiple displacement amplification (MDA). Deliverables include a) one 384-well microplate containing single cell MDA products, 10 uL per well, usually 0.5-5 micrograms gDNA per well; b) FACS data files; and c) MDA kinetics data files. For a standard S-101 service fee to be applicable, the following conditions must be met:

1. Samples do not contain biohazards or radioactivity.
2. Samples are suitable for FACS, i.e., cells are in aquatic solution and are less than 100 micrometers in diameter. Please see Preparation and Shipment on SCGC website for instructions on how to prepare various types of samples, such as aquatic, sediments, and soils.
3. Samples are cryo-preserved, following SCGC protocols (please see Preparation and Shipment on SCGC website).
4. FACS is based on SYTO-9 fluorescence (provided by SCGC), cell autofluorescence, or probes that are applied by SCGC customers prior to shipping to SCGC.
5. Only one sample and one sort gate is used per microplate, following SCGC's standard plate setup (see figures below).

IMPORTANT: Samples that have not been brought into aquatic suspension by SCGC customers may also be analyzed as a customized service. Please contact SCGC manager Brian Thompson for additional advice on how to produce FACS-compatible samples in your own lab or for a feasibility assessment and a quote for this work to be completed by SCGC.

High-speed fluorescence-activated cell sorting (FACS) is used to separate individual cells and to deposit them into 384-well microplates containing 600 nL 1x TE buffer per well. FACS offers a wide array of options for selective, automated cell sorting based on light scatter and fluorescence. The fluorescence may originate from native cell components (e.g., photosynthetic pigments) or be induced by stains (e.g., SYTO-9 for DNA), antibodies, FISH probes, fluorogenic cell activity probes (e.g., CTC), etc. For random cell sorting, SCGC's standard stain is a nucleic acids stain SYTO-9 (Invitrogen).

Single cell lysis is performed using cold KOH treatment (8), preceded by a series of five freeze-thaw cycles.

Single cell MDA is performed to obtain sufficient quantities of gDNA for downstream PCR, shotgun sequencing, or other analyses. MDA (9) produces high molecular weight copies of the genomic DNA (average amplicon length above 10 kbp). Differences in SCGC services from standard MDA protocols include DNA contaminant removal from reagents, instruments, workspaces, and consumables (2-7). Furthermore, we conduct real-time monitoring of the reaction kinetics in each well, which informs us about a) potential contamination of a specific microplate, and b) the success of MDA in a specific well. A row and three columns of positive and negative controls are included in each 384-well MDA microplate as a quality control measure (see figures below).

SAG generation success varies significantly among samples and depends on a variety of factors, such as a) preservation of intact cells and gDNA prior to cell sorting, b) successful cell discrimination from other particles during FACS, and c) successful single cell lysis (please see examples in Fig. 1), etc.

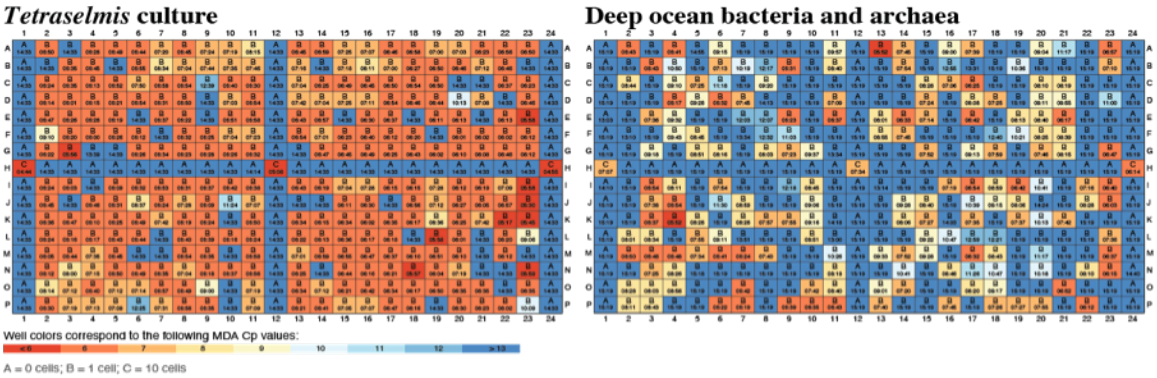

Fig. 1. Examples of MDA kinetics in microplates with divergent MDA success rates: a) cultured eukaryote alga *Tetraselmis*, and b) marine bacteria and archaea from 3,000 m depth. The MDA Cp values indicate time (hours) required to reach half of the maximal DNA-SYTO-9 fluorescence in each well. The MDA Cp is inversely correlated with the initial quantity of gDNA that is available to MDA polymerase.

SAG identification (S-102; S-103; S-104)

For tentative SAG identification, SCGC employs PCR amplification of small subunit (SSU) ribosomal RNA genes, followed by amplicon sequencing and subsequent database searches for closest relatives. These analyses are performed on the entire plate of SAGs that were produced by service S-101. SAG identification deliverables include: a) manually curated, partial SSU rRNA gene sequences of SAGs; b) BLASTn (10) against NCBI's non-redundant (nr) database output; and c) draft classification using RDP Classifier (11), SILVA Aligner (12), KeyDNATools, or a similar method. SCGC's standard PCR primers are listed in Table 2.

Table 2. PCR primers used in standard SCGC SAG identification.

Service	Forward primer	Reverse primer	References
S-102 Bacteria	27F: AGR GTT YGA TYM TGG CTC AG	907R: CCG TCA ATT CMT TTR AGT TT	(13-15)
S-103 Archaea	Arc_344F: ACG GGG YGC AGC AGG CGC GA	Arch_915R: GTG CTC CCC CGC CAA TTC CT	(16-18)
S-104 Eukarya	Euk528F: CCG CGG TAA TTC CAG CTC	EukB: GAT CCT TCT GCA GGT TCA CCT AC	(19, 20)

Sanger technology is used for PCR product sequencing from both ends. The two reads are quality-trimmed, assembled and curated. BLASTn (10) searches against nr are used to identify closest relatives in public databases. Draft classification is generated using RDP Classifier (11), SILVA Aligner (12), KeyDNATools (http://keydnatools.com), or a similar method.

The number of identified SAGs varies widely among samples and depends on the MDA success (discussed above), as well as on the suitability of PCR primers for the taxonomic groups present in the analyzed sample. SAG identification using alternative PCR primers may be performed as a customized service.

PCR-sequencing, customer primers (S-106)

PCR is performed using primers and conditions provided by SCGC customer. Sanger technology is used for PCR product sequencing from both ends. The two reads are quality-trimmed, assembled and curated. BLAST searches against nr are used to identify most similar sequences in public databases. These analyses are performed on the entire plate of SAGs that were produced by service S-101. Deliverables include: a) manually curated, partial sequences of PCR amplicons; b) results of BLASTn against NCBI's nr database.

SAG re-arraying (S-105)

SAGs are transferred from the original 384-well plates to 96-well plates. The SCGC customer defines transfer volumes, source wells, and destination wells. Prior to the transfer, the destination wells are pre-filled with 5-150 uL of either deionized water or 1x TE buffer, as specified by the SCGC customer. Deliverables include re-arrayed SAGs in a 96-well plate.

SCGC customers may be interested in a DNA concentration measurement, which can be performed simultaneously with S-105. This can be requested as a customized service.

SAG re-MDA (S-007)

In some cases, SCGC customers may need larger quantities of SAG gDNA than are available from the original MDA reaction (usually 0.5-5 ug). To achieve this goal, a second, larger-volume MDA reaction is performed, using products from the first single cell MDA reaction as the template. Deliverables include single cell MDA amplicons containing at least 100 micrograms gDNA, as well as DNA concentration measurements in the original and the secondary MDA products.

SERVICE GROUP: SAG GENOMIC SEQUENCING

Prokaryote SAG whole genome sequencing express (S-014)

This service includes sequencing library preparation, genomic sequencing, *de novo* assembly, and assembly quality control. Deliverables include original sequence reads, read QC (fastqc), assembled contigs, assembly statistics, principal component analysis of tetramer frequencies and the output of BLASTn against NCBI's nr database. Typically, this service is completed in 2-4 weeks. ***This service requires SAGs produced by S-101 as its input.***

On average, at least 5 million 2x150 bp or longer paired-end reads are generated per SAG using in-house MiSeq and NextSeq (Illumina) instruments. The obtained reads are pre-processed and *de novo* assembled using algorithms that are optimized for single cell MDA products (3, 4, 21, 22). A combination of tetramer homogeneity tests and blast searches against reference databases is used to detect potential DNA contaminants among the assembled contigs (3, 4, 21, 22). Benchmark data demonstrating SCGC SAG WGS pipeline performance is available here:
http://data.bigelow.org/~scgc/WGS_benchmark_data/

Please note that only partial genomes typically are recovered from individual cells. In our experience, genome recovery from SAGs of environmental microorganisms ranges from 0% to 100%, with an average of about 60% (7, 23). Low genome recovery may be caused by diverse factors, such as viral infections of the cell prior to sorting, incomplete cell lysis, DNA interactions with other cellular components, MDA and sequencing biases, and difficulties in de novo assembly. If multiple, closely related SAGs of interest are available, the following strategies may significantly improve the probability of high genome recovery: a) sequencing SAGs with lowest MDA Cp values, and b) sequencing multiple SAGs for a population co-assembly.

Genome annotation is not included in the S-014 service. However, SCGC can help with data submission to public annotation pipelines, such as IMG (http://img-stage.jgi-psf.org/cgi-bin/submit/main.cgi) and RAST (http://rast.nmpdr.org/).

SCGC's standard WGS services currently are limited to Bacteria and Archaea. However, WGS of viral and eukaryote SAGs may be performed as a customized service.

Prokaryote SAG WGS, small batch (S-015)

This service yields the same deliverables as S-014 but for a batch of up to 10 prokaryote SAGs. The same fee is applied for sequencing of batches smaller than 10 SAGs (alternatively, you may order individual SAG sequencing services S-014).

Prokaryote SAG WGS, medium batch (S-016)

This service yields the same deliverables as S-014 but for a batch of up to 48 prokaryote SAGs. The same fee is applied for sequencing of batches smaller than 48 SAGs (alternatively, you may order multiple small batches, service S-015).

Prokaryote SAG WGS, large batch (S-017)

This service yields the same deliverables as S-014 but for a batch of up to 384 prokaryote SAGs. The same fee is applied for sequencing of batches smaller than 384 SAGs (alternatively, you may order multiple small and/or medium batches, services S-015 and S-016).

SERVICE GROUP: MISCELLANEOUS

Consultation (S-011)

Basic support is included in the pricing of all SCGC services. If the SCGC customer requires more extensive help with study design and/or data interpretation (greater than two hours per project), we will request compensation for the associated labor costs via collaborative research grants or consultant fees. We may also request co-authorship on resulting publications for those SCGC scientists who are providing substantial, project-specific intellectual input.

Customized services (S-100)

SCGC offers a wide range of customized services, e.g., non-standard cell sorting, genomic sequencing of isolates and eukaryote SAGs, metagenomic and metatranscriptomic sequencing, bioinformatics support, method development, etc. Co-authorship on resulting publications may be requested for those SCGC scientists who are providing substantial, project-specific intellectual input. For more information, please contact SCGC manager Brian Thompson (bthompson@bigelow.org).

Cost estimate: Case study

Let's assume that an external SCGC customer from an academic institution wants to a) generate one 384-well plate of prokaryote SAGs, b) identify bacteria and archaea among those SAGs, and 3) perform whole genome sequencing of nine of these SAGs. The associated costs are summarized in Table 3. Deliverables for this project will be:

1. One 384-well microplate containing single cell MDA products (SAGs)
2. FACS and MDA kinetics data files
3. Fasta files of SSU rRNA genes of bacteria and archaea SAGs
4. Partial genome assemblies of nine SAGs and associated metadata

Table 3. Cost estimate for an SCGC project.

Service	Cat. #	Unit	Price per unit	# of units	Amount
SAG generation	S-101	384-well plate	$3,200	1	$3,200
Bacteria SAG identification	S-102	384-well plate	$2,600	1	$2,600
Archaea SAG identification	S-103	384-well plate	$2,600	1	$2,600
Prokaryote SAG WGS, small batch	S-015	10 SAGs	$9,900	1	$9,900
Total					**$18,300**

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