SOP of Sample Submission for GCM Projects

Version: 1.0

November 23th, 2017

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1. CTAB DNA Extraction Protocol

1) Add 200mg pellet obtained by centrifugation or liquid nitrogen grinding into 2ml microcentrifuge tube, Mix with 1ml of CTAB Extraction Buffer and vortex thoroughly.
2) Note: The amount of sample can be scale up appropriately to serve the requirements of different sequencing methods (details as given in SOP of Sample Submission for GCM Projects).
3) Transfer the sample to a 60 ℃ bath for 30 minutes.
4) Following the incubation period, centrifuge the homogenate for 5 minutes at 14,000 x g. Transfer supernatant to a new 2ml tube. Add 5 µl of RNase solution A and incubate at 32 ℃ for 20 minutes.
5) Add an equal volume of phenol and chloroform/isoamyl alcohol (24:1). Gently mix by turning the tubes for several times (about 5min.) until forming homogeneous emulsion, then centrifuge the sample for 10 min. at 14,000 x g to separate the phases. Transfer the aqueous upper phase to a new 2ml tube. Repeat this extraction for 2-3 times until the upper phase is clear.
6) Transfer the upper aqueous phase carefully to a precooled tube. Precipitate the DNA by adding 2/3 volume cold isopropanol and 10% 3M sodium acetate (NaAc) and incubate at -20 ℃ for 15 minutes or overnight.
7) Centrifuge the sample at 14,000 x g for 10 minutes. Decant the supernatant without disturbing the pellet and subsequently wash with 750 µl ice cold 70% ethanol. Decant the ethanol. Remove residual ethanol by drying in a SpeedVac.
8) Dry the pellet long enough to remove alcohol, but without completely drying the DNA. Dissolve DNA in100ul. The pellet may need warming in order to dissolve.

Materials Needed

1) CTAB buffer: 2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone,100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA
2) Centrifuge (up to 14,000 x g)
3) Isopropanol

4) 70% Ethanol

5) 2 ml centrifuge tubes

6) SpeedVac

7) 3M NaAc (pH 4.8)

2. Culture sample requirement

Take a single colony and inoculate with fresh medium (50-100mL) at suitable temperature overnight. OD600 at 0.6-0.8 is advisable (indicating exponential phase of bacterium). Collect the microorganism samples by centrifuge with culture medium removed, and rinse the samples by bacteria-free water or PBS for 1-3 times. Samples can be placed in a suitable size centrifuge tube wrapped with parafilm, and then shipped in dry ice. 1.5mL/ 2mLtube should be placed in a 50mLtube for better protection during transportation. If dry ice transportation is not available, samples must be re-suspended in fresh Culture medium and must be shipped at room temperature.

3. DNA sample requirement

Sample requirements: EDTA should not present in the buffer that dissolve DNA. Samples should not contain any particulates, chelating agents, divalent metal cations, denaturants and detergents, fluorescent dye (judge by naked eye) and non EB-gel resolving product. Samples should be not repeated freezing and thawing, stored in a high temperature or extreme PH solutions (PH < 6 or PH > 9). Samples are recommended shipped on dry ice.

Since Pacbio has higher sample quality requirements, in order to deliver the sample successfully, customers must achieve the above sample delivery standards and purify the sample with care to avoid polysaccharides, proteins and exonuclease residues.

Bacteria/Fungi De novo DNA Sequencing （Pacbio Platform）						
Sample Type	**Library Type**	**Mass**	**Concentration**	**OD**	**Integrity (AGE)**	**Sample Purity**
Genomic DNA	20KB Library	⩾5μg	⩾60ng/μL	OD260/280: 1.6-2.2 OD260/230: 1.6-2.2	The band shown on gel electrophoresis has little degradation, or of fragment size greater than 40kb.	No contamination with RNA, protein or saltions; colorless and transparent; non-sticky

Bacteria/Fungi De novo DNA Sequencing （ILLUMINA Platform）					
Sample Type	**Library Type**	**Mass**	**Concentration**	**Integrity (AGE)**	**Sample Purity**
Genomic DNA	500bp Library	⩾1μg	⩾12.5ng/μL	The band shown on gel electrophoresis has little degradation, or of fragment size greater than 20kb.	No contamination with RNA, protein or saltions; colorless and transparent; non-sticky.
Genomic DNA	PCR-free Library	⩾10μg	⩾30ng/μL		

Metagenomic Survey DNA Sequencing （ILLUMINA Platform）					
Sample Type	**Library Type**	**Mass**	**Concentration**	**Integrity (AGE)**	**Sample Purity**
Genomic DNA	270bp Library	⩾1μg	⩾12.5ng/μL	The band shown on gel electrophoresis has little degradation, or of fragment size greater than 20kb.	No contamination with RNA, protein or saltions; colorless and transparent; non-sticky.

Meta rDNA Amplicon Sequencing（ILLUMINA Platform）				
Sample Type	**Mass**	**Concentration**	**Integrity (AGE)**	**Sample Purity**
Genomic DNA	⩾0ng (above 50ng recommended)	⩾0ng/μL	Sample must be genomic DNA.	No contamination with RNA, protein or saltions; colorless and transparent; non-sticky.

4. Sample Submission to WDCM

The GCM project Sample Information Submission System is designed for electronic and standardized management of the sample information to ensure its integrity and effective utilization. It includes the functions of sample information's input, storage and query etc.

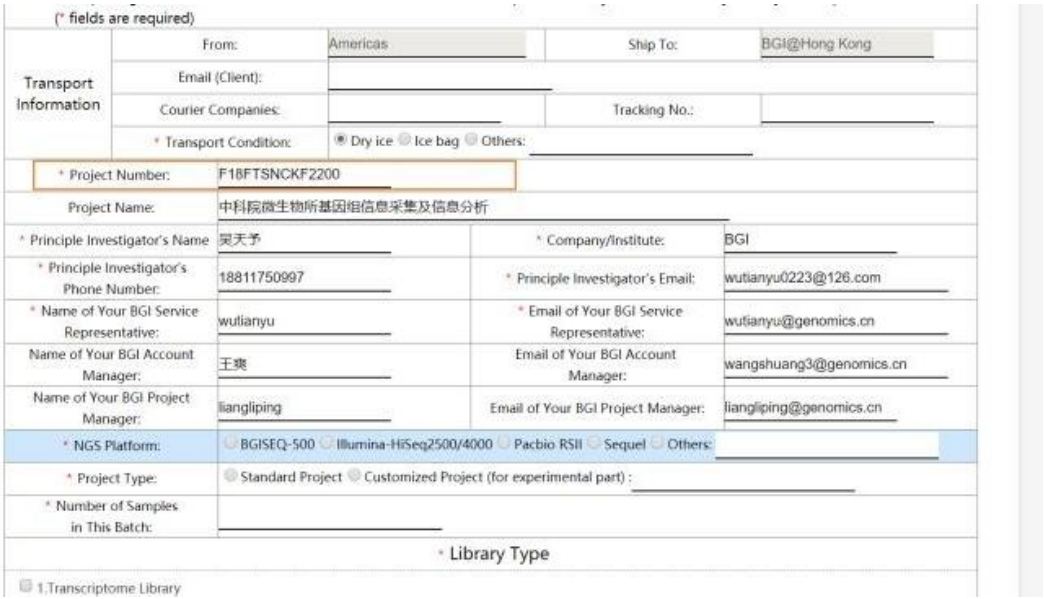
Step1.Filling out Sample Information Form

In preparation for shipping samples, please follow the instructions and take few minutes to fill out the attached sample information Form. Information includes the sample related metadata description.

Before shipping of the sample, would you please at first input the sample information through the online system www.sample.bgi.com of BGI

Please fill in the project number with : **F18FTSNCKF2200**

After you filling the project number, the following information will be automatically filled.



(* fields are required)

Transport Information	From:	Americas	Ship To:	BGI@Hong Kong
	Email (Client):			
	Courier Companies:		Tracking No.:	
	* Transport Condition:	Dry ice / Ice bag / Others:		
* Project Number:	F18FTSNCKF2200			
Project Name:	中科院微生物所基因组信息采集及信息分析			
* Principle Investigator's Name	吴天予	* Company/Institute:	BGI	
* Principle Investigator's Phone Number:	18811750997	* Principle Investigator's Email:	wutianyu0223@126.com	
* Name of Your BGI Service Representative:	wutianyu	* Email of Your BGI Service Representative:	wutianyu@genomics.cn	
Name of Your BGI Account Manager:	王爽	Email of Your BGI Account Manager:	wangshuang3@genomics.cn	
Name of Your BGI Project Manager:	liangliping	Email of Your BGI Project Manager:	liangliping@genomics.cn	
* NGS Platform:	BGISEQ-500 / Illumina-HiSeq2500/4000 / Pacbio RSII / Sequel / Others:			
* Project Type:	Standard Project / Customized Project (for experimental part) :			
* Number of Samples in This Batch:				

* Library Type

1.Transcriptome Library

Then would you please print the sample form through online system and send it together with the DNA sample.

Step2. Signed declaration letter

Declaration letter is expected to complete with the letterhead or logo of your organization

and brief sample information for the consideration of biosafety and clearance of Customs. A signature is required on the declaration letter. The demo declaration letter is in the appendix .

It is appreciated and required to attach the hard copy of sample information and signed declaration letter to the shipping package.

Step3.Sample Shipping

1) Cultures

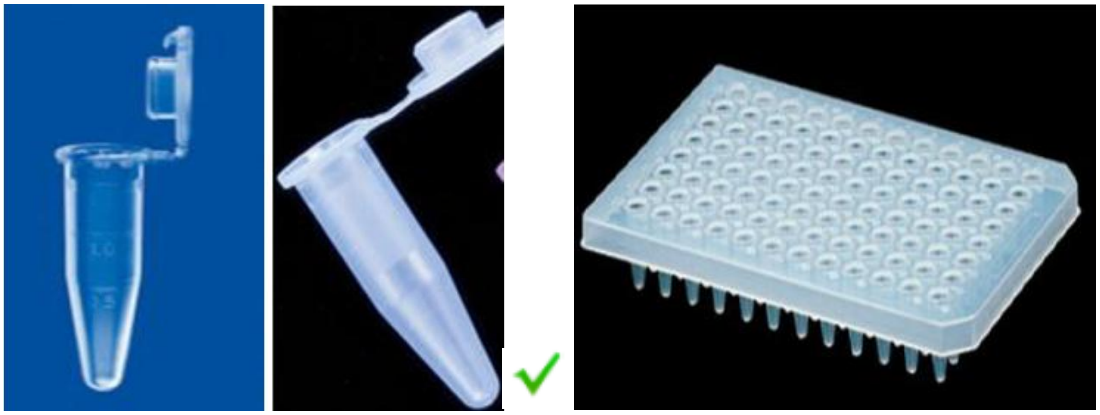
Shipping Address：

Consignee's Name	Institute of Microbiology, Chinese Academy of Science, Linhuan Wu
Consignee's Address	NO.1 Beichen West Road, Chaoyang District, Beijing 100101, China
Contact No.	+86-13811000502
Email	wulh@im.ac.cn

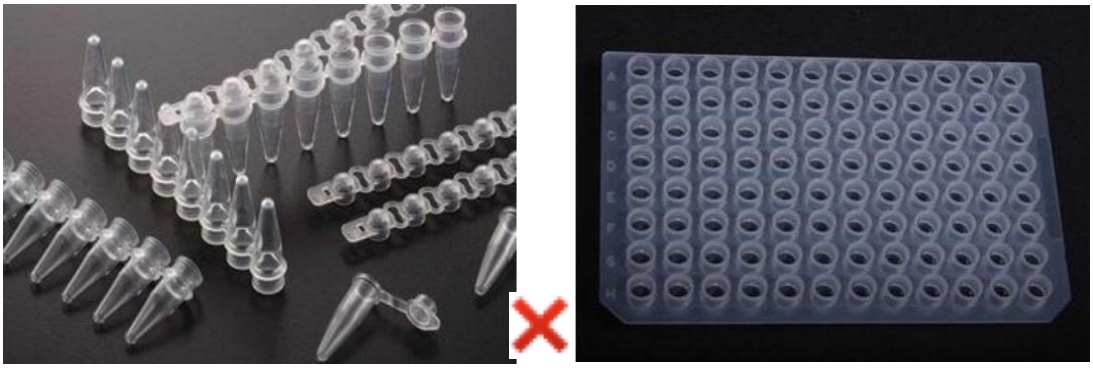
please use the POST system to send the sample to avoid the Chinese custom check.

2) DNA Samples

It is strongly recommended to collect all of the samples tubes (e.g.0.5ml) into a bigger tube (e.g. 5ml), which will prevent from being broken during shipping. Please pack the tube (s) and sufficient dry ice, 15 lbs for domestic shipping and 20 lbs for international shipping, into a foam container with a paper outer box. Filling up the empty space in the package also helps.



1.5mL Eppendorf tube or half skirted PCR plate is recommended



Don’t use single PCR tube, 8-well strip PCR tube or enclosed a PCR plate with domed cap.

Eppendorf tube placed in 50 mL centrifuge during sample Shipping

Shipping Address

The shipping address will be also shown on the completed sample information form automatically as long as client selects a correct SHIP TO facility in online sample system.

Consignee's Name	BGI Tech Solutions (Hong Kong) Co., Limited Sample Management Center ATTN: Ms. Wenyan XU
Consignee's Address	1/F, 16th Dai Fu Street, Tai Po Industrial Estate, Tai Po, Hong Kong
Contact No.	852-3952 2150/ 852-9683 9836
Email	bgihk.sample@genomics.cn
Remarks	Please note that Hong Kong has no postal/ zip code. To avoid delays/loss of samples due to incorrect routing, do not include "China" or "CN" in consignee's address. If "Country" is required, use "HongKong".

Recommended shipping date Samples

Samples	**Recommendedshipping day:**	**Recommended weight of dry ice**	**Recommended Shipping type**
Shipped to BGI@Hong Kong byclients directly	Monday or Tuesday	20 lbs	Internationalpriority shipping

Once the samples are received by WDCM or BGI, a detailed notification email will be sent to the clients.

Appendix 1

Declaration Letter

11/9/2017

To whom it may concern,

This is to certify that this shipment contains 10 DNA Samples that are for research purposes only. The samples were derived from microorganisms and were prepared in a lab. The materials are not hazardous, infectious, HIV positive, toxic or radioactive. The materials will be destroyed by

autoclave after experiments.

I hereby agree to send these materials to Institute of Microbiology, Chinese Academy of Sciences/BGI@HONGKONG genomics center for research purposes. Thank you for your attention.

Yours faithfully,

(Signature)

(Name)

(Title)