

Guidance notes

Genotyping by sequencing (GBS) service guidance notes

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1. Introduction

These guidance notes have been developed to assist with the submission of samples and supplementary information to LGC, Biosearch Technologies™ for a GBS service project.

GBS facilitates high-throughput discovery of SNPs and simultaneous genotyping in multiple DNA samples. It combines restriction enzyme-mediated complexity reduction with the high-throughput sequencing capacity of Illumina platforms to score random markers across an entire genome.

The GBS service is all inclusive, allowing for DNA extraction, fragmentation by restriction enzymes, library preparation, NGS run of pooled samples, and variant calling in the NGS dataset. Please note that customers can submit extracted DNA and be quoted revised pricing excluding extraction. Customers can also perform variant calling themselves and excluding this step from the all-inclusive service will impact pricing.

If you are a new customer, please ensure you have registered with the Biosearch Technologies Sequencing Shop prior to the start of your project by completing the [online registration form](#) under “New user account”.

For a free-of-charge consultation, please contact ngs@lqcgroupp.com.

Biosearch Technologies has secured a license for the commercial offering and use of GBS (sometimes known as SBG) technology across all species and geographies from KeyGene. Customers working with Biosearch Technologies are free to use derived data in any way and do not need any further license for the use of the GBS/SBG technology.

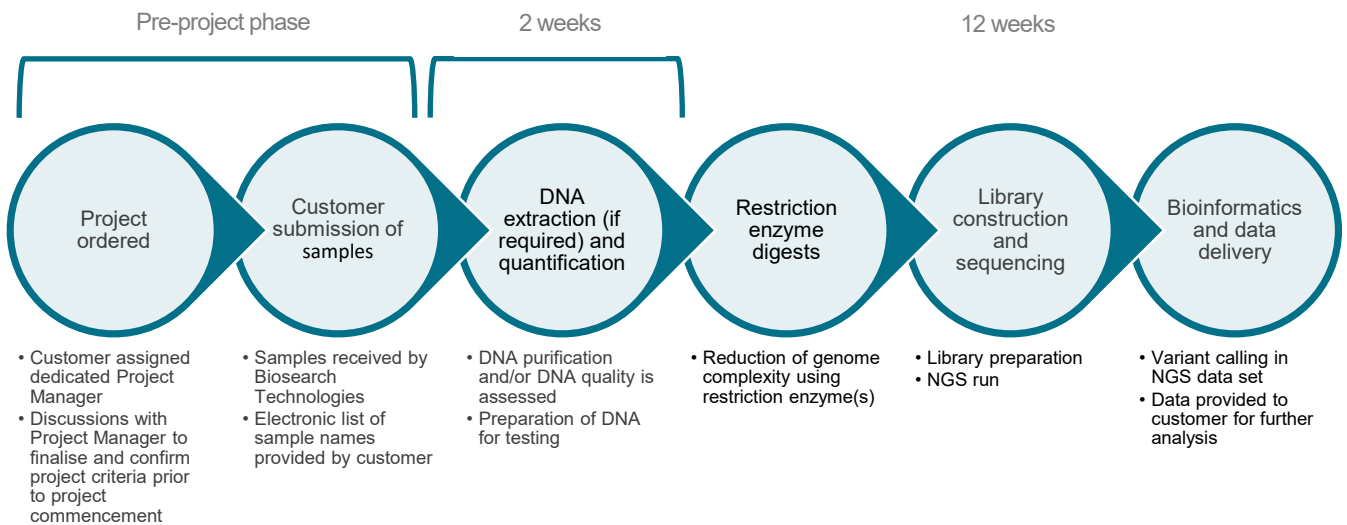
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2. Project workflow

Before commencing their GBS project, the customer will have a detailed discussion with their dedicated Project Manager to ensure all documentation, sample and shipping requirements are met.

Below outlines the details of the processes involved in the GBS service:



Faster turnaround times may be possible if requested. Please discuss this with our sequencing specialists (ngs@lqcgroupp.com) in advance of commencement of the project.

3. Sample submission

Please see the tables in Appendix A, B and C for information on documentation, DNA and tissue submission requirements.

3.1. Sample requirements

The minimum number of samples required for a GBS project is 24. There is not a maximum number, but GBS projects do not typically exceed 1920 samples.

For species that lack scientific publications regarding GBS, and/or that Biosearch Technologies has not previously used in an in-house GBS project, Biosearch Technologies recommends a small pilot project involving 6 samples prior to proceeding with a full GBS project.

Samples for a GBS service project can be submitted in the format of extracted DNA (see Appendix B) or tissue samples (see Appendix C). Prices are based on the receipt of samples in 96-well plate format; if sample transfer from individual tubes is required, additional charges will be incurred.

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3.2. Submitting tissue samples

Prior to submitting samples for purification, please ensure you have completed and submitted the [NGS sample submission form](#).

For GBS projects it is essential that Biosearch Technologies can obtain high molecular weight DNA. For this reason, storage and transport of the tissue samples is very important. Plant and fish tissue should be sampled using the relevant BioArk™ collection kits (see Appendix B).

For non-plant/non-fish tissue, sampled tissue should be placed immediately in 96-99% absolute ethanol or frozen at -20 °C following preparation. Tissue stored in ethanol can be shipped at room temperature. Fresh frozen tissue must be shipped on dry ice.

If submitting plant samples, please provide species and ploidy information.

Please note: the amount of DNA that can be extracted can vary due to a wide range of factors including sample condition and sample quality. Biosearch Technologies cannot guarantee the amount of DNA that will be obtained from submitted samples but will contact you to discuss further if there are any concerns.

3.2. Submitting DNA samples

- **For all DNA samples** please provide the following information for each sample:
 - DNA purification method and/or purification kit used, e.g. Biosearch Technologies [sbeadex™ chemistry](#) (preferred)
 - DNA concentration (ng/μL)
- **If submitting plant samples**, please provide the following information:
 - Species and ploidy

4. Data analysis and results

On completion of the project, a dataset containing the following is delivered to the customer:

- Raw reads in FASTQ format (sequencing data after base calling and de-multiplexing)
- Adapter-clipped reads
- Restriction enzyme site filtered reads

If data analysis has been purchased as part of your project, alignments, spreadsheets and VCF files containing the variant calls and sample genotype data are also provided

5. Retention and return of project samples

- All project material (samples, DNA and data) will be stored for up to 6 months (USA site) or 1 year (Berlin site), after the completion of the project.
- Should longer term storage be required or have any material returned, please discuss this with your Project Manager.
- Additional services outside of the GBS service are available. Please enquire with your Project Manager.

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6. Useful links and contacts

- Please refer to your order confirmation or invoice for the address of where to dispatch samples.
- For general GBS-related queries, please contact techsupport@lgcgroup.com.
- For further information on your specific GBS project, please contact your dedicated Project Manager.

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Appendix A. Forms for services

Application	Biosearch Technologies service	Forms to be completed and submitted for service project			New Biosearch Technologies customer	
		If submitting tissue samples	If submitting DNA	Other useful information	Complete form if you do not have a Biosearch Technologies customer number	Complete form if have not previously registered with our Sequencing Shop*
Next generation sequencing (NGS) (with or without DNA purification)	Genotyping by sequencing (GBS)	NGS sample submission form	NGS sample submission form	For details, please contact: ngs@lgcgroup.com	n/a	n/a

* For selected services, project results can only be accessed through our Biosearch Technologies Sequencing Shop. Please ensure you have registered with our Sequencing Shop in order to access your data.

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Appendix B. DNA submission requirements

Application	Biosearch Technologies service	Minimum DNA requirements					Preferred and accepted plastic-ware for shipping			Shipping requirements	
		Minimum sample number per batch	Concentration (per sample)	Volume (per sample)	Diluent requirements	Preferred/ accepted nucleic acid assessment	Plate/tube types	Plate seals	Plate/tube labelling	Packing and dispatch conditions	Other information
Next generation sequencing (NGS)	Genotyping by sequencing (GBS): nGBS/ddRAD/ GBS	n/a	600 ng (>20 ng/μL - 50 ng/μL)	≥30 μL	Tris (5mM, pH: 8,5) or TE	High-molecular weight (≥10 Kb)	<p>Tubes (<30 samples) 1.5 mL "safe-lock" (not screw cap)</p> <p>Plates Bacterial colonies or fungal tissue preserved in ethanol</p>	<p>Plates (skirted) 8-strip caps or heat-sealing (preferred)</p>	Each plate labelled with a unique identifier	Ambient (if in Tris/TE) or 4°C	Entry QC will be performed using agarose gel electrophoresis and fluorimetric measurement (e.g. Qubit)

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Appendix C. Tissue submission requirements

Please note the following important information:

- No biological material (of any type) should be shipped in 0.2 mL tubes or plates
- No BSL-III material is accepted at any Biosearch Technologies site
- No GMO seed or BSL-II material is accepted at the Middleton, WI, USA. If you wish to process these materials, please contact techsupport@gcggroup.com for alternative options. Non-viable GMP material (e.g. leaves) is accepted
- Samples may be inherently inhibitory, which is out of the control of Biosearch Technologies processes
- Any deviation from standard sample shipment guidelines (without prior discussion with Biosearch Technologies) may result in refusal of sample receipt or incur additional costs

Application	Sample type	Minimum sample requirements			Preferred and accepted plastic-ware for shipping				Shipping requirements	
		Sample number per batch	Volume/ quantity	Quality	Recommended sample collection tube/plate	Plate/tube types	Plate seals	Sample handling	Packing and dispatch conditions	Other information
Sequencing (Sanger ² or next generation sequencing)	Leaves	Dependent on size/nature of project - please discuss with your Project Manager 24 for GBS projects*1	4-9, 6 mm (diameter) leaf discs *3 Leaf material must be in discs or pre-cut into small pieces, otherwise additional processing costs will be incurred	Young leaf tissue, L3/L4 stage, avoiding midrib and axillary veins, consistent positions across plants	BioArk Leaf Kit (preferred)	BioArk Leaf Kit (Biosearch Technologies KBS-9370-001-L) (preferred) 96-well, 1.2 mL plate (preferred) 96-well, 2.2 mL plate (accepted)	BioArk Leaf Kit (preferred)	If not using the BioArk Leaf Kit, samples should be stored Dried at room temperature, or in 96-99% absolute ethanol	Shipment must contain purchase order number or signed quotation, for identification BioArk Leaf Kit Ambient Freeze-dried Ambient -20 °C Frozen Ethanol Ambient	Samples sent in tubes may incur an additional cost for sample transfer into a 96-well plate format If your species is: a) not on the Plant Species List b) precious/limited please discuss with your Project Manager as a pilot study/protocol optimisation may be required
Sequencing (Sanger ² or next generation sequencing)	Livestock tissue	Dependent on size/nature of project - please discuss with your Project Manager 24 for GBS projects*1	4 mm (cubic) solid tissue, 600 ng DNA	Fresh frozen	n/a	96-well, 2.2 mL Porvair plate (preferred) 96-well 1.2 mL plates (preferred)	Heat-sealing (preferred) Cap-mat sealing (preferred)	At -20 °C, or in 96-99% absolute ethanol	Shipment must contain purchase order number or signed quotation, for identification -20 °C Frozen Ethanol Ambient	Samples sent in tubes may incur an additional cost for sample transfer into a 96-well plate format If your species is precious/limited, please discuss with your Project Manager as protocol optimisation may be required

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Tissue submission requirements

Application	Sample type	Minimum sample requirements			Preferred and accepted plastic-ware for shipping				Shipping requirements	
		Sample number per batch	Volume/ quantity	Quality	Recommended sample collection tube/plate	Plate/tube types	Sample number per batch	Volume/ quantity	Quality	Other information
Sequencing (Sanger ^{*2} or next generation sequencing)	Aqua-culture	Dependent on size/nature of project - please discuss with your Project Manager 24 for GBS projects ^{*1}	4 mm (cubic) solid tissue, 600 ng DNA	n/a	BioArk Fish Kit (preferred)	BioArk Fish Kit (Biosearch Technologies KBS-9370-001-F) (preferred) 96-well, 2.2 mL Porvair plate (preferred) 96, deep-well 1.2 mL plate (preferred)	BioArk Fish Kit (preferred)	If not using the BioArk Fish Kit, samples should be stored at -20 °C, or in 96-99% absolute ethanol	Shipment must contain purchase order number or signed quotation, for identification BioArk Fish Kit Ambient -20 °C Frozen Ethanol Ambient	The BioArk Fish Kit has been validated for the collections of fish fins. Should an alternative sample type be desired, please discuss with your Project Manager Samples sent in tubes may incur an additional cost for sample transfer into a 96-well plate format If your species is precious/limited, please discuss with your Project Manager as protocol optimisation may be required

^{*1} If shipping samples to our Berlin site, a minimum sample number of 24 is required for purification projects

^{*2} Sanger sequencing custom-projects also available with a variety of tissue types (service includes DNA purification and Sanger sequencing). For Sanger re-sequencing of custom-projects, ensure >24 samples (<24 samples may incur an additional handling fee). For Targeted Resequencing projects, all tissue should be preserved in ethanol (absolute)

^{*3} The exact number of leaf discs will depend on various factors (age of leaf, size/scale of project, plant species etc.). For the exact number of leaf discs for your project, please discuss with your Project Manager.

^{*4} Other sample collection tube/plate types may be accepted. Please contact techsupport@lgcgroup.com for further information.

HMW = high molecular weight



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