



Guidance notes

SeqSNP HT 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 SeqSNP™ HT (high-throughput) project.

SeqSNP HT is a simplified targeted genotyping by sequencing (stGBS) service, which allows for genotyping of SNPs and small insertions/deletions (indels) via a mid-to-high-plex next generation sequencing (NGS) genotyping platform. It can provide an alternative for screening via fixed arrays, due to increased flexibility in marker selection and ease of number scalability.

Once customers have submitted their DNA, the SeqSNP HT service includes assay design and probe synthesis, DNA library construction, Illumina sequencing, and finally bioinformatics to allow for the calling of alleles. While DNA extraction is not included by default, please enquire for DNA extraction services.

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](#).

For a free-of-charge consultation, please contact seqsnph@lgcgroup.com.

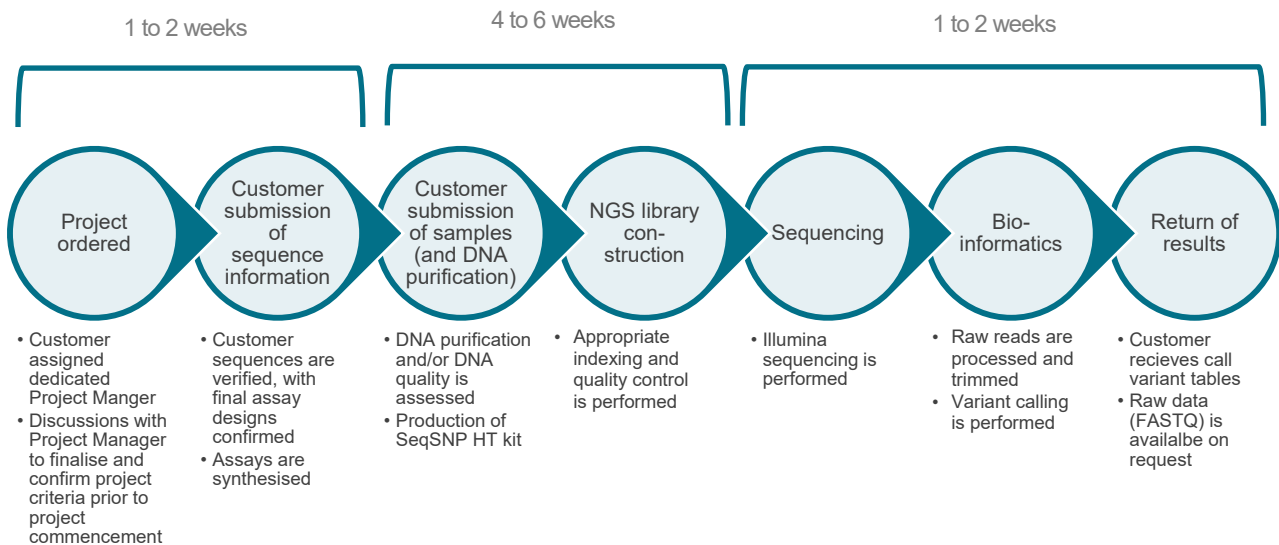
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2. Project workflow

Before commencing their SeqSNP HT 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 SeqSNP service:



All laboratory protocols are carried out according to Good Laboratory Practice (GLP) standards, including the inclusion of appropriate quality controls, as set out in our service provision licencing agreements.

3. Sample submission

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

Please confirm with your Project Manager the shipment address for your samples, prior to dispatch.

3.1 Submitting tissue samples

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

- **If submitting plant samples** please provide the following information:
 - Species and ploidy
 - Stage in breeding programme (if applicable)

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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
 - Stage in breeding programme (if applicable)

4. Sequence submission

Each SeqSNP HT project requires:

- Reference genome sequence (see Section 4.1)
- Polymorphism definition file (see Section 4.2)

Further details on acceptable sequence formats can be found on our [SeqSNP assay design data sheet](#).

Please send all information (as outlined below) to seqsnph@lgcgroup.com.

Please note, if you wish your oligonucleotides to be separated and screened against different batches of samples, please ensure that you discuss this with your Project Manager prior to the commencement of your project, as additional costs may be incurred.

4.1. Reference genome sequence

The reference genome sequence can be submitted as:

- A link to a publically available sequence, for example, the [human genome assembly](#) (preferred)
- The reference genome sequence in FASTA format (preferred)
- Shorter reference sequences, with at least 75 nucleotides of sequences flanking both upstream and downstream of the polymorphism (accepted only with prior discussion with your Project Manager)
- Transcriptomic sequences (please discuss with your Project Manager)

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4.2. Polymorphism definition file

The polymorphism definition file defines the positions of the SNPs/InDels to be included in your SeqSNP project (when compared against the reference genome sequence).

We accept the following formats:

- VCF (Variant Calling Format) (v4.0 or later) – see the following [VCF file guidance](#) and [VCF file example](#) (preferred)
- Microsoft Excel spreadsheet – see the following [Excel file example](#) (preferred)
- BED (Browser Extensible Data) – see the following [BED file guidance](#) and [BED file example](#) (accepted)

Please note:

- The customer is responsible for selecting the polymorphisms for interrogation in their SeqSNP HT project, as Biosearch Technologies are unable to advise on the selection on SNPs.
- Submission of VCF or Excel files (preferred) will allow us to verify that the polymorphisms identified are present in the reference genome. This is not possible with a BED file submission, though we do accept this file format.
- Due to scales of synthesis kits, we recommend a minimum of 48 polymorphisms (accepted) or 96 polymorphisms (preferred).
- The rate of design success will largely depend on various genomic factors (e.g. size/complexity of genome, ploidy of species etc.) Therefore, we recommend sending more polymorphisms (which have even density/spread across your target sequence) than are to be included in your project, to facilitate assay design. Your Project Manager will provide guidance on how many more polymorphisms are recommended for submission based on the specifics of your project. All assays will be confirmed with the customer prior to starting laboratory work.
- It is important that all sequence data (reference genome and polymorphism definition file) is correct. Biosearch Technologies cannot accept responsibility for any results generated as a result of incorrect sequence submission.

4.3. Flanking non-targeted polymorphisms file (optional)

This file will provide information on any known surrounding polymorphisms to those of interest. This may aid in the design process of the oligonucleotide probes, to identify areas to avoid which may compromise target enrichment.

Should you wish to submit a flanking, non-target polymorphisms file, please use the same file format as for the polymorphism definition file.

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5. Data analysis and results

- Following completion of the sequencing, data analysis will be performed to generate a call variant table, identifying the genotype.
- Raw reads, adaptor-clipped reads and quality trimmed reads will be provided to allow for your own analysis.
- If additional bioinformatics is required, please discuss this with your Project Manager.

6. 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 SeqSNP HT service are available. Please enquire with your Project Manager.

7. Useful links and contacts

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

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Appendix A: 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)	SeqSNP HT	96	30 ng/μL	20 μL	Tris/TE (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5)	Fluorimetrically-confirmed HMW (>10 Kb)	96-well plate	<u>Plates</u> 8-strip caps (accepted) or heat-sealing (preferred)	Each plate labelled with a unique identifier	Shipment must contain purchase order number or signed quotation, for identification Frozen on dry-ice (recommended if international dispatch) or ambient	All samples for an individual project in a single shipment Samples which fail to meet minimum requirements may be refused. Please discuss lower quality samples with your Project Manager, as data may be obtainable in specific experimental circumstances.

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Appendix B: Tissue submission requirements

Please note the following important information:

- No biological tissue 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 site. If you wish to process these materials, please contact techsupport@lgcgroup.com for alternative options. Non-viable GMP material (e.g. leaves) is accepted
- Overall genotyping quality and success rate can be detrimentally affected by poor quality starting material. Biosearch Technologies can't accept responsibility for lower genotyping call rate/SNP success rate if starting material is not optimal
- Any deviation from standard sample shipment guidelines (without prior discussion with Biosearch Technologies) may result in refusal of sample receipt or incur additional costs

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 ⁴	Plate/tube types	Sample number per batch	Volume/ quantity	Quality	Other information
Sequencing (Sanger* ² or next generation sequencing)	Leaves	Dependent on size/nature of project - please discuss with your Project Manager	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

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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 ⁴	Plate/tube types	Sample number per batch	Volume/ quantity	Quality	Other information
Sequencing (Sanger ² or next generation sequencing)	Seeds	Dependent on size/nature of project - please discuss with your Project Manager	Dependent on size/nature of project - please discuss with your Project Manager	n/a	BioArk Seed Kit (preferred)	BioArk Seed Kit (Biosearch Technologies KBS-9370-001-S) (preferred) 96-well, 2.2 mL Porvair plates (preferred) 96-well, 1.2 mL plates (accepted)	BioArk Seed Kit (preferred)	No special sample handling	Shipment must contain purchase order number or signed quotation, for identification BioArk Seed Kit 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
	Blood	Dependent on size/nature of project - please discuss with your Project Manager	200 µL - 500 µL	Fresh frozen EDTA, citrate or heparin	EDTA vacutainer PAXgene RNA Blood Tube (BD Bioscience 762165) PAXgene DNA Blood Tube (BD Bioscience 761165)	96-well, 2.2 mL plate (Porvair Sciences219030) (preferred) 96-well, 1.2 mL plates (accepted)	Heat-sealing (preferred) Cap-mat sealing (preferred)	No special sample handling	Shipment must contain purchase order number or signed quotation, for identification -20 °C Frozen on dry-ice	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
	Buccal swabs	Dependent on size/nature of project - please discuss with your Project Manager	One swab/sample	n/a	DNA Buccal Swabs (Isohelix SK-1S) Any appropriate buccal swab DNA collection kit	<u>Swabs</u> Sorted into boxes or bags (10 swabs/box or bag) <u>Tubes</u> Sorted into boxes	n/a	No special sample handling	Shipment must contain purchase order number or signed quotation, for identification Ambient	If your species is precious/limited, please discuss with your Project Manager as protocol optimisation may be required
	Saliva	Dependent on size/nature of project - please discuss with your Project Manager	One tube or disc/sample	n/a	Genotek Collection Kit (Oragene OG-500)	<u>Tubes</u> Sorted into boxes <u>Discs</u> Sorted into bags (10 discs/bag)	n/a	No special sample handling	Shipment must contain purchase order number or signed quotation, for identification Ambient	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 ⁴	Plate/tube types	Sample number per batch	Volume/ quantity	Quality	Other information
	Livestock tissue	Dependent on size/nature of project - please discuss with your Project Manager	4 mm (cubic) solid tissue, 600 ng DNA	Fresh frozen	n/a	96-well, 2.2 mL Porvair plate (preferred) 96, deep-well 1.2 mL plate (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 <u>Ethanol</u> 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
Sequencing (Sanger ^{*2} or next generation sequencing)	Aqua-culture	Dependent on size/nature of project - please discuss with your Project Manager	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 <u>Ethanol</u> 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

^{*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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