



Guidance notes



SeqSNP service guidance notes

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Not for use in diagnostic procedures.

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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™ project.

[SeqSNP](#) is a targeted genotyping by sequencing (targeted GBS) service, which allows for genotyping of SNPs and small insertions/deletions (indels) via a mid-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.

The SeqSNP service is all-inclusive, allowing for DNA extraction (alternatively customers can submit their extracted DNA), assay design and probe synthesis, DNA library construction, Illumina sequencing, and finally bioinformatics to allow for the calling of alleles.

If you are a new customer, please ensure you have registered with the LGC 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 seqsnp@lqcgroup.com.

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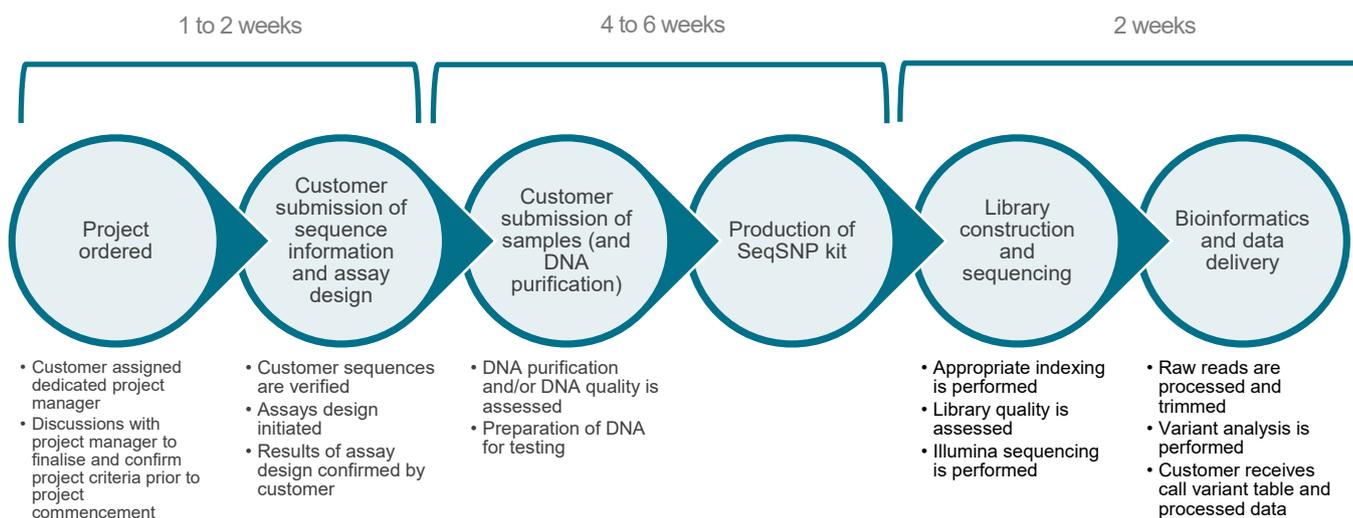
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2. Project workflow

Before commencing their SeqSNP 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:

3. Sample submission



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

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)

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. LGC 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)

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4. Sequence submission

Each SeqSNP project requires:

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

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)

A sequence file in FASTA format can contain several sequences. Each sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line must begin with a greater-than (“>”) symbol in the first column. The word following the “>” symbol is the identifier of the sequence, and the rest of the line is the description (optional). This can include the species, details on the function of the sequence, and sequence length.

FASTA example:

```
>AB000263 |acc=AB000263|descr=Homo sapiens mRNA for prepro cortistatin like peptide, complete cds.|len=368
ACAAGATGCCATTGTCCCCGGCCTCCTGCTGCTGCTGCTCTCCGGGGCCACGGCCACCGCTGCCCTGCC
CCTGGXAGGGTGGCCCCACCGGCCGAGACAGCGAGCATATGCAGGAAGCGGCAGGAATAAGGAAAAGCAGC
CTCCTGACTTTCTCGCTTGGTGGTTTGAGTGGACCTCCCAGGCCAGTGCCGGGCCCTCATAGGAGAGG
AAGCTCGGGAGGTGGCCAGGCGGCAGGAAGGCGCACCCCCCAGCAATCCGCGCGCCGGGACAGAATGCC
```

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 example](#) (preferred)
- Microsoft Excel spreadsheet – see the following [Excel file example](#) (preferred)
- BED (Browser Extensible Data) – see the following [BED file example](#) (accepted)

Please note:

- 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.
- BED file formatting uses 0-base numbering **by design**; this means that the first base is

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numbered as base zero. Other file formats use 1-base numbering, so this will require correcting if converting to BED format for submission. Please verify if your reference sequence is in 0-based or 1-based formatting. If it is 1-based formatting, co-ordinates will require adjusting to 0-base during BED formatting.

- We recommend a minimum of 48 polymorphisms to be included in your SeqSNP project due to the scales of the synthesis kits used. Our preference is to receive 96 polymorphisms per SeqSNP project.
- We recommend sending 20-50% more polymorphisms than are to be included in your project, to facilitate assay design. 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. LGC Biosearch Technologies cannot accept responsibility for any results generated as a result of incorrect sequence submission.

4.3. Flanking polymorphisms file (optional)

This file will provide information on any known surrounding polymorphisms flanking 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 polymorphisms file, please use the same file format as for the polymorphism definition file.

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 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 related queries, please contact techsupport@lgcgroup.com.
- For further information on your specific SeqSNP project, please contact your dedicated project manager.

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Appendix A: Forms for services

Application	LGC Biosearch Technologies service	Forms to be completed and submitted for service project		Other useful information	New LGC Biosearch Technologies customer	
		If submitting tissue samples	If submitting DNA		Complete form if you do not have an LGC Biosearch Technologies customer number	Complete form if you have not previously registered with our Sequencing Shop
Next generation sequencing (NGS) (with or without DNA purification)	SeqSNP (targeted GBS)	NGS sample submission form	NGS sample submission form	<ul style="list-style-type: none"> • Plant species list • Guidelines for sample shipment 	n/a	n/a

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Appendix B: DNA submission requirements

Application	LGC 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 (targeted GBS)	96	30 ng/μL	20 μL	Tris/TE (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5)	RNA-free Fluorimetrically-confirmed HMW (>10 Kb)	96-well plate	Plates Heat-sealing (preferred) 8-strip caps (accepted)	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.

*Based on human genome-sized organism. Higher DNA concentrations will be required for larger genomes.

**Large number of SNPs may require a larger volume.

HMW = high molecular weight

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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 LGC 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@lgcgroup.com for alternative options. Non-viable GMO material (e.g. leaves) is accepted
- Samples may be inherently inhibitory, which is out of the control of LGC Biosearch Technologies processes
- Any deviation from standard sample shipment guidelines (without prior discussion with LGC 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** or next generation sequencing)	Leaves	Dependent on size/nature of project - please discuss with your project manager	4-9, 6 mm (diameter) leaf discs***	Young leaf tissue, L3/L4 stage, avoiding midrib and axillary veins, consistent positions across plants	BioArk Leaf Kit (preferred)	BioArk Leaf Kit (LGC 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 freeze-dried, 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** 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 (LGC 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

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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	Plate seals	Sample handling	Packing and dispatch conditions	Other information
Sequencing (Sanger** or next generation sequencing)	Blood	Dependent on size/nature of project - please discuss with your project manager	200 µL- 500 µL	Fresh frozen EDTA, citrate or heparin	PAXgene RNA Blood Tube (BDBioscience 762165) PAXgene DNA Blood Tube (BDBioscience 761165)	96-well, 2.2 mL plate (Porvair Sciences 219030) (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 <u>-20 °C</u> 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
Sequencing (Sanger** or next generation sequencing)	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 Ship 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** or next generation sequencing)	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 Ship 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** or next generation sequencing)	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 <u>-20 °C</u> 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** 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 (LGC 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 <u>-20 °C</u> 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

*If shipping samples to our Berlin site, no minimum sample number is required for purification projects.

**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)

***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.



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