



SeqSNP service guidance notes

We are pleased to provide the following guidance on how LGC will work with you regarding your SeqSNP service project.

The SeqSNP service includes:

- Dedicated project manager
- LGC plant sample collection kit(s) (optional)
- DNA extraction service (optional)
- Probe design and probe library generation
- Sample library preparation
- NGS run of pooled samples
- Variant calling in NGS data set

The following topics are covered in this document:

Section 1: Project submission requirements

- Sample requirements
 - NOTE A: Submitting DNA samples
 - NOTE B: Submitting plant tissue
 - NOTE C: Submitting non-plant tissue
- Shipping conditions
- Documentation requirements

Section 2: Project workflow

Section 3: Important points to note

Section 4: Turnaround times

Section 5: Useful links and contacts

Section 1: Project submission requirements

Sample requirements

The minimum number of samples that can be submitted for a SeqSNP service project is 192. Samples for a SeqSNP project can be submitted in the format of extracted DNA ([see Note A](#)) or tissue samples (see [Note B](#) and [Note C](#)). DNA extraction is included in the project cost for specific sample types as detailed in [Note B](#) and [Note C](#). DNA samples should be submitted in 96- or 384-well plate format, and tissue samples should be submitted in 96-well plate format. Samples submitted in tubes may incur a surcharge for sample transfer (excludes blood or buffy coat samples).

If you are working with non-standard sample types, species or delivery formats that are not covered in this document, please contact LGC to discuss the options. Please note that additional costs may apply.

Note A: Submitting DNA samples

When submitting DNA for a SeqSNP project, samples should be submitted in 96- or 384-well plates; there is no requirement to leave any wells empty as controls, and samples can be formatted in rows or columns. Each plate submitted should be labelled, and the corresponding sample information detailed in the sample submission form (see [Documentation requirements](#) section).

LGC requires a minimum of 20 µL of DNA per individual sample, at a minimum concentration of 30 ng/µL (quantification by Qubit/PicoGreen).^{*} DNA must be submitted in solution. LGC requires high molecular weight DNA (average fragment length > 10 kb), RNA-free and eluted in Tris/TE. Samples that do not meet the quality requirements may be refused. DNA should be shipped on ice where possible to ensure that it remains cool during transit.

LGC recommends performing DNA extraction using our [sbeadex™ chemistry](#), that delivers nucleic acids in high yield, purity and quality.

^{*}If your DNA samples are less concentrated, please contact LGC to discuss. It may be possible for LGC to obtain good quality data with slightly less concentrated samples.

Note B: Submitting plant tissue

To submit plant leaf tissue to LGC, first determine whether or not your plant species has been previously extracted by LGC by consulting our current [plant species list](#).

- a. If your plant species is listed, please submit plant tissue using LGC's plant sample collection kit, following the sampling instructions provided with the kit. For SeqSNP projects, we require you to submit as many leaf discs as possible (taking care not to overfill the tube). The number of leaf discs required to yield sufficient DNA is highly dependent on the youth of the leaf sample and hence how rapidly the cells are dividing.

Please note: although your plant species may be listed, please be aware that the DNA requirements for SeqSNP projects (20 µL of 30 ng/µL) are higher than for services such as the KASP genotyping service. As DNA extraction efficiency is highly crop and sample type dependent, it is possible that our extraction protocol may require optimisation to ensure sufficient DNA is obtained for SeqSNP. This may involve a short pilot study on your species. If your plant samples are precious, and further sampling at a later date is not possible, please discuss this with LGC prior to commencement of your project. It may be advisable for LGC to optimise the extraction protocol on a small set of test samples prior to proceeding with the full set.

Please note that all 96 wells can be used for plant tissue – 2 wells do not need to be left empty as controls as would typically be required when using the kit for genotyping service projects. Each plate submitted should be labelled, and the corresponding sample information detailed in the sample submission form (see [Documentation requirements](#) section).

- b. If your plant species is not listed, you can either:
 - i. submit extracted DNA for your SeqSNP project. See [Note A](#) for further details. OR
 - ii. discuss a pilot extraction project with specialists at LGC. Protocol development is not included in the SeqSNP service project price. Please note that standard turnaround times and conditions may not be achievable if bespoke protocols are required within your project. If you are unsure, please discuss requirements for your species with LGC prior to commencement of your project.

Guidelines for sampling plant tissue

- Please ensure sampled tissue is of a consistent age across all plants sampled. For older plants with mature leaves, a larger number of leaf punches may be required (e.g. 7-9) than if young leaf tissue is sampled (e.g. 2-4)
- Please ensure that sampling is performed on consistent positions of each plant
- Avoid midrib and axillary veins on leaves
- Sample leaf material at L3/L4 stage (i.e. 2-3 leaves on the main shoot, and third/fourth leaf has appeared)

Guidelines if using LGC's plant sample collection kits

- Always re-package the plate in the corresponding box; the box and plate have corresponding barcodes
- Use a fresh leaf punch and mat for each collection kit
- Avoid puncturing the plastic bag when inserting the completed kit into the bag
- Do not re-open the plastic bag containing the completed sample kit

Once the collection has been completed, store all collection kits in the dark at room temperature.

Note C: Submitting non-plant tissue

If you would like to submit non-plant tissue samples such as whole blood, buffy coat or solid tissue for extraction, please discuss this with LGC prior to the commencement of your project. Your project manager will advise you of sample requirements specific to your project.

Shipping conditions

All samples for an individual SeqSNP project should be sent to LGC in a single shipment prior to the commencement of the project. It is essential to include your purchase order number or a signed quotation in your shipment to facilitate identification of the samples and to confirm your order for the service. DNA samples can be shipped on ice or at room temperature. If tissue samples are being submitted for extraction, please confirm the appropriate shipping conditions with LGC prior to shipping. If the plant sample collection kit is being used, please follow the detailed instructions provided in the kit manual.

Documentation requirements

In addition to the biological samples, LGC also requires the following documents in order to commence work on your project:

- A completed [Sample submission form](#). This will include sample names and plate layout information. In addition please provide your project manager with information regarding the DNA extraction method used and the concentration (ng/ μ L) of your submitted samples.
- A reference genome file (FASTA format) or details of the publically available reference genome sequence to be used.
- A BED file (.bed) containing SNP locations and information. 100 bp of flanking sequence is required upstream and downstream of each SNP.
- Purchase order or signed quotation
- If you are a new customer, please also register in our online shop (<http://shop.lgcgenomics.com>) to create your own unique LGC customer number. LGC cannot begin processing of your samples before a customer number is available.

Please send all documents to seqsnp@lgcgroup.com before shipping your biological samples to us.

Please be aware that if you do not supply the above documents, we cannot process your project, and the delivery of results will be delayed.

Section 2: Project workflow

Preliminary work: Assay design and library preparation (bioinformatics and laboratory):

- a. Assay design using customer-provided SNP sequence information
- b. Oligonucleotide synthesis
- c. Library preparation including indexing and quality control

Phase 1 – Preparation:

Step 1: Sample preparation (laboratory)

- d. Receipt of samples, sample barcode tracking and confirmation of sample receipt.
- e. DNA extraction (if required)
- f. Quality control of extracted or submitted DNA

Phase 2 – Processing:

Step 2: Sequencing (laboratory)

- g. Illumina NGS run of prepared libraries.

Phase 3 – Data analysis:

Step 3: Analysis of NGS reads (bioinformatics)

- h. Processing of NGS reads
- i. Variant calling in NGS data set (optional)
- j. Data supplied to the customer – Data will be delivered via USB stick or hard disc drive (depending on the amount of resulting data). Analysed results will consist of a table containing SNP data. Raw sequence data (FASTQ format) can be provided on request.

Section 3: Important points to note

1. **It is the responsibility of the customer to provide accurate sequence information at the start of a SeqSNP project.**
2. If you wish to re-use the oligo library for future projects please inform your project manager prior to submitting your SNP sequences. If possible please provide an estimation for how many reactions you will re-use the library; this may help us save cost for your future projects.
3. If submitting DNA, an additional charge may be incurred if DNA does not meet quality requirements. All submitted DNA samples will be quantified in-house (using Qubit) by LGC and it is this value that will be used to determine whether or not the samples are of sufficient concentration to be accepted.
4. It is possible that submitted DNA, or DNA extracted by LGC from submitted tissue, may meet the quality requirements, but that inhibitors may affect the quality of data obtained. Customers are warned that the presence of inhibitors is sample dependent and is outside of LGC's direct control.
5. If submitting tissue samples, an additional charge may be incurred if tissue does not meet quality requirements.
6. Pricing and conditions may vary depending on the technical requirements for specific organisms. Please enquire for a free-of-charge consultation with our sequencing specialists at seqsnp@lgcgroup.com.
7. Pricing is per sample and applies to a single work order (samples received in one batch). A minimum sample number of 192 samples applies.
8. Prior to the start of the project the target marker library is set up in collaboration between LGC and the customer. Please note that the project turnaround time starts after completion of the library setup which may take several weeks. It is therefore advisable to submit your reference sequence and SNP specification at the earliest possible convenient time.
9. SNP markers can be individually selected at the start of the project. Additional markers can be added during the project but please note that this will have time and cost implications. Please discuss this with LGC.
10. Typical applications of data generated include genomic selection and genomic prediction. Please note that guidance on breeding programmes is not included in this service. LGC may be able to advise on particular publications that can provide guidance; please discuss with our technical support team at tech.support@lgcgroup.com.
11. De novo SNP detection in the target region may be feasible dependent on the project setup. Please communicate with your project manager to enquire about the possibilities.
12. Each sample will carry an individual index which allows sorting of the reads after sequencing.
13. Any DNA samples remaining following completion of the project will be stored, unless otherwise specified, for 1 year after the end of the project.
14. All data generated is stored, unless otherwise specified, for up to 1 year after data delivery.
15. If you require additional work (e.g. repeated library preparation of certain samples, repeated data delivery or retrieval, extension of storage time), additional costs may apply.

Section 4: Turnaround times

The standard turnaround time for a SeqSNP project is 2 weeks, from receipt of sample to delivery of data. This excludes the preliminary assay design phase and any discussion following the data delivery. Please note that probe library design typically takes 4 to 6 weeks; it is therefore advisable to provide SNP sequences as early as possible to prevent delays to delivery of your project data. Please be aware that samples that are shipped from outside Europe may have slightly extended turnaround times due to internal transfer limitations.

Bespoke turnaround times are available on request. Please contact LGC to discuss this prior to commencing your project.

Section 5: Useful links and contacts

Plant sample collection kit	Please click this link for more details about LGC's Plant sample collection kit.
Plant species list	Please click this link to access a list of plant species that LGC has previously extracted DNA from.
DNA extraction services	Please click this link for more details on LGC's DNA extraction services.
Sample submission form (DNA extraction)	Please complete this form in advance of submitting your samples to LGC, if DNA extraction is required as part of your project. Completed forms should be sent to your project manager.
Sample submission and plate map templates	Please complete this form in advance of submitting your samples to LGC, if extracted DNA is being submitted.
LGC Genomics sequencing shop	Please click this link if you are a new customer and need to register online for your unique LGC customer account number.

Useful contacts

General enquiries:
seqsnp@lgcgroup.com

Price enquiries:
seqsnp@lgcgroup.com

Technical enquiries:
tech.support@lgcgroup.com

Queries relating directly to your ongoing project should be sent to your assigned project manager.

LGC is an international leader in the extended life sciences sector, including human healthcare, agri-food & the environment. LGC provides a comprehensive range of reference materials, proficiency testing schemes, genomics reagents and instrumentation, as well as research and measurement services. Its scientific tools and solutions enable organisations to advance research, develop new products and form an essential part of their quality and compliance procedures.

LGC Genomics quality management system is certified by DIN EN ISO 9001:2015.

Delivery of result: Scored genotyping data. Raw data can be provided on request.

Payment conditions: 30 days after date of invoice net.

Quotations are valid for 90 days, unless specified otherwise.

Offered net prices do not include shipping costs and taxes, where applicable. Customers within the European Union have to account for the VAT and need to provide the VAT identification number to LGC Genomics for correct invoicing.

All services offered are subject to LGC Genomics' Terms and Conditions.

www.lgcgroup.com/genomics • genomics@lgcgroup.com

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