

LGC Genomics Ltd Tel: Units 1 - 2 • Trident Industrial Estate Fax: Pindar Road • Hoddesdon • Herts Email: EN11 OWZ • UK Web: +44 (0)1992 470 757 +44 (0) 1992 470 687 genomics@lgcgroup.com www.lgcgroup.com/genomics

SSR Conversion Service Guidance

The following guidance notes outline how LGC will work with you regarding your SSR conversion service project.

The SSR conversion service includes:

- Dedicated project manager support
- LGC plant sample collection kit(s) (optional)
- DNA extraction service (optional)
- Long range PCR of the SSR region (3 kb per SSR)
- Illumina Sequencing
- Variant identification (SNP/InDel) and calling in NGS dataset
- KASP assay design service
- KASP genotyping service
- All required reagents and consumables

After we have received a Purchase Order for your first project, a dedicated project manager will be assigned to your project. For any specific enquiries regarding your conversion project, please contact your project manager directly. Any general enquiries should be sent to <u>info.uk@lgcgenomics.com</u>.

Sample submission requirements

Sample requirements

Sample requirements for an SSR conversion project are outlined in Table 1. For each SSR conversion project (containing between 1 and 80 SSR conversions), you will be required to submit a total of **48** unique samples. Of these 48 samples, **10** will be used for SNP/InDel discovery. The 10 samples to use will be defined by the customer. The discovered SNPs/InDels will be genotyped, using KASP, on all 48 samples. The samples for SNP discovery will need to be genetically diverse, non-related samples with contrasting phenotypes (in the case of trait specific SSR's). Please note that these samples will need to be of high quality.*

Sample requirements	Required	Notes
Total sample number	48 (including samples for SNP discovery)	Provided by customer.
Samples for SNP discovery	10	Please indicate these on the plate layout file. Note that these samples will need to be of high quality.*
Sample diversity	Diverse genetic samples	Applies to samples for SNP discovery. Note: Only one organism per project.
Control samples	Positive and negative control required	Please include controls for the target trait
No template control (NTC)	Yes	Please leave a minimum of two empty wells that will be used as NTCs
Accepted sample types	Extracted DNA or biological samples	See Table 2 for accepted biological sample types
Submission format	96-well plate format or LGC plant kit	If samples are provided in individual tubes, a surcharge for sample transfer may apply
LGC plant sample collection kit	Provided for plant samples only	Included (where applicable)
SSR sequence	Genomic location and sequence of the SSR	Please include +/- 1500 bp flanking sequence

Table 1. Overview of sample requirements for an SSR conversion project from LGC.

* If extracted DNA is submitted, please provide at least 1.1 µg DNA (per SSR) of high molecular weight (10 kb average fragment length or higher) in 96-well plate format. 1 µg is required for NGS, with an additional 10% required to facilitate



LGC Genomics Ltd Tel: Units 1 - 2 • Trident Industrial Estate Fax: Pindar Road • Hoddesdon • Herts EN11 OWZ • UK Web: +44 (0)1992 470 757 +44 (0) 1992 470 687 genomics@lgcgroup.com www.lgcgroup.com/genomics

subsequent KASP genotyping. Please contact your project manager to discuss detailed DNA requirements for your specific project.

DNA extraction is included in the project cost for specific sample types. Please see Table 2 for the sample types that we can accept.

If your project involves a plant species, please view our list of species that have been previously extracted and genotyped at LGC. If your plant species is listed, please submit leaf tissue using LGC's plant sample collection kit. Please contact your project manager for advice regarding the number of leaf punches required per sample for your specific project. If your plant species is not listed, you are requested to submit extracted DNA for your SSR project. Please provide at least 1.1 µg DNA (per SSR) of high molecular weight (10 kb average fragment length or higher) in 96-well plate format. 1 µg is required for NGS, with an additional 10% required to facilitate subsequent KASP genotyping. DNA can be submitted lyophilised or in solution. Please contact your project manager to discuss detailed DNA requirements for your specific project.

For some uncommon plant species, LGC may not have an established extraction protocol. Bespoke protocol development options may be available, but protocol development is not included in the 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.

Sample Type	Organism
Whole Blood	Human/Animal
Buffy Coat	Human/Animal
Serum	Human/Animal
Tissue	Human/Animal
Plant Tissue	Plant species that are included in our list of previously extracted and genotyped species

Table 2. Sample types accepted by LGC for a standard SSR conversion project. The volume / amount of sample provided needs to yield at least 1.1 µg (per SSR) of high molecular weight DNA (10 kb average fragment length or higher). Please contact your project manager to discuss the volume or amount of sample required for your specific project.

Documentation requirements

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

- A completed <u>Sample Submission Form</u>
- SSR sequence please provide the genomic location and the sequence of the SSR, including 1500 bp flanking sequence either side of the SSR. Any standard FASTA and text formats are acceptable.
- Sample information must be provided in one of the following formats:
 - If supplying samples in tubes, a manifest must be provided. The samples to be used for SNP discovery must be clearly identified. Please note that additional sample handling and transfer costs may apply when individual tubes are provided, and this may also increase standard turnaround times.
 - If samples are supplied in 96-well plate format, an LGC plate map must be provided. (see useful links section below)
- Clear identification of the samples to be used for SNP discovery in either the sample manifest or plate map.
- Purchase Order or signed quotation
- If you are a new customer, please also complete and send a New Customer Form, available on our website or in the useful links section below.

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.



LGC Genomics Ltd Tel: Units 1 - 2 • Trident Industrial Estate Fax: Pindar Road • Hoddesdon • Herts Email: EN11 OWZ • UK Web: +44 (0)1992 470 757 +44 (0) 1992 470 687 genomics@lgcgroup.com www.lgcgroup.com/genomics

Project workflow

Phase 1 (laboratory) – DNA extraction & SNP discovery:

- 1. Receipt of samples, sample barcode tracking and confirmation of sample receipt.
- 2. Samples and barcodes logged into Kraken[™] software (LGC's Laboratory Information Management System, LIMS)
- 3. DNA Extraction using Kleargene[™], sbeadex[™] or mag[™] technology (dependent on project requirements)
- 4. Long range PCR of the SSR region (3 kb per SSR), Illumina Sequencing & SNP calling* in NGS dataset
- 5. A list of identified SNPs is provided to you for your review.*

*If a larger than expected number of SNPs (as indicated on your quotation) is identified, you will be asked to select the markers for conversion or to inform us that you would like to design additional assays. Any additional assays can be designed at a discounted rate.

Phase 2 (laboratory) – Assay design and genotyping:

- 6. KASP assays are designed
- 7. KASP genotyping service performed on the 48 provided samples (technical validation of assays)
- 8. Scored genotyping data is provided

LGC Genomics offers consultation with you at every stage of the laboratory process to ensure that you are able to make the most effective use of our services. We also offer add-on services such as sample cherry picking, Whole Genome Amplification (WGA), and aliquoting. If you have any specific requirements for your project please do not hesitate to contact us and we will be happy to help you.

Phase 3 (customer-based) – functional validation of SNPs (not performed by LGC)

9. Functional validation study based on meta data, mapping population or field trial **performed by customer***

*PLEASE NOTE: LGC CANNOT VALIDATE THAT THE IDENTIFIED SNPS ARE ASSOCIATED TO THE SAME FUNCTION AS THE SSRS. For confirmation, a validation study is required. <u>This is not included in the SSR</u> marker conversion service package and it is expected that this will be performed by the end user. The submitted samples (see Section 1 sample requirements) provide sufficient data to guarantee the technical performance of assay design. However, the sample number genotyped within the service package does not guarantee the statistical power for trait linkage. Please consult with your statistician to confirm statistical power requirements. LGC does not offer breeding strategy advice or consultancy relating to biological processes related to the organism of study.

3. Additional information

Please take note of the following:

- 1. The presence of SNPs in SSRs or their flanking regions is a biological event that is possible but not certain. No guarantees can therefore be given that any SNPs will be present in these regions. Assay design and genotyping costs (Section 2 Phase 2) are refunded if no SNPs are found.
- 2. For plants, the SSR conversion service is only applicable to SSRs related to QTLs or associated traits (not applicable to multi-allelic fingerprinting).
- 3. For polyploid organisms the design of working KASP assays is possible but may not be feasible for all markers. If no assays can be designed, other options (e.g. sequencing-based analyses or real time assays) may be feasible. Please note that these options are not included in this service package. Gentoypes of invidual HET groups ie. AAACC cannot be assigned.
- 4. 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.
- 5. Additional SNPs that are identified in excess of the maximum defined number for the project are available for conversion at a package discounted rate.



LGC Genomics Ltd Units 1 - 2 • Trident Industrial Estate Pindar Road • Hoddesdon • Herts EN11 OWZ • UK

Tel:

Fax: Email:

Web:

+44 (0)1992 470 757 +44 (0) 1992 470 687 genomics@lgcgroup.com www.lgcgroup.com/genomics

Turnaround time

The following standard turnaround times apply:

2 weeks	
4 weeks	
2 weeks after selection of SNPs is provided	
2 weeks after assay design is completed	
10 weeks	
Performed by customer (field trial)	

Table 3. Standard turnaround times for SSR conversion projects.

Faster turnaround times may be possible if requested. Please discuss this with your project manager in advance of commencement of the project.

Useful links

Plant sampling kit	Please click this link for more details about plant sample collection kit
Applicable plant species list	Please click this link for more details about plant species
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 these form in advance of submitting your samples to LGC, if extracted DNA is being submitted.

Useful Contacts

Technical enquiries:		
Scientific Support Team		
Hoddesdon		
+44 1992 470757		
tech.support@lgcgroup.com		

Price Enquiries: Sales Support Team Hoddesdon +44 1992 470757 Orders.uk@lgcgroup.com

Order Enquiries

For general enquiries prior to commencement of your project, please email <u>info.uk@lgcgroup.com</u>. Queries relating directly to your project should be sent to the project manager who has been assigned to your project.

General information

LGC is an international leading provider of analytical, forensic and diagnostic services and reference standards (www.lgcgroup.com). The division LGC Genomics has over 23 years' experience in the international genomics sector and specific expertise in high quality, cutting-edge genomic and related services and products. LGC Genomics quality management system is certified by DIN EN ISO 9001:2008.

Delivery of result:A list of identified SNPs is provided after Phase 1 of the project. Additionally, scored
genotyping data is provided at the end of the project after Phase 2.Payment conditions:30 days after date of invoice net

Quotations are open for 30 days, unless specified otherwise. All services offered are subject to LGC Genomics' Terms and Conditions.