Manual

# DNA requirements for genotyping services

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BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

# Manual

DNA requirements for genotyping services

## Contents


1. Introduction ..... 3
2. Dry DNA samples ..... 3
3. Wet DNA samples ..... 4
4. Calculating the mass of DNA required according to genome size ..... 6
5. Whole genome amplification ..... 7
6. Further support ..... 7

# Manual

DNA requirements for genotyping services

## 1. Introduction

DNA can be shipped to LGC Biosearch Technologies in a dried-down or liquid format. Please refer to the relevant section below depending on how you would prefer to ship your samples:

The values stated here are intended as a guideline, and will vary based on the quality of your DNA samples.

When you send in your DNA samples, please include as much information as possible i.e. DNA concentration,method used for extraction, method used for DNA quantification.

***Note:*** it is always preferable to send in DNA at a higher mass or concentration than the minimum requirement. All DNA will be tested in-house before commencing your genotyping project to determine the most suitable dilution. Any unused DNA can be stored at Biosearch Technologies for future genotyping projects, or arrangements can be made to return surplus DNA to the customer.

A range of methods can be used for quantification of DNA samples, including spectrophotometry and PicoGreen. Spectrophotometers have a tendency to overestimate the quantity of DNA present whilst PicoGreen is more exact. If you have used PicoGreen for quantification, you may be able to send a lower quantity of DNA. For dry DNA (human): work on the basis of 5 ng per sample per reaction rather than 10 ng per sample per reaction. For wet DNA (human): work on the basis of 3.3 ng/µL per sample per reaction rather than 5 ng/µL per sample per reaction.

## 2. Dry DNA samples

The mass of DNA required per sample per SNP will differ depending on the genome size of your study organism. If you are working with human DNA (genome size ~3000 Mbp) or a species with a genome size in the range of 2000-3500 Mbp, we require 10 ng of good quality DNA per sample per SNP.

Please see the table below for a general guide to the amount of DNA required when sending in dried down samples. The exact amounts required will vary based on the starting material and the extraction procedures used. **When calculating the mass of DNA required for dried down samples, based on the number of SNPs to be run, always send sufficient DNA for five additional SNPs. This will account for potential losses during re-suspension of samples.**

| Genome size | Minimum mass of DNA required per sample per SNP | Example species |
|---|---|---|
| 100-750 Mbp | 2 ng<br>(N.B. this is the minimum mass that we accept) | *Oryza sativa* (rice), *Arabidopsis thaliana, Drosophila melanogaster* (fruit fly) |
| 750-2000 Mbp | 2.5-6.5 ng | *Solanum tuberosum* (potato), *Glycine max* (soybean) |
| 2000-3500 Mbp | 10 ng | *Homo sapiens* (human), *Zea mays* (maize/corn), *Helianthus annuus* (sunflower) *Mus musculus* (house mouse), *Danio rerio* (zebrafish) |
| 3500-5000 Mbp | 11-16 ng | *Lens culinaris* (lentil) |
| 5000-10000 Mbp | 16-33 ng | *Hordeum vulgare* (barley) |
| 10000-15000 Mbp | 33-50 ng | |
| 15000-20000 Mbp | 50-66 ng | *Allium cepa* (onion) |
| 20000-30000 Mbp | 66-100 ng | *Pinus cembra* (pine) |
| 30000-50000 Mbp | 100-166 ng | |

### 3. Wet DNA samples

The concentration of DNA required will vary based upon the genome size of your study organism. For human DNA, we require DNA at a minimum concentration of 5 ng/µL.

***Note:*** it is always advisable to send DNA at a higher concentration than the minimum requirement. This allows for a range of dilutions to be tested in-house and reduces the requirement for dead volumes. Any unused DNA can be stored at Biosearch Technologies for future genotyping projects, or arrangements can be made to return surplus DNA to the customer.

The table below gives a general guide to the concentration of DNA required, based on genome size of the study organism.

# Manual

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| Genome size | Minimum mass of DNA required per sample per SNP | Example species |
|---|---|---|
| 100-750 Mbp | 5 ng/µL<br>(N.B. this is the minimum concentration that we accept) | *Oryza sativa* (rice), *Arabidopsis thaliana, Drosophila melanogaster* (fruit fly) |
| 750-2000 Mbp | 5 ng/µL | *Solanum tuberosum* (potato), *Glycine max* (soybean) |
| 2000-3500 Mbp | 5 ng/µL<br>(N.B. this is the minimum concentration that we accept) | *Homo sapiens* (human), *Zea mays* (maize/corn), *Helianthus annuus* (sunflower) *Mus musculus* (house mouse), *Danio rerio* (zebrafish) |
| 3500-5000 Mbp | 5-11 ng/µL | *Lens culinaris* (lentil) |
| 5000-10000 Mbp | 11-22 ng/µL | *Hordeum vulgare* (barley) |
| 10000-15000 Mbp | 22-33 ng/µL | |
| 15000-20000 Mbp | 33-44 ng/µL | *Allium cepa* (onion) |
| 20000-30000 Mbp | 44-67 ng/µL | *Pinus cembra* (pine) |
| 30000-50000 Mbp | 67-111 ng/µL | |

Biosearch Technologies requires 1.5 µL DNA per sample per SNP at an appropriate concentration for the genome size of your study organism. This volume is calculated based on the minimum concentration. If higher concentrations are supplied, the volume requirement is reduced.

Due to laboratory handling procedures, we do not accept volumes of less than 20 µL. If you only have a small number of SNPs to run, you will still need to supply 20 µL of starting material. We also require a dead volume; this is an extra volume of DNA required for the automated pipetting process that we use. This volume will vary based on the total number of SNPs in your genotyping project. Please see the table below for an outline of the volumes of DNA to send.

***Note:*** If a large volume of DNA is required for your desired number of SNPs, please send this DNA at a higher concentration that the minimum acceptable concentration to reduce the volume required. This will enable the whole sample to be sent in one plate (maximum well volume 0.8 mL).

| Number of SNP SNPs to be run on your samples | Volume of wet DNA (µL) to be sent (exclusive of 'dead volumes') | Amount of additional DNA'dead volume' required (µL) for automation | Total volume of DNA required (µL)* |
|---|---|---|---|
| 1-5 | 10 | 10 | 20 |
| 6-10 | 15 | 10 | 25 |
| 11-25 | 12-38 | 20 | 32-58 |
| 26-50 | 39-75 | 20 | 59-95 |
| 51-100 | 77-150 | 30 | 107-180 |
| 101-200 | 152-300 | 30 | 172-330 |

*N.B. Ensure that the DNA is at or above the minimum concentration required for your study organism.

***Note:*** If you are supplying your DNA in greater concentrations than required for your number of SNPs, the DNA samples can be diluted in house. In these instances, the in-house dilution reduces the need for dead volumes.

### 4. Calculating the mass of DNA required according to genome size

If your study organism has a:

- Larger genome size – you will need to send a greater quantity of DNA. To calculate this, divide the genome size of your organism by the size of the human genome (3000 Mbp), and use the resulting number to multiply the amount of DNA that you need to send.
  e.g. *Triticum aestivum* (wheat): 15966 Mbp
  15966 Mbp/3000 Mbp = 5.3
  You will need 5.3 times as much wheat DNA per sample per SNP = 10 ng × 5.3 = 53 ng

- Smaller genome size – you can send a lower quantity of DNA. To calculate this, divide the human genome size (3000 Mbp) by the genome size of your organism and use the resulting number to divide the amount of DNA that you need to send.
  e.g. *Oryza sativa* (rice): 441 Mbp
  3000 Mbp/441 Mbp = 6.8
  You will need 6.8 times less rice DNA per sample
  per SNP = 10 ng/6.8 = 1.5 ng

***Note:*** we do not accept below 2 ng DNA for any species.

### 5. Whole genome amplification

If you do not have sufficient genomic DNA for the number of SNPs that you wish to run, it is possible to perform whole genomic amplification (WGA). WGA is a PCR technique that is used to produce large quantities of DNA from a small amount of starting material. There are a number of methods for WGA, and Biosearch Technologies favours use of the primer extension pre-amplification (PEP) technique. PEP employs the use of randomly synthesised 15-mer oligonucleotides, referred to as polyN15, that bind at sites throughout the genome and act as primers to enable DNA replication. Biosearch Technologies uses our in-house enzyme, Klear Taq, and buffer system to perform WGA reactions.

To perform WGA on your samples, Biosearch Technologies requires a minimum of 50 ng of genomic DNA per sample. If the quality of the starting material is good, the product is totally amplified to a concentration of 500-1000 times higher than that of the starting material.

### 6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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