Solutions for advancing cohort studies
Cohort study project support services

- Extraction
- SNP Discovery
- Genotyping
- Shipment, Sample tracking, project management
Extraction module

Samples → Pilot → Full service pickup (optional) → Extraction & QC

Shipment → Backup storage → Quantification & Normalization

High quality DNA ready to store & PCR

Extraction -> SNP Discovery -> SNP Genotyping
## Extraction service package

<table>
<thead>
<tr>
<th>Description</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td>Up to 10 mL</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td>Expected around 30 µg/ µL</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>Blood, saliva, others</td>
</tr>
<tr>
<td><strong>Quantification</strong></td>
<td>Full spectrophotometer graph (incl. OD 260/280) provided</td>
</tr>
<tr>
<td><strong>Normalisation</strong></td>
<td>Normalised fraction shipped to customer</td>
</tr>
<tr>
<td><strong>Backup</strong></td>
<td>Aliquots stored at LGC Genomics (optional)</td>
</tr>
<tr>
<td><strong>QC</strong></td>
<td>3 SNP genotyping (KASP) QC incl. gender test</td>
</tr>
<tr>
<td><strong>Certification</strong></td>
<td>Options for full ISO certified service</td>
</tr>
<tr>
<td><strong>Plate type</strong></td>
<td>Matrix 2D barcode storage racks included</td>
</tr>
<tr>
<td><strong>Shipment</strong></td>
<td>All-in shipment arrangements</td>
</tr>
</tbody>
</table>

**Extraction** → **SNP Discovery** → **SNP Genotyping**
Pilot and pick-up

1. Pilot study (12 - 48 samples)
2. Full service pick-up of samples (bulk or batch)
DNA extraction

3. DNA extraction
   - Extraction volumes: up to 10 mL (higher volumes possible)
   - Sample types: whole blood, buffy coats, serum, tissue, saliva or buccal swabs (other matrices possible)
4. Genotyping QC
   - 3 SNP Genotyping QC standard. Includes M/F assay for sample validation
   - 24 SNP Genotyping panel (also applicable for validation of e.g. NGS samples)
Quantification

5. Quantification of DNA / RNA concentration (UV measurement) (other quantification methods optional)
Normalisation

6. Normalisation to desired concentration and volume (typically of a <1ml fraction of total sample)
Backup

7. Backup of aliquots (typically 10%) in walk-in-freezer storage facility UK
Packaging and shipment

8. Shipment in industry standard 2D barcoded storage racks (Matrix)
Enhance productivity..., do more with less

- Cost savings can be used to increase sample number
- Real customer example:

<table>
<thead>
<tr>
<th>Sample number</th>
<th>In-house (core facility)</th>
<th>LGC Genomics</th>
</tr>
</thead>
<tbody>
<tr>
<td>973</td>
<td></td>
<td>4206</td>
</tr>
</tbody>
</table>

Extraction → SNP Discovery → SNP Genotyping
Cohort study project support services

- Extraction
- SNP Discovery
- Genotyping
- Shipment, Sample tracking, project management
SNP discovery module

DNA (selected samples) → Pilot → Shipment → GWAS arrays → Whole exome sequencing → Identified SNPs

Non-genetic biomarkers

Extraction → SNP Discovery → SNP Genotyping
SNP Discovery – Array technology

Array based SNP discovery

- Affymetrix GeneChip® DNA analysis solutions
  - 10,000 to 1.8 million markers
  - starting material only 100-500 ng of DNA

- Illumina Infinium & Infinium HD bead arrays
  - 50,000 to 5 million SNP markers
SNP discovery - NextGen sequencing

Roche GS FLX TITANIUM & Illumina HiSeq 2000 technology

- Whole exome enrichment
  - SureSelect Human All Exon v4+UTRs – 71Mb
  - Illumina TruSeq human all exome – 61MB
- Exome sequencing
  - HiSeq 2000
Cohort study project support services
Genotyping module

- Identified SNPs & InDel’s
- DNA (cohort)
- Pilot
- KASP genotyping
- Scored genotyping data

Extraction → SNP Discovery → SNP Genotyping
KASP™ chemistry

1) Assay components:
KASP uses three components: test DNA with the SNP of interest, KASP Assay mix containing two different, allele-specific, competing forward primers with unique tail sequences and one reverse primer; the KASP Master mix containing FRET cassette plus Taq polymerase in an optimised buffer solution.

2) Denatured template and annealing components – PCR round 1:
(allele-2 primer does not elongate)
(allele-1 primer binds and elongates)
(reverse primer elongates 5'-3')

In the first round of PCR, one of the allele-specific primers matches the target SNP and, with the common reverse primer, amplifies the target region.

3) Complement of allele-specific tail sequence generated – PCR round 2:
(Reverse primer binds, elongates and makes a complementary copy of the allele-1 tail.)

4) Signal generation – PCR round 3:
FAM-labelled oligo binds to new complementary tail sequence and is no longer quenched.

In further rounds of PCR, levels of allele-specific tail increase. The fluor labelled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal.
KASP genotyping in action

- We deliver over 1.5 Million PCR reactions / genotyping data point per day
Reference project

- **Name:**
  GEFOS ([http://www.gefos.org/](http://www.gefos.org/))

- **Location:**
  USA, Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom

- **LGC Genomics solutions provided (completed):**
  85,000 DNA samples, 83 SNP’s, 24 group world wide consortium

- **Reference:**
  Erasmus MC, Rotterdam
Reference project

• **Name:**
  Whitehall II ([http://www.ucl.ac.uk/whitehallII](http://www.ucl.ac.uk/whitehallII))

• **Location:**
  United Kingdom

• **LGC Genomics solutions provided (on-going):**
  NAP and SNP genotyping on 10,000 samples (several projects incl. DNA storage, sample tracking and sample handling)

• **Reference:**
  Prof. Dr. Aroon Hingorani (UCL):
  “For cohort genotyping LGC Genomics is unbeatable”
Reference project

• **Name:**
  EPIGEN (http://www.epigenchlamydia.eu)

• **Location:**
  Denmark, The Netherlands, Spain and the United Kingdom

• **LGC Genomics solutions provided (on-going):**
  NAP & WGA 5,000 serum + SNP Discovery (450 samples, Aros AB)
  + SNP Genotyping 10,000 samples

• **Reference:**
  Prof. Dr. Servaas Morre: “Facilitating a complete solution was decisive in outsourcing to LGC” [vs. BGI]
How can we help you?

• Tell us about your research goals and needs
• Get a project specific price quote
• Start a free pilot

www.lgcgenomics.com