Resequencing

Sample Amounts and Concentrations

Amount and Concentration of DNA	Container
Provide total DNA (≥75 ng per sample with ≥2 ng/µl; DNAse treated) in ≥20 µl	1-24 samples: tubes or 96-well PCR plate >24 samples: 96-well PCR plate

Remarks:

- If you would like to send samples for isolation, please refer to our user guide for DNA Isolation.
- DNA samples are preferably dissolved in DNase-free water or 10 mM Tris-HCl buffer (pH 7.5 – 8.5).
- DNA samples dissolved in the above buffers can be sent at ambient temperatures.

Sample Preparation

When preparing your samples, please ensure that your samples are placed in low DNA/RNA affinity plates (PCR plates) and that they are properly sealed (heat sealed or 8-cap strips).
Use 1.5 ml tubes only for less than 24 samples. Screw-capped tubes are the most robust and secure tubes (no accidental lid opening). If you are using snap-cap tubes, we recommend that you use Safe-Lock/SafeSeal tubes (less risk of accidental lid opening).

Library Preparation and Sequencing

Standard library preparation includes QC of your samples, library preparation and QC of the final library. For the Illumina DNA Tagmentation library, please provide > 75 ng DNA at a concentration of > 2 ng/µl (see our website for more details). Sequencing will be performed using convenient, customized Illumina Sequence Data Packages per sample, starting with 1 Gb reads sequenced in 2×150 bp read mode or longer. If applicable, please specify the ploidy, coverage, and genome size of the organism of interest.

Sample Processing

Typical coverages for Resequencing by Illumina.

Organism	Genome size (Mb; haploid)	Typical coverage/ Gb required
Methanococcus jannaschii (Archaea)	1.7	100 - 200 x / 0.1 - 0.2
E. coli K12 (bacteria)	4.6	100 - 200 × / 0.5 - 1
Yeast Saccharomyces cerevisiae (yeast)	12	80 - 100 × / 1 - 1.2
Caenorhabditis elegans (nematode worm)	97	80 - 100 × / 8 - 10
Arabidopsis thaliana (plant)	125	80 - 100 × / 10 - 13
Drosophila melanogaster (fruit fly)	180	80 - 100 × / 15 - 18
Danio rerio (zebrafish)	1400	30 - 50 × / 42 - 70
Homo sapiens (human)	3300	30 × / 99 (shallow); ≥80 × / ≥ 264 (deep)
Hordeum vulgare (barley)	4200	30 × / 126
Bufo bufo (toad)	5000	30 × / 150

Bioinformatic Analysis

The standard bioinformatics analysis includes mapping of the reads to the reference genome, determination of gene expression and statistical analysis (see also AppNote). In addition, alternative transcript isoform expression/alternative splicing and statistical analysis and gene fusion detection are performed. Pathway analysis is based on enriched genes and related terms (GO, KEGG and additional ontologies). For some model organisms with poor reference genomes and database entries, the analysis may be limited. For non-model organisms with reference genomes, individually optimized settings are applied.

Sample Shipment / Order Form Completion

Prior to shipping your sequencing samples to Microsynth, please proceed as follows to complete your order form:

1. Enter our webshop at https://srvweb.microsynth.ch/
2. Click on „**Illumina Sequencing**“ and afterwards on “**miCORE Resequencing**” in the green Analysis Services area
3. Fill in the order form and submit your order. Feel free to communicate any specific requirements or provide additional information using the comment field and file upload options.
4. Prepare your samples according to this User Guide or in case of isolation according to the DNA isolation User Guide.
5. If you have ordered DNA isolation along with NGS analysis, please select an appropriate Next Generation Sequencing entry point and select “Material for Isolation” for Sample Types.
6. Send your samples together with the printout to Microsynth AG according to the listed conditions, adding “NGS” or “Isolation” to the address.

Need More Information?

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