guidelines v1.04

ChIPed DNA submission guidelines for ChIP-seq

IN SHORT: Prepare your DNAs according to these guidelines and put them in our submission drawer at -20°, in minimum 5ul (more is better). In parallel, send the ChIPed submission excel form to gecf@epfl.ch . No info should be transmitted only orally.

How to submit your ChIPed DNA samples?

- Fill in the **ChIPed DNA submission excel form** found on our website and email it to us. All relevant information should be included in this form, nothing should be communicated only orally.

- The minimal QC to be provided is DNA quantification, ideally by qubit. Nanodrop can also be suitable for DNAs that are concentrated-enough to allow for a reliable measurement (above 30ng/ul).

- A profile analysis of the total input is mandatory. We can do it, or we can train you on our Fragment Analyzer for you to do it (recommended as it streamlines the workflow). Profiling the ChIPed DNA is recommended but not required.

- The GECF is an open access facility, therefore we cannot give guarantee regarding the confidentiality of data. If this is an issue, the easiest solution is to code your sample names.

- In parallel to submitting the form, please bring DNAs during regular office hours, on ice, in DNase-free PCR-grade 0.5ml-2ml microtubes, but not in PCR tubes/strips/PCR plates. Leave some space on the side of tubes for GECF codes.

- Indicated tube labels must match exactly the ones on your physical tubes. If available, labels of profiles must also match perfectly either tube labels or sample names.

- Avoid using “S1”, “S2” and “R1”, “R2” … in your samples name, as these codes are already appended by the sequencer to fastq files.

- If you need to **modify** or comment on your submission in any way, do it by email, even when you have already told us orally.

- Concentration or total ng amount submitted don’t need to be normalized/identical across samples.

- There are strong constraints on volume to submit, but a reasonable amount is between 10ul and 50 ul.

- There are strong constraints on ng amounts to submit, but the more the better. We can start a prep from 0.5ng without special protocol, and less can be possible (disclaimers apply). If you can submit more than 10ng, at least for total input samples.

ChIPed DNA preparation

- Measuring **DNA concentration** by nanodrop may be very inaccurate, in particular at low concentrations (<20ng/ul). Therefore, always report 260/280 and 260/230 ratios when nanodrop is used. Alternatively, a more accurate and sensitive method is Qubit DNA HS (available at GECF).

- Depending on your project/aims, the lower limit to the **DNA quantity** required for library prep will differ. Thus please contact us in advance to discuss it.

- **DNA elution** should ideally be done in PCR-grade H2O, Tris or "low-EDTA TE" (10mM Tris, 0.1mM EDTA).

- **DNA storage** should be done at -20°.

Recommendations for ChIP-seq experimental design

These recommendations are just a help to avoid most significant errors during Chip-seq, but for more in depth ones have a look at https://www.encodeproject.org/data-standards/chip-seq/

- When **designing your ChIP-seq experiment**, consider these points:

- Include a total input for each chromatin sample. In order to insure good total input data, we will determine the amount of total input DNA to use for library prep by considering the available ng amounts of the chipped samples:

Starting amount of Chipped DNA	**Starting amount of input DNA**
> 10 ng	Corresponding to amount of highest chipped sample
0.5-10 ng	10 ng
<0.5 ng	5 ng

- Validate antibody specificity in standard protein IP at least once at beginning of experiment.
- If available, validate antibody/method efficiency by qPCR on a known enriched target. If possible, we recommend doing it systematically before submitting samples for prep/sequencing.
- At least duplicate biological replicates are needed, but many/most bioinformaticians ask for **triplicates**, check that in advance.
- Facultative: have a cell line not expressing your IP target (not transduced, KO…)
- Facultative: for ChIP of a modified histone, have a control ChIP on the non-modified histone, to check that any difference is not simply due to difference in quantity of that histone but really due to difference of modification level.

Determining library prep method and sequencing parameters

- The choice of a library prep method depends directly on results from DNA quantification, and will be a shared decision between the users and the GECF.

- Single-end reads (SR) or paired-end (PE) reads? consensus is PE for chip-seq. This allows to identify clearly the bound region, which is not possible with just SR, as the ChIPed fragment could be +/-200nt in a typical library.

- Read length? If the targets are typically not thought to bind specifically to repetitive regions and if the user wants to minimize costs, PE37 is enough. If cost is not a critical issue, and/or if targets are repetitive, PE60 or PE75 is preferred as mapping will be better (fewer multiple mappings).

- Mio reads/sample? This strongly depends on the target ChIPed protein, and on the size of the genome. For mammalian TFs, a good starting point is 15-20 mio reads/sample. For the total inputs, the consensus is to sequence at least as much as for the ChIPed DNAs (some people sequence at same depth and some go for 2x more reads).

Bioinformatics

- We do not offer in depth bioinformatics analysis. If needed contact the EPFL bioinformatics facility.

Versions log

- v1.02: minor edits. Clarification regarding the input control starting amounts that we'll use for library prep. Clarified that the submission form itself must contain all information, and that DNA quantification is mandatory. Suggested that users perform profile analysis to streamline the workflow.

- v1.03: Clarified that samples must be brought to GECF in parallel of form submission. Clarified sequencing depth for total input controls. Clarified the method of determining total input starting amount. Minor edits.

- v1.04 (30.10.2025): Mentioned that we recommend checking enrichment by qPCR before each submission. Avoid R1, R2 in samples names. Commented on minimal volume and ng amount to submit.