ChIP/ MeDIP-enriched DNA Submission

Recommended sample amount:

20*-40ng IP enriched DNA at 200-400bp.

** 1) The minimum amount for each sample is intended for the whole experiment, including RNA quality control. If the minimum amount is unavailable, please consult our technical support by email at support@arraystar.com to make special arrangements.*

2) The minimum amount is for a single attempt. To minimize delays caused by experiment failures, it is recommended that twice the minimum amount above be supplied.

Recommended quality assessment of IP enriched DNA

DNA Concentration:

Quantification based on NanoDrop readings cannot be trusted when the DNA concentration is very low. We recommend to quantify IP-enriched DNA samples using a fluometric-based method such as Qubit® (Invitrogen), which is more precise and sensitive at low DNA concentrations.

DNA Purity:

DNA purity can be determined by NanoDrop ND-1000 readings of A_{260}:A_{280} and A_{260}:A_{230} ratios.

Specificity of IP:

Specificity of IP DNA can be assessed using Real-time quantitative PCR, to compare the enrichment efficiency of a known specific binding gene in the specific antibody and unspecific IgG-antibody immunoprecipitated fractions.

Note: Arraystar is not responsible for the enrichment specificity of IP experiment. Please validate the specificity of your IP experiment before submitting the samples.

1. Setup qPCR assay for assessment of IP Quality:

qPCR Assay	Input DNA	IP DNA	Mock IP (Non-Immune serum)
Positive Control Primer set*	√	√	√

* Positive Control Primer set: Primers design according to specific DNA methylation sites or TF/Histone/Protein binding sites

2. IP-qPCR Data Analysis (ΔΔCt method):

a) Normalize each IP DNA fractions' Ct value to the Input DNA fraction Ct value for the same qPCR Assay (ΔCt) to account for chromatin sample preparation differences. Calculate the % Input for each IP fraction:

$\%Input=2^{(CtInput-CtIP)} \times Fd \times 100\%$

Here, Fd is Input dilution factor.

For example, if 100ul sonicated sample is used for IP and 20ul sonicated sample is used as Input, Fd =1/5.

b) Adjust the normalized IP fraction Ct value for the normalized background (mock IP) fraction Ct value.

ΔΔCt [IP/mock IP] = ΔCt [normalized IP] - ΔCt [normalized mock IP]

3. Assessment of IP Quality:

a) The Input DNA Ct value should be less than 30.

b) The % Input for the mock IP DNA fraction should be less than 0.01%.

c) The IP DNA Ct value should be at least one cycle less than the mock IP DNA Ct value (Ct [mock] – Ct [IP] > 1.0) to be considered quantitatively above the background signal (noise) for the sample.

Shipping at room temperature

1. For DNA with QIAsafe™ DNA Tube (QIAGEN, Cat. #159104; Please refer to the supplier's instruction)

a) Determine the yield of the purified DNA and apply DNA solution into QIAsafe™ DNA Tube. For optimal protection, do not apply more than a total of 30ug of DNA per tube in a maximum volume of 100ul.

b) Gently add the DNA sample directly into the QIAsafe™ DNA Tube.

c) Thoroughly mix with gentle pipetting (avoid forming air bubbles).

d) For small volume (less than 20ul), air dry the sample in laminar flow hood for appropriate time.

Recommended drying times are given in the following table.

Sample volume(ul)	Approximate drying time(h)
5	2
5-10	6
10-20	10-12

For volumes ≥20ul, or for accelerated drying of all, use a vacuum concentrator (i.e. SpeedVac) without heat to ensure complete sample drying. Recommended drying times are given in the following table.

Sample volume(ul)	Approximate drying time(h)
10-20	0.5
20-30	1
30-50	1.5
50-100	2

e) Close tube, store or ship the DNA samples at room temperature.

Shipping on dry ice

1. For DNA in regular solution

a) Redissolve the DNA in Nuclease-free water or TE buffer.

b) Store at -80°C or -20°C. Ship on dry ice.