Antibody certification report

Replicate #1	
Total reads	24,890,602

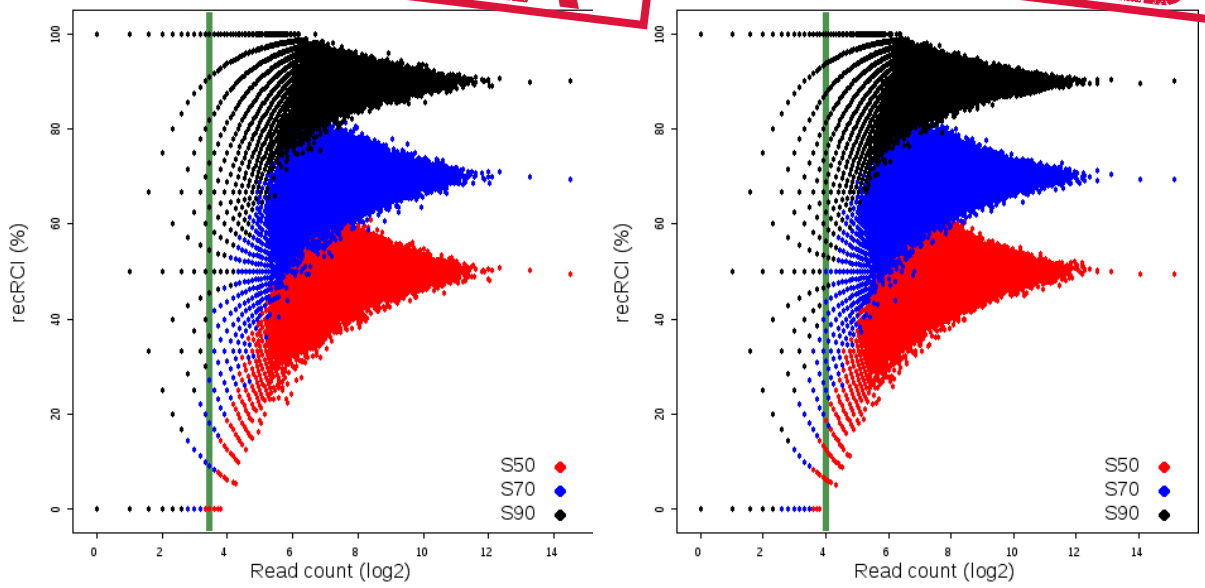
Replicate #2	
Total reads	46,589,190

QC parameters	
Sampling percentages	50, 70, 90
Windows size (bp)	500
Sampled strand(s)	both
Genome assembly	hg19
Background subtraction	On

Results			
QC values		denQC (50%)	simQC
rep. #1	2.5%	0.785	8.398
	5%	2.981	3.754
	10%	7.542	1.941
rep. #2	2.5%	0.574	5.390
	5%	1.836	2.889
	10%	4.278	1.585

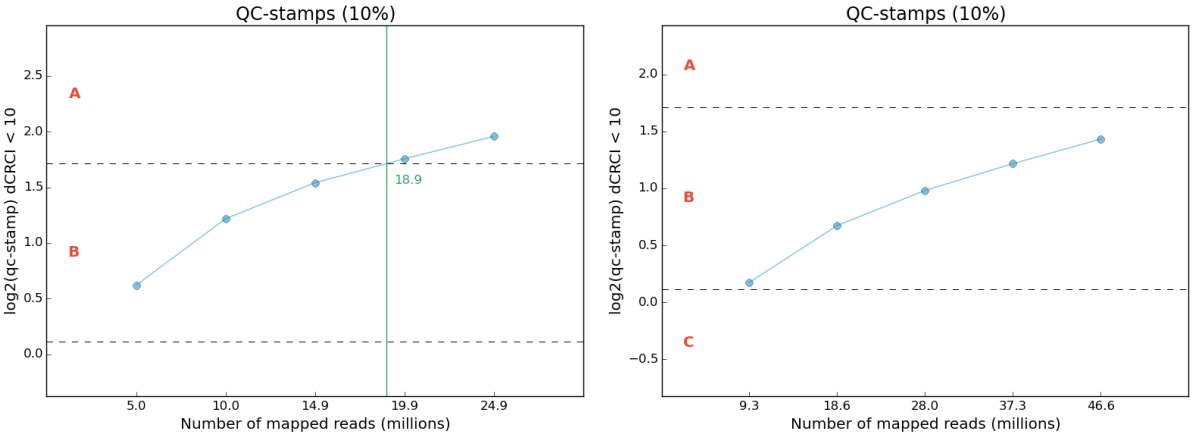
Global QC certification AAA

Global QC certification AAB



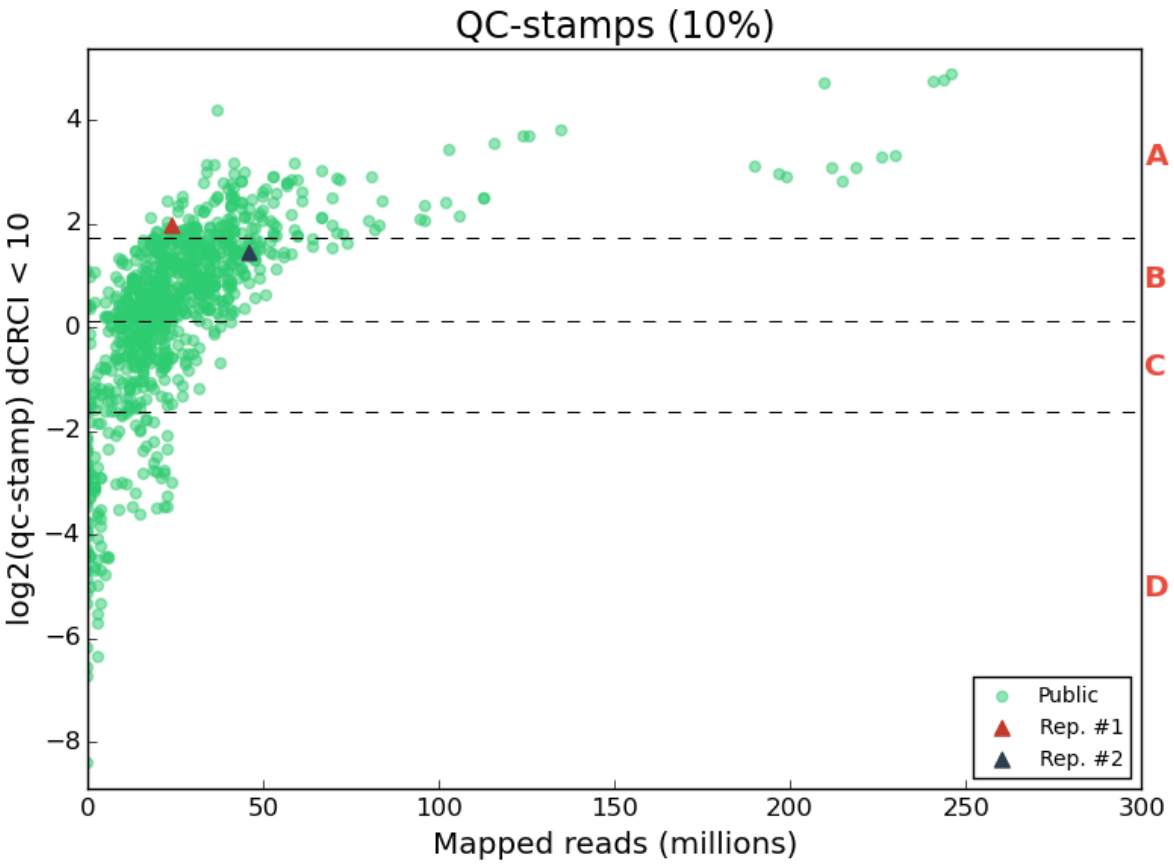
Scatterplot illustrating changes in the read-counts intensity per genomic window (500nts bin length) when using a fraction of the total mapped reads (TMRs) for reconstructing the enrichment profile. The x-axis corresponds to the read counts per bin in the original enrichment profile (in log2) and the y-axis makes reference to the recovered read count intensity (recRCI) for the sampling conditions used (in %). TMRs were random sampled at three different subset fractions; s90 or 90%, s70 or 70% and s50 or 50% of TMRs, respectively. The dark-green vertical line depicts the read-count intensity background thresholds used (Poisson distribution model; p-value threshold = 0.995).

Optimal sequencing depth estimation



Estimation of the minimal sequencing depth required for reaching the maximal quality grade. This analysis is performed by re-computing quality grades from subsequent decreasing fractions of the initial total mapped reads (TMRs). The x-axis represents the mapped reads used for computing the illustrated quality descriptor (20%, 40%, 60%, 80% and 100% of TMRs). The y-axis depicts the computed quality descriptor (QC-stamps) assessed for the different mapped-reads conditions.The vertical green line reveals the predicted minimal sequencing depth required to reach a "A" QC-stamp.

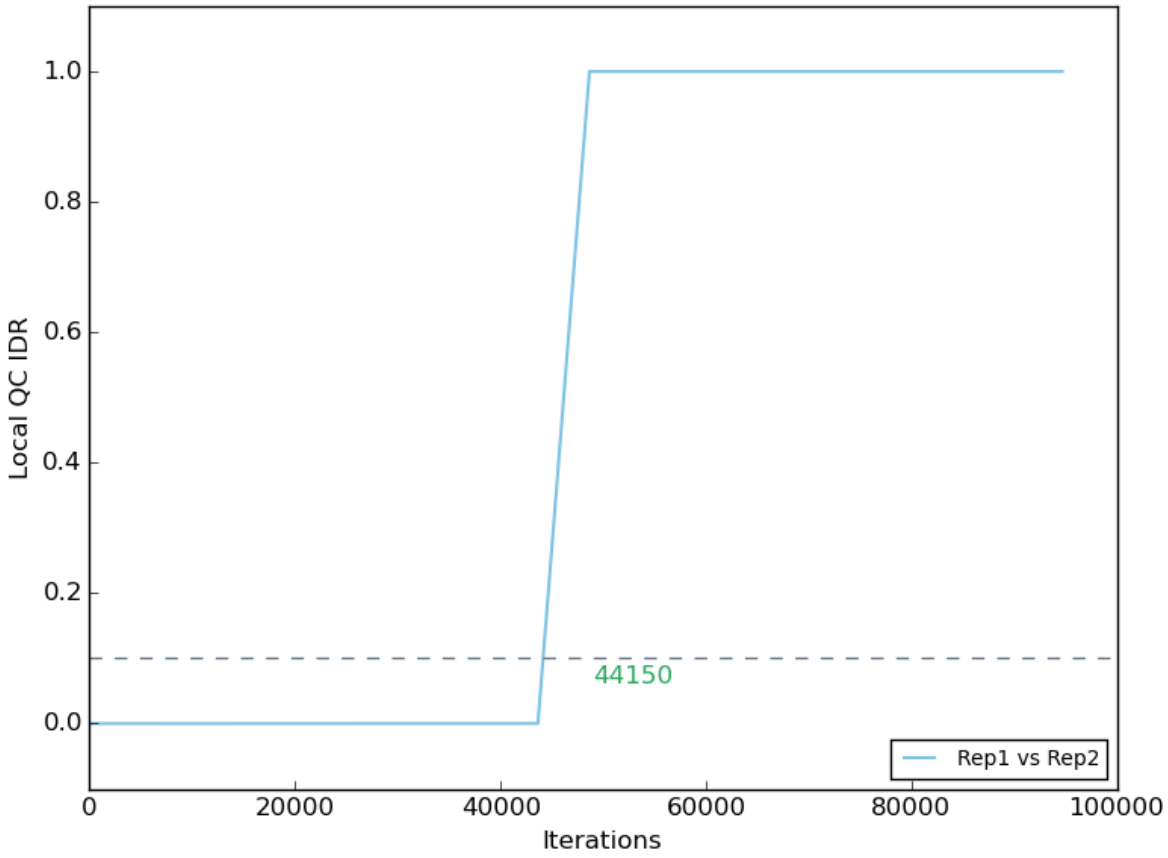
Computed QC-stamps positioning relative to the NGS-QC database content



Comparison of the assessed quality grade with those computed for publicly available datasets currently hosted in the NGS-QC database (ngs-qc.org). Note that while the publicly available entries correspond to the same antibody target, the antibody sources and batch numbers may differ.

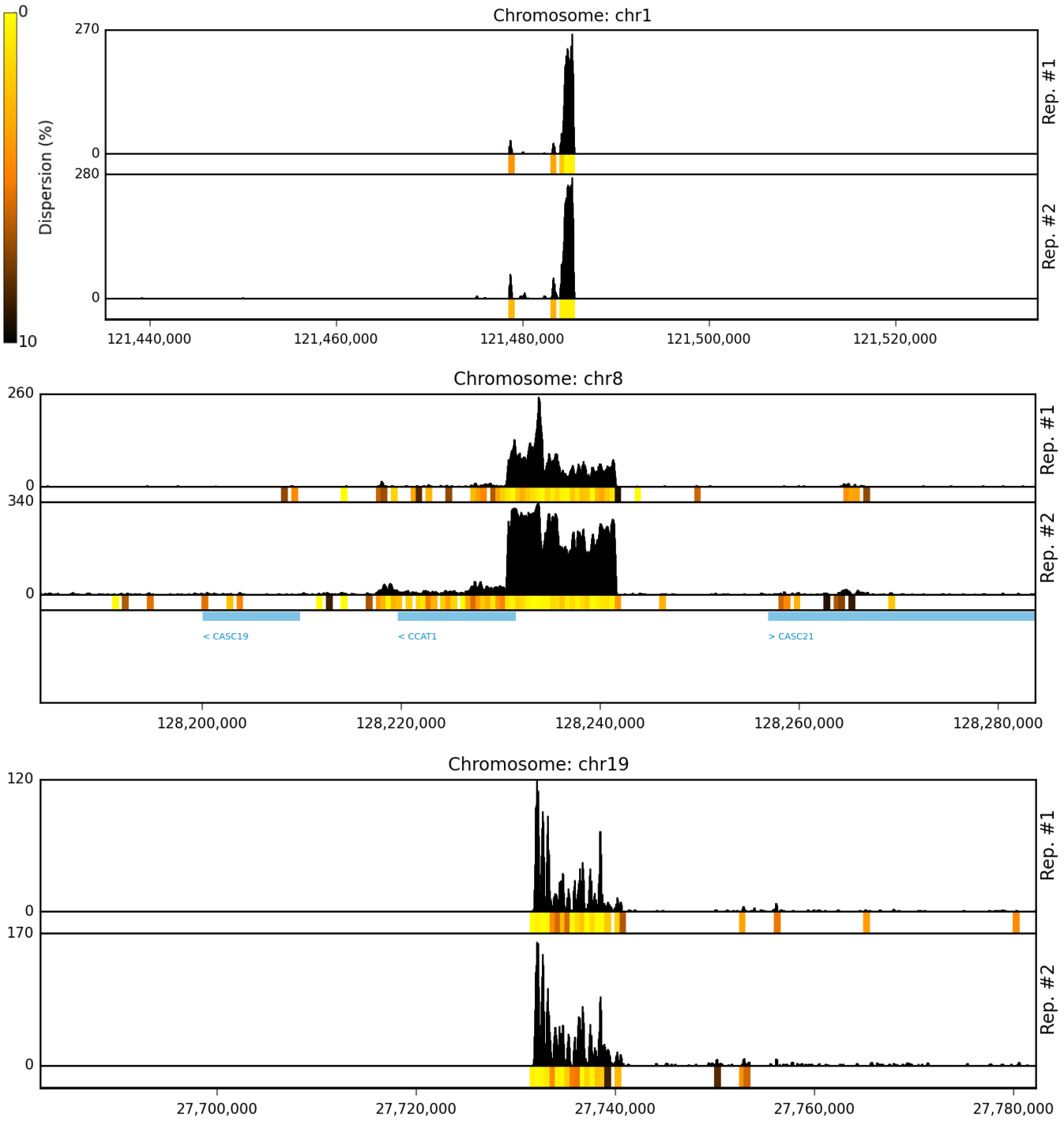
Local QC Irreproducibility discovery rate (local QC-IDR)

Concordance among biological ChIP-seq replicate assays were previously assessed by the Irreproducibility discovery rate assay (Q. Li et al; Ann. Appl. Stat; 2011). In the same manner, we have established an IDR assay based on the comparison of the genomic regions (500nts length) presenting the least read count intensity dispersion (dRCI<10%) levels following the described random TMR subsampling procedure. This measurement per genomic region is also referred to as local QC dispersion; a more detailed description is available at ngs-qc.org.
Briefly, genomic regions displaying a dRCI<10% from both genomic replicates are paired according to overlaps of their genomic coordinates. Genomic regions present in only one of the replicates ("outliers") are also retained in the paired list but a penalty dRCI score is added (penalty dRCI is 15 for outlier regions).
Genomic regions are ranked for the lowest absolute difference between dRCI values per paired genomic regions; subsequently the local QC IDR, defined as the fraction of outlier regions retrieved per 5000 sorted genomic region events, is computed in a sliding window manner starting with the top ranked positions.

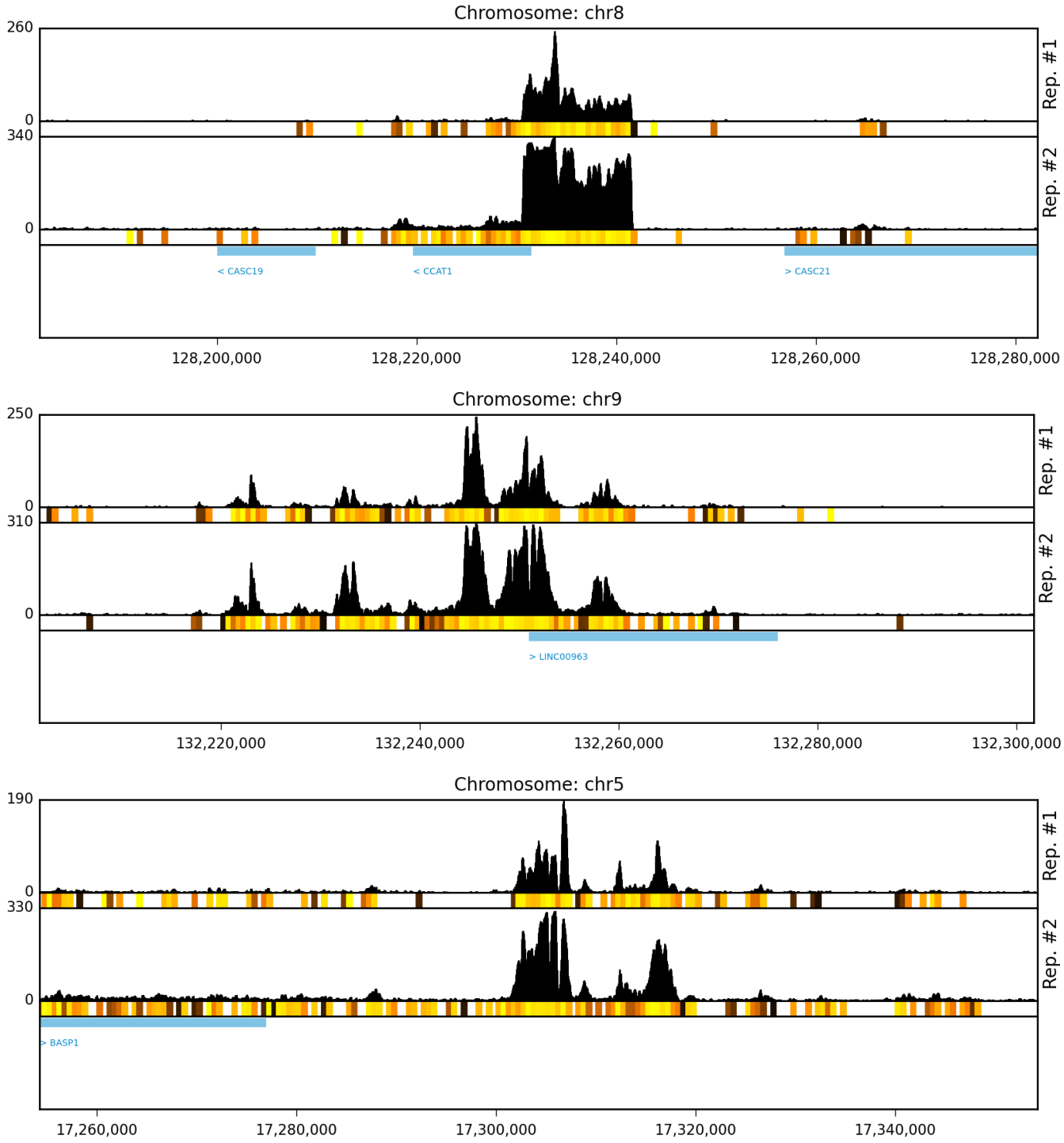


In the plot the x-axis corresponds to the ranked genomic regions and the y-axis to the computed local QC IDR; i.e. to the degree of irreproducibility among compared local QC indicators per replicate. The horizontal dashed line defines the number of genomic regions with local QC IDR levels below 0.1. Importantly, it defines the number overlapping genomic regions among biological replicates having optimal dRCI levels. In the pages 4 and 5 of this report examples of several regions with their associated local genomic QC (illustrated by heat maps) are displayed in the context of read count intensity profiles.

Example of genomic regions per biological replicate



Read-counts intensity profile illustrated in the context of its corresponding local QC indicators (heatmap; from 0 to 10% read-counts intensity dispersion). The lower the dispersion, the better is the robustness quality of the genomic region in view and, as illustrated, it correlates well with the enrichment patterns. The y-axis corresponds to read-count intensity levels and the x-axis to genomic coordinates. Blue bars depict annotated genes when present in the region shown. Note that "clonal reads" were removed in this analysis.



Read-counts intensity profile illustrated in the context of its corresponding local QC indicators (heatmap; from 0 to 10% read-counts intensity dispersion). The lower the dispersion, the better is the robustness quality of the genomic region in view and, as illustrated, it correlates well with the enrichment patterns. The y-axis corresponds to read-count intensity levels and the x-axis to genomic coordinates. Blue bars depict annotated genes when present in the region shown. Note that "clonal reads" were removed in this analysis.

Antibody certification procedure

Cell culture

HeLa cells were grown in DMEM 1g/L glucose, 5% Fetal Calf Serum and 40µg Gentamicin up to a density of 15-20 millions cells/15cm plates. Cells were fixed for 30min with paraformaldehyde (1% in 1XPBS). Fixation was quenched with 0.2M glycine (in 1XPBS), then cells were washed three times with 1XPBS, collected and stored at -80°C.

Chromatin Immunoprecipitation

Sonication

40 million cells were sonicated in 500µL of Lysis Buffer (1% Na-deoxychlorate, 50mM TrisHCl pH8, 140mM NaCl, 1mM EDTA, 1% Triton X-100) containing 5-times diluted Protease Inhibitor Cocktail (PIC; Roche Diagnostic; 1 tablet solubilized in 10ml Lysis Buffer). Sonication was performed with a Bioblock Scientific instrument (Vibra Cell 75043; 40 cycles, 30s ON end 59s OFF; 38% power). Chromatin fragmentation was evaluated by agarose gel electrophoresis as follows: 20µL of sonicated chromatin was diluted with 20µL 1xTE (10mM Tris-HCl pH 8, 1mM EDTA) and 5µL 5M NaCl was added. Diluted chromatin was incubated at 100°C for 30 min, centrifuged at 12,000 rpm and the supernatant was loaded onto a 2% agarose gel.

Chromatin Immunoprecipitation

25µL of ChIP-IT Protein G Magnetic Beads (ActiveMotif) were incubated with 5µg of H3K27ac antibody (pAb) as following antibody supplier's information (Catalog No: 39133, Lot No: 01613007) in 100µL PIC-containing Lysis Buffer. After two hours at 4°C on a rotating shaker, chromatin from 3 million cells were added and the final volume was adjusted to 500µL with PIC-containing Lysis Buffer and incubation on a rotating shaker was continued overnight at 4°C.

The immunoprecipitated chromatin was recovered by magnetic bead separation, followed by multiple washing steps on a custom liquid handling platform (TECAN EVO75). Specifically, the washing is performed as follows: (1) Low salt washing (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM TrisHCl pH 8, 150mM NaCl); (2) High salt washing (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM TrisHCl pH 8, 500mM NaCl); (3) LiCl-washing (0.25M LiCl, 1% IGEPAL CA630, 1%% Na-deoxycholate, 1mM EDTA, 10mM Tris pH 8) and (4) 1xTE washing.

The immunoprecipitated chromatin was eluted and de-crosslinked in 100µL of elution buffer (1% SDS, 100mM NaHCO3, 250mM NaCl, 0.2mg/ml Proteinase K) and incubated for 4 hours at 65°C. The eluted chromatin was supplemented with 200µL H2O and 300µL phenol/chloroform/isoamyl alcohol (25/24/1) mix was added. After two extraction steps, the aqueous phase was subjected to ethanol precipitation in presence of 1µL GlycoBlue (Invitrogen; 15mg/ml). The precipitated material was re-suspended in 45µL H2O; 5µL was used for validation by quantitative PCR, the remaining 40µL were used for DNA library preparation.

DNA Library preparation and Massive parallel sequencing

The DNA library preparation for massive parallel sequencing was performed according to standard procedures (NEXTFlex ChIP-Seq Kit (Biooscientific)) adapted to automation by our custom liquid handling platform (TECAN EVO75). Prior to DNA sequencing library preparation was monitored using a Tapestation (Agilent). Samples were sequenced on an Illumina HiSeq2500 platform following standard procedures.

NGS-QC certification

The antibody certification report is based on the concepts described as part of the quality control system developed in H. Gronemeyer's team. (Mendoza-Parra MA et Al. Nucleic Acids Res. 2013 Nov; 41(21)). Briefly the quality assessment is based on the use of subsets of the total mapped reads (TMRs) for reconstructing enrichment profiles which are then compared with the original profile. Thus, the assessed quality grades describe the differences among compared profiles (original profile reconstructed from the total mapped reads versus those reconstructed with fractions of the TMRs) beyond expectation; i.e. a proportional decrease in the read counts intensity due to the use of a subset of the TMRs. For further details please visit our dedicated website (www.ngs-qc.org) where a detailed tutorial is available.