Technologies to study spatial genome organisation

Expansion of Table 1 in https://academic.oup.com/bfg/advance-article/doi/10.1093/bfgp/elz019/5586926

Method	Info	How?	Advantages	Disadvantages
In situ Hi-C	DNA-DNA proximity ligation is performed in intact nuclei, generating much denser Hi-C maps.	Crosslink cells, use a 4-cutter restriction enzyme to digest the DNA, fill the overhangs and incorporate biotinylated nucleotides, ligate the blunt-end fragmnets, shear the DNA, pulldown the biotinylated ligation junctions with streptavidin beads and sequence.	- High (kbp) resolutions due to 4-cutter - Variations allow application to samples with limited cell numbers (Low-C) - Reduces frequency of spurious contacts due to random ligation in dilute solution.	- Requires extreme sequencing depth - Mostly captures pairwise interactions.
Micro-C	Use micrococcal nuclease instead of restriction enzymes, which enables nucleosome resolution of chromatin contact maps.	Crosslink cells, digest using MNase, mononucleosomal end repair.	- High (nucleosome) resolution.	- Unreliable for capturing long-range interactions - Only captures pairwise interactions.
DNase Hi-C	Use DNase I instead of restriction enzymes, producing libraries of higher resolution.	Crosslink cells, lyse to liberate nuclei, treat with DNase I, end-repair chromatin ends and sequence.	- High (sub-kbp) resolution	- Bias towards DNase I hypersensitive sites - Only captures pairwise interactions.
ChIA-PET	Examine all loci bound by a specific protein. Combines ChIP with 3C.	Crosslink cells, sonicate DNA, use antibody of choice to enrich protein-of-interest-bound chromatin fragments. Continue as above.	- Good at detecting long-range interactions - Can focus on contacts from a factor of choice	- Requires large cell numbers - Generates short reads
CHi-C	Choose baits of interest for Hi-C.	As above, but includes a pull down step to only capture the interactions involving the bait of interest.	- Can be adapted for low cell counts - Can choose loci of interest (baits).	- Probes can be costly - Mostly captures pairwise interactions
HiChIP	Discover protein-centric *in situ* chromatin loops. Similar to ChIA-PET but with less input material	DNA contacts are established *in situ* in the nucleus before lysis, ChIP is performed on the contact library to directly capture long-range interactions associated with a protein of interest. Then use paired-end sequencing.	- Focused on contacts from a factor of choice - Works will low cell counts	- Dependent on antibody quality - Variable data complexity.
TSA-seq	Allows the distance of every gene from specific nuclear landmarks to be measured simultaneously.	The nuclear structure of interest is tagged with horseradish peroxidase which generates a highly reactive molecular called tyramide that labels the DNA. The closer the DNA is to the structure, the more it will be labelled.	- Provides information on physical distances	- Confined to particular nuclear features of interest.
Tri-C/ MC-4C	Tri-C characterises concurrent chromatin interactions at individual alleles.	Tri-C libraries are generated using an enzyme (NlaIII) selected to create relatively small DNA fragments (~200 bp) for the target fragment. The fragments are religated, MC-4C then uses Cas9 digestion of the sequence of interest.	- Long sequencing reads - Captures multi-way interactions	- Relatively low throughput - MC-4C hass a complicated Cas9 step.

i3C	Developed to capture native interactions in intact nuclei in the absence of fixation from either one viewpoint (i4C), within one extended locus (iT2C) or genome-wide (iHi-C)	Collect living intact nuclei, treat with restriction endonuclease, ligate cohesive DNA ends and sequence.	- No fixation - Captures native interactions of improved signal quality	- Sparse genome-wide matrices - Requires novel analysis tools.
GAM (geome architecture mapping)	Captures genome-wide interactions in a ligation free manner. Can also perform imaging on the cell splices before DNA sequencing to choose only those of interest (e.g. those in a particular cell type).	Cells are fixed and their nuclei are randomly sliced into thin nuclear section using microdissection. The DNA in these nuclear sections is isolated, amplified and sequenced. Loci in close spatial proximity will end up in the same slice more often than loci far away.	- No ligation step - Captures multi-way and longer-range interactions - Allows selection of target cells in a complex tissue.	- Requires equipment for microdissection of nuclei - Biases from whole-genome amplification.
SPRITE	Split-pool recognition of interactions by tag extension removes the need for ligation.	Splits crosslinked chromatin complexes into 96-well plates and subjects them to consecutive rounds of barcoding, mixing and resplitting so that DNA sequences in the same complex (close to each other) end up with the same unique barcode combination.	- No ligation - Captures multi-way and longer-range interactions	- Relies on the efficiency of barcode ligation.
ChIA-Drop	Single-molecular precision of chromatin contact data. Done in a droplet.	Use chromatin immunoprecipitation to select a chromatin sample, load this sample onto a microfluidics device to generate gel-bead-in-emulsion (GEM) droplets. Thus, each droplet contains uniqure DNA barcodes.	- No ligation - Captures single-molecule interactions.	- Laborious analysis - Requires 10X Genomics sequencing platform.
DamC	Combines DNA-methylation based detection of chromosomal interactions with NGS and a biophysical model of methylation kenetics.	Use a TALE-iD approach of a fusion of TALE DNA-binding domains with Dam methylases that is electrotransported into human cells. In DamC, chromosome folding is inferred by modelling Dam-induced methylation profiles in living cells.	- Detection of *in vivo* interactions	- Requires cell manipulation - Low throughput.
Multiplexed FISH	24-colour karyotyping technique.	Multiplex labelling of all chromosomes in the genome in a combinatorial fashions, such that each homologous pair of chromosomes is uniquely labelled. Microscopic visualisation and digital acquisition of each fluorophore. Superimpose images, enabling individual chromosomes to be classified based on the fluor composition.	- Higher resolution compared to conventional FISH	- Lower throughput compared to genomics