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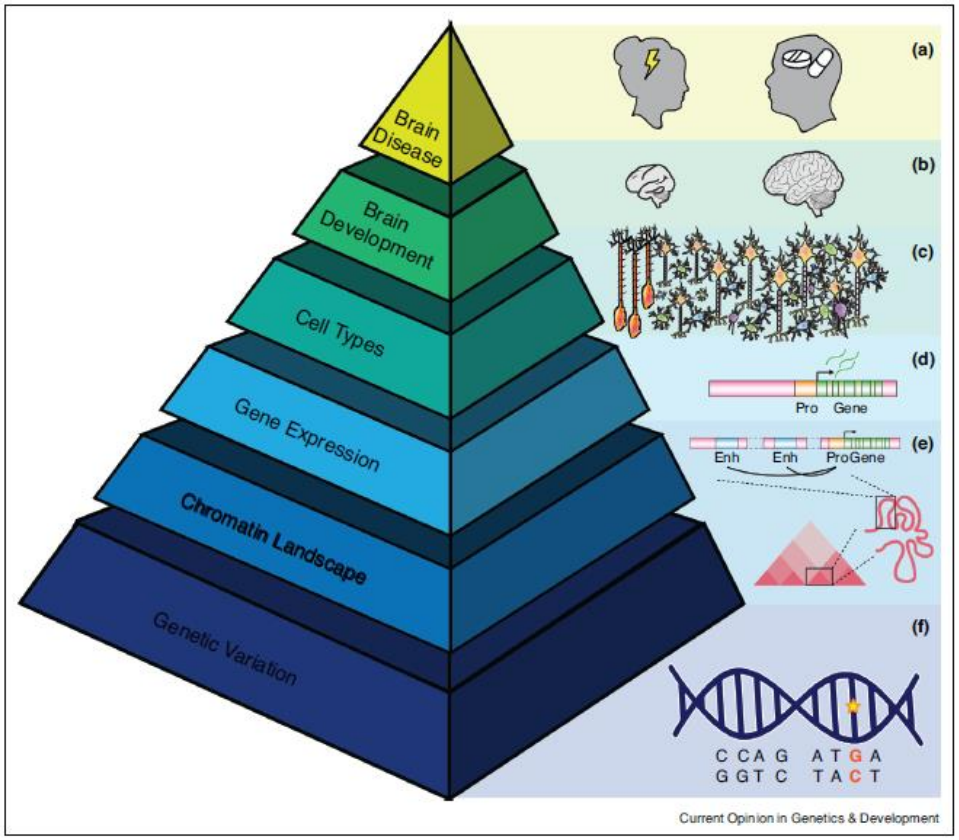
Current Opinion in Genetics & Development

Regulatory landscape in brain development and disease

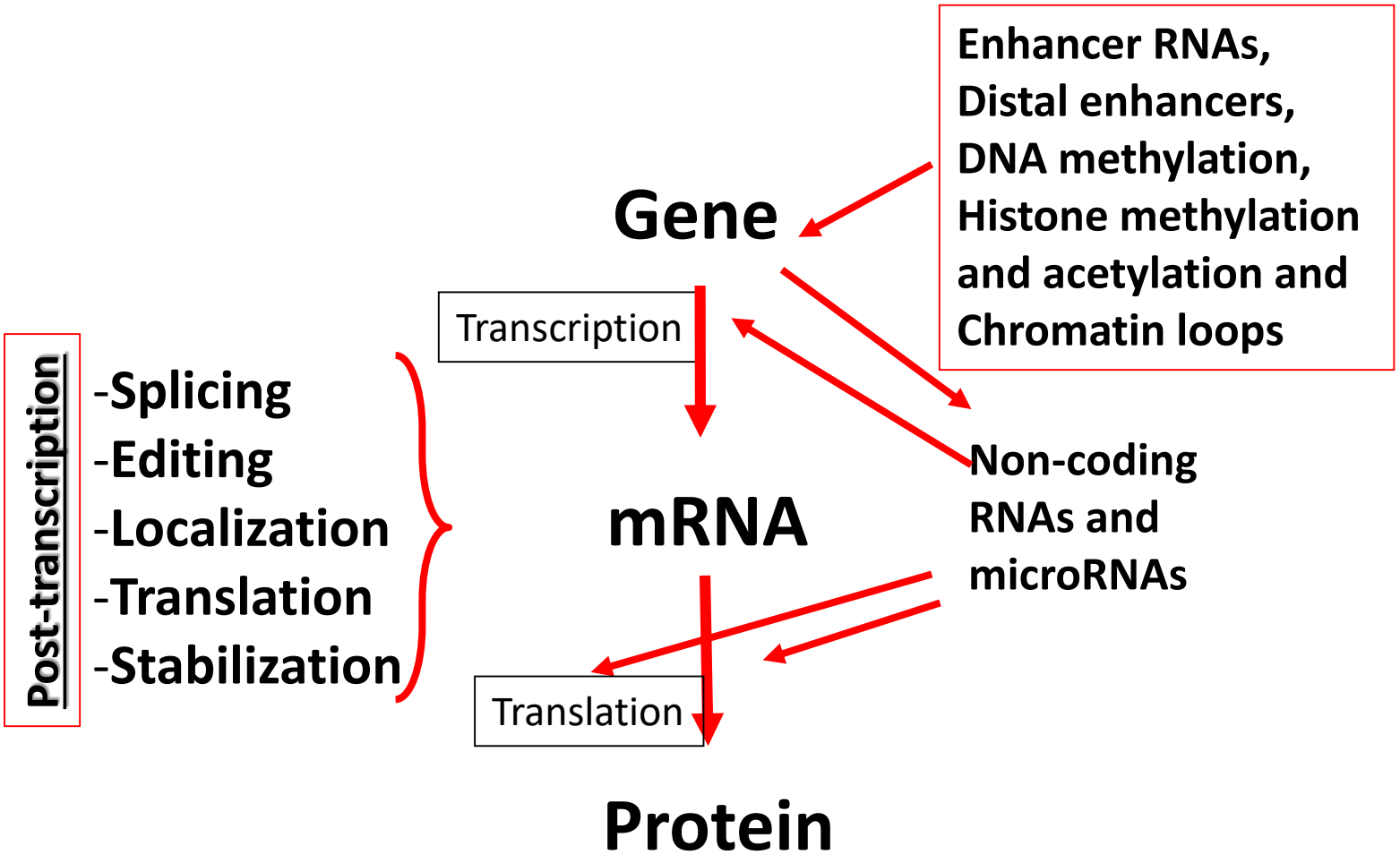
Keeley Spiess[1] and Hyejung Won[1,2]

Figure 1

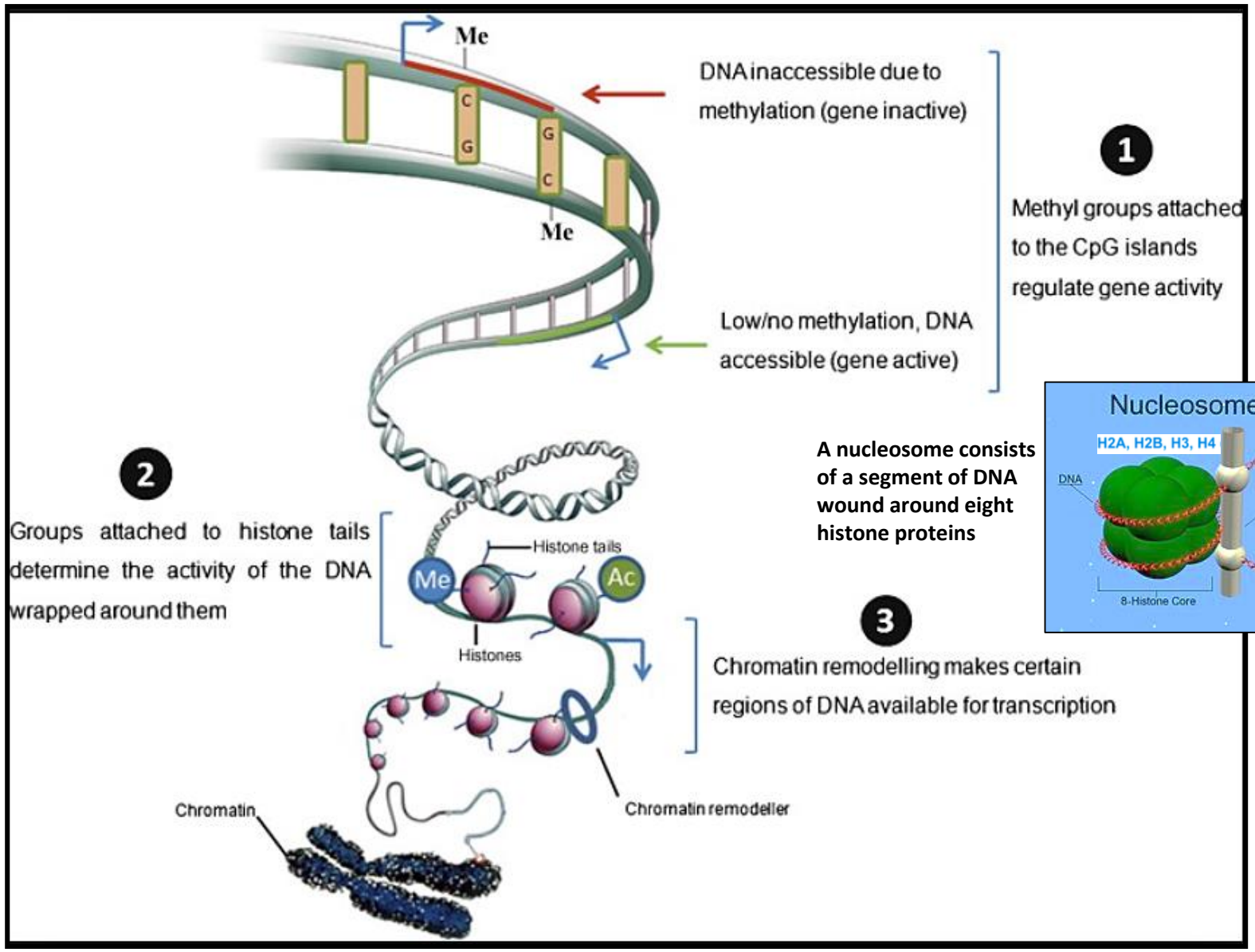


The regulatory landscape of the brain serves as a basis for understanding the molecular, cellular, and developmental underpinnings of brain disease. Genetic variation **(f)** can lead to changes in chromatin architecture **(e)**, which in turn affect gene regulation. Gene expression **(d)** changes in one or multiple cell types **(c)** can perturb brain development **(b)**. Alterations in any of these layers contributing to brain function throughout the lifespan of an individual can result in a variety of brain diseases **(a)**. Enh, enhancer; Pro, promoter.

Current view of Gene expression

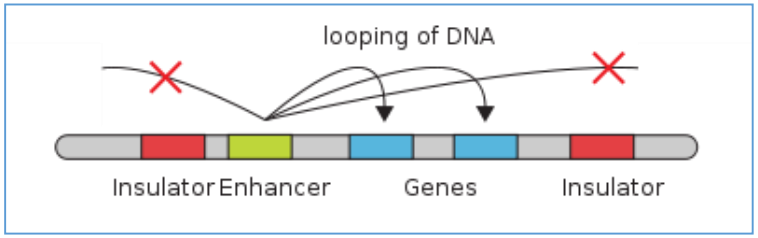


Epigenetic mechanisms regulate gene transcription



General Biology overview: Transcription factors, enhancers and insulator proteins regulate the transcriptional complex

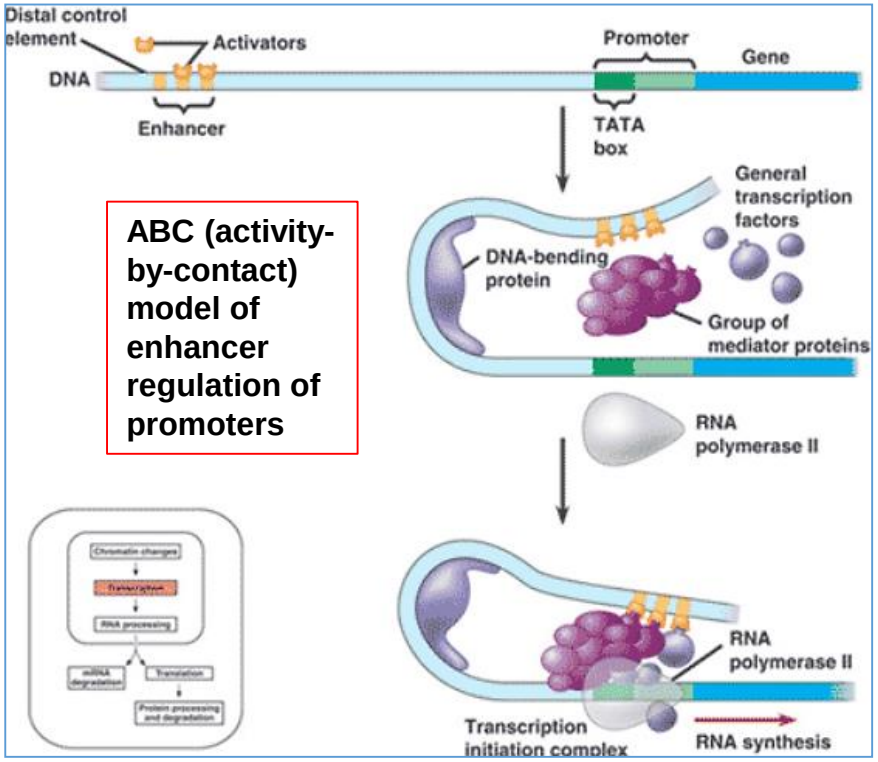
DNA is not linear but organized in high level 3D structures



An enhancer is a short (50-1500 bp) region of DNA that can be bound by proteins (activators) to increase the likelihood that transcription of a particular gene will occur. An enhancer may be located upstream or downstream of the genes it regulates

An insulator is a genetic boundary element that limits the interaction between enhancers and promoters. Insulators thus determine the set of genes an enhancer can influence.

Insulators have also been found to cluster at the boundaries of topologically associating domains (TADs) and may have a role in partitioning the genome into "chromosome neighborhoods"



ABC (activity-by-contact) model of enhancer regulation of promoters

Enhancers are bound by the histone acetyl-transferase p300-CBP and transcription factors bind to the promoter and the enhancer associated proteins and enhancer RNAs increase the activity of the promoter.

Genome-Wide Quantitative Enhancer Activity Maps Identified by STARR-seq

Cosmas D. Arnold, Daniel Gerlach, Christoph Stelzer, Łukasz M. Boryń, Martina Rath, Alexander Stark*

Genomic enhancers are important regulators of gene expression, but their identification is a challenge, and methods depend on indirect measures of activity. We developed a method termed STARR-seq to directly and quantitatively assess enhancer activity for millions of candidates from arbitrary sources of DNA, which enables screens across entire genomes. When applied to the *Drosophila* genome, STARR-seq identifies thousands of cell type–specific enhancers across a broad continuum of strengths, links differential gene expression to differences in enhancer activity, and creates a genome-wide quantitative enhancer map. This map reveals the highly complex regulation of transcription, with several independent enhancers for both developmental regulators and ubiquitously expressed genes. STARR-seq can be used to identify and quantify enhancer activity in other eukaryotes, including humans.

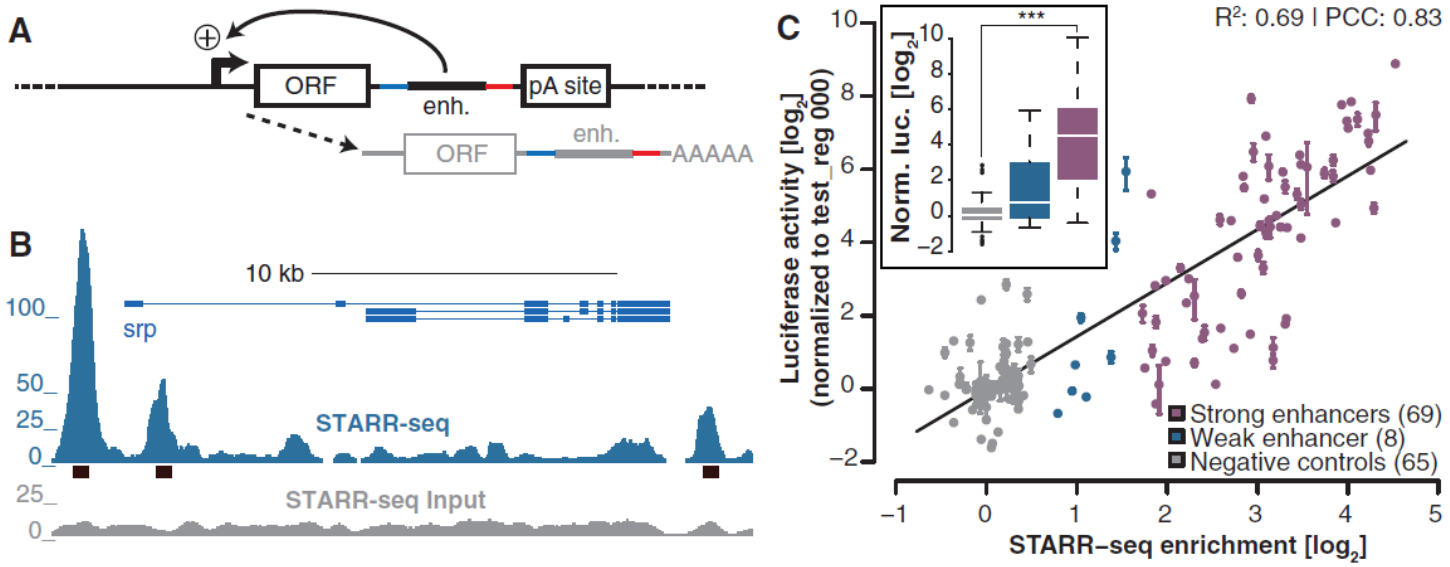


Fig. 1. STARR-seq genome-wide quantitative enhancer discovery. (**A**) STARR-seq reporter setup [enh., enhancer candidate; ORF, open-reading frame (here: GFP); pA site, polyadenylation site; +, transcriptional activation]. (**B**) STARR-seq (blue) and input (gray) fragment densities in the *srp* locus. Black boxes denote predicted enhancers ("peaks"). (**C**) STARR-seq and luciferase signals are linearly correlated: R^2, coefficient of determination and Pearson correlation coefficient (PCC or *r*). [Error bars indicate two independent biological replicates; (Inset) the same data as a boxplot; $***P \leq 0.001$, Wilcoxon rank-sum test; $n = 65$, 8, and 69.]

STARR-seq: **S**elf-**t**ranscribing **a**ctive **r**egulatory **r**egion sequencing

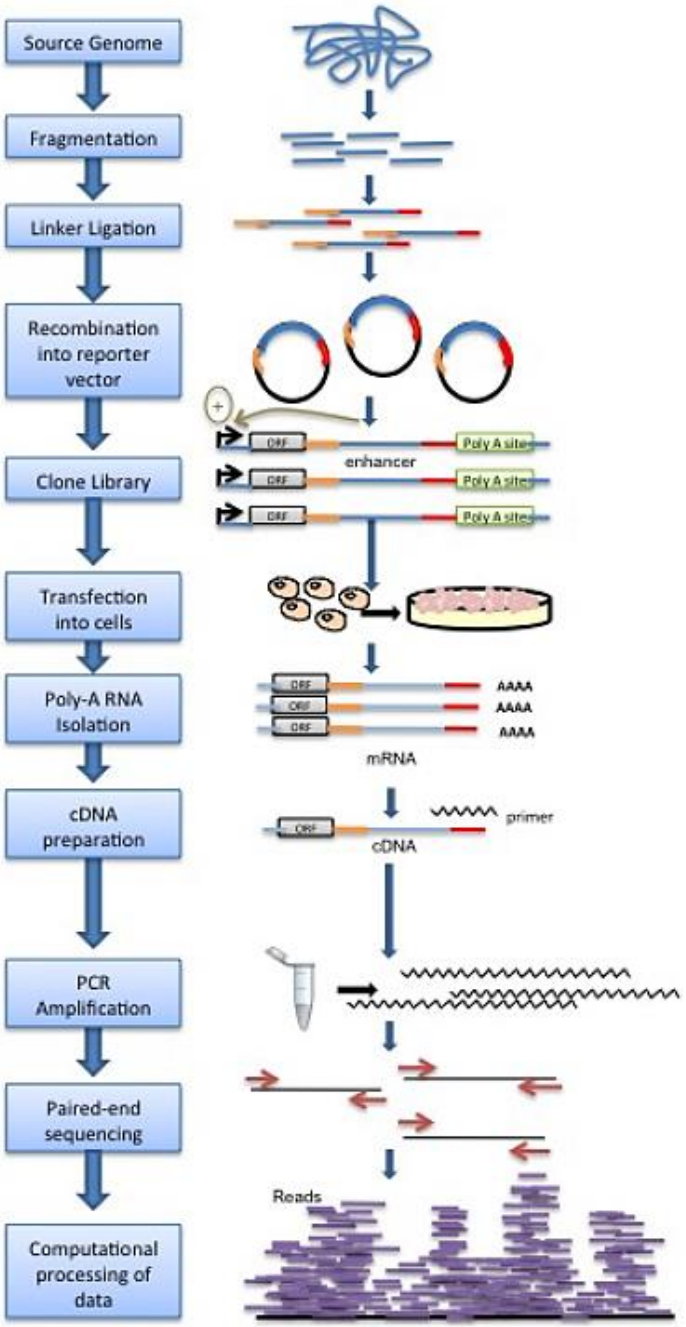
Massively parallel quantification of the regulatory effects of noncoding genetic variation in a human cohort

STARR-seq

Christopher M. Vockley,[1,2,8] Cong Guo,[2,3,8] William H. Majoros,[2,4,8] Michael Nodzenski,[5] Denise M. Scholtens,[5] M. Geoffrey Hayes,[6] William L. Lowe Jr.,[6] and Timothy E. Reddy[4,7]

We report a novel **high-throughput method to empirically quantify individual-specific regulatory element (i.e. enhancer) activity at the population scale.** The approach combines targeted DNA capture with a high-throughput reporter gene expression assay. As demonstration, we measured the activity of more than 100 putative regulatory elements from 95 individuals in a single experiment. In agreement with previous reports, we found that most genetic variants have weak effects on distal regulatory element activity. Because haplotypes are typically maintained within but not between assayed regulatory elements, the approach can be used to identify causal regulatory haplotypes that likely contribute to human phenotypes. Finally, we demonstrate the utility of the method to functionally fine map causal regulatory variants in regions of high linkage disequilibrium identified by expression quantitative trait loci (eQTL) analyses.

Genome Research (2015) 25:1206–1214 Published by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/15; www.genome.org



STARR-seq Methodology

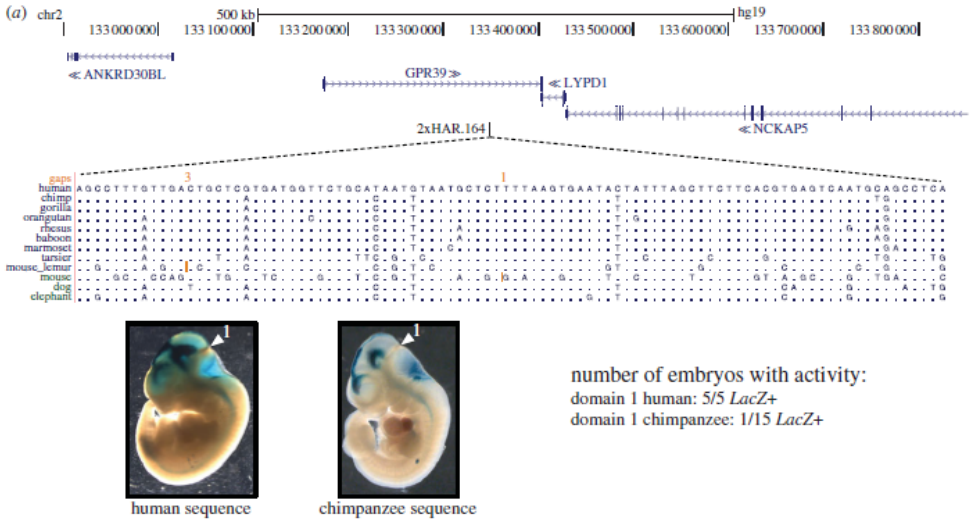
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

rstb.royalsocietypublishing.org

Many human accelerated regions are developmental enhancers

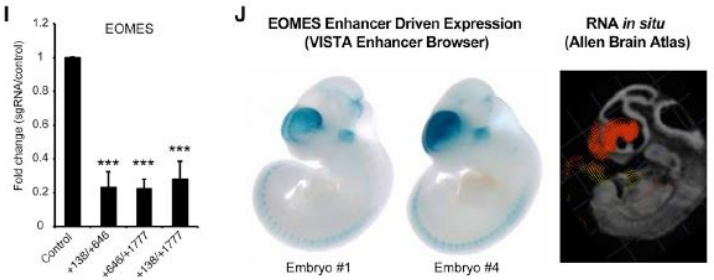
John A. Capra[1,†], Genevieve D. Erwin[1,3], Gabriel McKinsey[2], John L. R. Rubenstein[2] and Katherine S. Pollard[1,4]

[1]Gladstone Institutes, and [2]Nina Ireland Laboratory of Developmental Neurobiology, Genetics and Development,

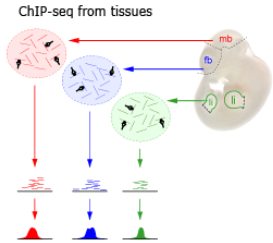


number of embryos with activity:
domain 1 human: 5/5 *LacZ*+
domain 1 chimpanzee: 1/15 *LacZ*+

T-box transcription factor (TF) Eomes



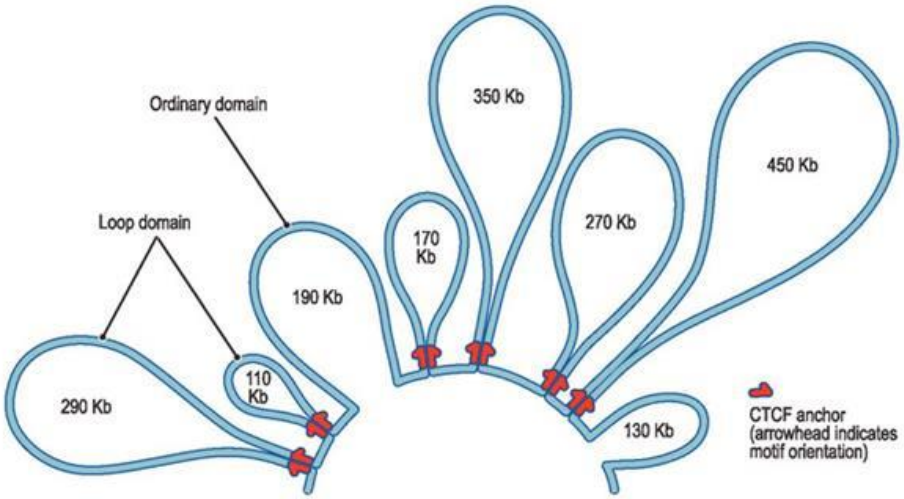
VISTA predicted EOMES enhancer reporter (LacZ staining) (https://enhancer.lbl.gov/) and RNA in situ hybridization for EOMES (http://developingmouse.brain-map.org/) at E11.5. Reporter signal and EOMES RNA expression display a similar pattern with enrichment in the telencephalon.



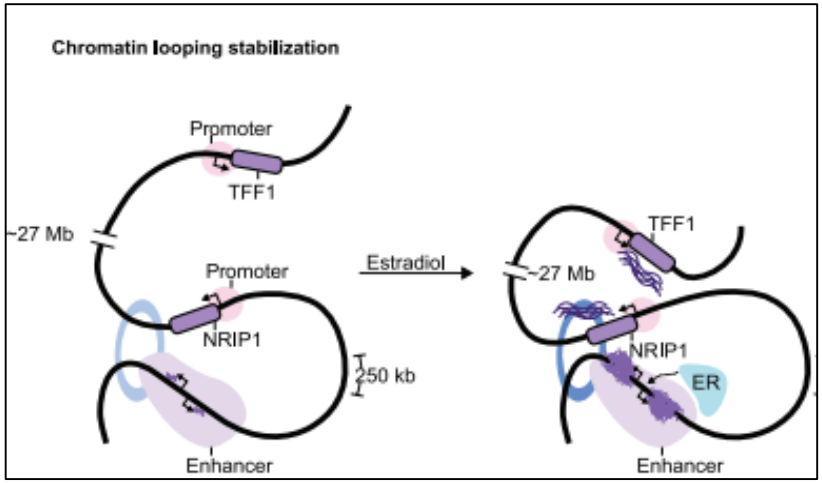
Lawrence Berkeley National Laboratory

General Biology overview:
Current view of chromosome architecture revealed by 3D and Hi-C methods

Chromatin loops in the β hemoglobin gene



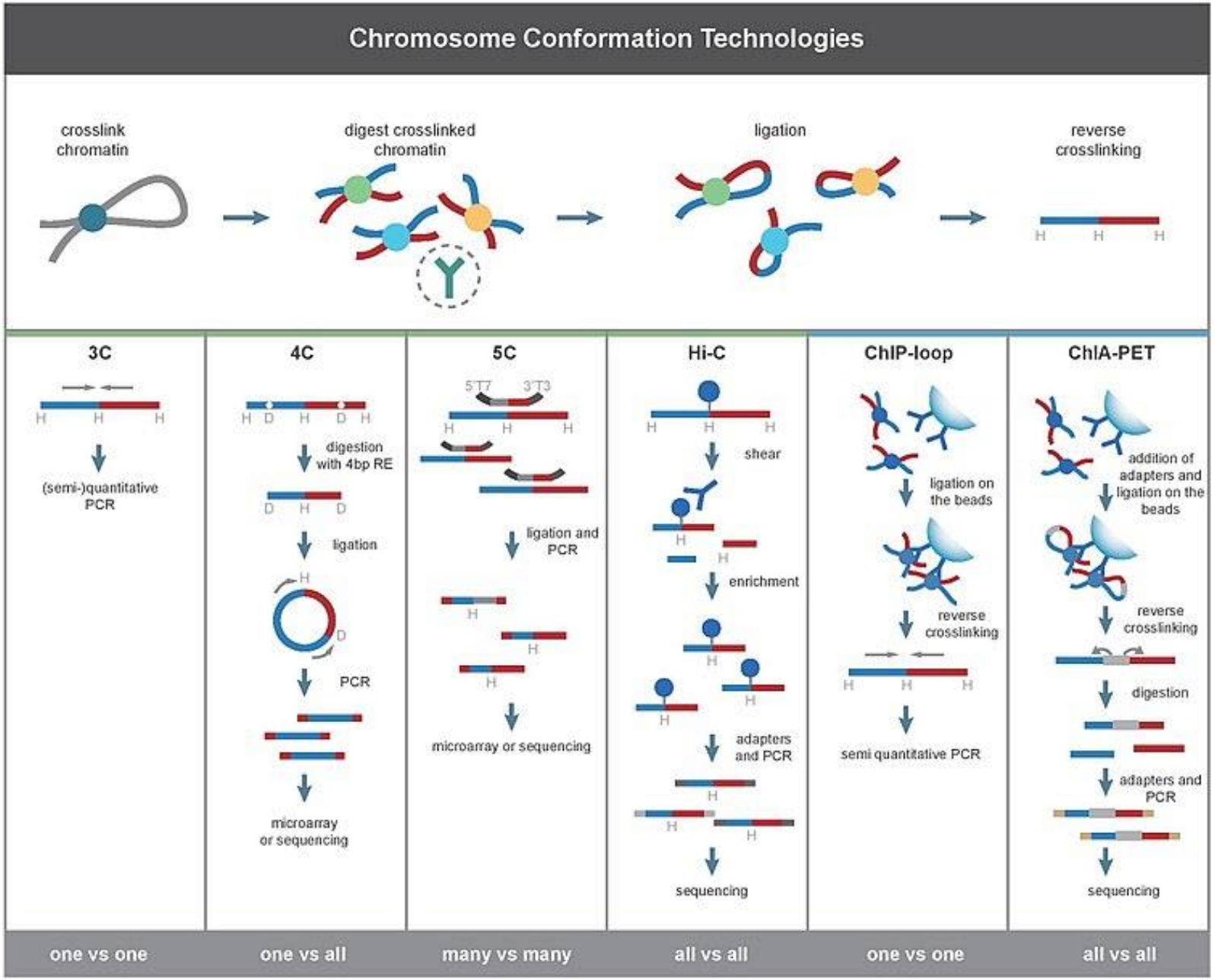
http://jonlieffmd.com/blog/vast-complexity-of-chromatin-3d-shapes



Enhancer RNAs: Insights Into Their Biological Role

Cortés-Fernández de Lara et a, Epigenetics insights 2019

The first chromatin found to have an unusual looping 3D structure is a particular group of five genes making the beta subunit of hemoglobin. The insulator protein CTCF holds together long-range interactions of different sections of the chromatin near the **TADs**.



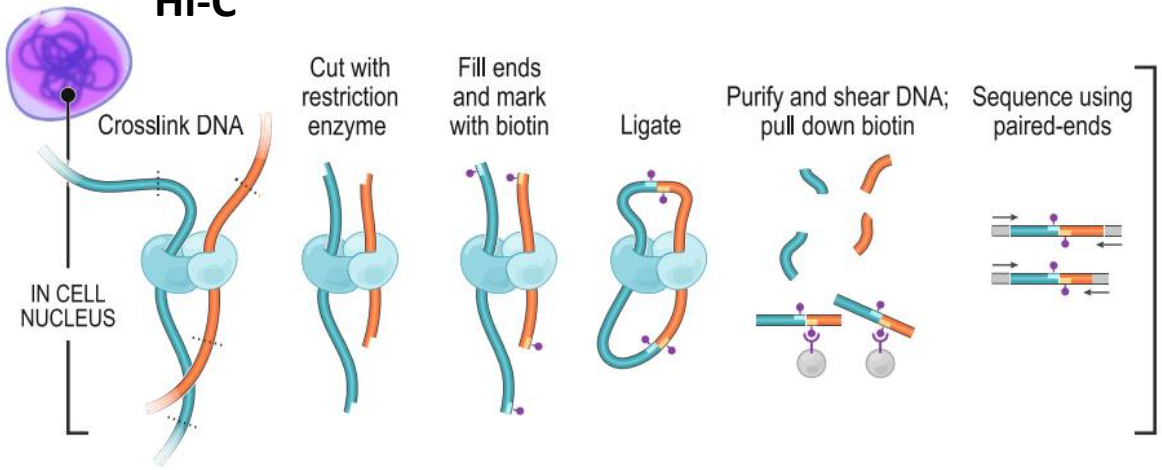
https://en.wikipedia.org/wiki/Chromosome_conformation_capture

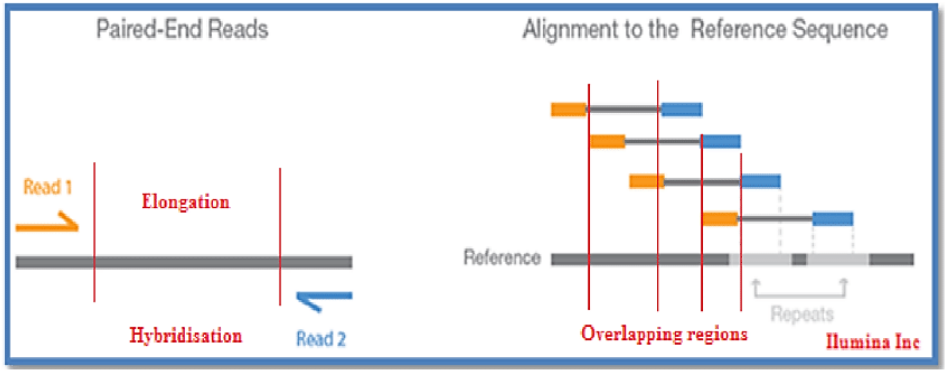
Table 1

Chromatin architecture in the human brain tissue

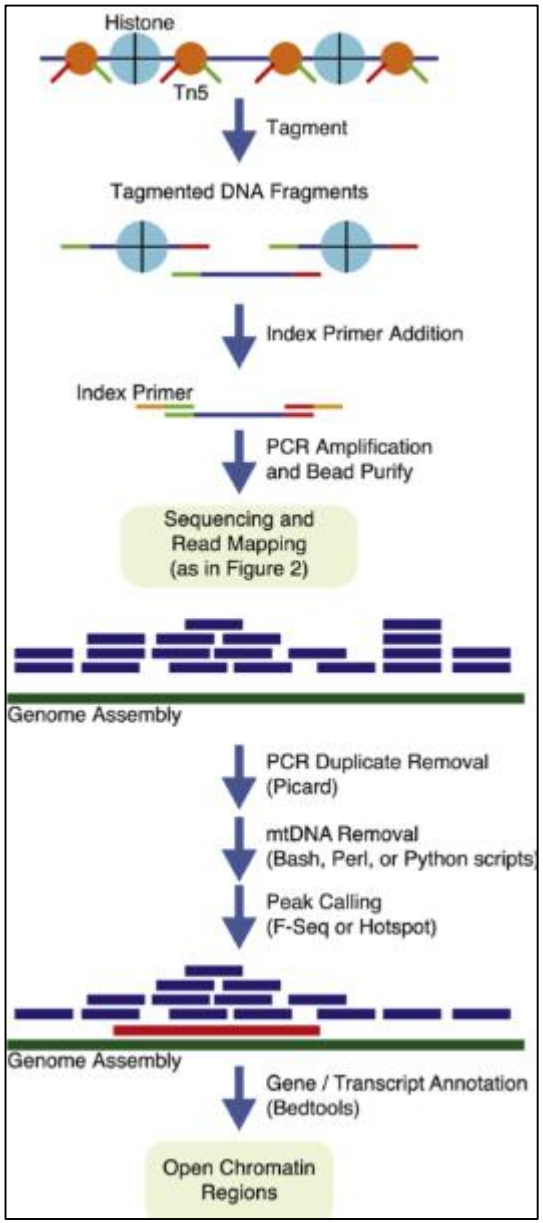
Technique	Measures
ChIP-seq	Histone modification and transcription factor (TF) binding sites
ATAC-seq	Chromatin accessibility
Hi-C	Three-dimensional chromatin interaction

Hi-C

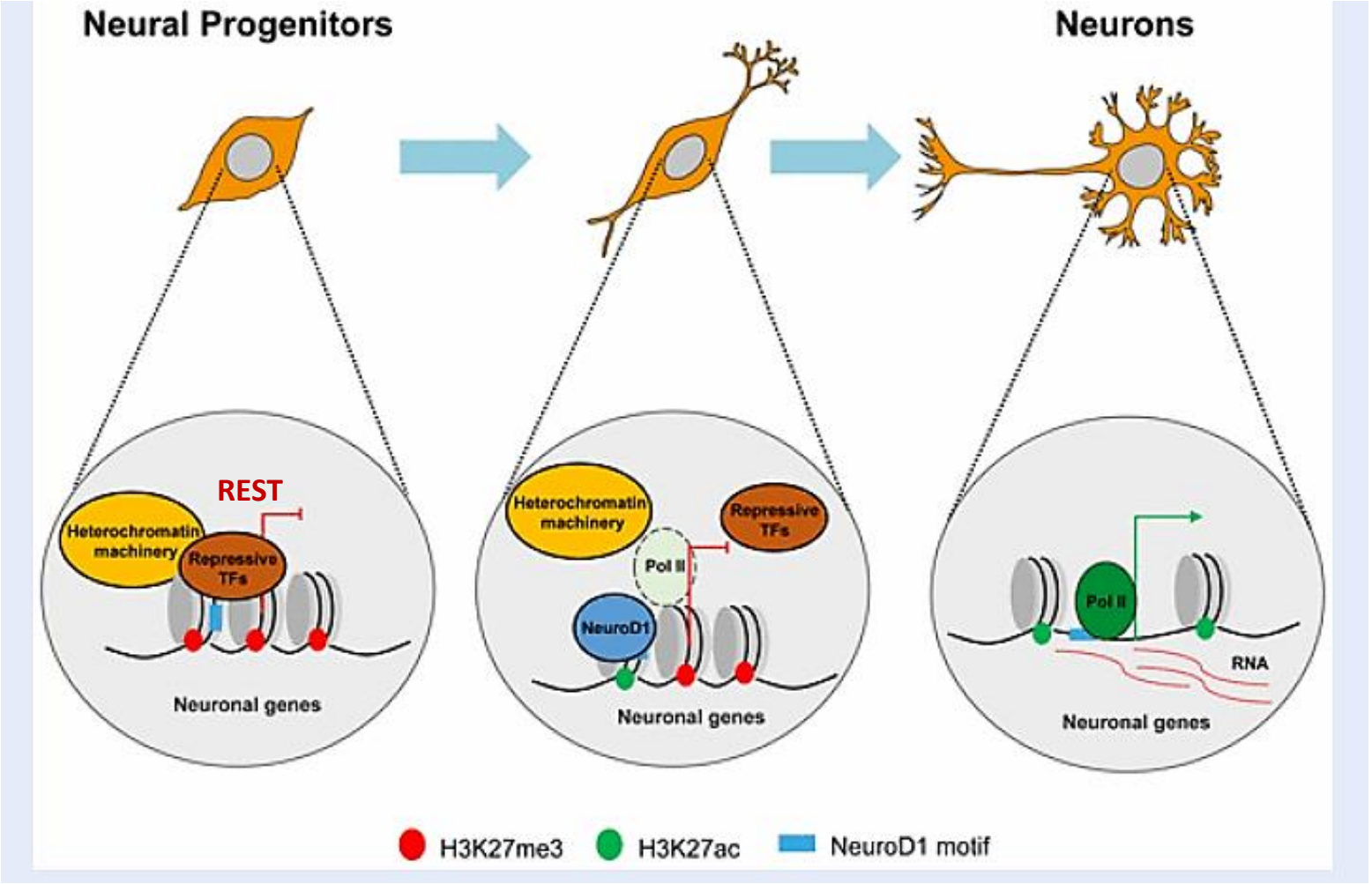




ATAC-seq

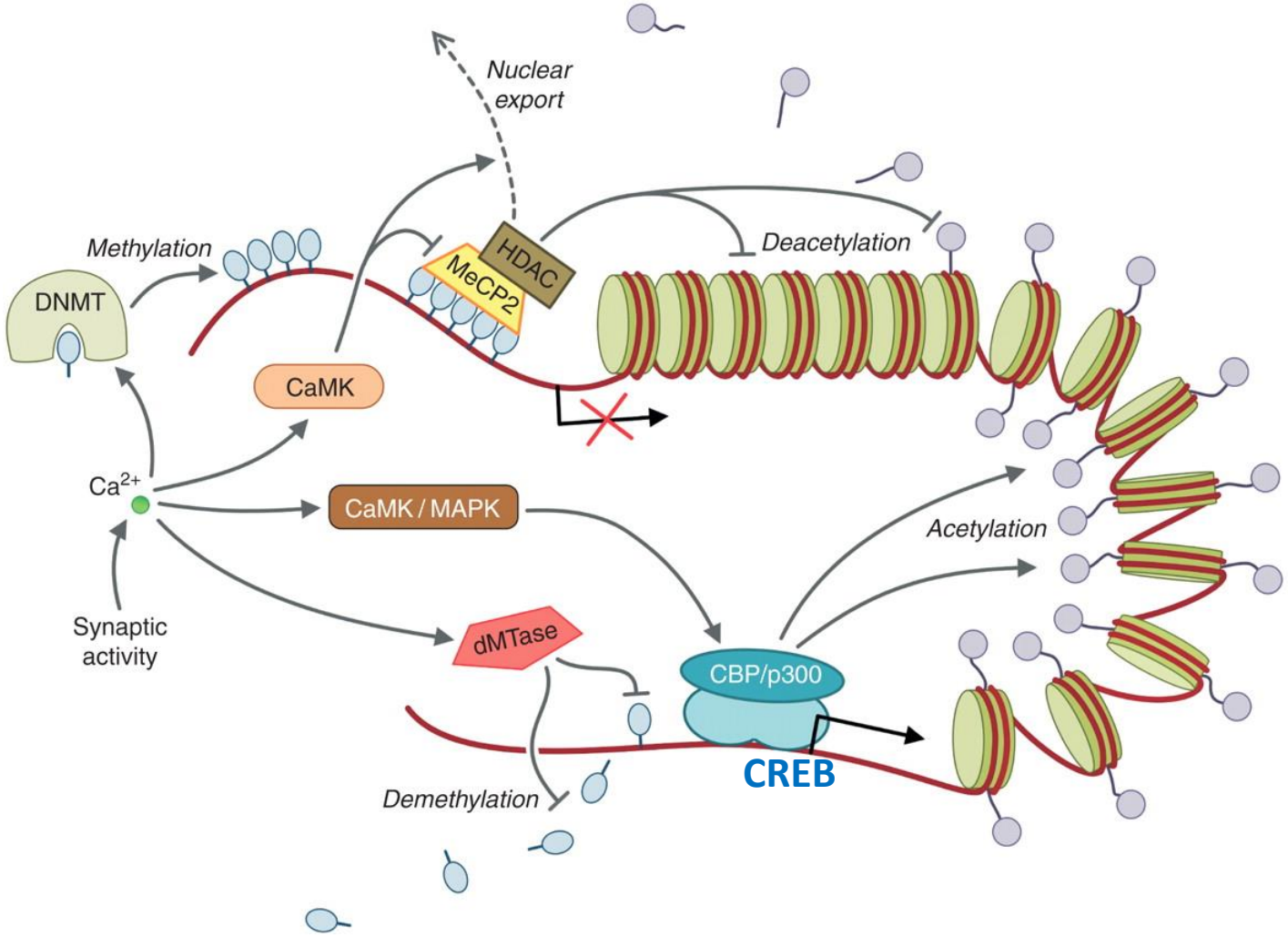


The transcription factor NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program



Abhijeet Pataskar et al EMBO J 2016

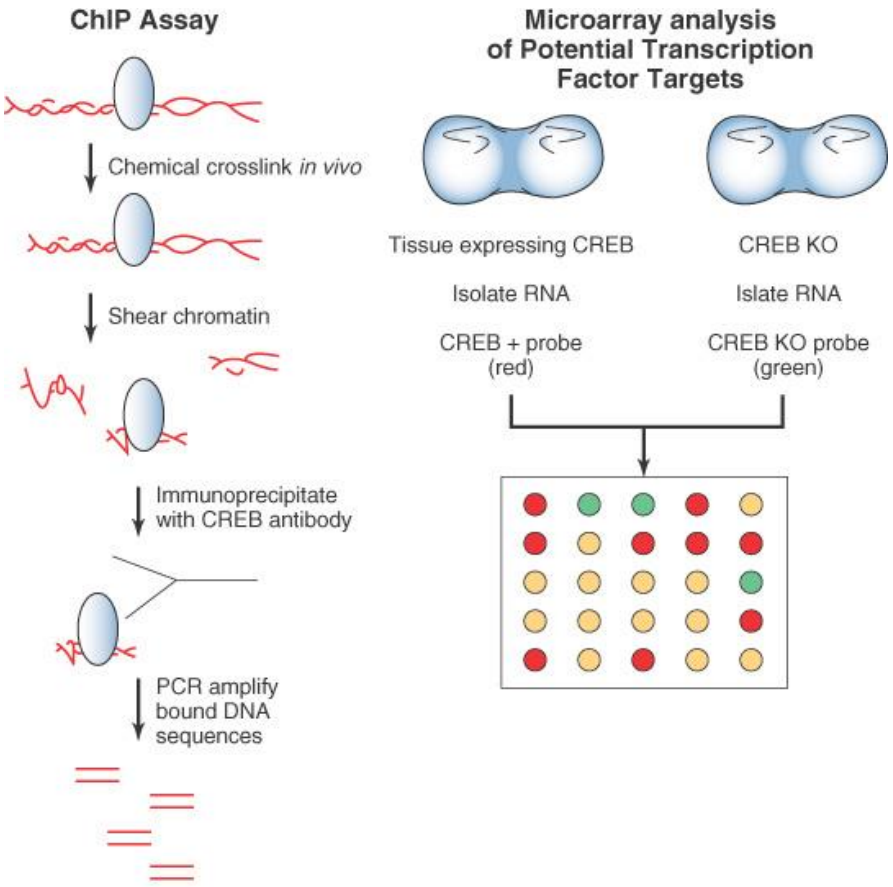
Epigenetic mechanisms in synaptic activity and Ca2+-dependent transcriptional regulation



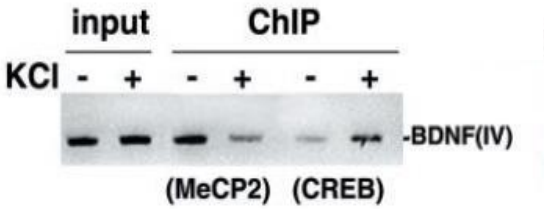
Anna M. Hagenston, and Hilmar Bading Cold Spring Harb Perspect Biol 2011;3:a004564

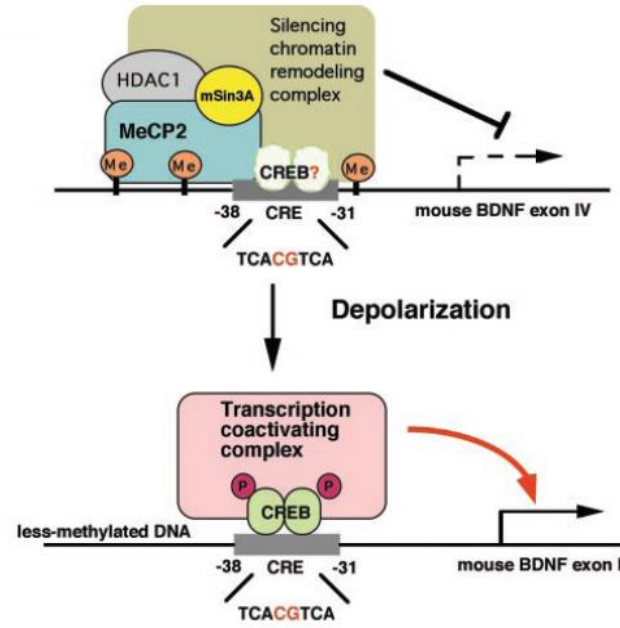
Chromatin IP method to determine which genes are bound to transcription factors

Chromatin Immuno-Precipitation (ChIP) assay



ChIP assays demonstrate reciprocal association of MeCP2 and CREB with the BDNF exon IV promoter



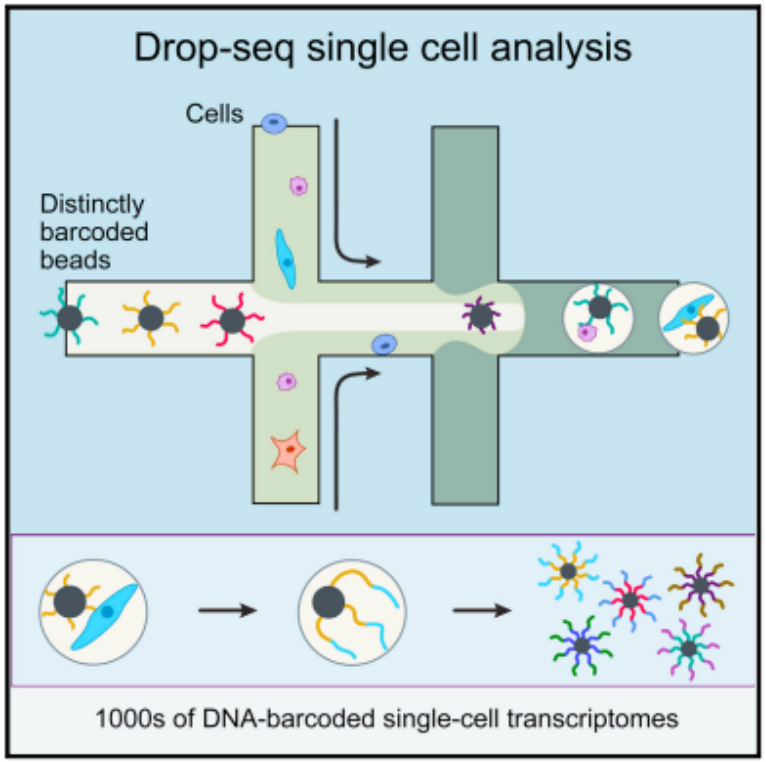


Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

Evan Z. Macosko,[1,2,3,*] Anindita Basu,[4,5] Rahul Satija,[4,6,7] James Nemesh,[1,2,3] Karthik Shekhar,[4] Melissa Goldman,[1,2] Itay Tirosh,[4] Allison R. Bialas,[8] Nolan Kamitaki,[1,2,3] Emily M. Martersteck,[9] John J. Trombetta,[4] David A. Weitz,[5,10] Joshua R. Sanes,[9] Alex K. Shalek,[4,11,12] Aviv Regev,[4,13,14] and Steven A. McCarroll[1,2,3,*]

Cell 161, 1202–1214, May 21, 2015

Graphical Abstract



Authors

Evan Z. Macosko, Anindita Basu, ..., Aviv Regev, Steven A. McCarroll

Correspondence

emacosko@genetics.med.harvard.edu (E.Z.M.),
mccarroll@genetics.med.harvard.edu (S.A.M.)

In Brief

Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.

https://www.sciencedirect.com/science/article/pii/S0092867415005498

Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets

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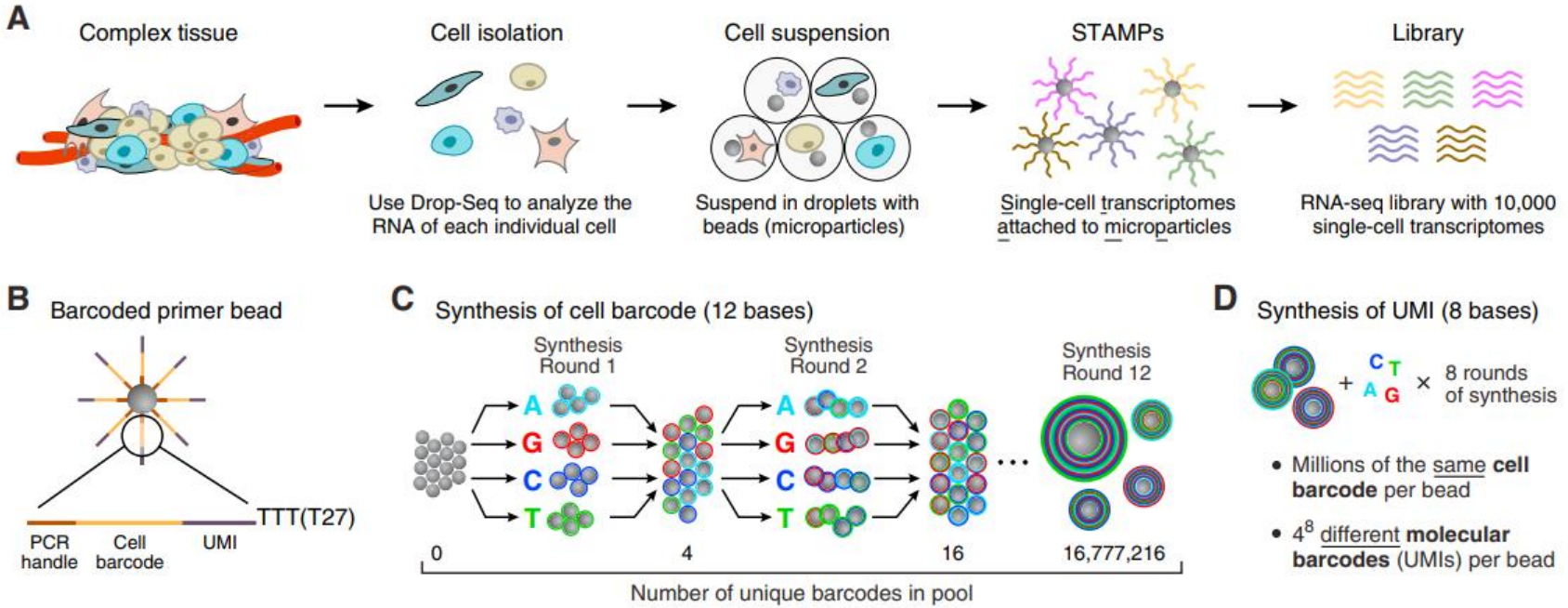

https://www.sciencedirect.com/science/article/pii/S0092867415005498

65536

Resource

Cell

Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells

Cell 161, 1187–1201, May 21, 2015

Authors

Allon M. Klein, Linas Mazutis, ...,
David A. Weitz, Marc W. Kirschner

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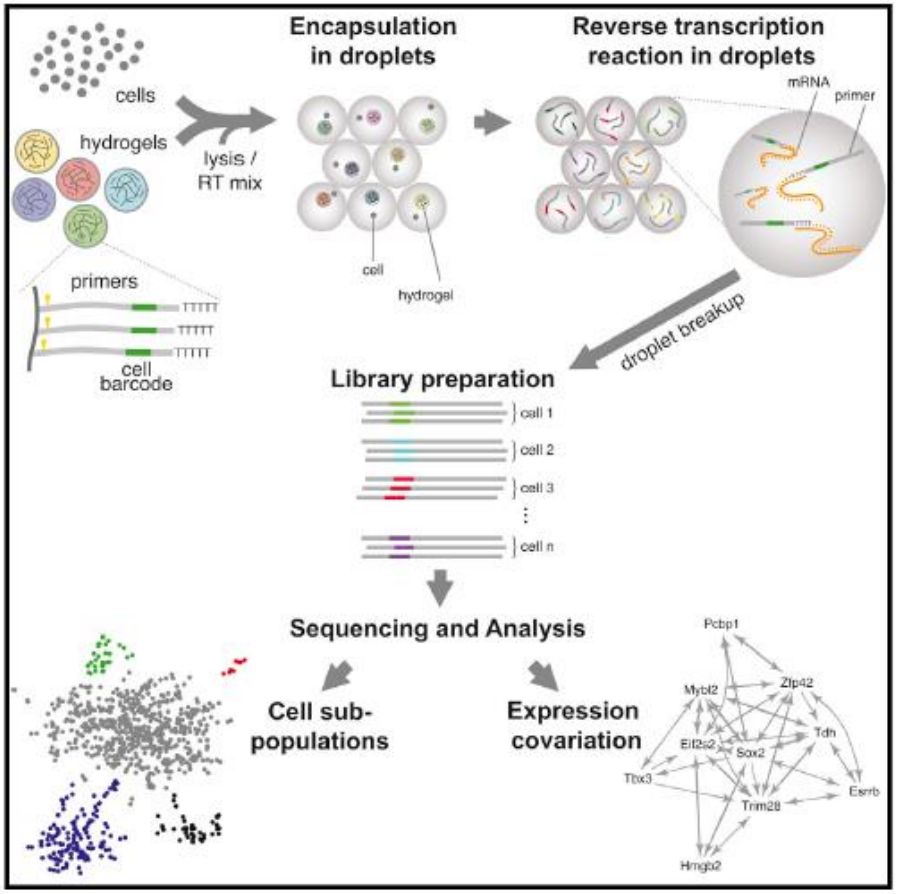
weitz@seas.harvard.edu (D.A.W.),
marc@hms.harvard.edu (M.W.K.)

Graphical Abstract



Highlights

- Cells are captured and barcoded in nanolitre droplets with high capture efficiency
- Each drop hosts a hydrogel carrying photocleavable combinatorially barcoded primers
- mRNA of thousands of mouse embryonic stem and differentiating cells are sequenced
- Single-cell heterogeneity reveals population structure and gene regulatory linkage (and differentiation trajectories)

https://www.sciencedirect.com/science/article/pii/S0092867415005000?via%3Dihub

Klein, et al, 2015

Validation of single cell capture and barcoding

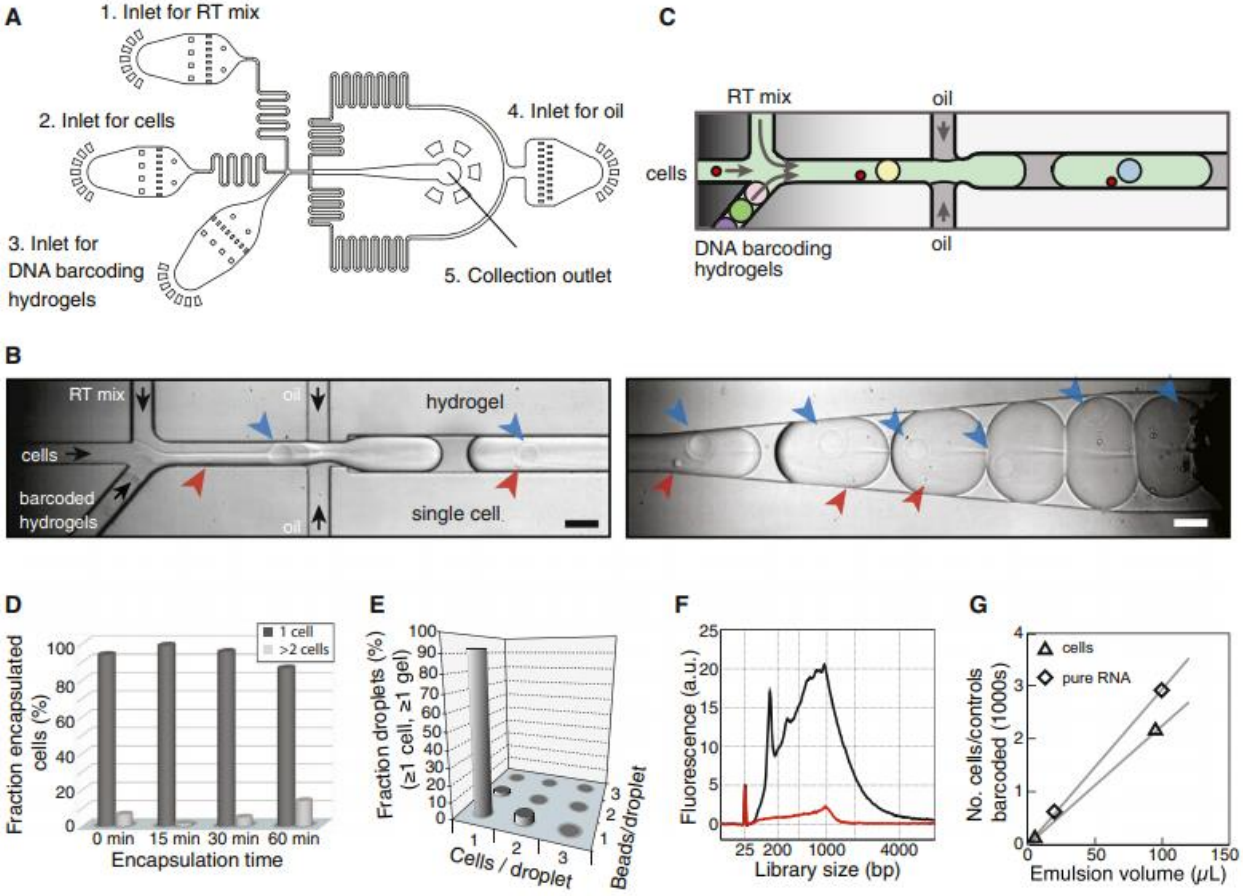


Figure 3. A Droplet Barcoding Device

(A) Microfluidic device design, see also Figure S2.

(B and C) Snapshots of encapsulation (left) and collection (right) modules, see also Movies S1 and S2. Arrows indicate cells (red), hydrogels (blue), and flow direction (black). Scale bars 100 μm.

(D) Droplet occupancy over time.

(E) Cell and hydrogel co-encapsulation statistics showing a high 1:1 cell:hydrogel correspondence.

(F) BioAnalyzer traces showing dependence of library abundance on primer photo-release.

(G) Number of cells/controls as a function of collection volume.

https://www.sciencedirect.com/science/article/pii/S0092867415005000?via%3Dihub#mmc7

Single-cell genomics identifies cell type–specific molecular changes in autism

Dmitry Velmeshev[1,2]*, Lucas Schirmer[1,3,4], Diane Jung[1,2], Maximilian Haeussler[5], Yonatan Perez[1,2], Simone Mayer[1,2,6], Aparna Bhaduri[1,2], Nitasha Goyal[1,2,7], David H. Rowitch[1,3,8,9], Arnold R. Kriegstein[1,2]*

Velmeshev et al., Science 364, 685–689
17 May 2019

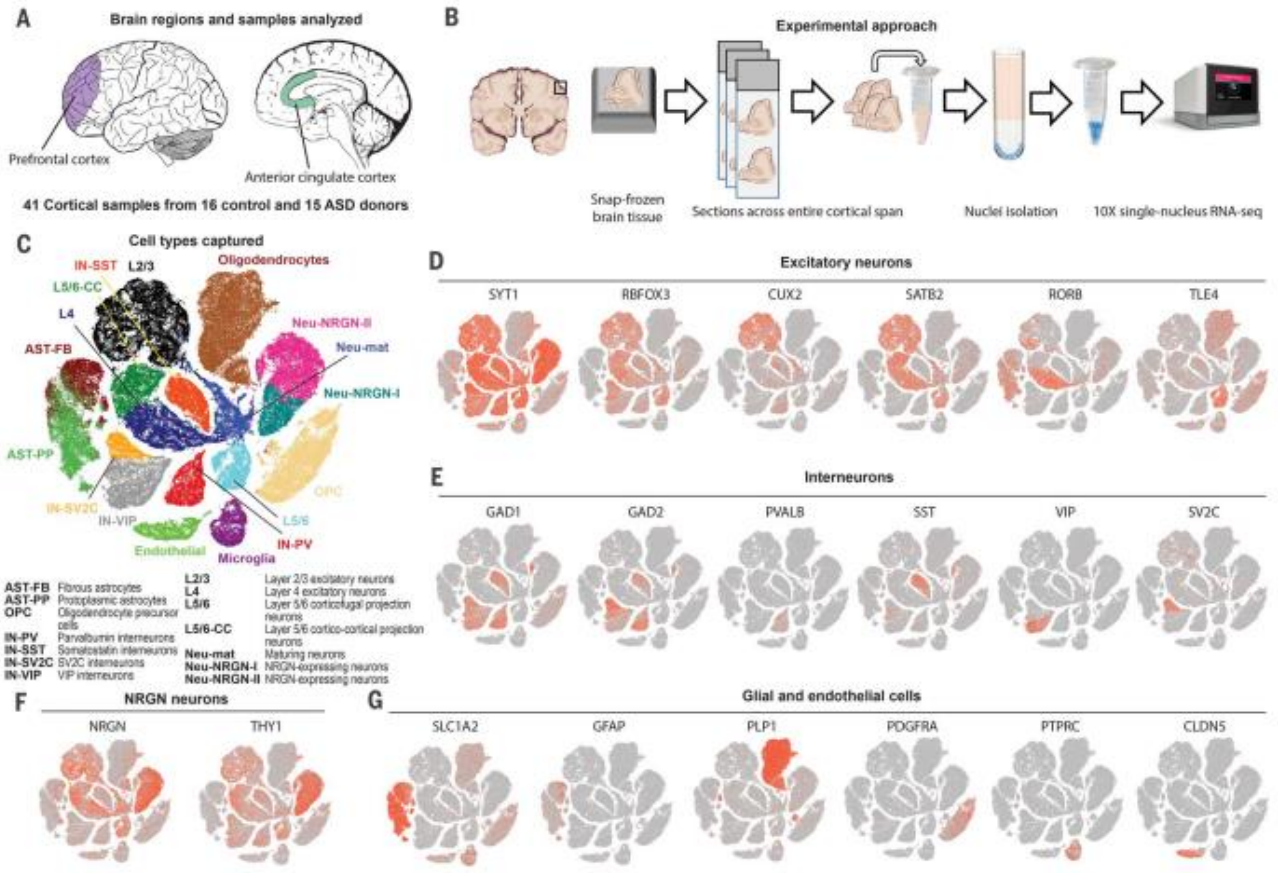


Fig. 1. Overview of the experimental approach and snRNA-seq dataset. (**A**) Cortical regions analyzed with snRNA-seq including the PFC and ACC regions. (**B**) Experimental approach to snap-frozen tissue sample processing and nuclei isolation. (**C**) Unbiased clustering of snRNA-seq data. Cell types were annotated according to expression of known marker genes. (**D**) Expression of excitatory neuronal subtype markers. (**E**) Inhibitory neuronal subtype marker expression. (**F**) Markers of NRGN-expressing neurons. (**G**) Markers of glial cell types and endothelial cells.

t-Distributed Stochastic Neighbor Embedding" or t-SNE – Laurens van der Maaten lvdmaaten.github.io › tsne is a technique for dimensionality reduction that is particularly well suited for the visualization of high-dimensional datasets.

Velmeshev et al., Science 364, 685–689 17 May 2019

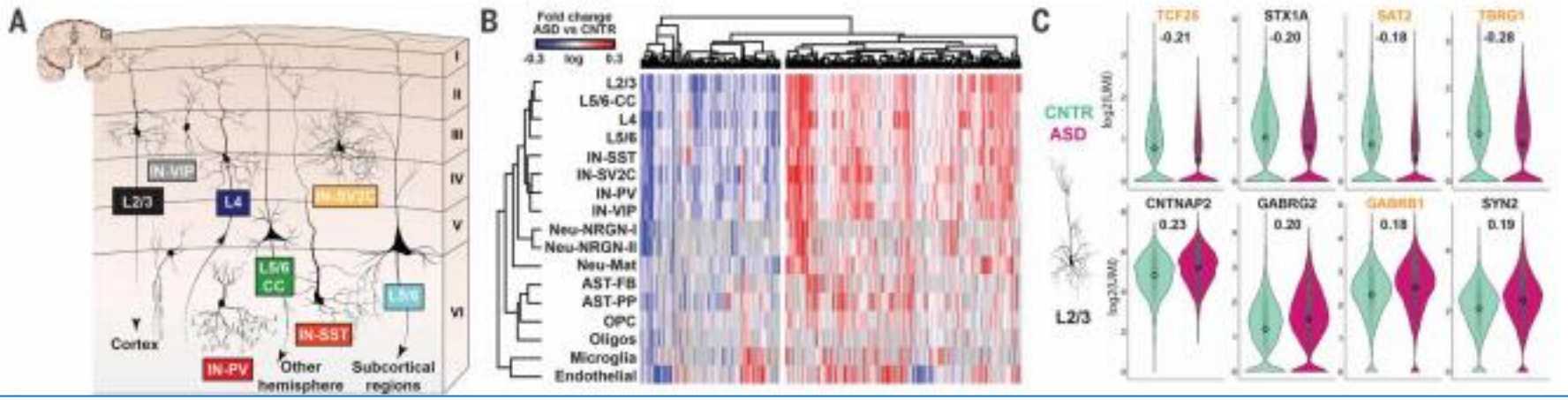
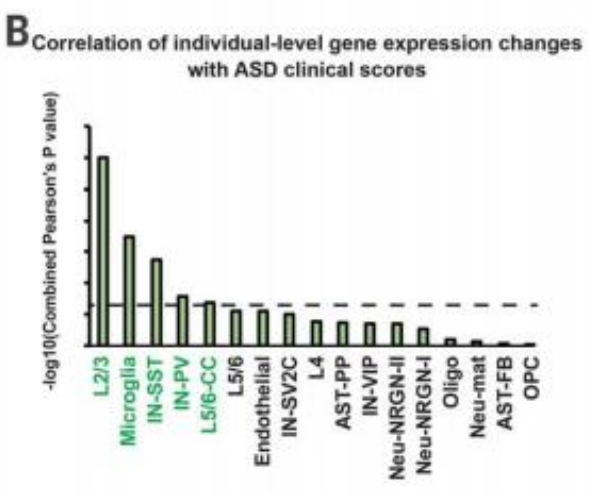
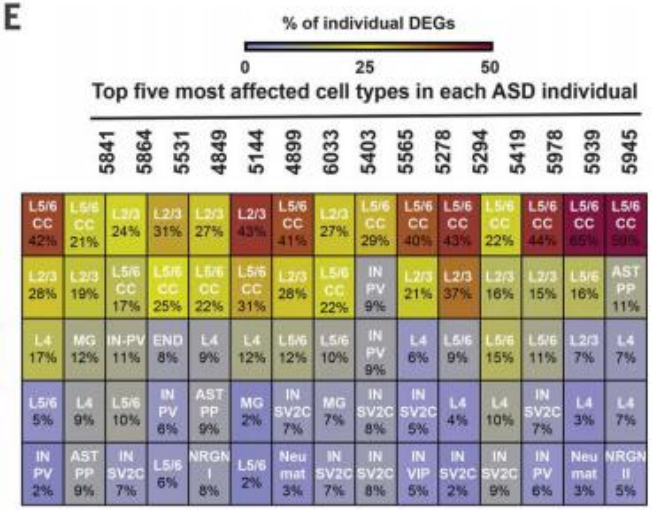


Fig. 3. Gene expression changes across specific cell types in ASD. (A) Schematic of cortical neurons with known layer localization. (B) Hierarchical clustering based on log-transformed relative (fold) changes [versus control (CNTR)] of DEGs in each cell type. (C) Violin plots for top genes differentially expressed in L2/3 neurons in ASD. Genes dysregulated in sporadic epilepsy are indicated in orange.

Fig. 4. Correlation of cell type–specific gene dysregulation in ASD patients with clinical severity. (B) Cell types ranked by correlation of DEGs with combined clinical scores. Cell type DEGs in green were significantly ($P < 0.05$) correlated with clinical severity. (E) Analysis of cell types most enriched for transcriptional changes in individual ASD patients.

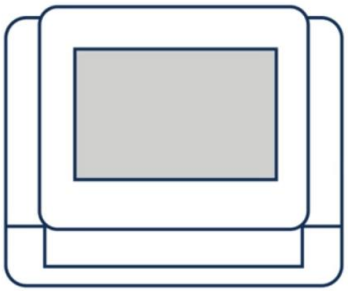




Overall, they found that synaptic signaling of upper-layer excitatory neurons and the molecular state of microglia are preferentially affected in autism.

The Chromium Single Cell Gene Expression Solution

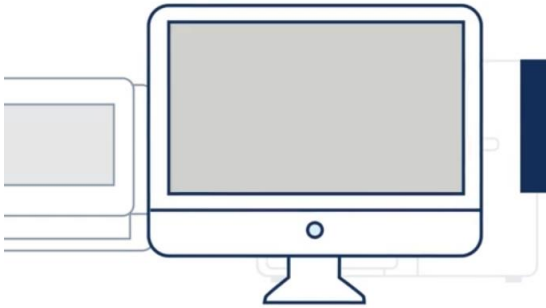
Uncover cell-to-cell gene expression variability and identify rare cell types in heterogeneous samples



Flexible throughput

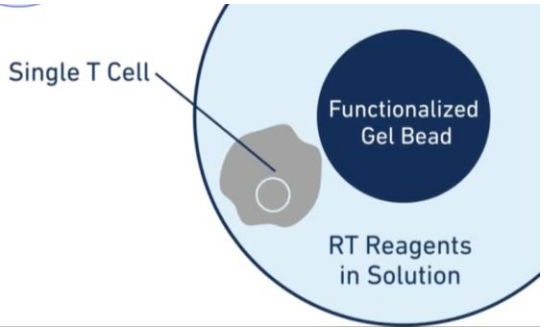
Automated barcoding then library construction

Chromium System

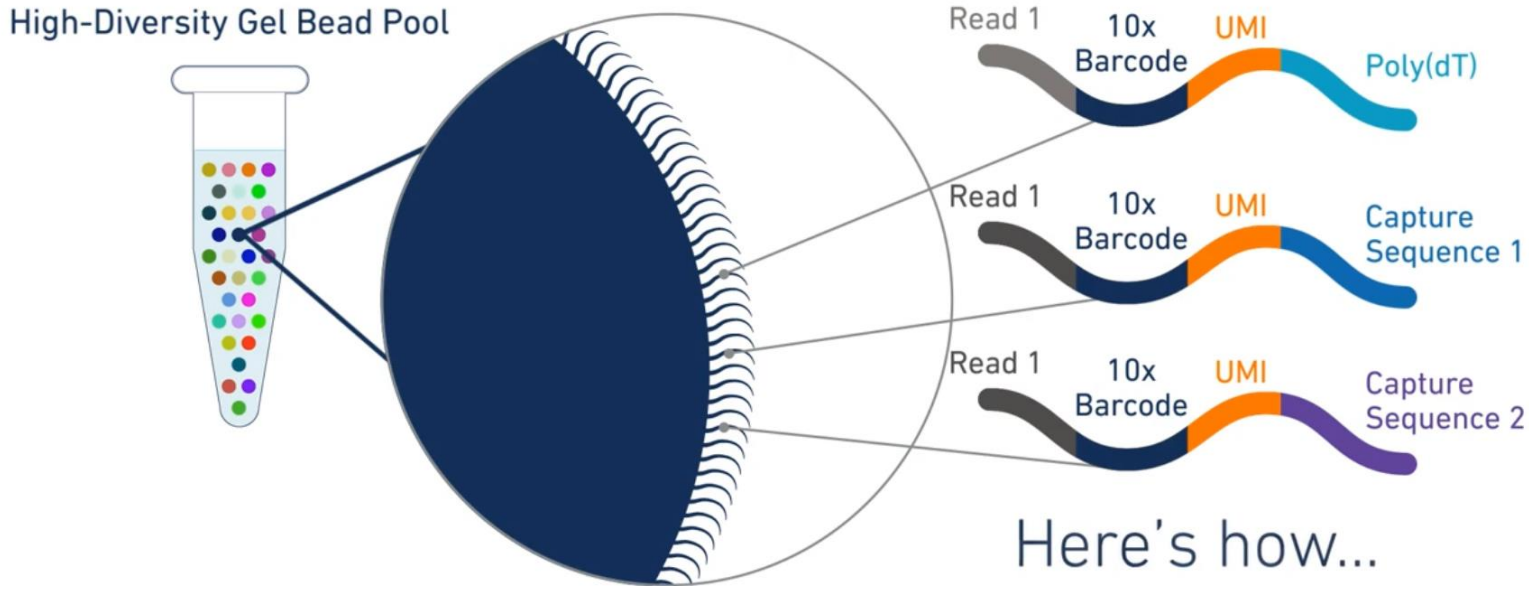


Turn-key analysis & visualization

10x Software Tools



10x Next GEM Technology samples a pool of ~3.6 million 10x Barcodes to separately index each cell's transcriptome



Here's how...

10x GENOMICS

Chromium controller

https://www.10xgenomics.com/solutions/single-cell/

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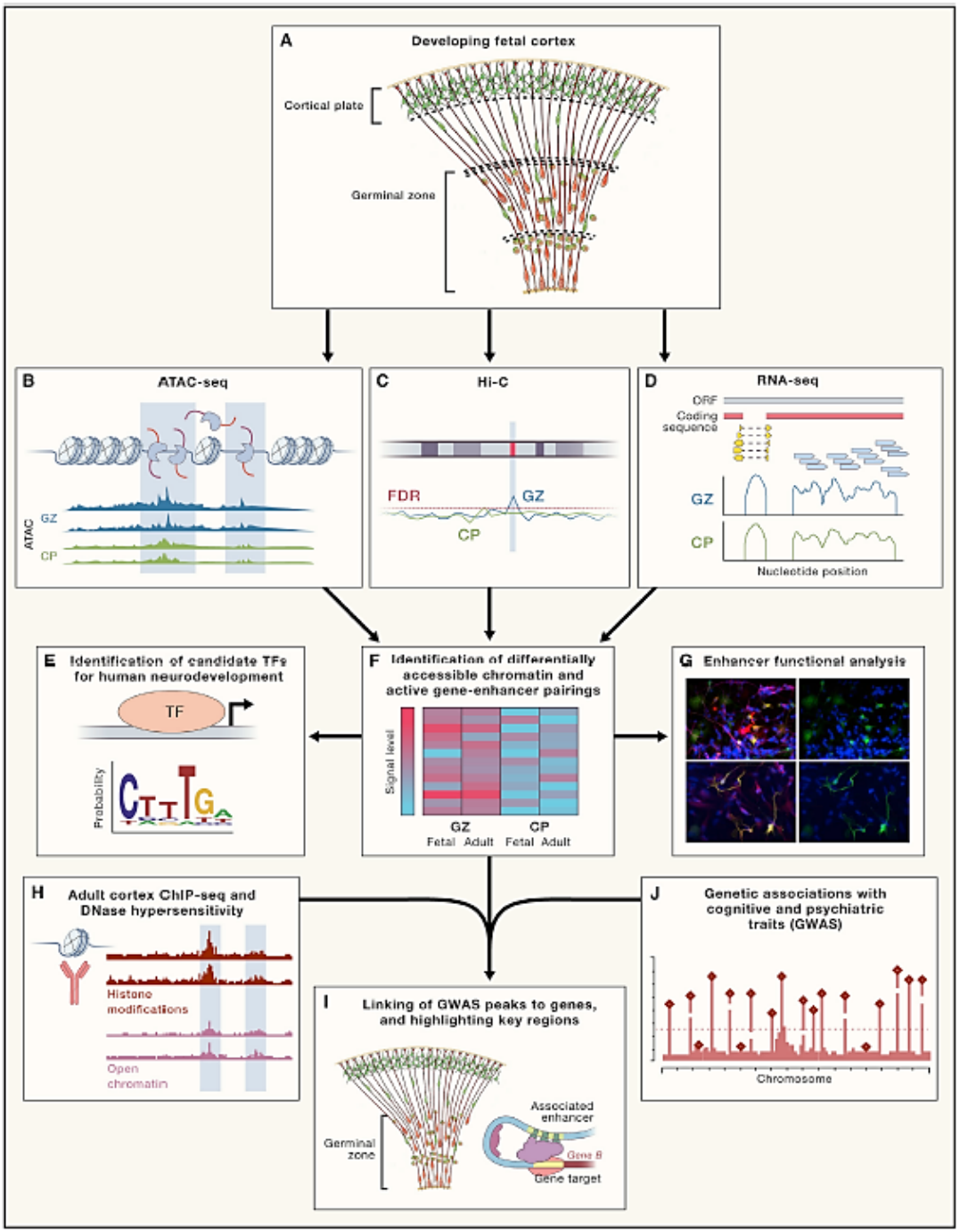
Weaving New Insights for the Genetic Regulation of Human Cognitive Phenotypes

Bernard Mulvey[1] and Joseph D. Dougherty[1,]

Cell 172, 2018, p. 11-13

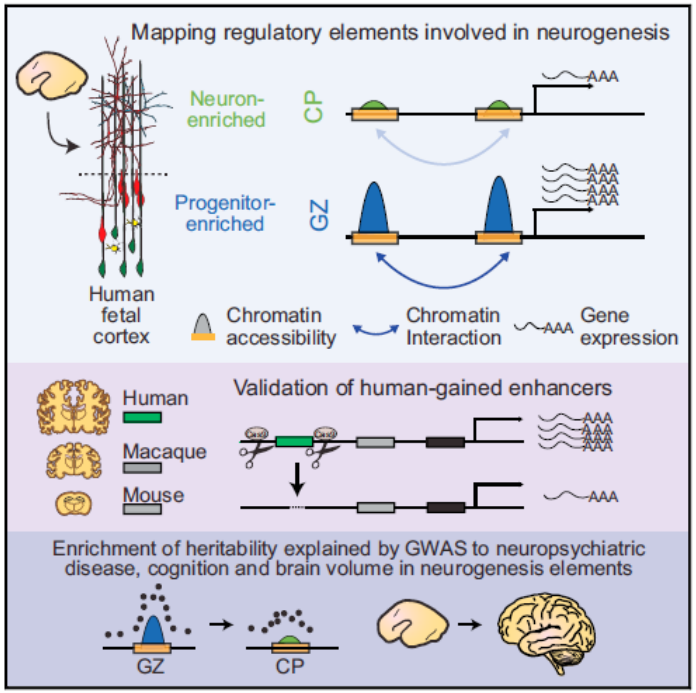


de la Torre-Ubieta et al., 2018, Cell *172*, 289–304
January 11, 2018 © 2017 Elsevier Inc.
https://doi.org/10.1016/j.cell.2017.12.014

Cell

The Dynamic Landscape of Open Chromatin during Human Cortical Neurogenesis

Graphical Abstract



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In Brief

A high-resolution map of non-coding regulatory elements driving human cortical neurogenesis reveals uniquely human enhancers linked to common genetic variants associated with cognitive function.

Highlights

1) They defined non-coding regions regulating gene expression in developing human cortex
2) Human-gained enhancers preferentially regulate genes expressed in outer radial glia
3) A distal human-gained enhancer regulates FGFR2 during neocortical development
4) Genetic variation influencing cognition and brain size act during neurogenesis

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ARTICLE

https://doi.org/10.1038/s41467-020-19319-2 OPEN

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Common schizophrenia risk variants are enriched in open chromatin regions of human glutamatergic neurons

Mads E. Hauberg[1,2,3,4,5,13], Jordi Creus-Muncunill[6,13], Jaroslav Bendl[1,2,7,13], Alexey Kozlenkov[1], Biao Zeng[7], Chuhyon Corwin[6,7,8], Sarah Chowdhury[6,7,8], Harald Kranz[9], Yasmin L. Hurd[1,2,10], Michael Wegner[11], Anders D. Børglum[3,4,5], Stella Dracheva[1,2,12], Michelle E. Ehrlich[6,7,8], John F. Fullard[1,2,7] & Panos Roussos[1,2,7,12]✉

NATURE COMMUNICATIONS | (2020) 11:5581 | https://doi.org/10.1038/s41467-020-19319-2 | www.nature.com/naturecommunications