

Transcriptomics Practical Setup

Umar Ahmad, Ph.D.

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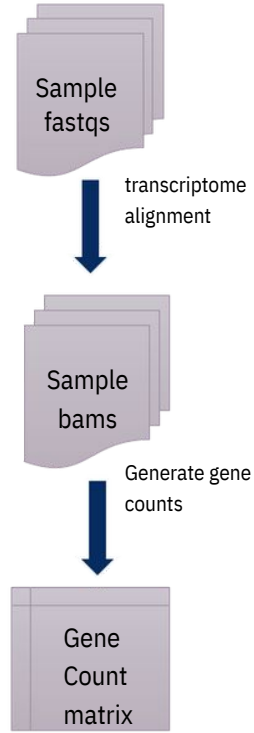
Faculty Member, Sa'adu Zungur University

 @babasaraky



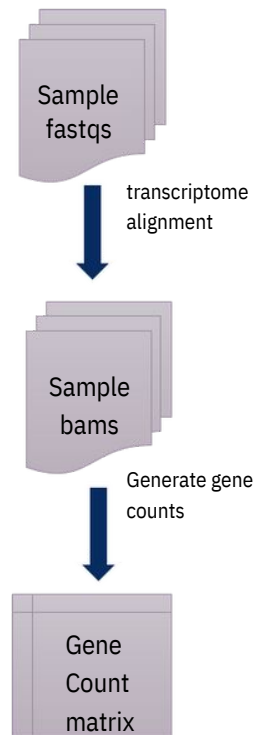
Transcriptomics pre-processing workflow

Preprocessing



Transcriptomics pipeline/workflow

Preprocessing

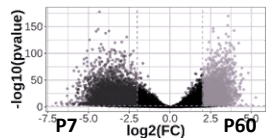


Analyses

Clustering



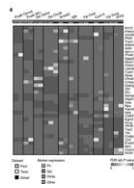
Differential Expression



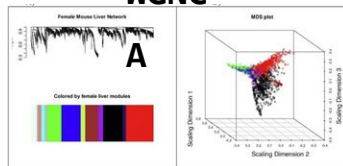
Functional Enrichment



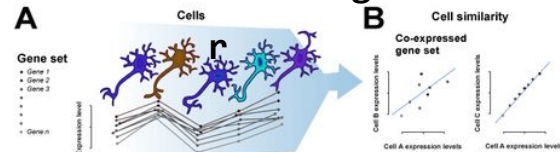
Coregulated Gene Expression



WGNC

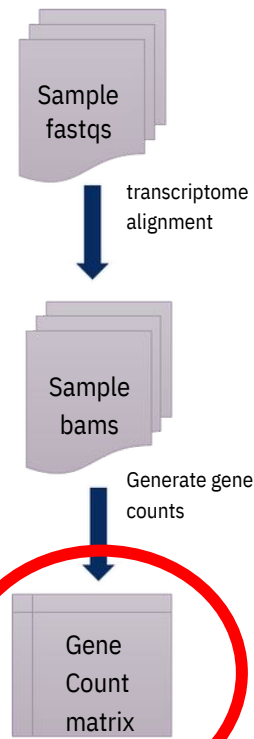


MetaNeighbo



Transcriptomics pipeline/workflow

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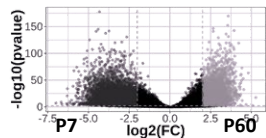


Analyses

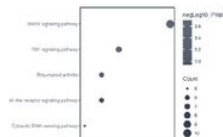
Clustering



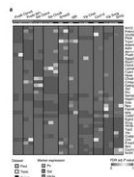
Differential Expression



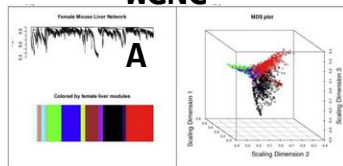
Functional Enrichment



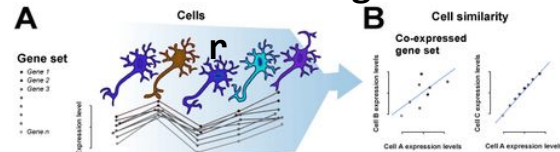
Coregulated Gene Expression



WGNC



MetaNeighbor








ARTICLE

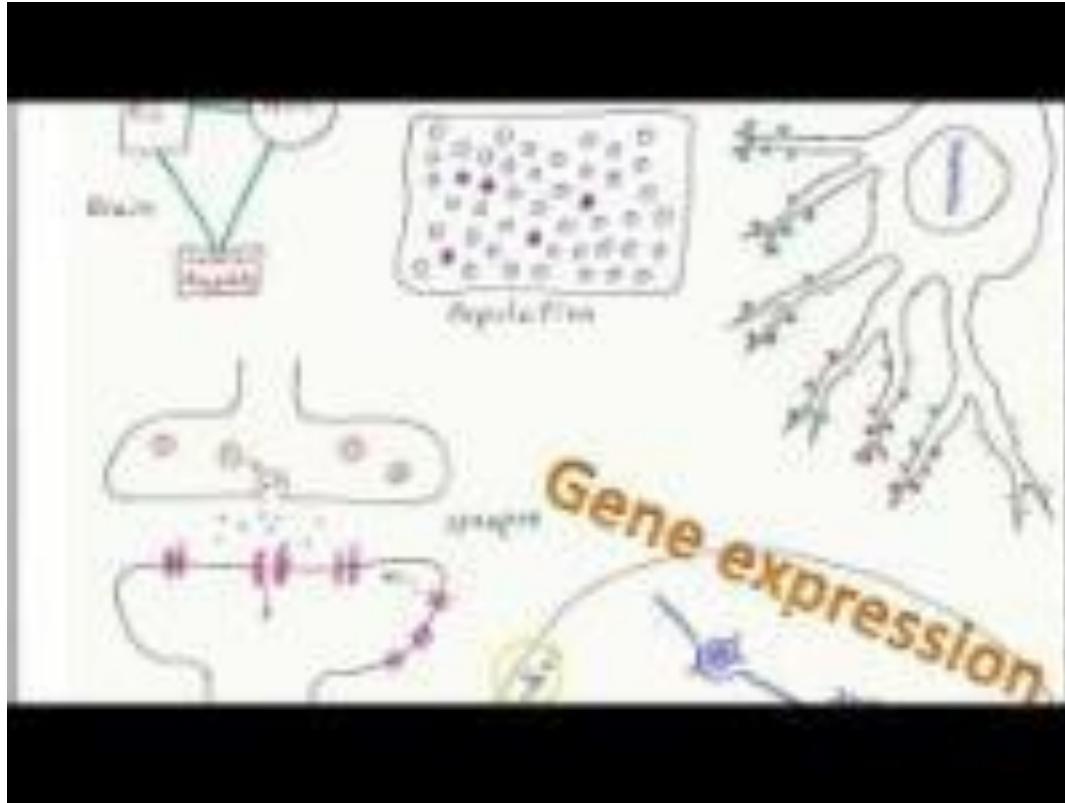
<https://doi.org/10.1038/s41467-019-09960-x>

OPEN

Engram-specific transcriptome profiling of contextual memory consolidation

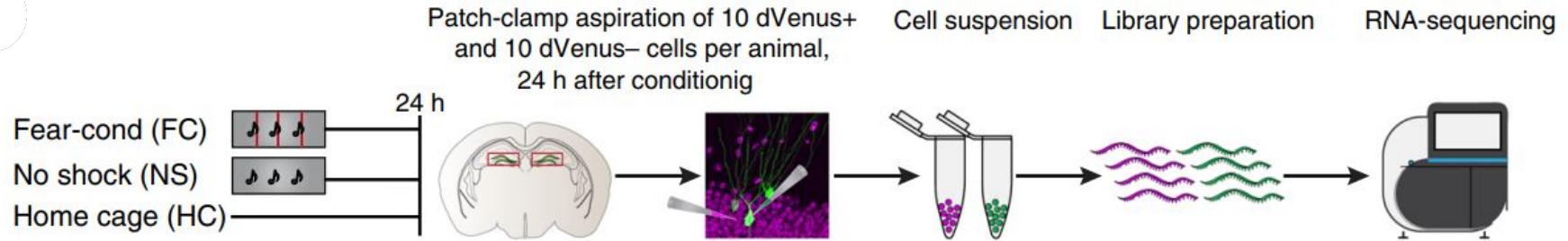
Priyanka Rao-Ruiz ^{1,2}, Jonathan J. Couey¹, Ivo M. Marcelo^{1,3}, Christian G. Bouwkamp¹, Denise E. Slump¹, Mariana R. Matos², Rolinka J. van der Loo², Gabriela J. Martins^{3,4}, Mirjam van den Hout ⁵, Wilfred F. van Ijcken ⁵, Rui M. Costa^{3,4}, Michel C. van den Oever ² & Steven A. Kushner ¹

What is an engram??



Engram-specific transcriptome profiling
of contextual memory consolidationPriyanka Rao-Ruiz^{1,2}, Jonathan J. Couey¹, Ivo M. Marcello^{1,3}, Christian G. Bouwkamp¹, Denise E. Slump¹,
Mariana R. Matos², Rolinka J. van der Loo², Gabriela J. Martins^{3,4}, Mirjam van den Hout⁵,
Wilfred F. van Icken⁵, Rui M. Costa^{3,4}, Michel C. van den Oever² & Steven A. Kushner¹

Experimental design to isolate cells that participate in fear learning engram



Accessing the data through NCBI GEO

**NCBI**

**GEO**
Gene Expression Omnibus

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GEO help: Mouse over screen elements for information.

Scope: Format: Amount: GEO accession:

Series GSE129024 [Query DataSets for GSE129024](#)

Status

Public on Mar 30, 2019

Title

Engram-specific transcriptome profiling of contextual memory consolidation

Organism

[Mus musculus](#)

Experiment type

Expression profiling by high throughput sequencing

Summary

Sparse populations of neurons in the dentate gyrus (DG) of the hippocampus are causally implicated in the encoding of contextual fear memories. However, engram-specific molecular mechanisms underlying memory consolidation remain largely unknown. Here we perform unbiased RNA sequencing of DG engram neurons 24h after contextual fear conditioning to identify transcriptome changes specific to memory consolidation. DG engram neurons exhibit a highly distinct pattern of gene expression, in which CREB-dependent transcription features prominently (P=5.2x10⁻¹³), including *Atf3* (P=2.4x10⁻⁴¹), *Penk* (P=1.3x10⁻¹⁵), and *Kcnq3* (P=3.1x10⁻¹²). Moreover, we validate the functional relevance of the RNAseq findings by establishing the causal requirement of intact CREB function specifically within the DG engram during memory consolidation, and identify a novel group of CREB target genes involved in the encoding of long-term memory.

Overall design

Biological replicates: Fear conditioned: n=14. No shock controls: n=4. Home cage controls: n=3. The contents 10 dVenus+ and 10 dVenus- cells were aspirated from each animal (biological replicate)

Contributor(s)

Rao-Ruiz P, Couey JJ, Marcelo IM, Bouwkamp CG, Slump DE, Matos MR, van der Loo RJ, Martins GJ, van den Hout M, van Dicken WF, Costa RM, van den Oever MC, Kushner SA

Citation(s)

Rao-Ruiz P, Couey JJ, Marcelo IM, Bouwkamp CG et al. Engram-specific transcriptome profiling of contextual memory consolidation. *Nat Commun* 2019 May 20;10(1):2232. PMID: 31110186

Submission date

Mar 29, 2019

Last update date

May 23, 2019

Contact name

Steven Kushner

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s.kushner@erasmusmc.nl

Organization name

Erasmus MC

Citation(s)

der Loo RJ, Martins GJ, van den Hout M, van Dicken WF, Costa RM, van den Oever MC, Kushner SA
Rao-Ruiz P, Couey JJ, Marcelo IM, Bouwkamp CG et al. Engram-specific transcriptome profiling of contextual memory consolidation. *Nat Commun* 2019 May 20;10(1):2232. PMID: 31110186

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State/province

Zuid Holland

ZIP/Postal code

3015 GD

Country

Netherlands

Platforms (1)

[GPL17021](#) Illumina HiSeq 2500 (Mus musculus)

Samples (38)

[GSM3690851](#) Fear Conditioned, dVenus+, MouseNr 01 [G01]
[GSM3690852](#) Fear Conditioned, dVenus+, MouseNr 03 [G03]
[GSM3690853](#) Fear Conditioned, dVenus-, MouseNr 01 [NG01]

Relations

BioProject

[PRJNA529794](#)

SRA

[SRP189843](#)

Download family

Format

SOFT formatted family file(s)

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MINIML formatted family file(s)

[MINIML !\[\]\(4cf4858f0f33d9147b4f89d7334365ec_img.jpg\)](#)

Series Matrix File(s)

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Supplementary file

Size

Download

File type/resource

[GSE129024_RAW.tar](#)

3.2 Mb

[\(http\)\(custom\)](#)

TAR (of TXT)

[GSE129024_counts_per_gene_sample.txt.gz](#)

647.9 Kb

[\(ftp\)\(http\)](#)

TXT

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Raw data are available in SRA

Processed data provided as supplementary file

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Accessing the data through NCBI GEO

NCBI GEO Accession Display for GSE129024

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Scope: Self | Format: HTML | Amount: Quick | GEO accession: GSE129024

Series GSE129024 Query DataSets for GSE129024

Status: Public on Mar 30, 2019
 Title: Engram-specific transcriptome profiling of contextual memory consolidation
 Organism: *Mus musculus*
 Experiment type: Expression profiling by high throughput sequencing
 Summary: Sparse populations of neurons in the dentate gyrus (DG) of the hippocampus are causally implicated in the encoding of contextual fear memories. However, engram-specific molecular mechanisms underlying memory consolidation remain largely unknown. Here we perform unbiased RNA sequencing of DG engram neurons 24h after contextual fear conditioning to identify transcriptome changes specific to memory consolidation. DG engram neurons exhibit a highly distinct pattern of gene expression, in which CREB-dependent transcription features prominently ($P=5.2 \times 10^{-13}$), including *Atf3* ($P=2.4 \times 10^{-41}$), *Penk* ($P=1.3 \times 10^{-15}$), and *Kcnq3* ($P=3.1 \times 10^{-12}$). Moreover, we validate the functional relevance of the RNAseq findings by establishing the causal requirement of intact CREB function specifically within the DG engram during memory consolidation, and identify a novel group of CREB target genes involved in the encoding of long-term memory.

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Submission date: Mar 29, 2019
 Last update date: May 23, 2019
 Contact name: Steven Kushner
 E-mail(s): s.kushner@erasmusmc.nl
 Organization name: Erasmus MC

Citation(s): der Loo RJ, Martins GJ, van den Hout M, van Dicken WF, Costa RM, van den Oever MC, Kushner SA
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 Street address: Dr. Molewaterplein 40
 City: Rotterdam
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 ZIP/Postal code: 3015 GD
 Country: Netherlands

Platforms (1): GPL17021 Illumina HiSeq 2500 (*Mus musculus*)

Samples (38):
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 GSM3690853 Fear Conditioned, dVenus-, MouseNr 01 [NG01]

Relations
 BioProject: PRJNA529794
 SRA: SRP189843



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SOFT formatted family file(s)	SOFT
MINIML formatted family file(s)	MINIML
Series Matrix File(s)	TXT

Supplementary file	Size	Download	File type/resource
GSE129024_RAW.tar	3.2 Mb	(http/custom)	TAR (of TXT)
GSE129024_counts_per_gene_sample.txt.gz	647.9 Kb	(ftp/http)	TXT

SRA Run Selector

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Accessing the metadata

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Accession: PRJNA529794 Search

Filters List

- 1 ☐ AvgSpotLen
- 2 ☐ Bases
- 3 ☐ Bytes
- 4 ☐ Cell_type
- 5 ☐ source_name
- 6 ☐ Treatment

Common Fields

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LibrarySource	TRANSCRIPTOMIC
Organism	Mus musculus
Platform	ILLUMINA
ReleaseDate	2019-04-01
SRA Study	SRP189843
Strain	C57BL/6J
Tissue	Hippocampal Dentate Gyrus granule neurons

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Found 38 Items Search within results Q Clear

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Accessing the metadata

NCBI SRA Run Selector Log in to NIH

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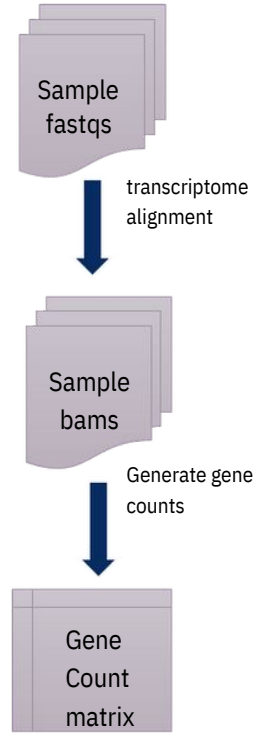
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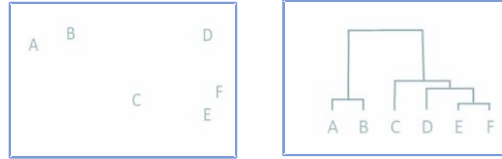
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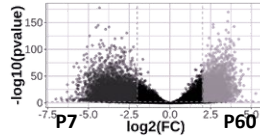


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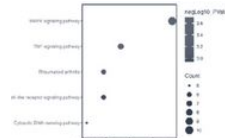
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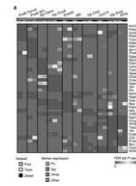
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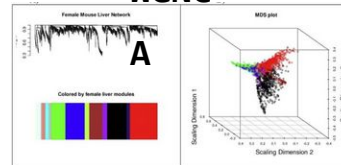
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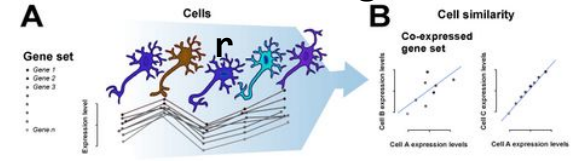
Coregulated Gene Expression



WGNC



MetaNeighbo



Questions?



Thank you for listening!