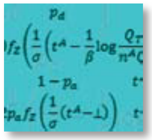
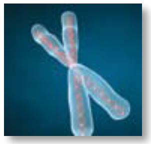
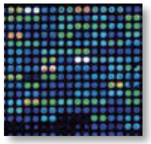




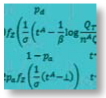
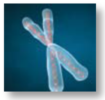
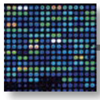
Clinical 24-chromosome PGS using microarrays for Day 5 transfer decisions

David S. Johnson, PhD
Senior V.P. for Research

GENE SECURITY NETWORK

Outline

- Introduction to Gene Security Network
- Overview of GSN's commercial clinical array technology for PGS
- Clinical case studies
- Clinical outcome
- Future directions

My past experience

Genome-Wide Mapping of in Vivo Protein-DNA Interactions

David S. Johnson,[1*] Ali Mortazavi,[2*] Richard M. Myers,[1†] Barbara Wold[2,3†]

In vivo protein-DNA interactions connect each transcription factor with its direct targets to form a gene network scaffold. To map these protein-DNA interactions comprehensively across entire mammalian genomes, we developed a large-scale chromatin immunoprecipitation assay (ChIPSeq) based on direct ultrahigh-throughput DNA sequencing. This sequence census method was then used to map in vivo binding of the neuron-restrictive silencer factor (NRSF; also known as REST, for repressor element–1 silencing transcription factor) to 1946 locations in the human genome. The data display sharp resolution of binding position [±50 base pairs (bp)], which facilitated our finding motifs and allowed us to identify noncanonical NRSF-binding motifs. These ChIPSeq data also have high sensitivity and specificity [ROC (receiver operator characteristic) area ≥ 0.96] and statistical confidence ($P < 10^{-4}$), properties that were important for inferring new candidate interactions. These include key transcription factors in the gene network that regulates pancreatic islet cell development.

Although much is known about transcription factor binding and action at specific genes, far less is known about the composition and function of entire factor-DNA interactomes, especially for organisms with large genomes. Now that human, mouse, and other large genomes have been sequenced, it is possible, in principle, to measure how any transcription factor is deployed across the entire genome for a given cell type and physiological condition. Such measurements are important for systems-level studies because they provide a global map of candidate gene network input connections. These direct physical interactions between transcription factors or cofactors and the chromosome can be detected by chromatin immunoprecipitation (ChIP) (1). In ChIP experiments, an immune reagent specific for a DNA binding factor is used to enrich target DNA sites to which the factor was bound in the living cell. The enriched DNA sites are then identified and quantified.

For the gigabase-size genomes of vertebrates, it has been difficult to make ChIP measurements that combine high accuracy, whole-genome completeness, and high binding-site resolution. These data-quality and depth issues dictate whether primary gene network structure can be inferred with reasonable certainty and comprehensiveness, and how effectively the data can be used to discover binding-site motifs by computational methods. For these purposes, statistical robustness, sampling depth across the genome, absolute signal and signal-to-noise ratio must be good enough to detect nearly all in vivo binding locations for a regulator with minimal inclusion of false-positives. A further challenge in genomes large or small is to map factor-binding sites with high positional resolution. In addition to making com-

putational d this dictates tation relati such as tran and exons, (2). Finally actome me tinely and way to dete ics in respo genetic mu turned to u ing to gain selection or positional a

The Cl from other ChIPArray, (SACO) (3 produced, a 1A) and, un plasmid libr assays, the the genome rather than For exampl ness by an A nucleotide-l of roughly needed for genome. In and so av hybridizatio constraints r 50% of de hybrids is secondary s is feasible f being restri genome tilin

ChIPSe quencing pl Illumina an counting as cations is to

[1]Department of Genetics, Stanford University School of Medicine, Stanford, CA, 94305-5120, USA. [2]Biology Division, California Institute of Technology, Pasadena, CA 91125, USA. [3]California Institute of Technology Beckman Institute, Pasadena, CA 91125, USA.

*These authors contributed equally to this work.

†To whom correspondence should be addressed. E-mail: woldb@its.caltech.edu (B.W.); myers@shgc.stanford.edu (R.M.M.)

Science AAAS

www.sciencemag.org SCIENCE VOL 316 8 JUNE 2007

GENOME RESEARCH

Systematic evaluation of variability in ChIP-chip experiments using predefined DNA targets

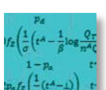
David S. Johnson, Wei Li, D. Benjamin Gordon, Arindam Bhattacharjee, Bo Curry, Jayati Ghosh, Leonardo Brizuela, Jason S. Carroll, Myles Brown, Paul Flicek, Christoph M. Koch, Ian Dunham, Mark Bieda, Xiaoqin Xu, Peggy J. Farnham, Philipp Kapranov, David A. Nix, Thomas R. Gingeras, Xinmin Zhang, Heather Holster, Nan Jiang, Roland D. Green, Jun S. Song, Scott A. McCuine, Elizabeth Anton, Loan Nguyen, Nathan D. Trinklein, Zhen Ye, Keith Ching, David Hawkins, Bing Ren, Peter C. Scacheri, Joel Rozowsky, Alexander Karpikov, Ghia Euskirchen, Sherman Weissman, Mark Gerstein, Michael Snyder, Annie Yang, Zarmik Moqtaderi, Heather Hirsch, Hennady P. Shulha, Yutao Fu, Zhiping Weng, Kevin Struhl, Richard M. Myers, Jason D. Lieb and X. Shirley Liu

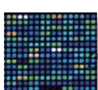
Genome Res. 2008 18: 393-403; originally published online Feb 7, 2008;
Access the most recent version at doi:10.1101/gr.7080508

CSH PRESS GENOME RESEARCH

Distinct DNA methylation patterns characterize differentiated human embryonic stem cells and developing human fetal liver

Alayne L. Brunner, David S. Johnson, Si Wan Kim, et al.





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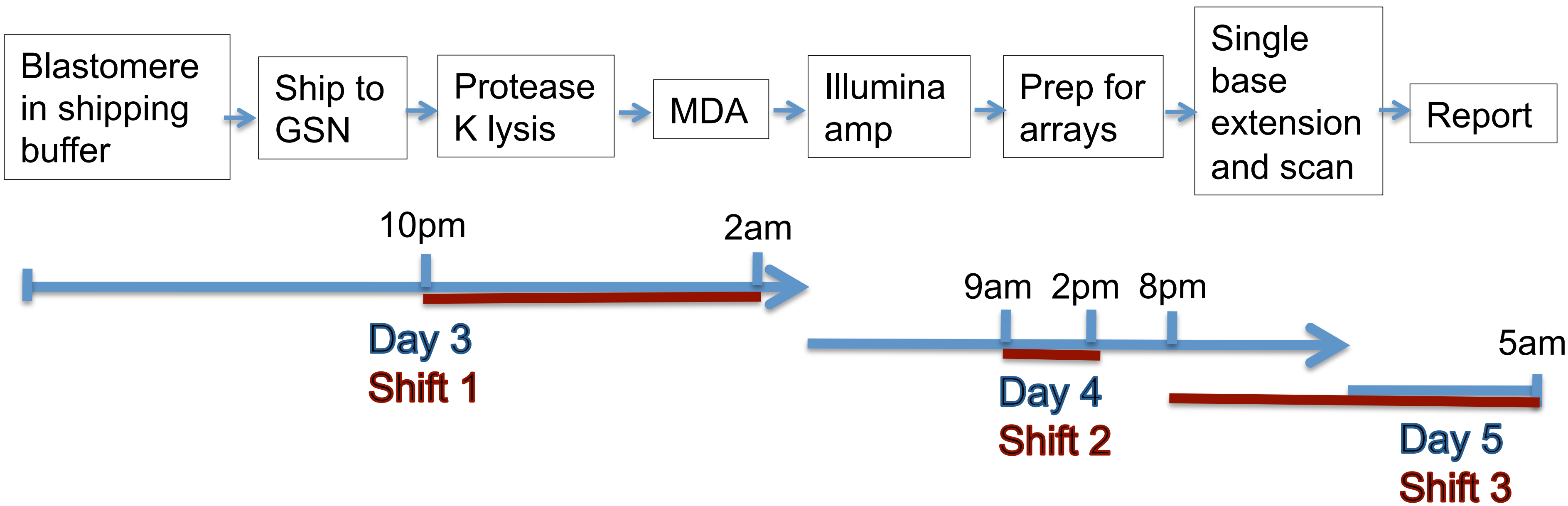
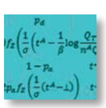
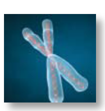
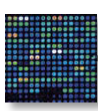
Overview of Gene Security Network

- Lab (CLIA-certified)
  - Five genomics technicians
  - Two Clinical Laboratory Scientists (CA-licensed)
  - One clinical lab manager
- Statistics
  - Five algorithm developers
- Software development
  - Four J2EE engineers
  - One database developer
- Clinical support
  - PGD Director
  - Three certified genetic counselors
  - Medical Geneticist (ABMG certified)

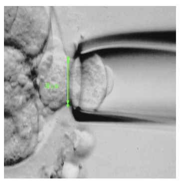
GSN's molecular technology

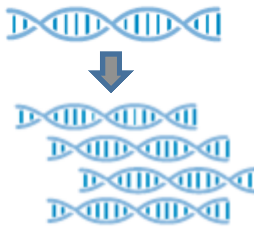


GSN's bioinformatics technology

Noisy Single Cell Array Measurements from MDA





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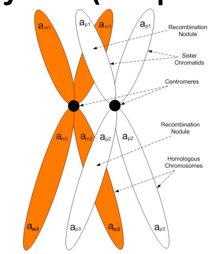
Microarray Measurements on Father & Mother

+

Data from Human Genome Project (HapMap)



Parental Support

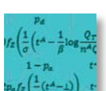
$$P(\hat{n}|D,M,F) = \frac{\sum_{(n^M,n^F)\in\hat{n}} P(n^M)P(n^F)P(D|n^M,n^F,M,F)}{\sum_n \sum_{(n^M,n^F)\in n} P(n^M)P(n^F)P(D|n^M,n^F,M,F)}$$

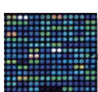
"Cleaned" Single Cell Data

1. 24 Chromosome PGS
2. Monogenic disease testing & 24 chromosome PGD

Summary of technical capabilities

Criterion	FISH	CGH	GSN arrays
Detects aneuploidy across 24 chromosomes	✖	✔	✔
Partial aneuploidy (large deletions & additions)	✔	✔	✔
Detection of haploidy and polyploidy	✔	✖	✔
Detection of UPD	✖	✖	✔
Parental origin of trisomies and monosomies	✖	✖	✔
Detection of DNA contamination	✖	✖	✔
Individual confidences for accuracy of each call on alleles and chr. copy numbers	✖	✔	✔
Detection of both mitotic and meiotic copy errors	✔	✔	✔
Screening multiple disease loci in parallel	✖	✖	Preclinical
Aneuploidy on 24 chromosomes at same time as disease loci	✖	✖	Preclinical



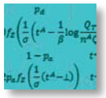
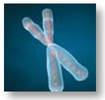
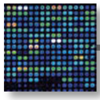


Case study 1: egg donor

Patients
- 45-year old father
- 24-year old egg donor
- 45-year old mother
    - Conceived naturally at age 42
    - Six subsequent IVF cycles with no pregnancies using her own eggs
    - First donor IVF cycle was Trisomy 16, spontaneously aborted
    - Second donor IVF cycle had triplets with 2-embryo transfer, underwent reduction, had infection, lost child

Clinical Decision: Transferred two euploid embryos using GSN's data (4/16 euploid)

Outcome: Ongoing single pregnancy at 22 weeks

Case study 1: egg donor

embryo id	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	sex	Day 3 morph	transfer	Day 5 ploidy	Day 5 morph
1	-P	E	E	-M	-M	-P	E	-P	E	E	0	E	-P	E	-P	E	E	-P	E	E	E	E	XX	1			B
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		46XX	HB
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX	1	YES		HB
4	-M	0	-M	-P	E	0	-P	-M	0	-M	-M	0	-M	0	0	-M	-M	0	-M	-M	-P	-P	X	1			ARR
5	E	+M	E	E	+M	E	E	E	+M	+P	+M	E	E	E	E	E	E	E	E	+M	E	E	XXY	1		48XX, +5,9	B
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2			B
7	E	E	E	E	NC	E	E	E	E	E	NC	E	E	E	E	E	E	E	E	E	E	E	NC	1		46XX	HB
8	-M	-M	-M	E	-M	-M	-M	0	E	E	0	-M	-M	-M	-M	-M	-M	E	E	-M	-M	E	Y	1			COMP
9	-P	E	-M	E	-M	-M	-M	-P	E	E	E	E	E	E	-M	E	E	E	E	E	E	E	XY	1			HB
10	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XY	1	YES		HB
11	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX	1			HB
12	0	0	-P	0	0	-P	-P	E	0	-P	0	-P	-P	0	-P	0	0	-P	0	0	0	0	X	1		56XY, +1,2,3,7,8, 9,10,13,14, 15,16,21,22 ; -4,6,20	HB
13	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XY	1			HB
14	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XXY	1		46XX	HB
15	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XXY	1		46XX	HB
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2		46XX	HB

Ploidy call notation:
+M maternal trisomy
+P paternal trisomy
-M maternal loss
-P paternal loss
E euploid
0 nullisomy
NC no call

D5 notation:
B blastocyst
HB hatching blastocyst
COMP compacted
ARR arrested

D3 notation:
1 best morphology
4 worst morphology

- 4/16 euploid Day 3
- 5/7 euploid Day 5

GSN GENE SECURITY NETWORK

Case study 2: recurrent pregnancy loss

<u>Patients</u>:

- 39-year old father
- 40-year old mother
    - 1st natural pregnancy was trisomy 13, lost at week 15
    - 2nd natural pregnancy lost, blighted ovum

<u>Clinical Decision</u>: Transferred 2 euploid embryos using GSN's data (3/10 blastomeres were euploid)

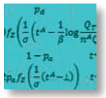
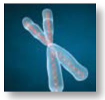
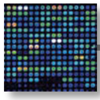
<u>Outcome</u>: Ongoing twin pregnancy at 25 weeks

Case study 2: recurrent pregnancy loss

embryo id	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	sex
1	-P	-P	-M	-M	E	-M	E	-P	-P	+M	-P	-M	E	-P	E	-P	E	-M	-P	-M	E	-M	XY
2	E	-M	E	E	E	E	E	-P	-M	E	E	E	E	-M	E	-P	E	E	-M	E	E	-M	XY
3	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX
4	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX
5	E	E	E	E	E	+M	E	+M	E	E	E	+M	E	E	E	E	-M	E	E	E	E	-M	XX
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	+M	XY
7	E	-P	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX
8	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	-M	-M	XX
9	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XY
10	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	-M	E	E	E	E	E	-M	XY

Notation:
+M maternal trisomy
+P paternal trisomy
-M maternal loss
-P paternal loss
E euploid

Summary:
• 11 paternal loss monosomies, 16 maternal loss monosomies (50-50 across ~1000 blastomeres)
• 4 maternal gain trisomies, 0 paternal gain trisomies (95-5 across ~1000 blastomeres)

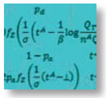
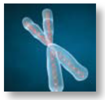
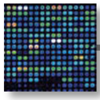
Case study 3: oligozoospermia

<u>Patients</u>:

- 26-year old father
    - Severe oligozoospermia
- 29-year old mother
    - 1^{st} pregnancy was natural
    - 2^{nd} and 3^{rd} pregnancies lost to miscarriage at 5 weeks

<u>Clinical Decision</u>: Transferred 2 euploid embryos using GSN's data (3/7 blastomeres were euploid)

<u>Outcome</u>: Ongoing pregnancy at 6 weeks

Case study 3: oligozoospermia

embryo id	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Sex
1	+M	E	E	E	+P	+M	E	+P	E	E	E	E	E	E	+P	+M	E	+M	E	+P	E	+M	XY
2	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XY
3	E	E	E	E	E	E	E	E	E	E	E	E	+P	E	E	E	E	E	E	E	E	E	XXY
4	E	E	E	E	E	E	E	E	E	E	E	E	E	+P	E	-M	E	E	E	E	E	E	XX
5	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX
6	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX
7	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	XX

Notation:
+M maternal trisomy
+P paternal trisomy
-M maternal loss
-P paternal loss
E euploid

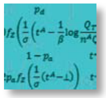
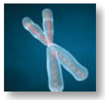
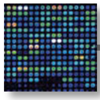
- Typically 95% of trisomies are maternal in origin (~1000 blastomeres measured)
- Here 5/10 trisomies are paternal in origin
- (not statistically significant)

Case study 4: blastocyst biopsy

<u>Patients</u>:
- 32-year old mother
- 34-year old father
- No children, no prior pregnancies, no fertility problems
- Mother undergoing chemo and radiation therapy for Hodgkin's lymphoma
- Elected for IVF with blastocyst freezing for transfer at a later date

<u>Results</u>: Seven out of eight trophectoderm biopsies were euploid. One embryo had maternal trisomies on chromosomes, 8, 9, and 15.

<u>Outcome</u>: Seven euploid blastocysts frozen for later use.

GSN
GENE SECURITY NETWORK
14

Overall

	GSN	Control Arm data from published studies:					
		Average of all Studies	Hardarson 2008	Staessen 2004	Rubio 2005	Staessen 2008	Munne 1999
# of Embryo Transfers	38	81	53	121	24	89	117
# of Embryos transferred	64	196	95	338	50	89	408
Mean Maternal Age	34	N/A	40	≥37	≥38	30	38.5
Pregnancy rate	61% ± 7.9%	41% ± 5.5%	30.2%[a]	32.2%[b]	50%[c]	58.4% [d]	40.2%[e]
Implantation rate	45% ± 8.1%	14% ± 2.4%	18.9%	11.5%	16.0%	N/A	13.7%
On-going pregnancy (≥12 weeks) / Live Birth rate	50% ± 8.1%*	32% ± 5.2%	18.9%**	24.0%**	45.8%*	41.5%**	36.8%*

[a] 16 HCG+/53 ET; [b] 39 HCG+/121 ET; [c] 12 HCG+/24 ET; [d] 42 HCG+/89 ET; [e] 47 HCG+/117

* On-going pregnancy rate per Embryo Transfer; ** Live Birth rate per Embryo Transfer

Advanced Maternal Age

	GSN	Control Arm data from published studies:				
		Average of all Studies	Hardarson 2008	Staessen 2004	Rubio 2005	Munne 1999
# of Embryo Transfers	13	79	53	121	24	117
# of Embryos transferred	21	223	95	338	50	408
Mean Maternal Age	40	N/A	40	≥37	≥38	38.5
Pregnancy rate (per ET)	54% ± 13.8%	36% ± 5.4%	30.2%[a]	32.2%[b]	50%[c]	40.2%[d]
Implantation rate	48% ± 13.7%	14% ± 2.3%	18.9%	11.5%	16.0%	13.7%
On-going pregnancy (≥12 weeks) / Live Birth rate	38% ± 12.8%*	30% ± 5.1%	18.9%**	24.0%**	45.8%*	36.8%*

[a] 16 HCG+/53 ET; [b] 39 HCG+/121 ET; [c] 12 HCG+/24 ET; [d] 47 HCG+/117

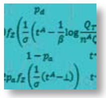
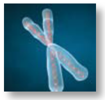
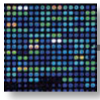
* On-going pregnancy rate per Embryo Transfer; ** Live Birth rate per Embryo Transfer

Younger Maternal Age

	GSN	Control Arm data from published studies: Staessen 2008
# of Embryo Transfers	25	89
# of Embryos transferred	43	89
Mean Maternal Age	30	30
Pregnancy rate (per ET)	64% ± 9.6%	58% ± 5.2%[a]
Implantation rate	44% ± 9.9%	N/A
On-going pregnancy (≥12 weeks) / Live Birth rate	56% ± 9.9%*	42% ± 5.2%**

[a] 52 HCG+/89 ET

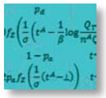
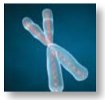
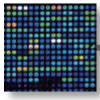
* On-going pregnancy rate per Embryo Transfer; ** Live Birth rate per Embryo Transfer

GSN GENE SECURITY NETWORK

Future directions

- Planning a large randomized prospective clinical trial
- Ongoing NIH grant to examine concordance between Day 3 and Day 5 biopsies
- Clinical validation of 24-chromosome screening with single locus disease screening

Acknowledgments

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- Stanford IVF (California, USA)
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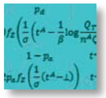
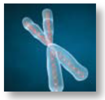
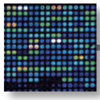
- Reproductive Endocrinology Associates of Charlotte (North Carolina, USA)
- Reproductive Care Center (Utah, USA)
- Huntington Reproductive Center (California, USA)
- Gene Security Network (California, USA)

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