

Functional Genomic Screens in the RNAi Platform

Broad Institute of MIT and Harvard



Functional Genomic Screens in the RNAi Platform

‘Functional genomics’

→ Gene function in context.... in cells

“What to expect if you go down this road”

- I. Overview
- II. Perturbations
- III. Planning a screen – the idea & all the key ingredients
- IV. Optimization/Execution
- V. Data analysis
- VI. Gene function, putting it all together: “Figures 3-7”

What's the gene that causes 5q- syndrome?

What is 5q- syndrome? - myelodisplasia

5q- syndrome patients:

Severe anemia

- Reduced erythroid development
- Normal numbers of megakaryocyte
- Hypolobated micromegakaryocytes
- Normal/elevated platelets
- Normal/low neutrophils
- Occasional progression to AML

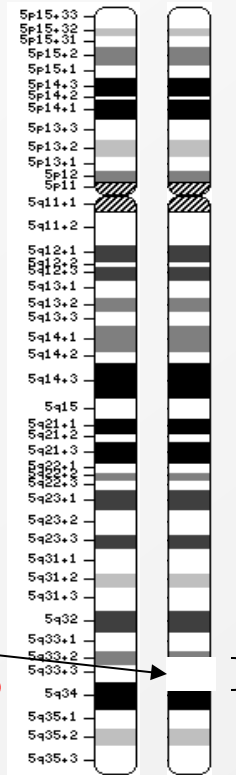
Distinct haematological disorder with deletion of long arm of No. 5 chromosome

van Den Berghe, *Nature*, **251**, 437-438 (**1974**)

Deletion (1.5Mb)

40 genes, which one?

(Haploinsufficiency)



**A 40-year hunt among 40 genes
....but no luck**

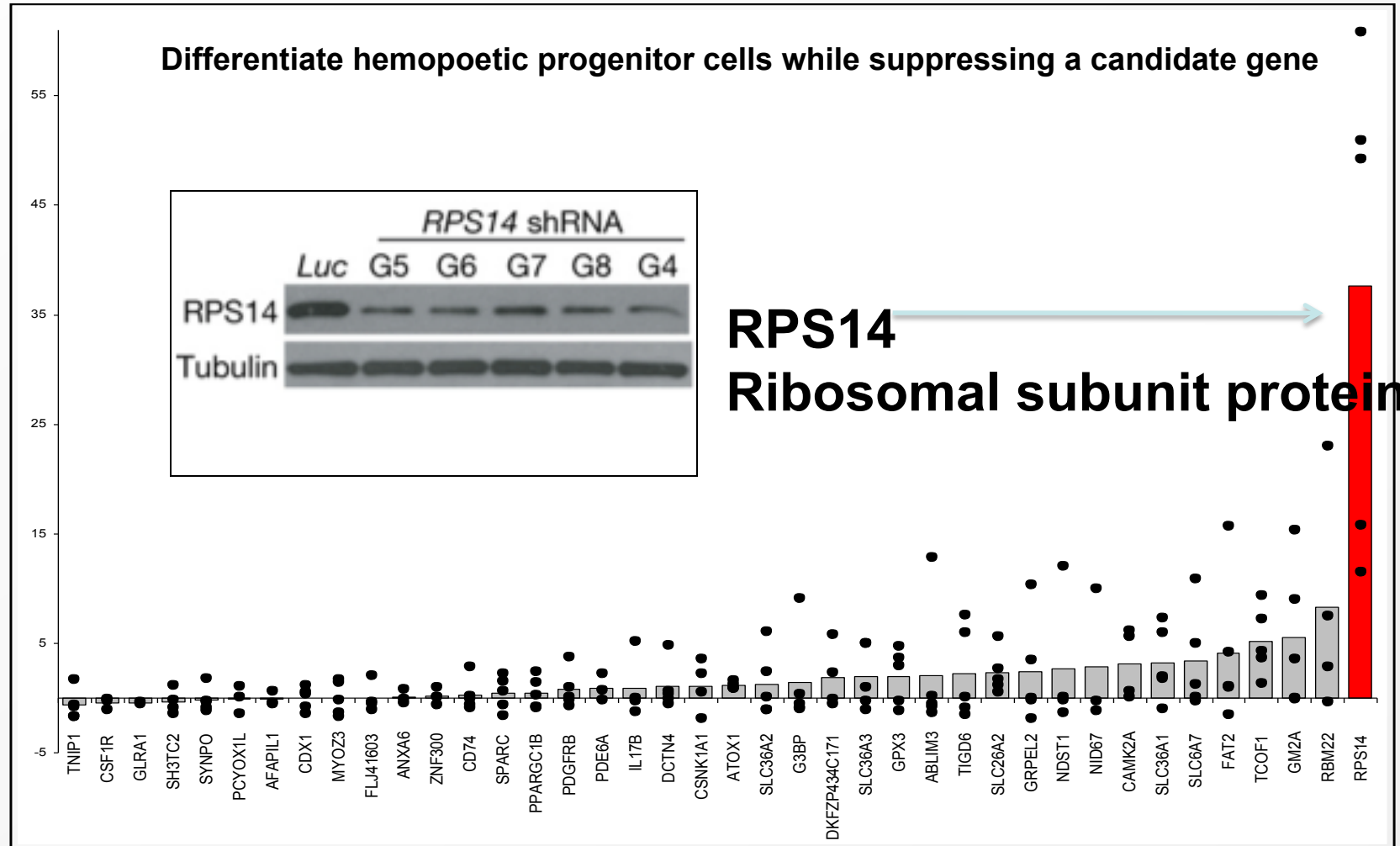
Ben Ebert, Todd Golub
Nature 2008

Focused screen of common deleted region

A key to screen design: a model for disease

Hemopoietic progenitor differentiation - increased megakaryocyte/erythrocyte ratio

Ratio Megakaryocyte/Erythroid



RPS14
Ribosomal subunit protein

Hairpin targets

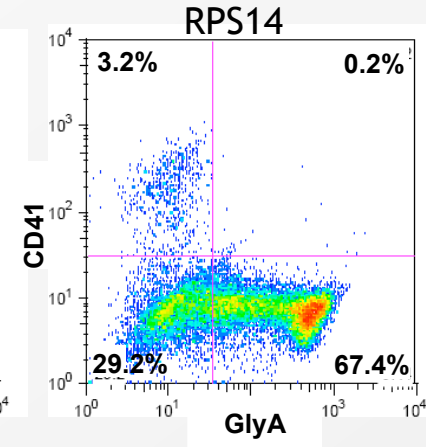
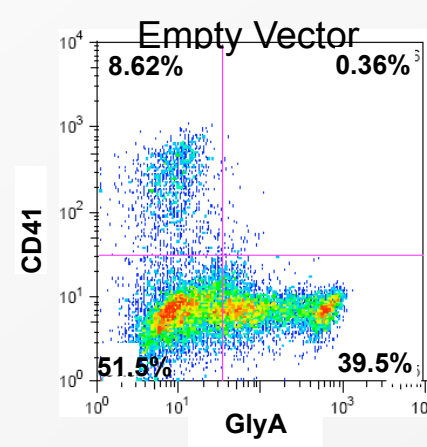
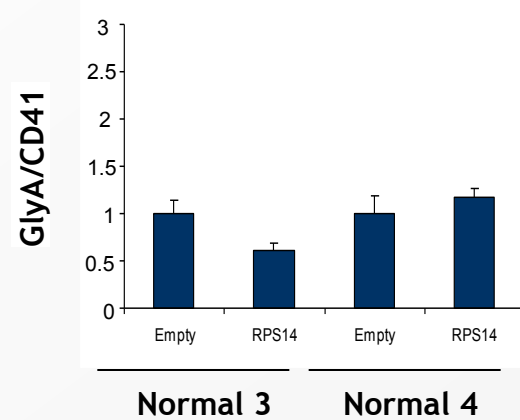
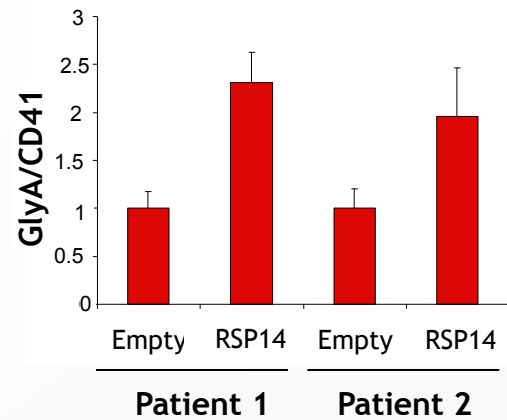
Ben Ebert, Todd Golub

RPS14 overexpression 'reverts' 5q- patient cells

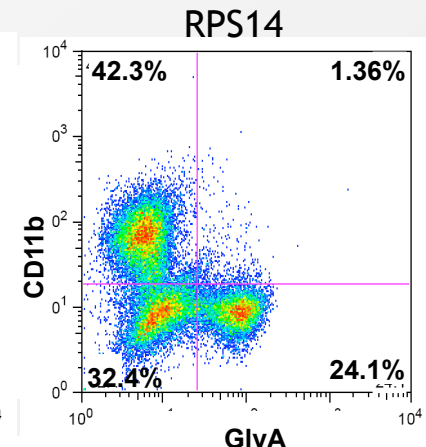
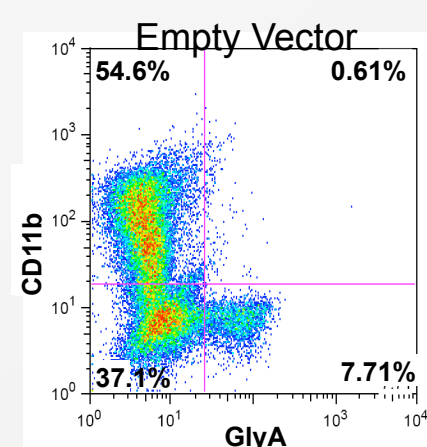
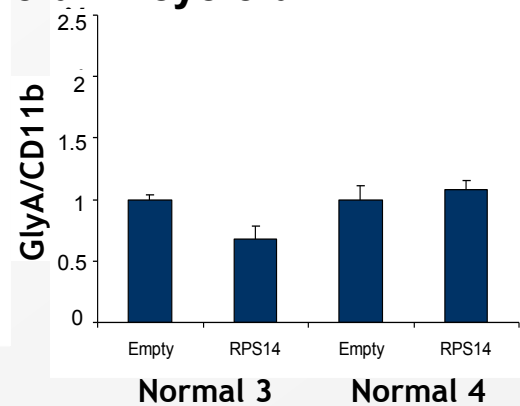
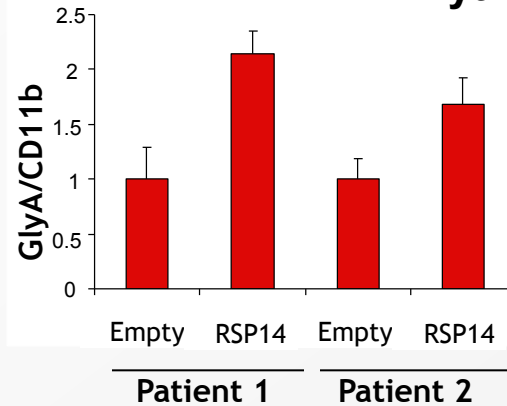
5q- patients

Non-5q- MDS patients

Erythroid / megakaryocyte

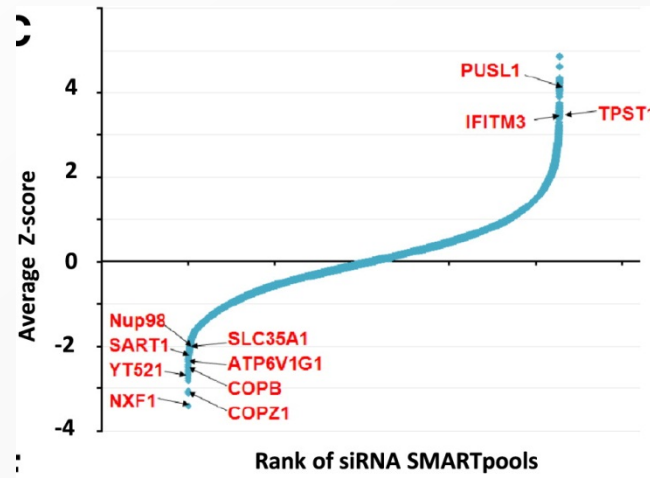


Erythroid / myeloid



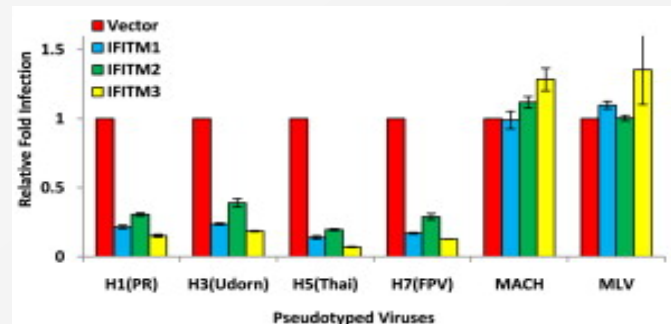
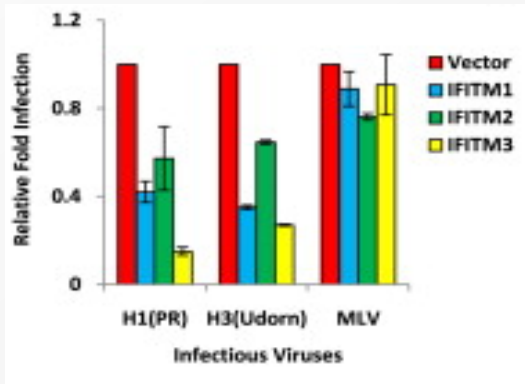
Which host cell genes restrict flu infection?

Knock down genes and monitor flu infection levels in U2OS cells



IFIT3 was noted as an interferon-induced gene.

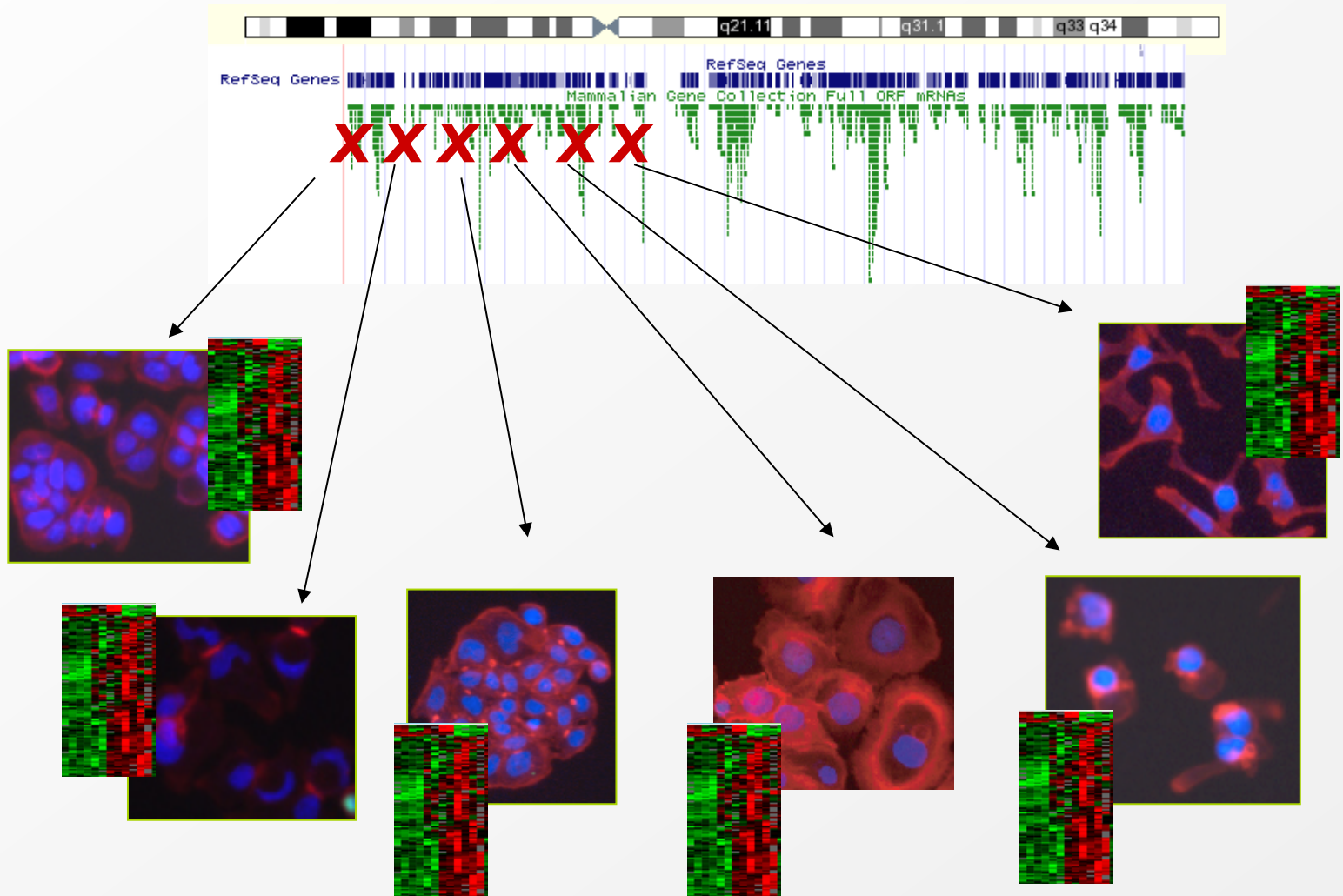
Follow-up shows IFITM family are all flu restriction factors.



Brass,
Farzan,
Elledge
Cell 2009

Systematic gene function discovery:

- Perturb each gene and look for change in cell behavior
- Knockdown, overexpression, re-engineer, etc.



Functional genomics

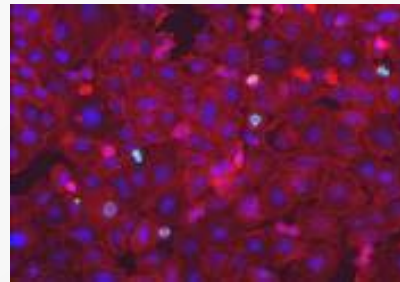
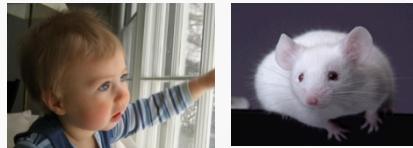
→ Probe function by perturbing gene activity in cells

It can be complicated...

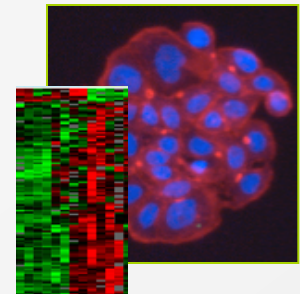
- Multiple functions per gene
- Multiple isoforms, protein modifications
- Context-dependence functions:
cell type, cell state, environment

Cell-based loss-of-function screens

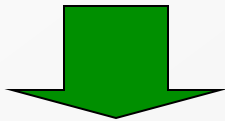
Human and mouse



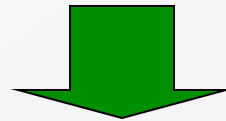
What happens to the cells?



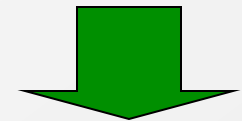
Need:



Reagents to perturb every gene



Good tissue culture model



Good readouts of cell state

Screen projects



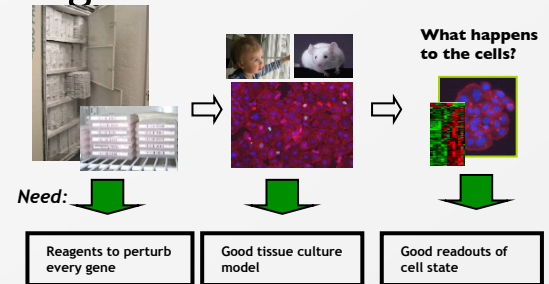
Define project

- Define question: “Find genes that do XYZ”
- Define biological model system
- Define assays to read out phenotypes of interest

Primary screen – feasibility and execution

- Optimize model system, assay(s); positive and negative controls
- Select gene set to interrogate

Execute pilot and primary screen – select hits



Follow up on interesting genes/pathways

- Confirm assay result
- Confirm target gene specificity – multiple RNAi reagents, target KD
- Elaborate the biological effects,
e.g. mechanism generality/context, biomedical sig?

The screen is just one early step of a project

Gene perturbations

Genome-scale in mammalian cells

- **RNA interference** – last 10 years
- **Overexpression** – possible for decades, rare in practice
- **Insertional mutagenesis in haploid cells** – a few years
- **Other strategies: (scale varies)**

**Genome engineering: Zn fingers, TALENS, CRIPR
miRNA sponges, antisense**

Disease models


- Cancer
 - Barbie, D. A., P. Tamayo, et al. (2009). "Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1." *Nature* 462: 108-12.
 - Boehm, J. S., J. J. Zhao, et al. (2007). "Integrative genomic approaches identify IKBKE as a breast cancer oncogene." *Cell* 129: 1065-79.
- Anemia
 - Ebert, B. L., J. Pretz, et al. (2008). "Identification of RPS14 as a 5q- syndrome gene by RNA interference screen." *Nature* 451: 335-9.
- Osteoporosis
 - Jones, D. C., M. N. Wein, et al. (2006). "Regulation of adult bone mass by the zinc finger adapter protein Schnurri-3." *Science* 312: 1223-7.
- Mitochondrial Dysfunction
 - Pagliarini, D. J., S. E. Calvo, et al. (2008). "A mitochondrial protein compendium elucidates complex I disease biology." *Cell* 134: 112-23.
- Immunology and systems immunology
 - Amit, I., M. Garber, et al. (2009). "Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses." *Science* 326(5950): 257-63.
 - Oberdoerffer, S., L. F. Moita, et al. (2008). "Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL." *Science* 321: 686-91.

Challenges to the systematic functional annotation of the genome

Cell-based screening:

- Tissue culture model, phenotypic assay

Genetic perturbations issues, for example RNAi:

- *Delivery – efficiency, effects* 
- *Gene knockdown effectiveness*
- *Specificity: Off-target, non-specific effects*
- *Follow-up bottleneck*
- *Cost*



Partly an ‘art’ – experience helps a lot!

Publications using the TRC library in direct collaboration or consultation with the Broad RNAi Platform 2006-2010

- Amit, I. et al. (2009) "Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses." **Science** 326: 257-63.
- Barbie, D. A. et al. (2009) "Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1." **Nature** 462: 108-12.
- Barretina, J. et al. (2010) "Subtype-specific genomic alterations define new targets for soft-tissue sarcoma therapy." **Nature Genetics** 42: 715-721.
- Bilodeau, S. et al. (2009) "SetDB1 contributes to repression of genes encoding developmental regulators and maintenance of ES cell state." **Genes & Dev** 23: 2484-2489.
- Boehm, J. S. et al. (2007) "Integrative genomic approaches identify IKBKE as a breast cancer oncogene." **Cell** 129: 1065-79.
- Ebert, B. L. et al. (2008) "Identification of RPS14 as a 5q- syndrome gene by RNA interference screen." **Nature** 451: 335-9.
- Eguchi, J. et al. (2008) "Interferon regulatory factors are transcriptional regulators of adipogenesis." **Cell Metab** 7: 86-94.
- Firestein, R. et al. (2008) "CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity." **Nature** 455: 547-51.
- Frohling, S. et al. (2007) "Identification of driver and passenger mutations of FLT3 by high-throughput DNA sequence analysis and functional assessment of candidate alleles." **Cancer Cell** 12: 501-13.
- Gohil, V. M. et al. (2010) "Mitochondrial and nuclear genomic responses to loss of LRPPRC expression." **J Biol Chem** 285: 13742-7.
- Johannessen, C.M. et al. (2010) "COT drives resistance to RAF inhibition through MAP kinase pathway reactivation." **Nature** 468:968-972
- Jones, D. C. et al. (2006) "Regulation of adult bone mass by the zinc finger adapter protein Schnurri-3." **Science** 312: 1223-7.
- Jones, T.R. et al. (2009) "Scoring diverse cellular morphologies in image-based screens with iterative feedback and machine learning." **PNAS** 106:1826-31.
- Kagey, M.H. et al. (2010) "Mediator and cohesin connect gene expression and chromatin architecture." **Nature** 467: 430-435.
- Kim, S. Y. (2010) "CK1epsilon is required for breast cancers dependent on beta-catenin activity." **PLoS One** 5: e8979.
- Moffat, J. et al. (2006) "A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen." **Cell** 1246: 1283-98.
- Oberdoerffer, S. et al. (2008) "Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL." **Science** 321: 686-91.
- Pagliarini, D. J. et al. (2008) "A mitochondrial protein compendium elucidates complex I disease biology." **Cell** 134: 112-23.
- Pavri, R. et al. (2010) "Activation-Induced Cytidine Deaminase Targets DNA at Sites of RNA Pol. II Stalling by Interaction with Spt5." **Cell** 143:122-133.
- Perocchi F. et al. (2010) "MICU1 encodes a mitochondrial EF hand protein required for Ca²⁺ uptake." **Nature**, 467: 291-6.
- Root, D. E. et al. (2006) "Genome-scale loss-of-function screening with a lentiviral RNAi library." **Nat Methods** 3: 715-9.
- Sancak, Y. et al. (2007) "PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase." **Mol Cell** 25: 903-15.
- Savina, A. et al. (2009) "The small GTPase Rac2 controls phagosomal alkalization and antigen crosspresentation selectively in CD8(+) dendritic cells." **Immunity** 30: 544-55.
- Scholl, C. et al. (2007) "The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis." **J Clin Invest** 117: 1037-48.
- Scholl, C. et al. (2009) "Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells." **Cell** 137:821-34.
- Smolen, G.A. et al. (2010) "A genome-wide RNAi screen identifies multiple RSK-dependent regulators of cell migration" **Genes & Dev.** 24: 2654-2665.
- Sugiana, C. et al. (2008) "Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease." **Am J Hum Genet** 83:468-78
- Vasudevan, K. M. et al. (2009) "AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer." **Cancer Cell** 16: 21-32.
- Vicent, S. et al. (2010) "Wilms tumor 1 regulates KRAS-driven oncogenesis and senescence in mouse and human models." **J Clin Invest** 120:3940-3952.
- Yang, W. S. et al. (2008) "Inhibition of casein kinase 1-epsilon induces cancer-cell-selective PERIOD2-dependent growth arrest." **Genome Biol** 9: R92.
- Zhang, Q. et al. (2009) "Control of cyclin D1 and breast tumorigenesis by the Egln2 prolyl hydroxylase." **Cancer Cell** 16(5): 413-24.

RNAi Platform – what do we do?

- *Genomic screening projects – fct. genomics expertise*
- *R & D in functional genomics tools, strategies*
- *Infrastructure for maintaining, distributing, employing gene perturbation reagents*
- *Libraries, reagents creation*

The Broad RNAi Platform

~35 People

Production of shRNA
+ ORF libraries; others

Screening collaborations

Informatics and
computational biology

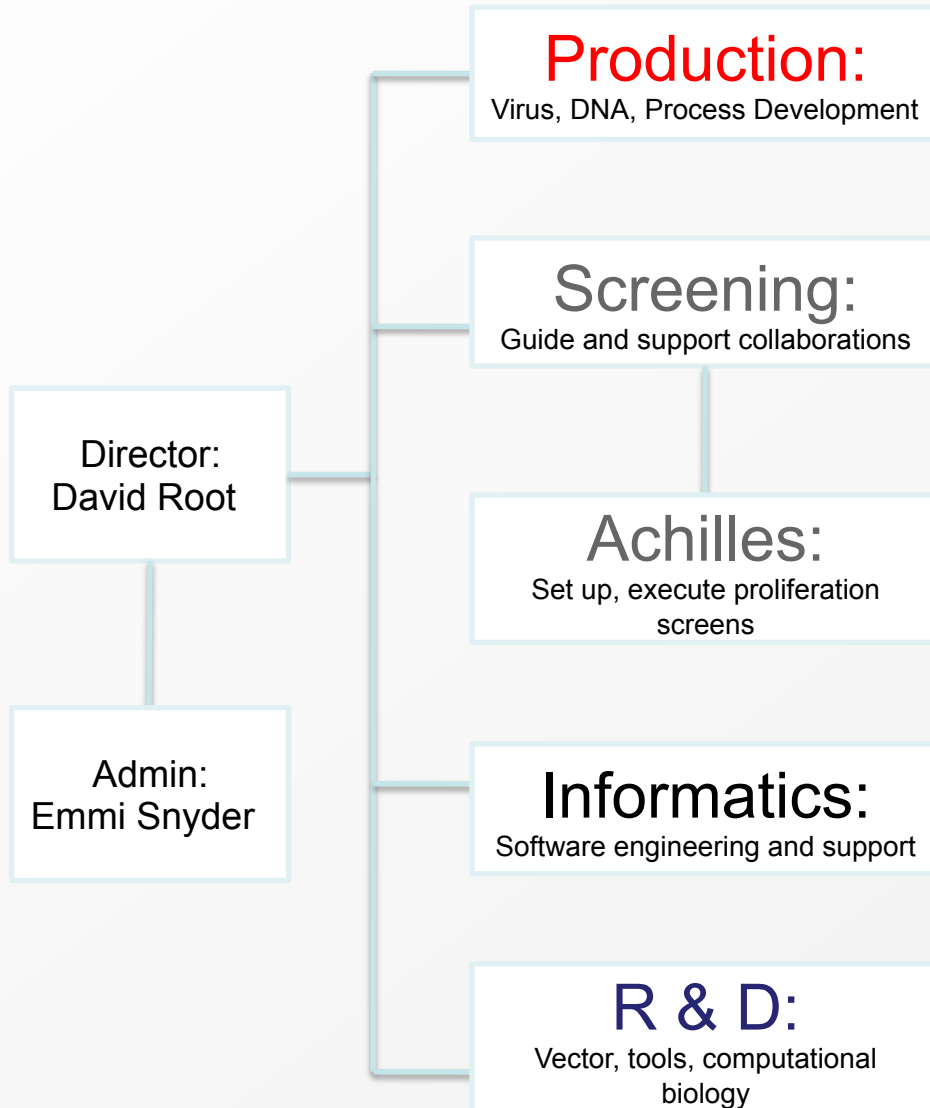
Research on functional
genomics methods; design
of new reagents

Researchers
in Broad community

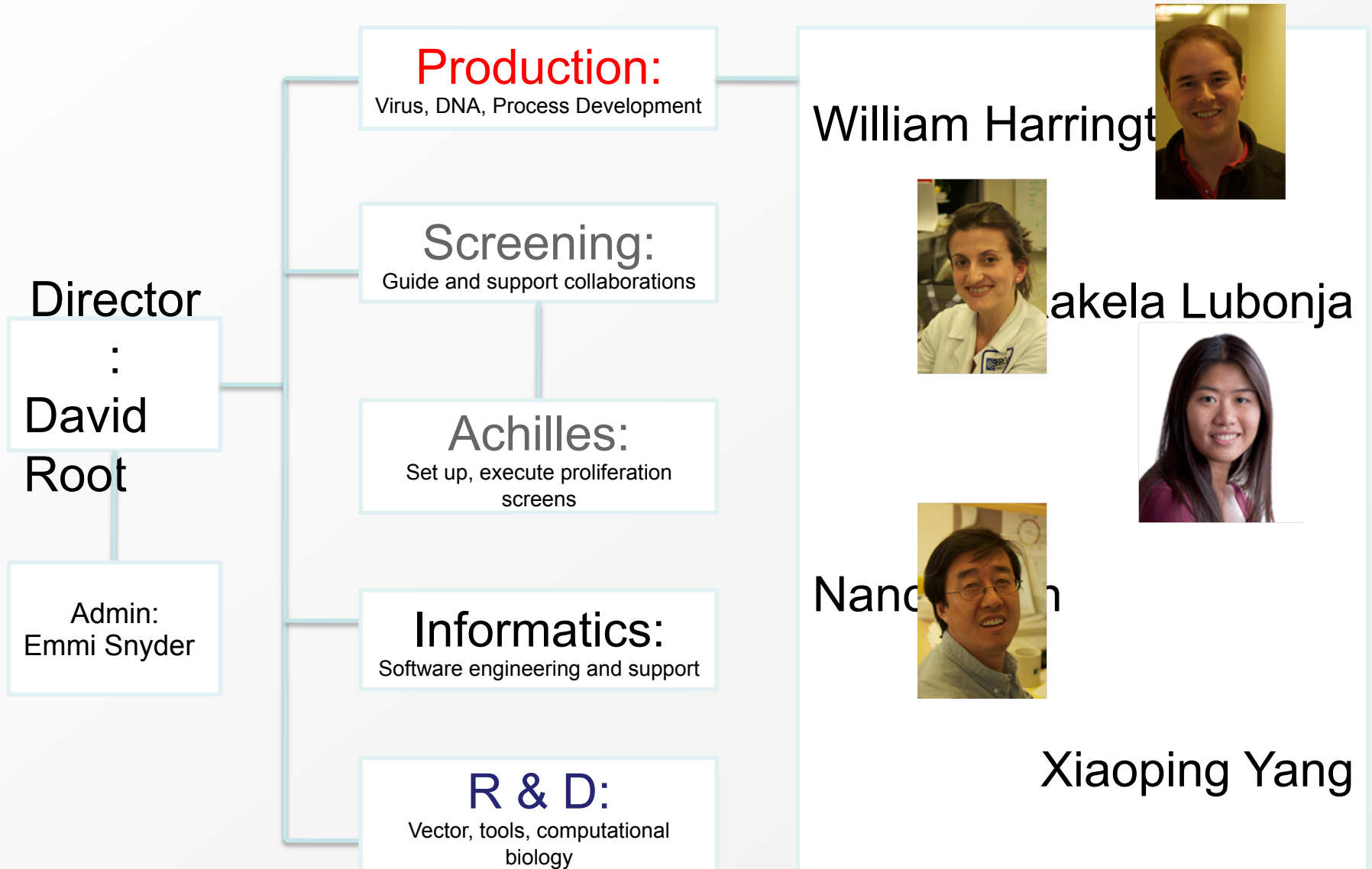
The research world



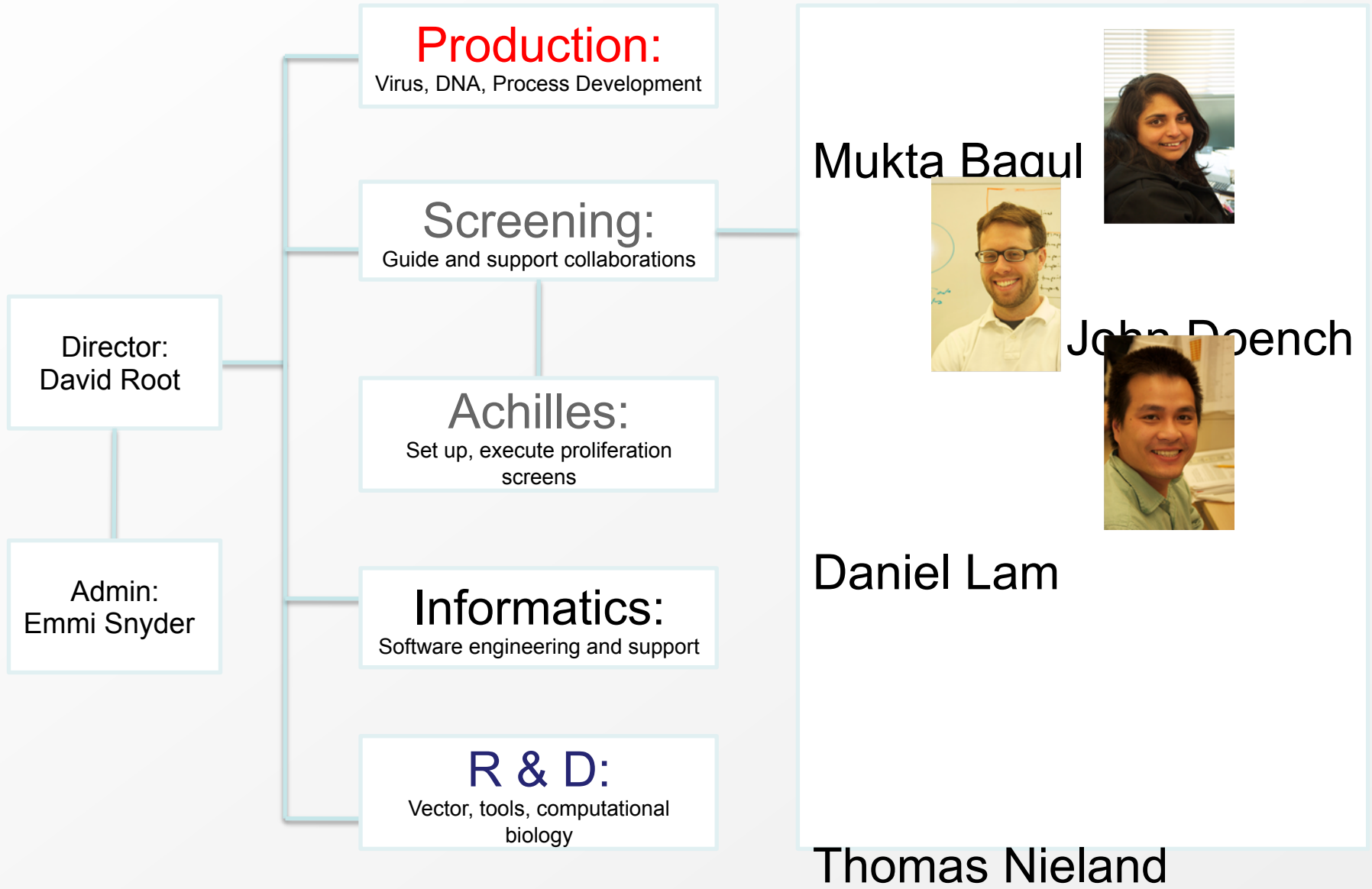
Platform Organization



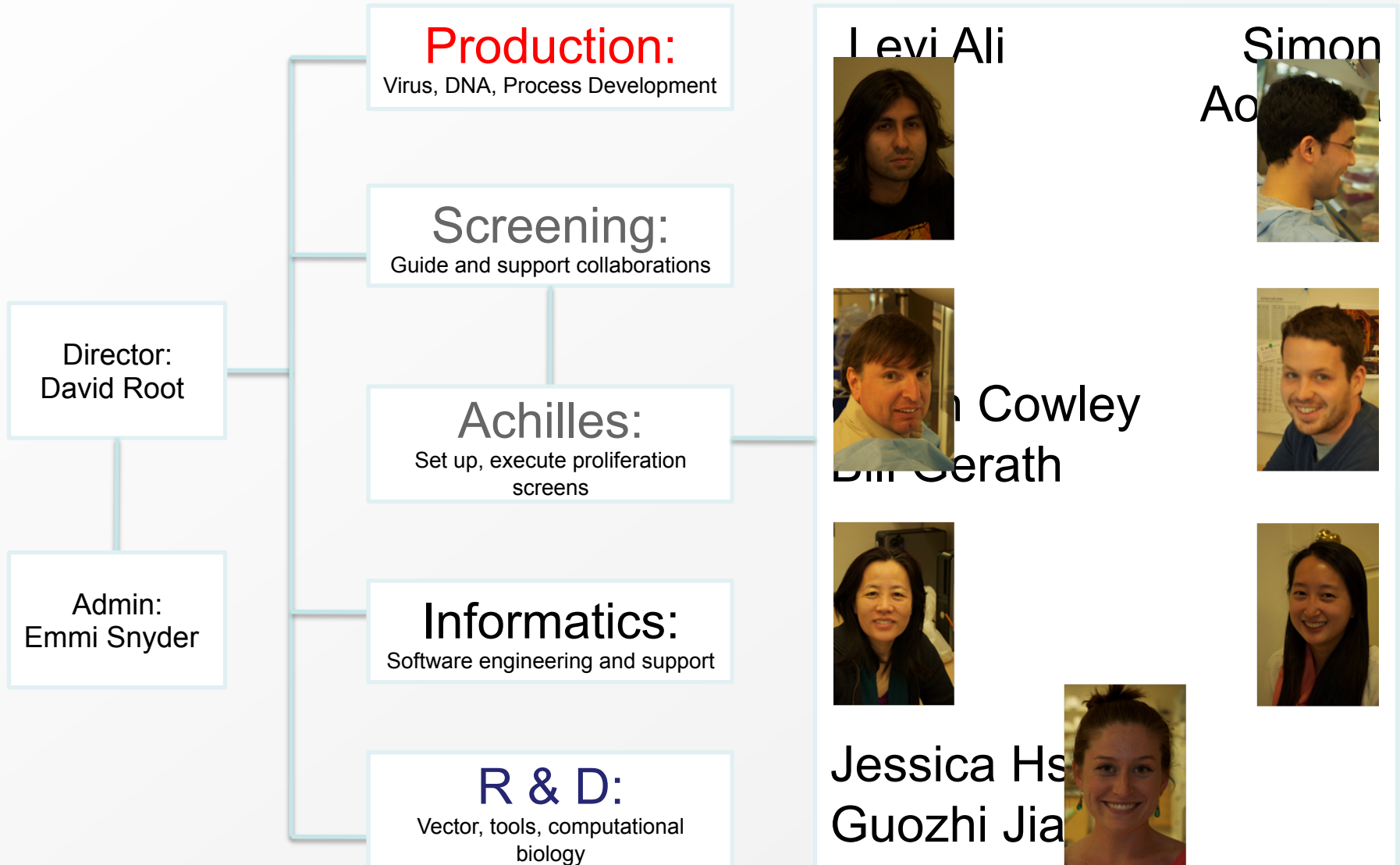
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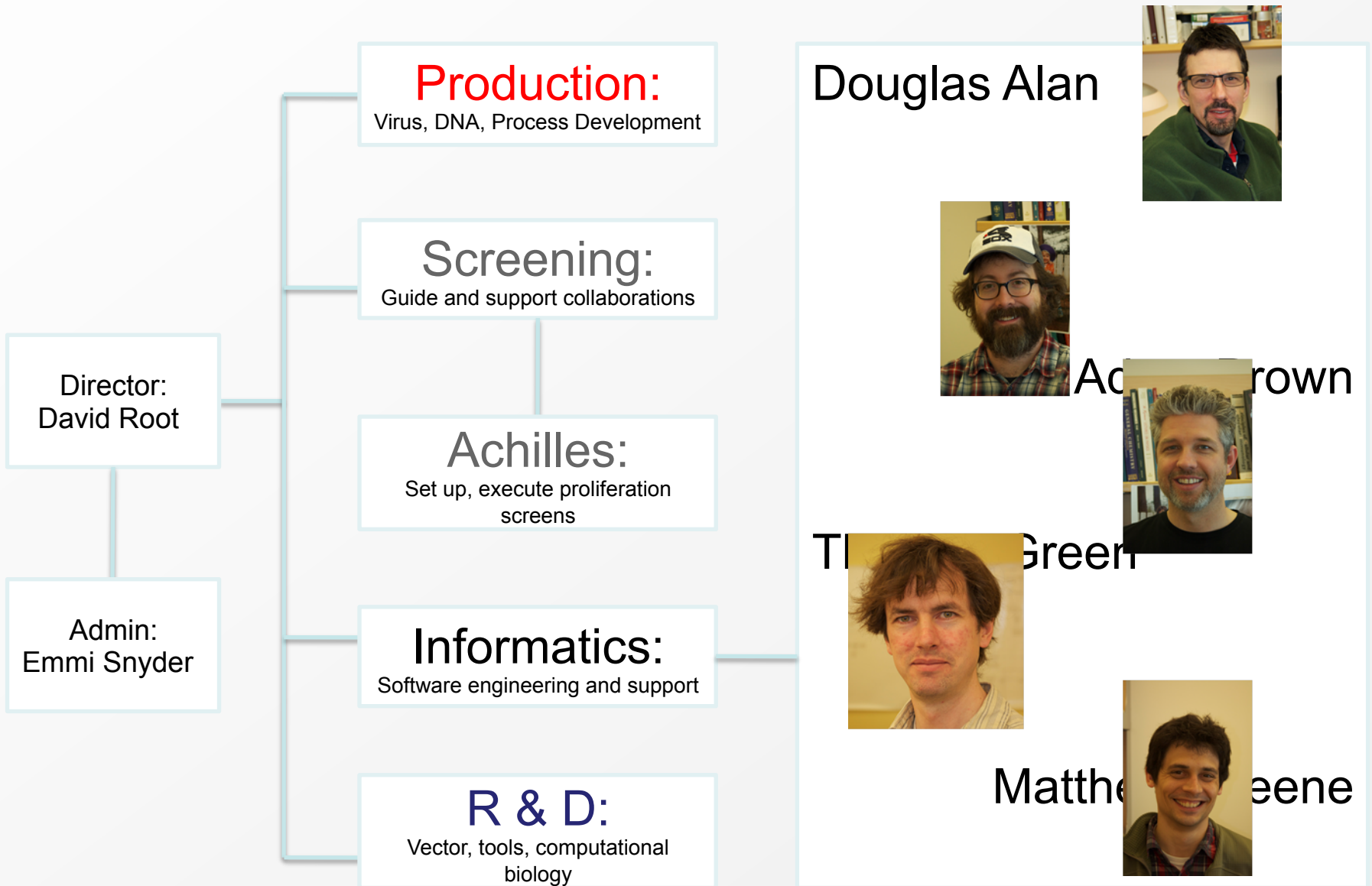
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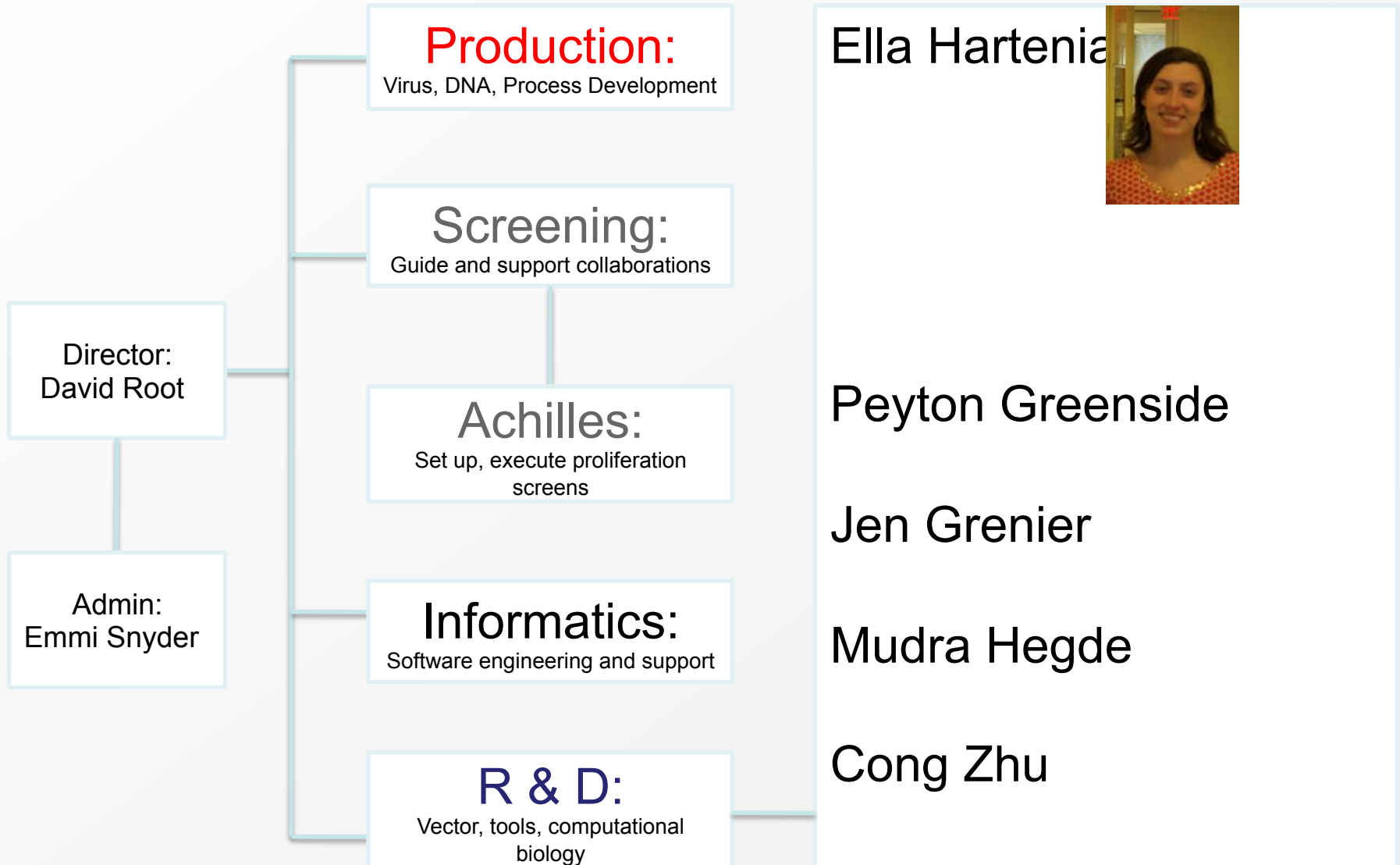
Platform Organization



Platform Organization



Platform Organization



RNAi Platform

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<https://iwww.broadinstitute.org/rnai/db/>

Come see us about:

- Screen project ideas – gene discovery
- How to use gene perturbation tools for non-screen based projects
- Projects to investigate new strategies, technologies for fct. genomics