BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Knott, Simon Robert Vincent

eRA COMMONS USER NAME (credential, e.g., agency login): KNOTTS

POSITION TITLE: Faculty Research Scientist I

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Queen's University, Kingston, Ontario, Canada	BSc (Hon)	05/2004	Computer Sciences
Queen's University, Kingston, Ontario, Canada	MSc	08/2006	Computer Sciences
University of Southern California, Los Angeles, CA	PhD	05/2011	Computational Biology
Cold Spring Harbor Laboratory	Postdoc	08/2016	Functional Genomics

A. Personal Statement

I combine computational biology and functional genomics to elucidate the molecular mechanisms that drive cancer progression. Subsequent to graduating with a PhD in Computational biology from the University of Southern California, I joined Dr. Gregory Hannon's lab as a Hope Funds for Cancer Postdoctoral Fellow at Cold Spring Harbor Laboratory (CSHL). There, my initial focus was on elucidating the sequence determinants of short hairpin RNA (shRNA) efficacy. After developing a computational algorithm that identified and employed these features to predict potent targeting molecules for any gene, I lead a team through a set of functional genetic screens to validate the accuracy of the tool. This work lead to both a high-profile publication describing the resource and the commercialization of corresponding human and mouse shRNA libraries. Since then, others have harnessed the prokaryotic CRISPR/Cas9 system for functional studies in mammalian cells. I have recently developed algorithms and expression vectors that together maximize the efficacy of CRISPR targeting molecules. Once again, these reagents have been experimentally validated for their superiority to existing tools. This work was recently the subject of a second "resource" publication and these tools have also been commercialized.

While developing and applying these reagents to a variety of applications, I became interested in tumor heterogeneity and metastasis. To study these disease aspects, I developed a method to virally barcode individual cells within complex populations, so that they could be tracked throughout the various stages of disease progression. In an initial set of experiments, I lead a team to apply this method to study mechanisms of cancer cell intravastation into the vascular. This work culminated with a publication in the journal Nature, where we described how cells enter the blood through a non-invasive mechanism termed vascular mimicry. I have subsequently led a second team through a set of *in vivo* shRNA screens to elucidate the drivers of cancer cell extravasation from the vasculature. This work has identified both systemic free asparagine levels and the asparagine biosynthetic capacity of cancer cells as key drivers of metastasis.

As a principal investigator, I apply computational biology and functional genomics to study the hetero-cellular interactions within tumors that drive resistance to established and experimental therapies. The lab is currently focused on identifying these interactions as they relate to anti-angiogenic therapies and immune checkpoint blockade. To this end, we are applying single-cell profiling methodologies to interrogate murine models of resistance and patient tumor material. Following the identification of novel cellular interactions and corresponding molecular pathways, we apply our novel CRISPR reagents to perturb their function, so that their impact on treatment response can be studied. The overall goal of the laboratory is to improve patient response to treatment by discovering novel ways of interrupting these inhibitory cellular relationships.

a. **Knott SRV***, Wagenblast E*, Kim SY, Soto M, Khan S, Gable AL, Maceli AR, Dickopf S, Erard N, Harrell C, Perou CM, Wilkinson JE, Hannon GJ. Asparagine availability governs metastasis in a model of breast cancer. *In review*. (*first author).

b. Erard N*, **Knott SRV***, Hannon GJ. A CRISPR resource for individual, combinatorial, or multiplexed gene knockout. *Mol Cell*. 2017, 56(6):796-807. (*first author)

c. Wagenblast E, Soto M, Gutiérrez-Ángel S, Hartl CA, Gable AL, Maceli AR, Erard N, Williams AM, Kim SY, Dickopf S, Harrell CJ, Smith AD, Perou CM, Wilkinson JE, Hannon GJ & **Knott SRV**. A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. *Nature*. 2015, 520:358-362.

d. **Knott SRV***, Maceli A*, Erard N*, Chang K*, Marran K, Zhou X, Gordon A, El Demerdash O, Wagenblast E, Kim S, Fellmann C, Hannon GJ. A computational algorithm to predict shRNA potency. *Mol Cell.* 2014, 56(6):796-807. (*first author)

B. Positions and Honors

Positions and Employment

2016-	Assistant Professor, Cedars-Sinai, Los Angeles CA
2014-2016	Research Investigator, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2013-	Consultant, Transomic Technologies Inc., Huntsville, AL
2012-2014	Hope Funds For Cancer Research Postdoctoral Fellow, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2011-2012	Postdoctoral Scientist, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Honors

2017-	Winnick Scholar Award, Cedars-Sinai internal award for translational science
2012-2015	Hope Funds for Cancer Research Postdoctoral Fellow Fellowship
2011	Harrison Kurtz Award, Most outstanding PhD, University of Southern California
2006-2011	Center for Excellence in Genomics PhD Studentship

C. Contribution to Science

1. My earliest contributions to the scientific literature came while I was a PhD candidate studying the regulatory mechanisms governing DNA-replication initiation in *S. cerevisiae*. Replication occurs on a dynamic chromatin template that is actively engaged in orthogonal processes involving protein-DNA interactions. For each of these processes to proceed unimpeded, they must be spatially and temporally coordinated. Eukaryotes achieve this harmonization by implementing a heritable schedule of replication initiation events at loci called replication origins. During my PhD, I developed molecular and computational methods to measure this schedule on a genome-wide scale. I applied these methods to elucidate the role that the histone deacetylase RPD3 plays in regulating origin firing times to coordinate replication and transcription. In addition I identified the precise protein complex that RPD3 acts from to regulate origin initiation. Later in my graduate studies I identified a pair of Forkhead-box transcription factors (Fkh1 and Fkh2) as key regulators of origin firing. Both have been shown to play critical roles in cell cycle dependent transcriptional regulation, however neither had been implicated as a regulator of replication. Functional analyses demonstrated that Fkh1 and Fkh2 bind and recruit replication origins into foci within the nucleus where other proteins required for initiation are enriched. In these foci, origins gain early access to these factors, which in turn promotes their firing. Since the publication of this research, several studies have shown that genome integrity is diminished in regions where this regulation is altered, leading to genetic disease and evolution.

a. **Knott SRV**, Peace JM, Ostrow AZ, Gan Y, Rex AE, Viggiani CJ, Tavaré S, Aparicio OM. Forkhead transcription factors establish origin timing and long-range clustering in S. cerevisiae. *Cell.* 2012, 148(1-2):99-111.

b. Viggiani CJ, **Knott SRV**, Aparicio OM. Genome-wide analysis of DNA synthesis by BrdU immunoprecipitation on tiling microarrays (BrdU-IP-chip) in *Saccharomyces cerevisiae. CSH Protoc* 2010, 2:2010.

c. **Knott SRV**, Viggiani CV, Aparicio OM. To promote and protect: Coordinating DNA replication and transcription for genome stability. *Epigenetics.* 2009, 4(6):362-365.

d. **Knott SRV**, Viggiani CV, Aparicio OM, Tavaré S. Strategies for analyzing highly enriched IP-chip datasets. *BMC Bioinformatics.* 2009a, 10:305.

e. **Knott SRV**, Viggiani CV, Tavaré S, Aparicio OM. Genome-wide replication profiles indicate an expansive role for Rpd3L in regulating replication initiation timing or efficiency, and reveal genomic loci of Rpd3 function in *Saccharomyces cerevisiae*. *Genes Dev.* 2009b, 23(9):1077-1090.

2. When I moved to Cold Spring Harbor Laboratory for my postdoctoral work, I sought to translate the multidisciplinary skillset I had acquired during my PhD into identifying cancer therapeutics, through the use of functional genomic screens. Multiplexed loss-of-function studies, which use shRNAs to elicit target gene suppression, have become a powerful and commonly drawn weapon in the "war on cancer". Early in the development of this technology, the sequence determinants governing shRNA efficacy were unknown, leading to sub-optimal screen results. To remedy this, I applied feature selection and machine learning to a large set of shRNA potency measurements to identify the nucleotide combinations that were most predictive of strength. More importantly, I developed a computational algorithm, based on these variables, for the *in silico* prediction of shRNA efficacy. Through a series of large-scale functional studies, this tool was shown to be superior to others that had been employed to design pre-existing libraries. Based on these results, genome-wide human and mouse shRNA collections were then constructed using this algorithm. These libraries are currently distributed through a commercial partner (Transomic Technologies Inc.) and they are in use by more than 300 academic and industrial labs worldwide.

   Recently, the prokaryotic CRISPR/Cas9 system has been harnessed for functional studies in mammalian cells. Here, the ablation of target function relies on frame-shift mutations resulting from error-prone end joining at Cas9-induced double strand breaks (DSB). Others have shown that repair resolution is, at least partially, driven by recombination between short homologous sequence-pairs flanking the Cas9 cut site. In order to select more effective target sites, I have developed an algorithm that, based on flanking sequences, is capable of predicting the likelihood of frame-shift inducing repair at any DSB. Further, I have determined that a greater suppression of target function can be achieved by focusing Cas9 to evolutionarily conserved regions within genes. This strategy increases the likelihood of non-frame-shift mutation having a detrimental impact on protein function. Functional studies have confirmed the utility of these strategies, and I have applied them to design genome-wide human and mouse CRISPR libraries. These are now in construction and will be distributed commercially to the scientific community.

   a. Erard N*, **Knott SRV***, Hannon GJ. A CRISPR resource for individual, combinatorial, or multiplexed gene knockout. *Mol Cell*. 2017, 56(6):796-807. (*first author)

   b. **Knott SRV***, Maceli A*, Erard N*, Chang K*, Marran K, Zhou X, Gordon A, El Demerdash O, Wagenblast E, Kim S, Fellmann C, Hannon GJ. A computational algorithm to predict shRNA potency. *Mol Cell.* 2014, 56(6):796-807. (*first author)

3. While applying the functional tools detailed above to various models of cancer progression, I became interested in how tumor heterogeneity impacts metastasis and drug resistance. To study these aspects of the disease, I developed a strategy that combines Fluorescence-Activated Cell Sorting and viral barcoding, to enable the tracking of individual cells, within heterogeneous populations, throughout

disease progression. I then led a team to develop a model of breast cancer heterogeneity using this method. Within this model, distinct clones displayed specialization, for example, dominating the primary tumor, contributing to metastatic populations, or showing tropism for entering the lymphatic or vasculature systems. Gene expression profiling revealed that clones which intravasate to the vasculature express two secreted proteins, Serpine2 and Slpi. *In vivo* functional studies showed that these proteins are necessary and sufficient to promote vascular mimicry (VM), where tumor cell lined networks form to provide nutrients while simultaneously providing cells with an escape route to the blood. Finally, we found that the anticoagulant activity of both Serpine2 and Slip likely promotes perfusion of the extravascular networks they promote. Since this time, phase I clinical trial has been launched that is designed to assess the safety of an inhibitor of VM in patients with untreatable cancers.

a. Wagenblast E, Soto M, Gutiérrez-Ángel S, Hartl CA, Gable AL, Maceli AR, Erard N, Williams AM, Kim SY, Dickopf S, Harrell CJ, Smith AD, Perou CM, Wilkinson JE, Hannon GJ & **Knott SRV**. A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. *Nature*. 2015, 520:358-362.

4. The study detailed above was focused on understanding drivers of intravasation. In a search for drivers of the later stages of metastasis, I performed an *in vivo* functional screen that assayed for the ability of cells to exit the vasculature and colonize lung metastases. The screen was focused on genes that differentiated circulating breast tumor cells in their ability to perform this phenotype. Within the candidate gene list identified in the screen, Asparagine Synthetase (Asns) was the most prognostic of patient relapse. Asns is responsible for the intracellular conversion of Asparatate into Asparagine. Silencing of Asns resulted in cells having a reduced capacity to invade *in vitro* and a significantly decreased ability to form metastases. Increasing asparagine availability through *ex vivo* supplementation or enforced expression of Asns, resulted in increased invasiveness. Further, when asparagine was depleted *in vivo*, through the administration of l-asparaginase, a significant reduction of metastases was observed. Finally, Asns silencing in combination with l-asparaginase treatment resulted in complete ablation of metastases in the lung. The silencing of Asns causes a reduction in the abundances of asparagine-rich proteins. Epithelial-to-mesenchymal transition (EMT) promoting proteins, which have been implicated in disease spread, were enriched in the asparagine-dependent set due to their high asparagine content. Further, we determined that the majority of mammalian EMT promoting orthologs are enriched for asparagine content. To my knowledge this is the first reported instance of EMT being regulated by the availability of a single amino acid.

a. **Knott SRV***, Wagenblast E*, Kim SY, Soto M, Khan S, Gable AL, Maceli AR, Dickopf S, Erard N, Harrell C, Perou CM, Wilkinson JE, Hannon GJ. Asparagine availability governs metastasis in a model of breast cancer. *In review*. (*first author).

C. Research Support

Completed Research Support

Hope Funds for Cancer Research Postdoctoral Fellowship 12/01/12/-11/30/15
HFFCR
Combinatorial shRNA screens to identify synergistic drivers of disease progression in Pancreatic Cancer.
Role: Postdoctoral Fellow

Ongoing Research Support

Cedars-Sinai Medical Institute-Faculty Startup Package, Knott (PI) 09/01/16-08/30/21
CSRI
Functional genomics approaches to study tumor heterogeneity and metastasis
Role: Principal Investigator

Winnick Scholar Research Award 03/10/17-02/28/19
CSRI
Clonal analysis of hetero-cellular interactions in breast and ovarian cancer
Role: Principal Investigator