NIST-ABMS
Workshop to Develop a Metrology Roadmap for Synthetic Biology

July 12, 2013, Imperial College, London, United Kingdom

Hosted by the National Institute of Standards and Technology - Advances in Biomedical Measurement Science (ABMS) Program

Co-hosted by Imperial College, the National Academy of Sciences, and the BioBricks Foundation

Organizers
Sarah Munro, NIST-ABMS Program
Marc Salit, NIST-ABMS Program

Participants
Catherine Ainsworth, Imperial College
Jacob Beal, BBN Technologies
Parag Chitnis, National Science Foundation
Vincent Danos, University of Edinburgh
Kim de Mora, iGEM Foundation
Camille Delebecque, Synbio Consulting
Rob Dickinson, Imperial College
Alistair Elfick, University of Edinburgh
Drew Endy, Stanford University & BioBricks Foundation
Douglas Friedman, National Academy of Sciences
Emma Frow, University of Edinburgh
India Hook-Barnard, National Academy of Sciences
Richard Johnson, Global Helix & BioBricks Foundation
Linda Kahl, BioBricks Foundation
Jason Kelly, Ginkgo Bioworks
Steve Laderman, Agilent Technologies, Inc.
Carlos Olguin, Autodesk
Megan Palmer, Synberc
Randy Rettberg, iGEM Foundation
Lynn Rothschild, NASA
Max Ryadnov, National Physical Laboratory
Ben Sheridan, BSI
Reshma Shetty, Ginkgo Bioworks
Christina Smolke, Stanford University

Executive Summary

The NIST-ABMS Workshop to Develop a Metrology Roadmap for Synthetic Biology was held at Imperial College, London, United Kingdom on July 12, 2013 immediately following the Sixth International Meeting on Synthetic Biology (SB 6.0). The intention of this workshop was to develop the critical components necessary for developing an actionable synthetic biology metrology roadmap. The first component is a prioritized list of essential metrology infrastructure – new measurement technologies, reference materials, reference data, reference methods, and validated predictive models. The second component is a list of the biological engineering capabilities that will be enabled by building this metrology infrastructure. An actionable roadmap will enumerate the developments in measurement science and infrastructure, and will identify how these advances will improve our ability to engineer biological systems.

This workshop report summarizes the observations of the 26 workshop participants, a diverse group from academic, government, industry, and non-profit organizations. This report does not necessarily reflect the perspectives of the organizations that the participants represent. Furthermore, it was agreed that this report would be shared broadly to solicit input from synthetic biology stakeholders who could not be present at this initial meeting.

The charge to the workshop participants was to collaboratively describe the aforementioned components needed for a synthetic biology metrology roadmap. The metrology chapter of the International Technology Roadmap for Semiconductors (ITRS) was held up as model to guide the discussion. The presentation describing the charge to the workshop, the invitation letter, and the agenda are provided at the end of this document.

The top 3 identified metrology infrastructure needs were:

1. Measurement technology and method development for high-throughput analysis at the single-cell level, with the specific objective of measuring diversity of function within a cell population.

2. DNA fabrication standards that will provide metrics beyond cost per base and error rate of gene synthesis, such as cost per variant, turn time, or validated function

3. Standards for comparing performance of engineered biological systems

Two distinct, but linked, ways of assessing progress of the field and impact of developing metrology resources were identified:

- Applied capabilities that are directly related to commercial applications of engineered biological systems
- Foundational capabilities that may or may not support current or future commercial applications

Both types of capabilities should be included in a comprehensive and actionable metrology roadmap, although there was not agreement over the relative importance of the two categories.

We welcome your feedback on this initial report and we solicit your suggestions for concrete actions that can take us another step forward toward building an actionable metrology roadmap for synthetic biology.

I. Prioritizing metrology infrastructure needs

A non-exhaustive list of potential metrology infrastructure needs was elicited from workshop participants. This list includes needs for reference materials, data, and methods as well as new measurement technologies. The diverse group also discussed sociological, intellectual property, and procedural considerations for measurement science needs in synthetic biology. The list of concepts provided by participants is shown in Table 1 and is rank-ordered based on participant votes. Each participant had an equal number of votes that they could distribute amongst the entire list as desired. Prior to voting the list of concepts were grouped into the categories, Design, Build, Test, Scale, and Use and if participants proposed similar concepts those concepts were grouped together for voting.[*] It was noted that "simulate" is a key component of the design-build-test cycle. Better measurements will lead to improved simulation capabilities with validated predictive models and accelerate the design-build-test cycle. It was also noted that the list of metrology needs is meant to build a roadmap for the synthetic biology community; it is not work that would be done solely by metrology institutes such as the National Institute of Standards and Technology (NIST) or the National Physical Laboratory (NPL).

The highest priority need was measurement technology and method development for the purpose of characterizing diversity of function in cell populations. Ideally these measurement technologies would enable high-throughput, rapid, single cell analysis techniques, and would provide the characteristics of measurement comparability (traceability and measurement uncertainty).

The second and third most highly ranked metrology needs were standards for DNA fabrication and standards for comparison of engineered cells. These standards could be a combination of reference materials, reference data, and reference methods. For DNA fabrication a "SYNPACK" benchmark was proposed, similar to the computing benchmark LINPACK. It was agreed that current metrics, such as cost per base, are insufficient and new metrics for DNA fabrication such as cost per variant, turn time, or functional validation of sequences are needed. For comparison of engineered strains, reference methods to quantify small molecule productivity and yields are needed. This type of comparison is critical for choosing which strains will be best to scale in an industrial process.

[*] Reshma Shetty and Sarah Munro organized the list of measurement needs

Other high priority needs included measurements of metabolites (e.g. *in vivo* biosensors), fundamental processes (e.g. transcription) and cellular resources and loads. Ideally these types of measurements would be *in vivo* and transferable – not limited to a single class of metabolites or a species of organisms.

II. Identifying biological engineering capabilities that would be enabled by new metrology infrastructure

Participants considered what improved biological engineering capabilities could be linked to metrology infrastructure developments. These were in two categories, applied capabilities directly related to current commercial applications of engineered biological systems and foundational capabilities that may or may not have immediate or future commercial impacts. There was tension between a desire to focus a roadmap on the near-term applied capabilities and a desire to describe foundational capabilities that may or may not have immediate commercial impacts. Concern was also expressed that it may be too early to delineate any capability indicators, because we may push research efforts in the wrong direction. By limiting scope to current synthetic biology industrial applications we might miss the opportunity to foster a phase-change for the industry by exploring foundational engineering capabilities. Conversely if we encourage impractical and abstract target capabilities with unclear applications, we could hinder the growth of both the current, and future, synthetic biology industry paradigms. It is possible that by acknowledging these issues and considering both types of capabilities in a metrology roadmap for synthetic biology we can avoid these potential pitfalls.

Biological engineering capabilities that could be enumerated and quantified in a metrology roadmap include:

- Achieving commercially viable synthetic biology production methods for chemical intermediates, e.g. top 12 biomass-derived intermediates identified in 2004 DOE report.[1] Important metrics to consider for comparison with non-biological methods for deriving these chemical intermediates include achieving economic parity (or better), as well as land use and environmental footprint.
- Expansion to novel material science and biomedical applications beyond the existing small molecule and pharmaceutical markets, such as links between biological materials and electronics, identifying and producing new natural products for medical applications, or cell-based therapeutics.
- A "phase-change" in the breadth and scale of the synthetic biology industry
    - Expansion of the types of organisms that can be engineered, beyond the small cohort of currently used organisms

[1] Werpy, T., Petersen, G., Aden, A., et al. (2004). Top Value Added Chemicals From Biomass. Volume 1-Results of Screening for Potential Candidates From Sugars and Synthesis Gas, 76. Retrieved from http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA436528

    - Movement from large-scale centralized production toward distributed production
- DNA synthesis capabilities – how much, how fast, how accurate?
- Efficiency of DNA integration into an engineered biological system
- Precision of expression control
- Number of orthogonal regulatory elements
- Number of input channels/computational scale/output channels
- How long does it take to build a genetic program?
- Evolvability/Mutation robustness – control of mutation rates would have implications for biosafety and industrial scale production
- Control of population diversity – applications in control of the human microbiome and environmental engineering, etc.

Conclusion

During this initial workshop participants worked to develop two critical components of a metrology roadmap for synthetic biology; prioritizing metrology infrastructure needs and identifying biological engineering capabilities that would be enabled by new metrology infrastructure. Input from the broader synthetic biology community is requested. We solicit both your suggestions for further refinement of the content of this report and specific actions that would bring us closer to developing a metrology roadmap.

Table 1. Identified and prioritized measurement needs from participants

Type	Measurement Need	Votes
Test	Representations for genetic (function) diversity (population composition and evolution thereof)	11
	HT + single cell measurements that can scale	
	Turn-key non-invasive imaging and tracking of large #s of single cells	
	High-throughput single cell measurements of protein or other chemical concentrations	
	Establish comparability for flow cytometry	
	High throughput 'omics, preferably single cell	
	Direct noninvasive rapid single-cell quantitative measurements of RNA, protein and metabolites	
Build	"SynPack" – DNA Fab benchmarks, $, time, accuracy (analogue to LINPACK benchmarks for computing)	10
	Standards for assessing the performance of gene synthesis beyond cost per bp and error rate Cost per rational junction Cost per variant – how to account for level of diversity Turn time	
Scale	Comparable methods for measurement of strain productivity & yield for small molecules	8
	Standards for measuring and benchmarking the performance of strains that make small molecules	
Test	Direct measure of RNA pol along DNA in live cells	7
	Standard unit of gene expression – like a kg or meter, perhaps several to compare against different chassis – *E. coli*, yeast, mouse	
Test	Rapid, quantitative measurement of novel metabolites (when don't know exact chemical structure)	6
	Genetically encoded sensors to all primary and secondary metabolites,	
	Biosensors for in vivo monitoring and sensing of many kinds and multiplexed	
Test	Measurement for the load a construct places on a cell	5
	Measurement for maximum available cellular resources	
Use	Incentives to adopt metrology and standards	4
Test	Standard breadboard system that scales complexity	3
	Standard cell-free solution for characterization	
	Need standard environment for measurement, just like STP	
	Unpack black box of expression Measure each step, single molecule under known conditions, [NTP], temp, linear or circular DNA, binding, initiation, degradation	

Type	Measurement Need	Votes
Design Use	Reliable methods/tools for checking IP status of biological components and designs	3
	Standards for disclosure of patents that cover ("read on") standards adopted or being considered for adoption by the SB research community	
Build Test	Standard open vector backbone library, standard starter kit of pre-instrumented organism	3
Test	Gene expression reference objects covering top 5 operational variables	3
Use	Safety & efficacy measurements for deliberate environmental release of organisms	3
	Characterization of environmental effects/functions including and beyond organismal fitness, gene flow	
Design	Definitions of terms in synthetic biology, i.e. what is a part, etc.	3
	Ontological framework and characterization data for gene functions (that go way beyond species origin, if any)	
Design	Data types for part characterization	2
Design	Design rules and associated data and measurements	2
Use	Metrology for assessing the biosafety level of parts & devices & genes (regulatory science)	1
	Standard function-level classification of virulence pathogenicity at the genetic function level, so we don't do risk/safety screening based on species origin alone	
Use	Standard for "best practice in responsible innovation"	1
	Best practice for incorporating consumer/user needs into upstream design	
Use	Measurements to bridge + link academic/company – for-profit divides	1
Use	The 'meaning' of biological information needs to be agreed + understood and also machine readable	1
Use	Can we define Shannon Entropy of biological systems? If not can we define the biological equivalent/analogue for the Shannon Entropy	1
Use	Evolvable standards – our understanding of biology is not fixed	–
Design	Characterization DNA "Commons": Units, methods, interoperable/shared, reference materials/data sheets	–
Design	Need standard genetic background	–
	Standard strain use: in vitro work is very valuable, but much work will remain in vivo (in which case correct strains need to be used)	
Test	"Stimulate –> Response" test methodologies at the single cell level, multi-cell level	–

Type	Measurement Need	Votes
Test	Measurement of toxicity a construct places on a cell	–
Scale	Set of standard fermentation conditions	–
	Set of standard feedstock, medium formulations	
Use	What would be the end product i.e. reference methodology versus reference materials? - Is the priority to have intercomparison studies? Would useful reference materials be molecules (genes, proteins) or sources (cell lines)?	–
Test	Automation – so we can collect very large amounts of data, only with enough data may we get to a good level of understanding	–
Test	Reference methods for analysis, calibration, & controls, e.g. removing background auto-fluorescence – large variations with strain	–

NIST/ABMS Workshop to Develop a Metrology Roadmap for Synthetic Biology

Co-hosted by
Imperial College London
BioBricks Foundation
National Academy of Sciences

Who are we?

National Institute of Standards and Technology

- U.S. National Measurement Institute
  - U.S. Department of Commerce
  - Advance "measurement science, standards, and technology"
  - Non-regulatory
- 6 research laboratories

Advances in Biomedical Measurement Science "ABMS" Project

LELAND STANFORD JUNIOR UNIVERSITY
DIE LUFT DER FREIHEIT WEHT
1891

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

Founding Affiliates:

Agilent Technologies

life technologies™

Worldview

Systematic approach that lets me know how confident I can be in the results; when I know how confident I can be, I can make good decisions.

- Traceability
- Measurement Uncertainty
- Method Validation

CHARGE TO THE WORKSHOP

We could be wrong about all of this.

Disclaimer

Takeaways

- Consensus "Canonical" Engineering Process
  - organizing tool for communication
  - framework for identifying metrology needs
- List of Metrology Needs
  - current, existing measurements that need…
    - characterization
    - improvements
    - comparability
  - new measurements
    - things we can't measure today that we need
- Possible SB Engineering Performance Parameters
  - Complexity of a refactored genetic program?
  - Size of a perfect synthetic DNA object?
  - Turn time for design, build and test of a new design?
  - How many designs can be cycled in parallel?
  - How many D-B-T cycles before something works?
  - How reusable is a genetic object/program/module?
    - Consideration of *Practices*

ITRS Metrology Roadmap

one chapter in a larger technology roadmap

INTERNATIONAL
TECHNOLOGY ROADMAP
FOR
SEMICONDUCTORS

2011 EDITION

METROLOGY

THE ITRS IS DEVISED AND INTENDED FOR TECHNOLOGY ASSESSMENT ONLY AND IS WITHOUT REGARD TO ANY COMMERCIAL CONSIDERATIONS PERTAINING TO INDIVIDUAL PRODUCTS OR EQUIPMENT.

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Detailed measurement needs and projections…

Table MET2 Metrology Technology Requirements

Year of Production	*2009*	*2010*	*2011*	
Flash ½ Pitch (nm) (un-contacted Poly)(f)	38	**24**	**22**	
Microscopy				
Inline, nondestructive microscopy process resolution (nm) for P/T=0.1	**3.8**	**2.4**	**2.2**	
Microscopy capable of measurement of patterned wafers having maximum aspect ratio/diameter (nm) (DRAM contacts) [A]	**17**	**>20**	**>20**	
	60	**50**	**40**	
Materials and Contamination Characterization				
Real particle detection limit (nm) [B]	**20**	**18**	**16**	
Minimum particle size for compositional analysis (dense lines on patterned wafers) (nm)	**17**	**15**	**13**	
Specification limit of total surface contamination for critical GOI surface materials (atoms/cm^2) [C]	**5.00E+09**	**5.00E+09**	**5.00E+09**	**5.0**
Surface detection limits for individual elements for critical GOI elements (atoms/cm^2) with signal-to-noise ratio of 3:1 for each element	**5.00E+08**	**5.00E+08**	**5.00E+08**	**5.0**

Manufacturable solutions exi

Semiconductor Fab Technology Requirements

Table MET3 Lithography Metrology (Wafer) Technology Requirements

Year of Production	2009	2010	2011	2012	2
Flash ½ Pitch (nm) (un-contacted Poly)(f)	38	24	22	20	
DRAM ½ Pitch (nm) (contacted)	45	40	36	32	
MPU/ASIC Metal 1 (M1) ½ Pitch (nm) (contacted)	54	45	38	32	
MPU Printed Gate Length (GLpr) (nm) ††	47	41	35	31	
MPU Physical Gate Length (GLph) (nm)	29	27	24	22	
ASIC/Low Operating Power Printed Gate Length (nm) ††	54	47	41	35	
ASIC/Low Operating Power Physical Gate Length (nm)	32	29	27	24	
ASIC/Low Standby Power Physical Gate Length (nm)	38	32	29	27	
MPU Etch Ratio GLpr/GLph (nm)	1.6039	1.5296	1.4588	1.4237	1.
Wafer minimum Overlay control DRAM single litho tool (nm)	9.0	8.0	7.1	6.4	
Wafer overlay output metrology uncertainty (nm, 3 σ)* P/T=.2	1.8	1.6	1.4	1.3	
Gate (MPU Physical Gate Length)					
Printed gate CD control (nm), Uniformity (variance) is 12% of CD. Allowed lithography variance = 3/4 total variance of physical gate length *	3.0	2.8	2.5	2.3	
Wafer CD metrology tool uncertainty (nm) * 3σ at P/T = 0.2 for isolated printed and physical lines [A]	0.60	0.55	0.50	0.46	
Etched Gate Line Width Roughness (nm, 3 σ) < 8% of CD **	2.32	2.12	1.94	1.77	
Wafer CD metrology tool uncertainty for LWR (nm), P/T=0.2	0.46	0.42	0.39	0.35	
Dense Line (Flash 1/2 pitch, un-contacted poly)					

What are the SB Performance Parameters? (*Technology Requirements*)

- How do they evolve?
  - what new applications do the evolved parameters enable?
- What metrology needs to happen to enable this evolution?

Shape of the day

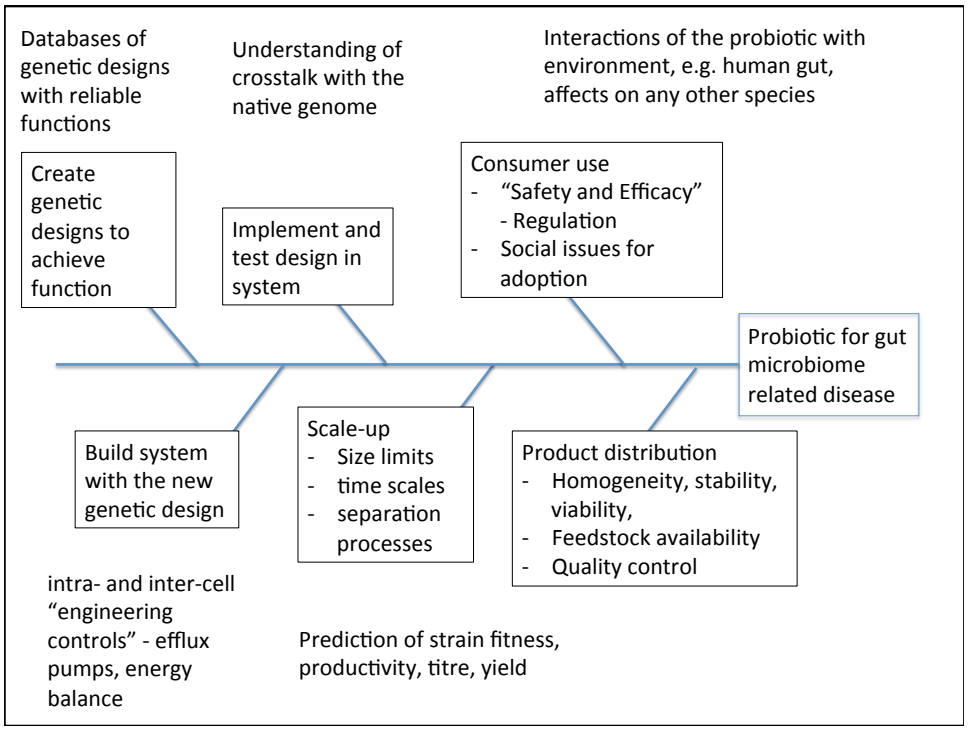
- Opening remarks, charge to the workshop
- Synthetic biology (SB) engineering processes
- Consensus SB engineering process
- Identify measurement strengths & gaps
- **Break**
- Prioritize list of measurement gaps
- How should we quantify our ability to engineer biology?
  - *What does success look like?*
- Working **lunch** discussion
  - *What would an ideal SB engineering process look like?*
- Close the Loop
  - *How will new metrology impact our ability to engineer biology?*

SB Engineering Process

- describing the engineering process for SB in consensus, generic way…
  - will let us identify design rules
  - will let us talk about what measurements are needed at what point in the process, and what difference they'll make.

What does an SB Engineering Process look like?

- Process diagram of how you would get to creating a product via synthetic biology
- You could be abstract or use an existing product or something novel
  - Coat made by bacteria that consists of cellulose
- What are the steps (and cycles) that are needed to get there?
- Don't worry too much about it being artistic, you could just use text instead.
- What is common across different types of industrial SB processes?
- Converge to a consensus model
- Then we can identify measurements and standards that we need to create to enable the industry to grow.



Dear colleagues,

There have been numerous calls to develop metrology for synthetic biology – infrastructure such as new measurement technologies, reference materials, reference data, reference methods, and validated predictive models. We invite you to join us for an informal, invitation-only workshop to identify and prioritize metrology needs. The workshop will be held following SB6.0 at Imperial College, London, UK on Friday July 12, 2013 from 8:00am – 2:00 pm.

The intended outcome of this workshop is an actionable metrology roadmap – a set of goals for metrology resources in synthetic biology. This roadmap should detail how meeting these goals will improve our ability to engineer biology. To ensure an effective and targeted discussion we request that each participant present one slide describing their vision of the process of engineering a biological system for a commercial application. Please expand upon the design-build-test paradigm to describe additional critical steps needed to create a commercially viable synthetic biology product or technology, such as scaling production or product distribution. By integrating your diverse and valuable perspectives to describe a consensus synthetic biology enterprise process model we can begin to identify the gaps and the most needed metrology infrastructure.

We hope you can join us in what we believe is a critical step toward growing our global bioeconomy – building a synthetic biology metrology roadmap. If you cannot attend please feel free to suggest a different participant from your organization for us to invite to this workshop. Please RSVP at your earliest convenience and email your slide to sarah.munro@nist.gov by July 3, 2013.

Best regards,

Marc Salit, Ph.D. and Sarah A. Munro, Ph.D.

Biosystems and Biomaterials Division

National Institute of Standards and Technology

Advances in Biomedical Measurement Science Program

Stanford University

NIST-ABMS Workshop to Develop a Metrology Roadmap for Synthetic Biology

Co-hosted by
National Academy of Sciences
BioBricks Foundation
Imperial College London

Sherfield Hall, SALC 6
Friday July 12, 2013
08:00 AM – 02:00 PM

08:00 – 08:30	Opening remarks and charge to the workshop
08:30 – 09:30	Participants present their typical synthetic biology (SB) engineering processes
09:30 – 09:50	Break
09:50 – 10:30	Derive consensus SB engineering process
10:30 – 11:00	Use consensus process as a framework to identify measurement strengths & gaps
11:00 – 11:15	Prioritize list of measurement gaps
11:15 – 12:00	How should we quantify our ability to engineer biology? *What does success look like?*
12:00 – 01:00	Working lunch discussion *What would an ideal SB engineering process look like?*
01:00 – 02:00	Close the Loop *How will new metrology impact our ability to engineer biology?*