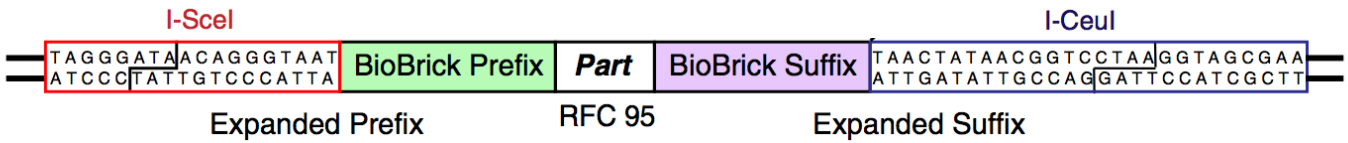
The case for decoupling assembly and submission standards to maintain a more flexible registry of biological parts



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Presented by Heejo Choi
Week 12 | 2015 April 29

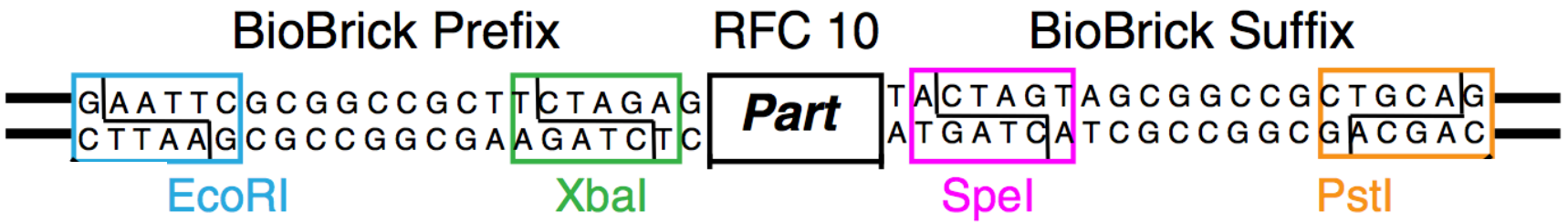
BioBrick's Request for Comments (RFC) Process hosts discussions for the development of standards

- BioBricks Foundation manages the Registry of Standard Biological Parts
  - Submission standards – requirements on DNA sequence
  - Assembly standards – procedure to combine biological parts

- RFCs communicate requests for new standards
  - Propose a standard
  - Describe protocols
  - Comment, extend, or replace previous RFC

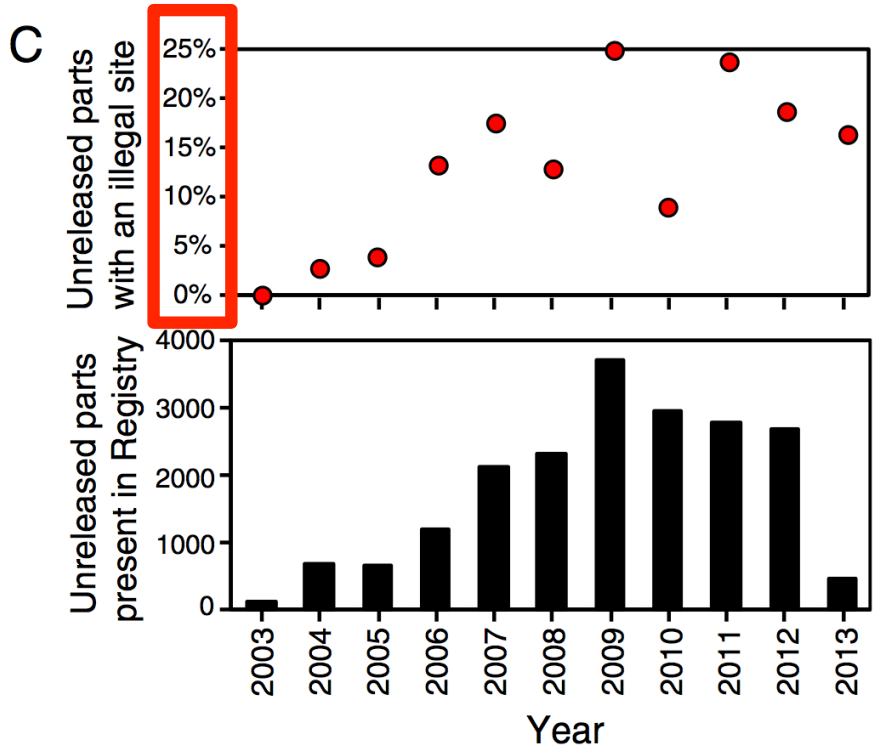
- 100+ RFCs documented today

Current Standard: RFC 10 constitutes a combined assembly and submission standard



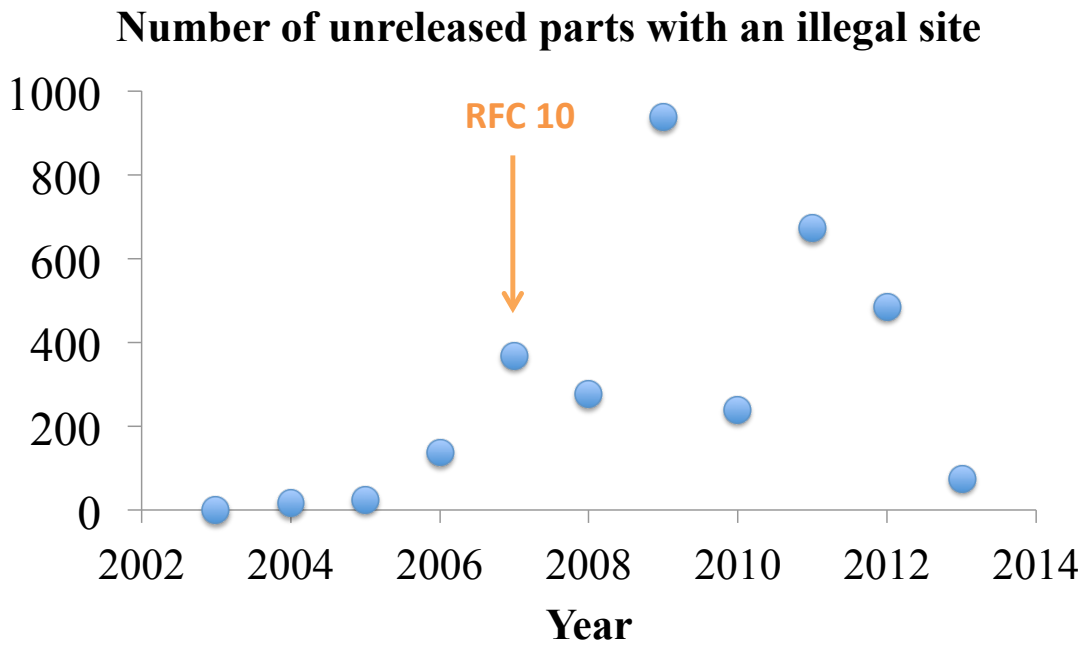
- Parts must be flanked by prefix and suffix sequences containing restriction enzyme sites for assembly
- These illegal restriction sites must not be present within the sequence

Recent trends suggest a growing number of unreleased parts due to an illegal site



- Data represents parts submitted by July 29, 2013
- "suggest a significant and growing burden" to adhere RFC 10

Recent trends weakly suggest a growing number of unreleased parts due to an illegal site



Data represents parts submitted by July 29, 2013

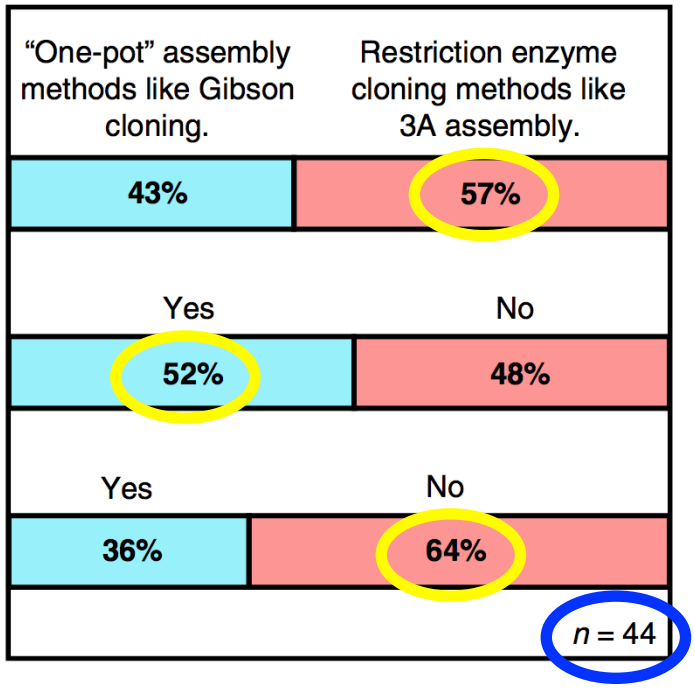
Adapted from Figure 1C

RFC 95 may alleviate the burden of removing illegal restriction enzyme sites prior to part submission

What DNA assembly methods does your iGEM team most commonly use?

Has your team used site-directed mutagenesis to remove illegal restriction sites from a part to submit it to the Registry?

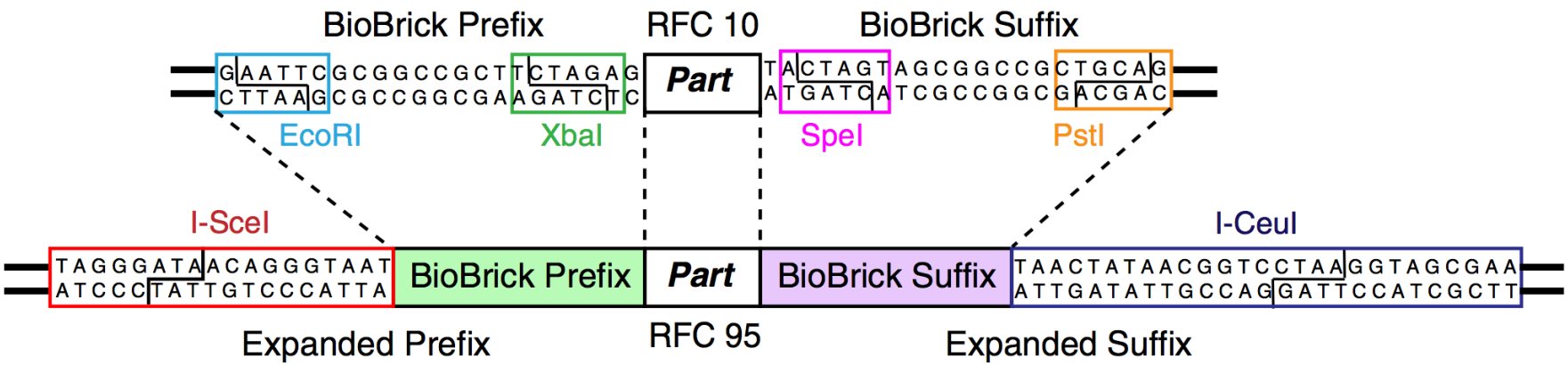
Has your team decided not to submit a part to the Registry due to the presence of illegal restriction sites?



- ~1850 participants (*iGEM 2013 Annual Review*)
- < 3% survey participation

RFC 95 proposes a submission standard decoupled from assembly

A



- Homing endonucleases recognize (and cleave) longer target sequences
  - Less likely to find illegal sites within the Part
- RFC 95 can be backwards compatible with RFC 10
  - Flanking current restriction enzyme sites with homing endonuclease sites

Assumptions

The group's claims rest on the following **assumptions**:

- Field is currently limited by the number of available parts
  - Perhaps expanding number of available parts is not the limiting factor
  - True challenge is defining the quality, characterization, and documentation of parts (Kwok 2010)

- Transition from RFC 10 to RFC 95 is larger than implied
  - RFC 95 is not always backwards-compatible with RFC 10
  - Illegal sites within the part must still be removed
  - Majority of teams use restriction enzyme cloning methods

Concerns and Suggestions

Concerns:

- Survey data rests on < 3% response
- Paper may be ahead of its time (field is not limited by the number of available parts)

Suggestions:

- Acquire more data points for survey (Figure 2)
- Clarity of how data in Figure 1C
- Consider mandatory questionnaire for a representative random pool
- Ask direct questions:
  - "Would your team prefer to use RFC 95 as the standard rather than RFC 10?"
  - "Does your team feel the Registry needs more standard parts?"

Significance of data curation & information accessibility

- Registry of Standard Biological Parts is ever growing
  - Philosophy of “Get, Give & Share”
- Standardization ensures compatibility of parts when creating longer, more complex parts
- Curation process is limited by quality part characterization
  - Exemplified by diverse degree of detail for logic gate entries in SynBioLGDB

Significance of anticipating change

"For a genetic parts repository and registry to remain relevant as technology progresses, it should anticipate these changes and adapt its methods to complement them."

Future Work

- RFC 98 (rapid assembly of RFC 10 compatible parts in a single reaction)
- RFC 104 (rapid assembly of RFC 10 parts without using restriction enzymes)

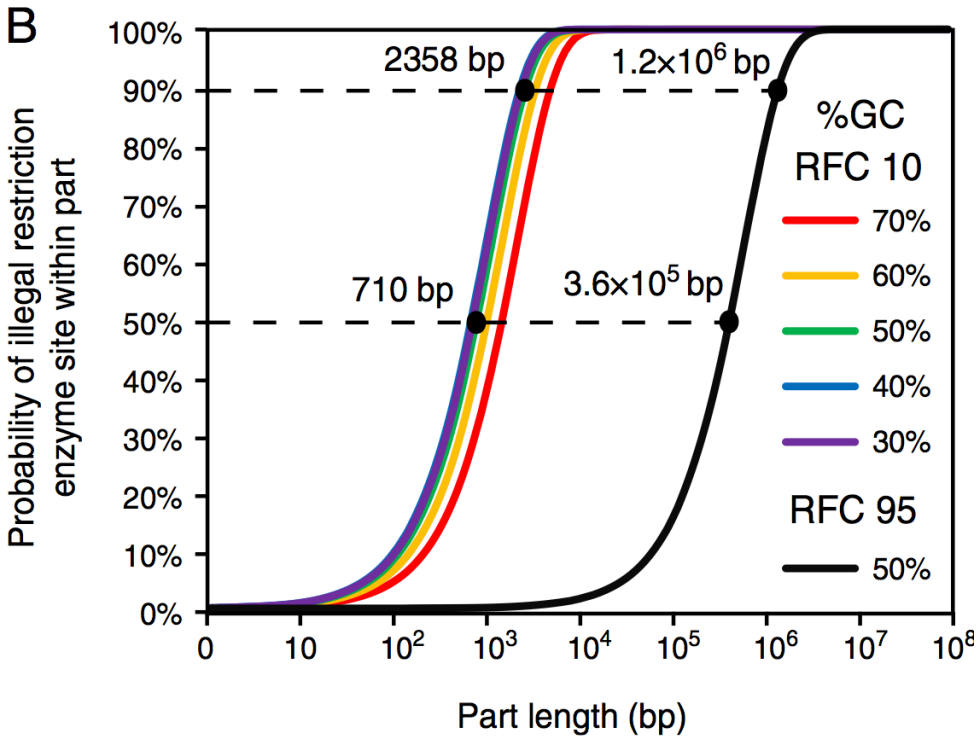
The Backstory from M. Hammerling (2012 UT Austin iGEM Team)

- Submitted parts were rejected due to illegal sites

- "While we made it to the international competition at MIT that year, the team was unwilling to completely re-engineer a perfectly functional and well-characterized biological part to comply with what we viewed as an arbitrary sequence constraint. This limited how much success we were able to achieve at the international level that year. "

- "…introduce a new submission standard that would fix this problem and allow teams to focus on developing novel, useful parts rather than mutating the sequences of their parts to comply with the current submission standard."

Utilizing homing endonuclease sites reduces the probability of encountering a illegal site within part



- Restriction enzyme sites (6-8 bp)
- Homing endonuclease sites (15-30 bp)

Most used rank	Part	Length (bp)	P(illegal site in part)
1	RBS	12	2-3%
2	Terminator	129	4-12%
9	GFP	720	50%

from Top 10 Most Used Parts on the Registry (http://parts.igem.org/Frequently_Used_Parts)