Towards a High-Level Programming Language for Standardizing and Automating Biology Protocols

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Genetic Control of Surface Curvature

Utpal Nath, Brian C. W. Crawford, Rosemary Carpenter,
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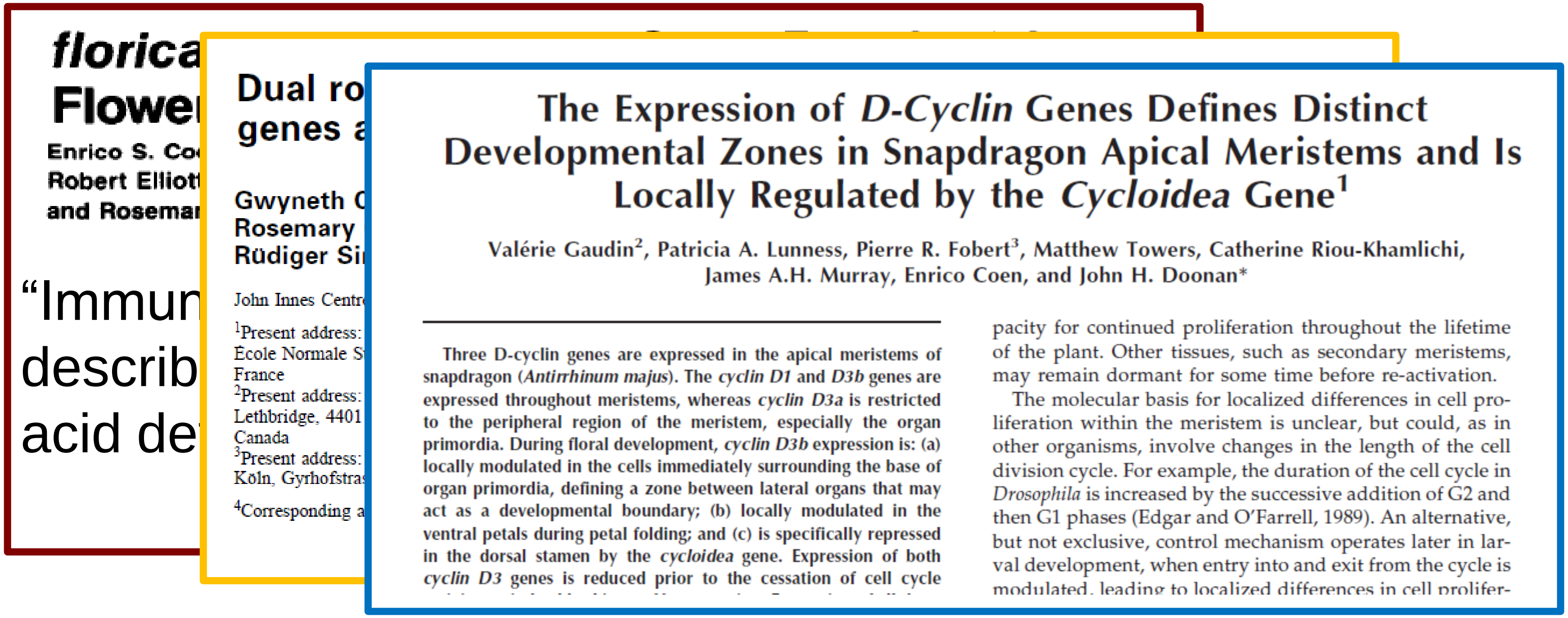
Material and Methods

***In situ* Hybridization.** The methods used for tissue preparation, digoxigenin-labelling of RNA probes, and *in situ* hybridisation were as described previously (*S13*). The probe used



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[4]Corresponding a

The Expression of *D-Cyclin* Genes Defines Distinct Developmental Zones in Snapdragon Apical Meristems and Is Locally Regulated by the *Cycloidea* Gene[1]

Valérie Gaudin[2], Patricia A. Lunness, Pierre R. Fobert[3], Matthew Towers, Catherine Riou-Khamlichi,
James A.H. Murray, Enrico Coen, and John H. Doonan*

Three D-cyclin genes are expressed in the apical meristems of snapdragon (*Antirrhinum majus*). The *cyclin D1* and *D3b* genes are expressed throughout meristems, whereas *cyclin D3a* is restricted to the peripheral region of the meristem, especially the organ primordia. During floral development, *cyclin D3b* expression is: (a) locally modulated in the cells immediately surrounding the base of organ primordia, defining a zone between lateral organs that may act as a developmental boundary; (b) locally modulated in the ventral petals during petal folding; and (c) is specifically repressed in the dorsal stamen by the *cycloidea* gene. Expression of both *cyclin D3* genes is reduced prior to the cessation of cell cycle

pacity for continued proliferation throughout the lifetime of the plant. Other tissues, such as secondary meristems, may remain dormant for some time before re-activation.

The molecular basis for localized differences in cell proliferation within the meristem is unclear, but could, as in other organisms, involve changes in the length of the cell division cycle. For example, the duration of the cell cycle in *Drosophila* is increased by the successive addition of G2 and then G1 phases (Edgar and O'Farrell, 1989). An alternative, but not exclusive, control mechanism operates later in larval development, when entry into and exit from the cycle is modulated, leading to localized differences in cell prolifer-

Problems with Existing Descriptions of Protocols

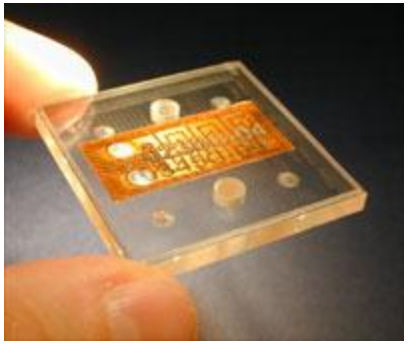
- **Incomplete**
  - Cascading references several levels deep
  - Some information missing completely
- **Ambiguous**
  - One word can refer to many things
  - E.g., “inoculate” a culture
- **Non-uniform**
  - Different words can refer to the same thing
  - E.g., “harvest”, “pellet down”, “centrifuge” are equivalent
- **Not suitable for automation or for programming standard biological parts**

Towards a High-Level Programming Language for Biology Protocols

Goal: in scientific publications, replace textual description of methods used with code

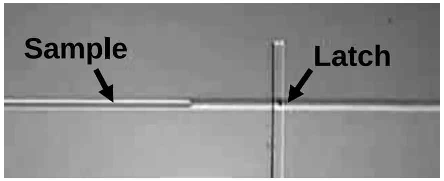
1. Enable automation
via microfluidic chips

2. Improve reproducibility
of manual experiments

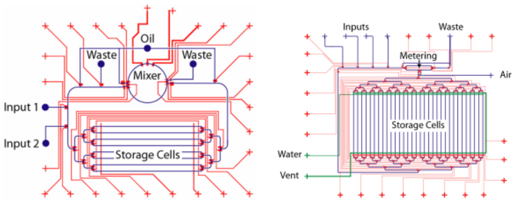


Contributions to Date

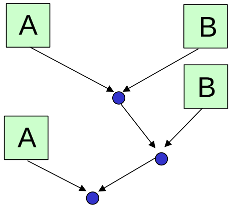
- **Microfluidics:** first manipulation of discrete samples using soft-lithography [LabChip’06]



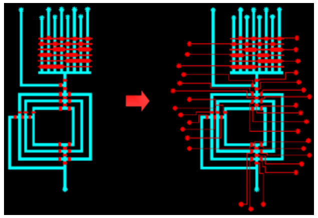
- **Programming:** first mapping of single ISA across different chips [DNA’06, NatCo’07]



- **Optimization:** first efficient algorithm for complex mixing on chip [DNA’06, NatCo’07]



- **Computer Aided Design:** first tool that routes channels, generates GUI [MIT’09]



- **Work in Progress:** programming language for expressing and automating broad class of experiments

The BioStream Language

- **BioStream is a protocol language for reuse & automation**
  - Portable
  - Volume-independent
- **Initial focus: molecular biology**
  - Mixing
  - Heating / cooling
  - Cell culture
  - Centrifugation
  - Electrophoresis
  - Timing constraints
- **Implemented as a C library**
  - Used to express 15 protocols
  - Initial backend: emit readable instructions for human
- **Validation in progress**
  - Intern at Indian Institute of Science
  - Would represent first biology experiment grounded in architecture-independent programmed description

Language Primitives

- **Declaration / measurement / disposal**
  - declare_fluid
  - declare_column
  - measure_sample
  - measure_fluid
  - volume
  - discard
  - transfer
  - transfer_column
  - declare_tissue
- **Combination / mixing**
  - combine
  - mix
  - combine_and_mix
  - addto_column
  - mixing_table
- **Centrifugation**
  - centrifuge_pellet
  - centrifuge_phases
  - centrifuge_column
- **Temperature**
  - set_temp
  - use_or_store
  - autoclave
- **Timing**
  - wait
  - time_constraint
  - store_until
  - inoculation
  - invert_dry
- **Detection**
  - ce_detect
  - gas_chromatography
  - nanodrop
  - electrophoresis
  - mount_observe_slide
  - sequencing

Example: Plasmid DNA Extraction

I. Original protocol *(Source: Klavins Lab)*

Add 100 ul of 7X Lysis Buffer (Blue) and mix by inverting the tube 4-6 times. *Proceed to step 3 within 2 minutes.*

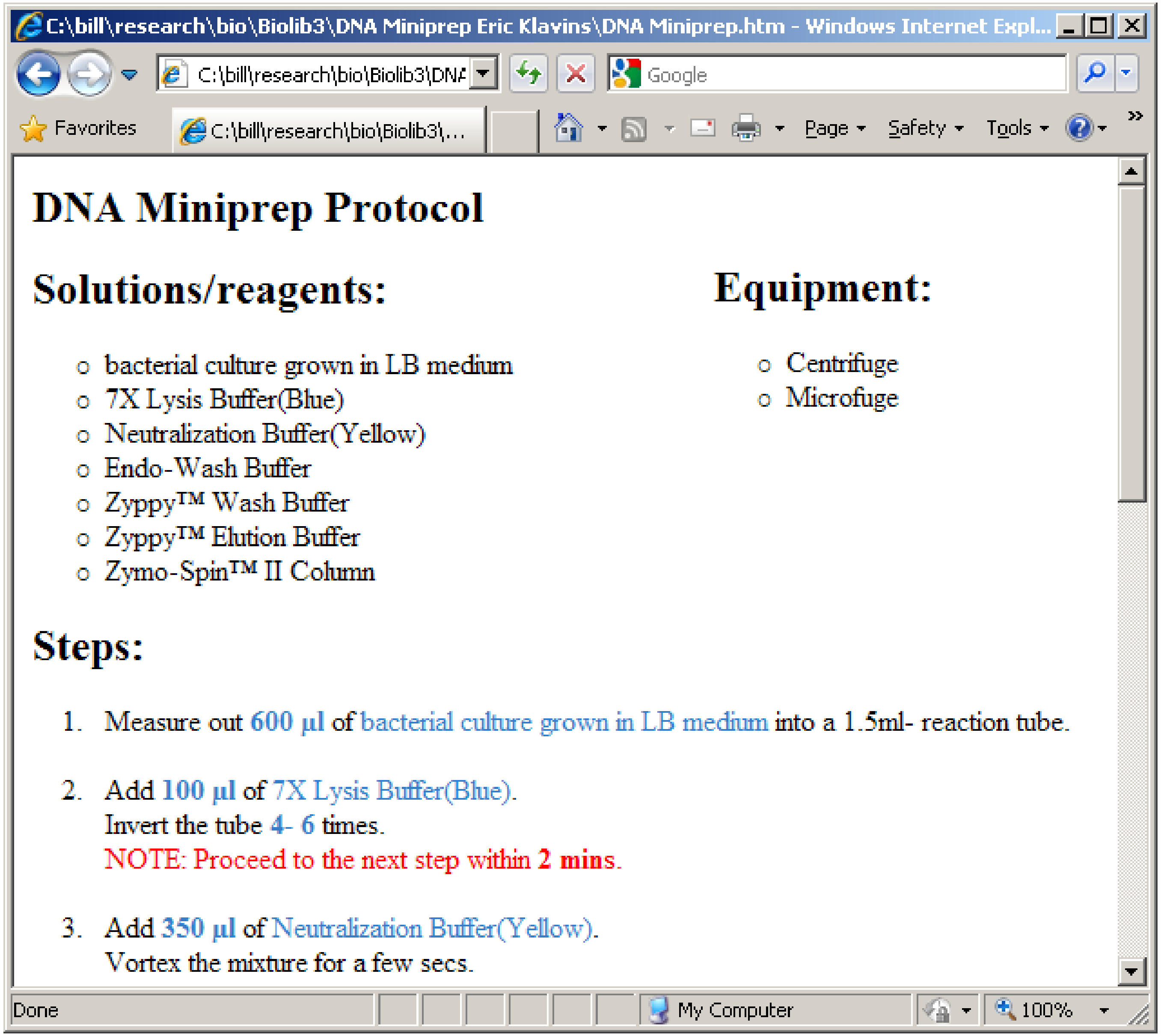
II. BioStream code

```
FluidSample f1 = measure_and_add(&f0, &lysis_buffer, 100*uL);
FluidSample f2 = mix(&f1, INVERT, 4, 6);
time_constraint(&f1, 2*MINUTES, next_step);
```

III. Auto-generated text output

Add 100 ul of 7X Lysis Buffer (Blue).
Invert the tube 4-6 times.
NOTE: Proceed to the next step within **2 mins.**

Example: Plasmid DNA Extraction



DNA Miniprep Protocol

Solutions/reagents:

- bacterial culture grown in LB medium
- 7X Lysis Buffer(Blue)
- Neutralization Buffer(Yellow)
- Endo-Wash Buffer
- Zyppy™ Wash Buffer
- Zyppy™ Elution Buffer
- Zymo-Spin™ II Column

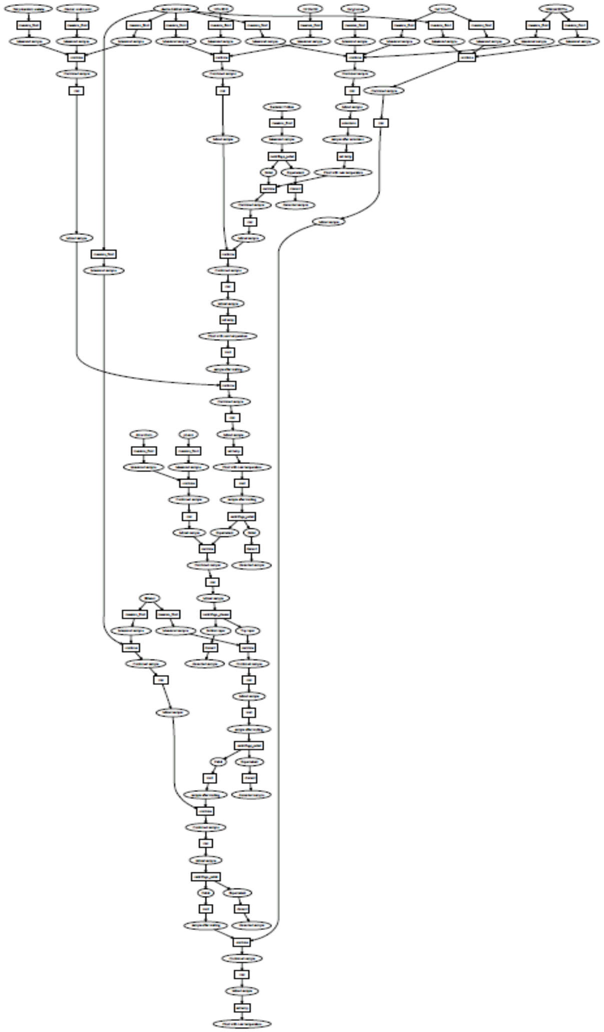
Equipment:

- Centrifuge
- Microfuge

Steps:

1. Measure out **600 µl** of bacterial culture grown in LB medium into a 1.5ml- reaction tube.
2. Add **100 µl** of 7X Lysis Buffer(Blue).
   Invert the tube **4- 6** times.
   NOTE: Proceed to the next step within **2 mins**.
3. Add **350 µl** of Neutralization Buffer(Yellow).
   Vortex the mixture for a few secs.

Auto-Generated Dependence Graph



1. Standardizing Ad-Hoc Language

- **Need to convert qualitative words to quantitative scale**
- **Example: a common scale for mixing**
    - When a protocol says “mix”, it could mean many things
    - Level 1: tap
    - Level 2: stir
    - Level 3: invert
    - Level 4: vortex / resuspend / dissolve

2. Separating Instructions from Hints

- **How to translate abstract directions?**
  - “Remove the medium by aspiration, *leaving the bacterial pellet as dry as possible*.”

```
Centrifuge(&medium, ...);
hint(pellet_dry)
```

→ Aspirate and remove medium.
Leave the pellet as dry as possible.

- **Separating instructions and hints keeps language tractable**
  - Small number of precise instructions
  - Extensible set of hints

3. Generating Readable Instructions

- **In typical programming languages- minimal set of orthogonal primitives**
- **But can detract from readability**

Original: “Mix the sample with 1uL restriction enzyme.”

BioStream with orthogonal primitives:

```
FluidSample s1 = measure(&restriction_enzyme, 1*uL);
FluidSample s2 = combine(&sample, &s1);
mix(s2, tap);
```

Measure out 1ul of restriction enzyme.

Combine the sample with the restriction enzyme.

Mix the combined sample by tapping the tube.

3. Generating Readable Instructions

- **In typical programming languages- minimal set of orthogonal primitives**
- **But can detract from readability**

Original: “Mix the sample with 1uL restriction enzyme.”

BioStream with compound primitives:

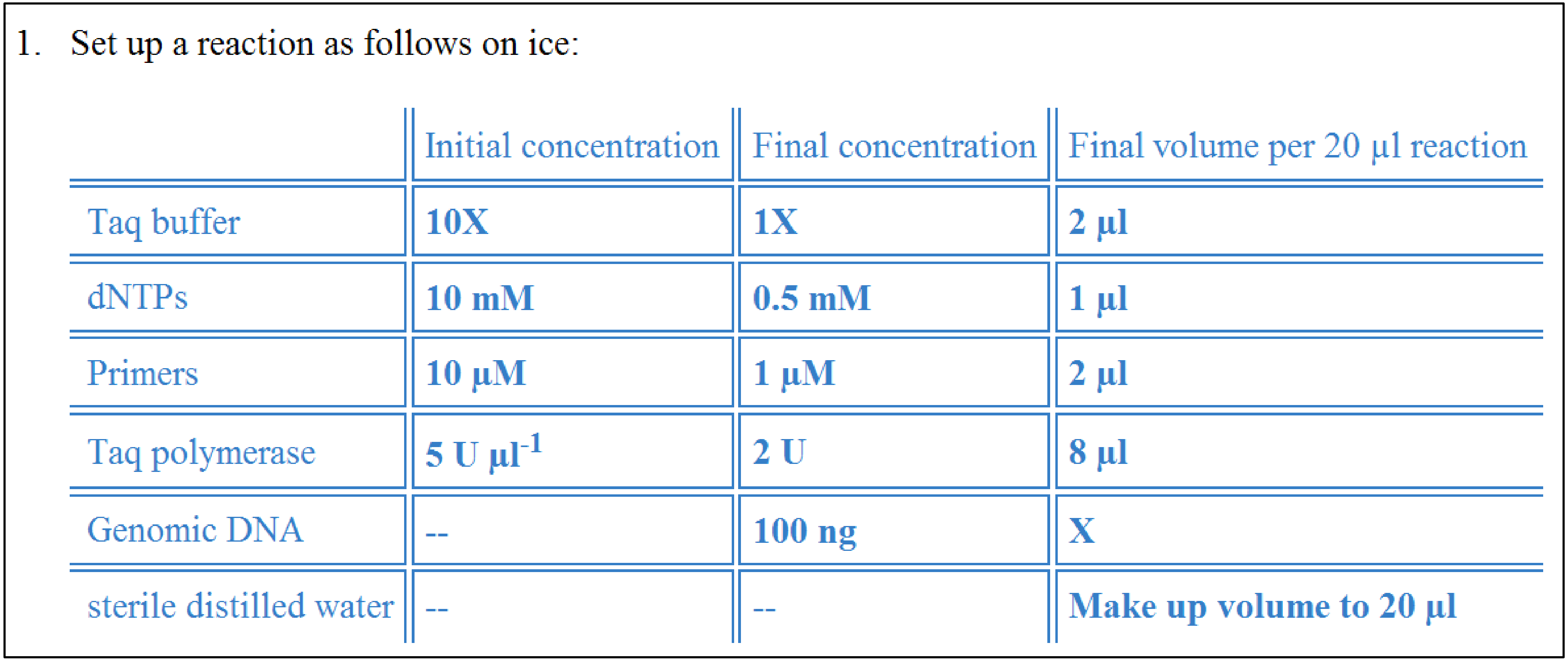
```
combine_and_mix(&restriction_enzyme, 1*uL,
                &sample, tap);
```

Add 1uL restriction enzyme and mix by tapping the tube.

✓ Define a standard library that combines primitive operations

3. Generating Readable Instructions

```
mixing_table_pcr(7,20,array_pcr,initial_conc,
                 final_conc,vol);
```



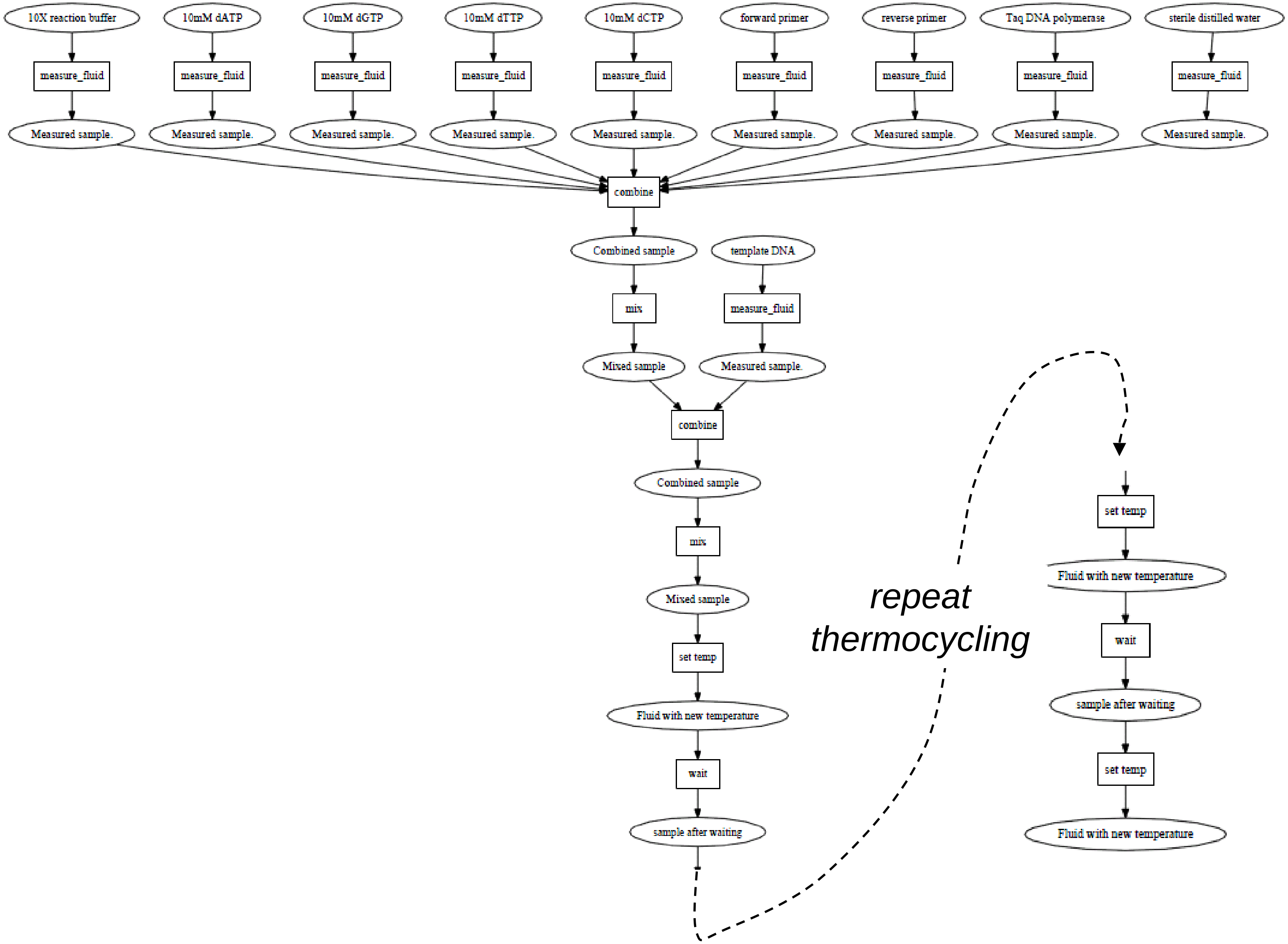
1. Set up a reaction as follows on ice:

	Initial concentration	Final concentration	Final volume per 20 µl reaction
Taq buffer	**10X**	**1X**	**2 µl**
dNTPs	**10 mM**	**0.5 mM**	**1 µl**
Primers	**10 µM**	**1 µM**	**2 µl**
Taq polymerase	**5 U µl^{-1}**	**2 U**	**8 µl**
Genomic DNA	--	**100 ng**	**X**
sterile distilled water	--	--	**Make up volume to 20 µl**

Benchmark Suite

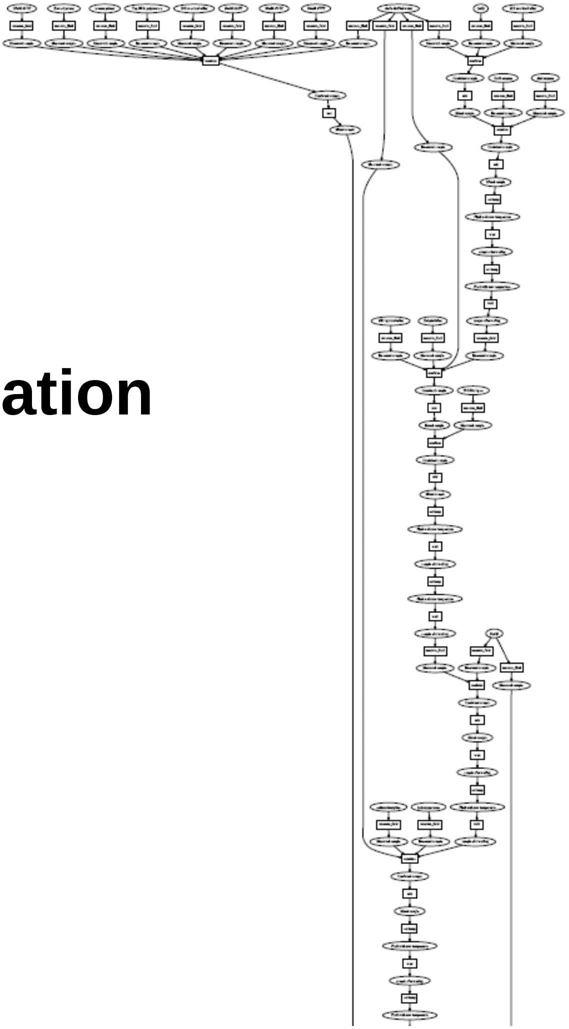
Name	Source	Lines of Code
★ Alkaline DNA Miniprep (Animal)	Textbook	114
AllPrep RNA/Protein (Animal)	Qiagen kit	180
Immunolocalization	Lab notes	127
DNA Sequencing	Published paper	162
Molecular barcodes methods	Published paper	267
SIRT1 Redistribution	Published paper	220
Splinkerette PCR	Published paper	248
Touchdown PCR	Published paper	65
Transcriptional instability	Published paper	187
★ DNA Miniprep (Bacterial)	Class notes	102
★ Restriction enzyme digestion	Class notes	55
★ Restriction enzyme ligation	Class notes	67
★ DNA Extraction (Plant)	Lab notes	481
Plant RNA isolation	Lab notes	137
Plasmid purification	Qiagen kit	158
TOTAL		**2570**

Example: PCR



repeat thermocycling

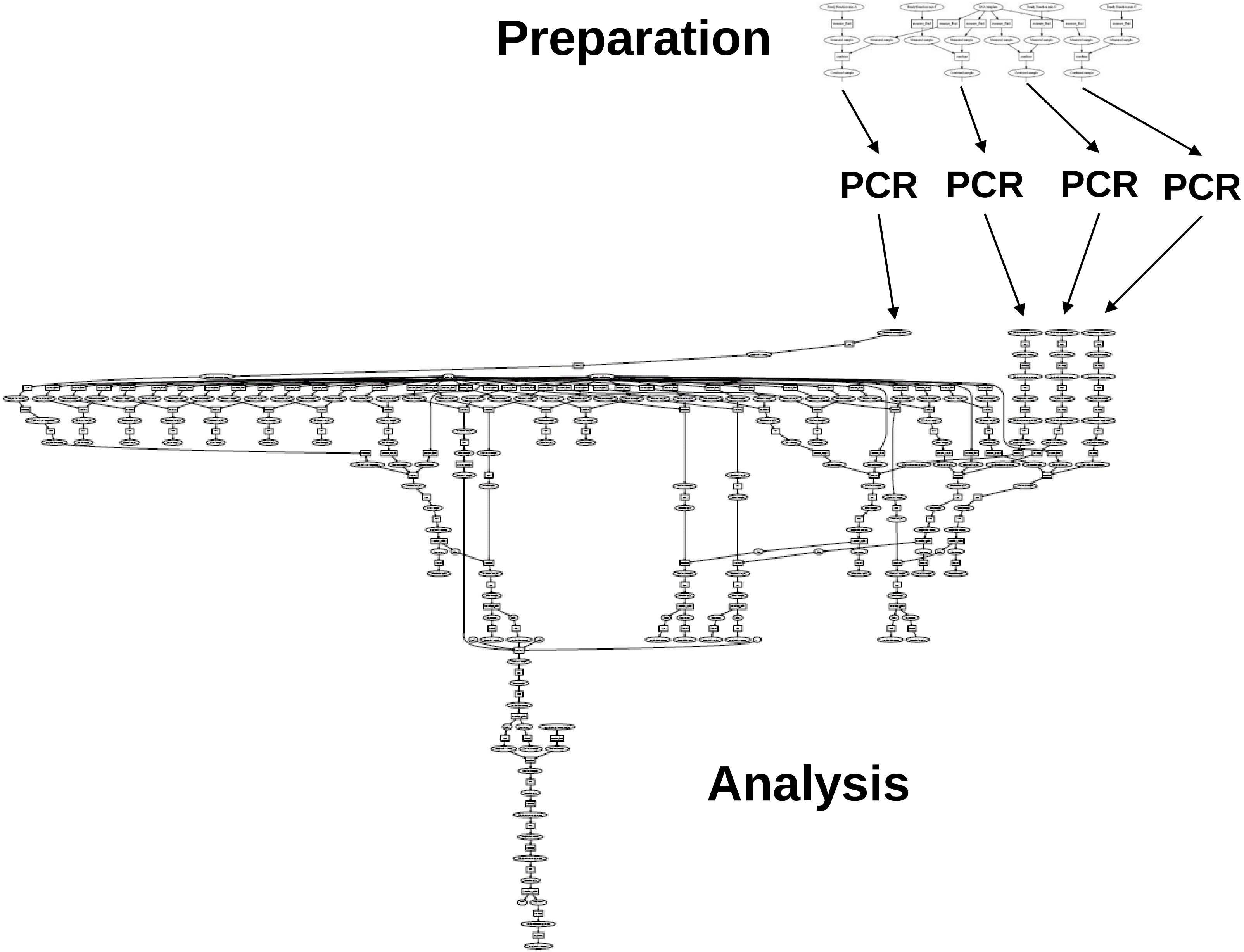
Example: Molecular Barcodes



Preparation

+ PCR (2)

Example: DNA Sequencing

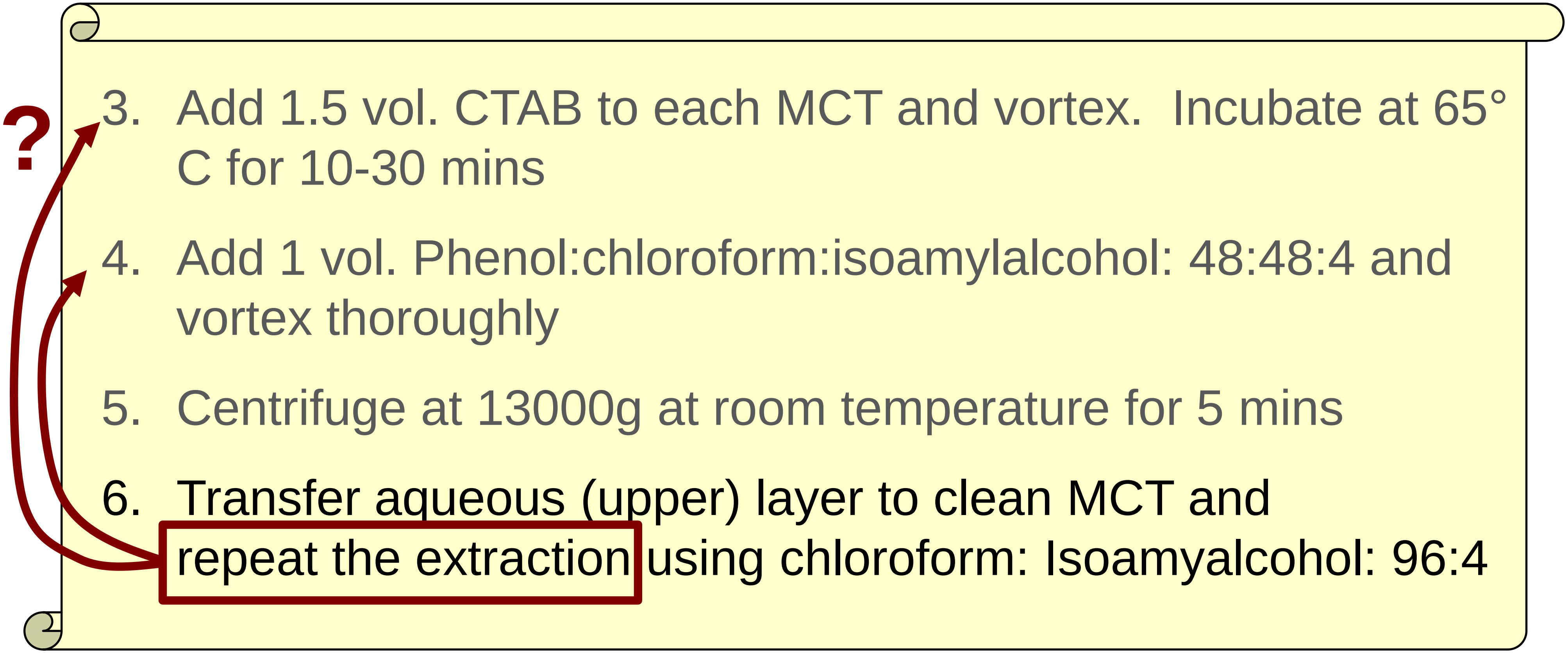


Preparation

PCR **PCR** **PCR** **PCR**

Analysis

Exposing Ambiguity in Original Protocols

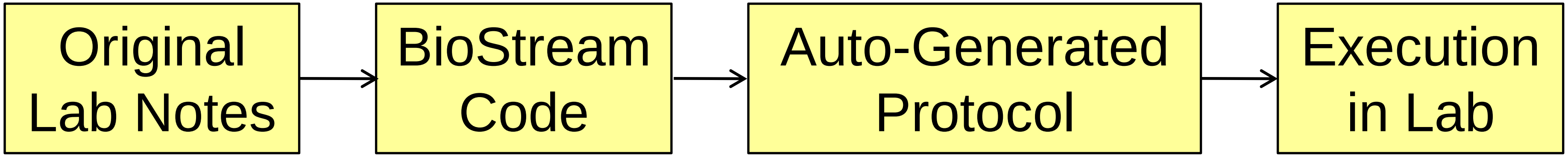


3. Add 1.5 vol. CTAB to each MCT and vortex. Incubate at 65° C for 10-30 mins
4. Add 1 vol. Phenol:chloroform:isoamylalcohol: 48:48:4 and vortex thoroughly
5. Centrifuge at 13000g at room temperature for 5 mins
6. Transfer aqueous (upper) layer to clean MCT and repeat the extraction using chloroform: Isoamyalcohol: 96:4

- **Coding protocols in precise language removes ambiguity and enables consistency checking**

Validating the Language

- **Eventual validation: automatic execution**
  - But BioStream more capable than most chips today
  - Need to decouple language research from microfluidics research
  - Also validate in a synthetic biology context
- **Initial validation: human execution**
  - In collaboration with Prof. Utpal Nath's lab at IISc
  - Target Plant DNA Isolation, common task for summer intern



Biologist is never exposed to original lab notes

- **To the best of our knowledge, first execution of a real biology protocol from a portable programming language**

Future Work

- **Adapt the language to biologists**
  - ***Currently looking for collaborators to use the language!***
  - Focus on ‘natural language’ authoring rather than programming
  - Share language and protocols on a public wiki
- **Backends for BioStream**
  - Generate graphical protocol
  - Program a part of/ complete synthetic biological system to perform a given protocol/function
- **Automatic scheduling**
  - Schedule separate protocols onto shared hardware, maximizing utilization of shared resource (e.g., thermocycler)

Related Work

- **EXACT:** EXperimental ACTions ontology as a formal representation for biology protocols [Soldatova et al., 2009]
- **Aquacore:** ISA and architecture for programmable microfluidics, builds on our prior work [Amin et al., 2007]
- **Robot Scientist:** functional genomics driven by macroscopic laboratory automation [King et al., 2004]
- **PoBol:** RDF-based data exchange standard for BioBricks

Conclusions

- **A high-level programming language for biology protocols is tractable and useful**
  - Improves readability
  - Enables automation
- **Vision: a defacto language for experimental science**
  - Replace ad-hoc language with precise, reusable description
  - Download a colleague’s code, automatically map to your microfluidic chip or lab setup
- **Seeking users and collaborators!**
  1. Send us your protocols
  2. We code them in BioStream
  3. You inspect standardized protocol, optionally validate it in lab

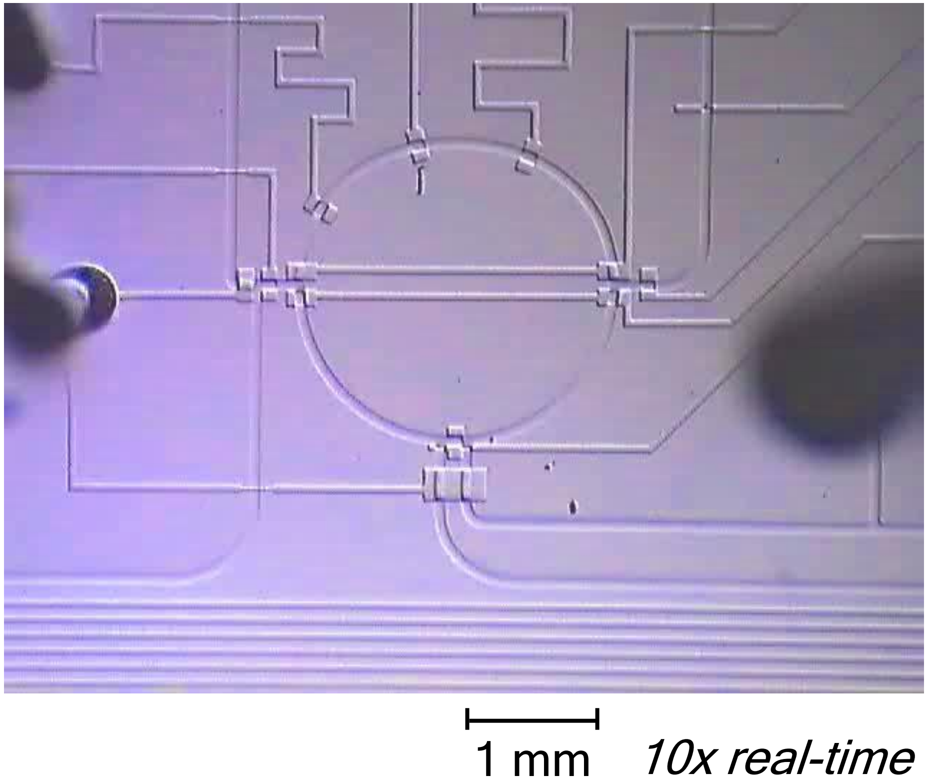
Acknowledgements

- Dr. Utpal Nath, Indian Institute of Science
- Mansi Gupta, Subhashini Muralidharan, Sushmita Swaminathan, Indian Institute of Science
- Dr. Eric Klavins, University of Washington

Extra Slides

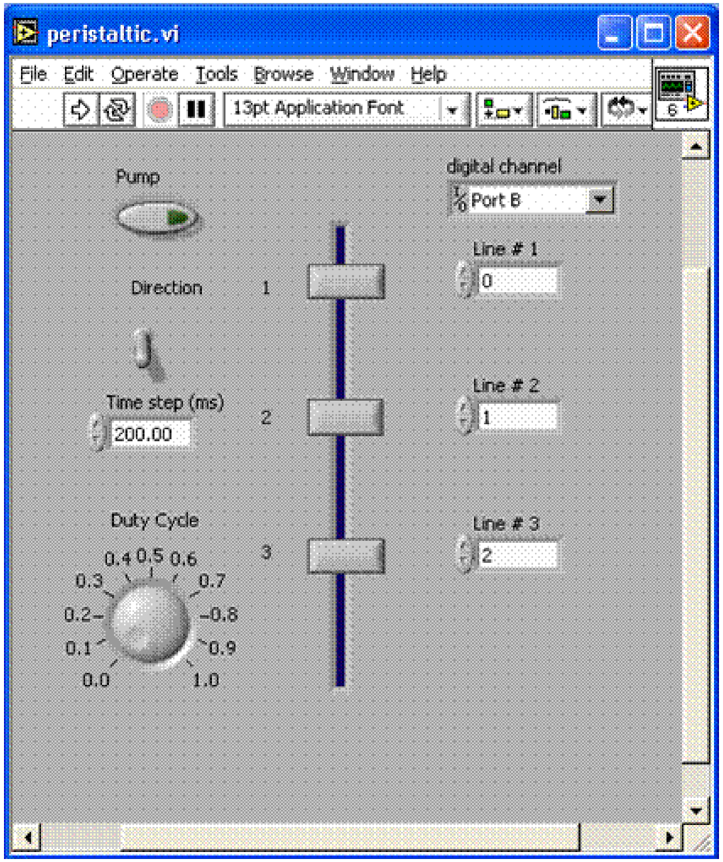
Microfluidic Chips

- **Idea: a whole biology lab on a single chip**
  - Input/output
  - **Sensors**: pH, glucose, temperature, etc.
  - **Actuators**: mixing, PCR, electrophoresis, cell lysis, etc.



- **Benefits:**
  - Small sample volumes
  - High throughput
  - Low-cost
- **Applications:**
  - Biochemistry   - Cell biology
  - Biological computing -Synthetic biology

Current Practice: Expose Gate-Level Details to Users



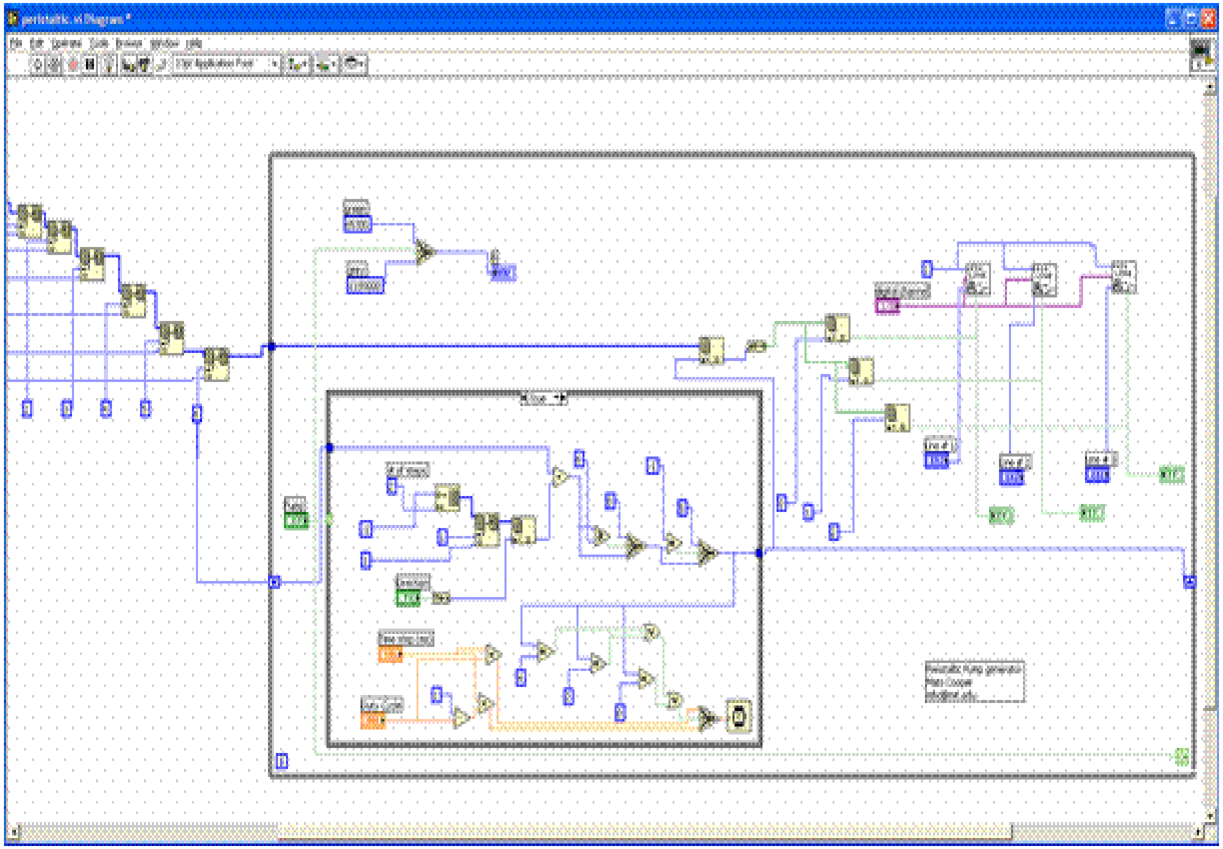
digital channel: Port B

Time step (ms): 200.00

Line # 1: 0

Line # 2: 1

Line # 3: 2

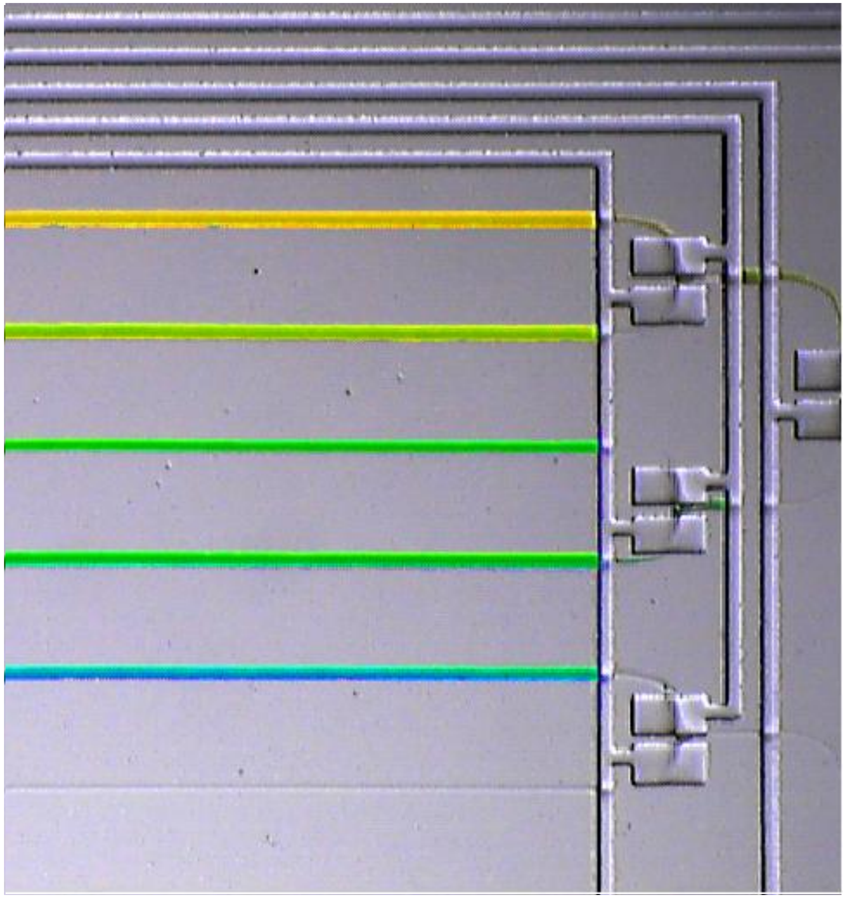


- **Manually map protocol to the valves of the device**
  - Using Labview or custom C interface
  - Given a new device, start over and do mapping again

Our Approach: “Write Once, Run Anywhere”

- **Example: Gradient generation**

```
Fluid yellow = input (0);
Fluid blue = input(1);
for (int i=0; i<=4; i++) {
    mix(yellow, 1-i/4, blue, i/4);
}
```



450 Valve Operations

- **Hidden from programmer:**
  - Location of fluids
  - Details of mixing, I/O
  - Logic of valve control
  - Timing of chip operations

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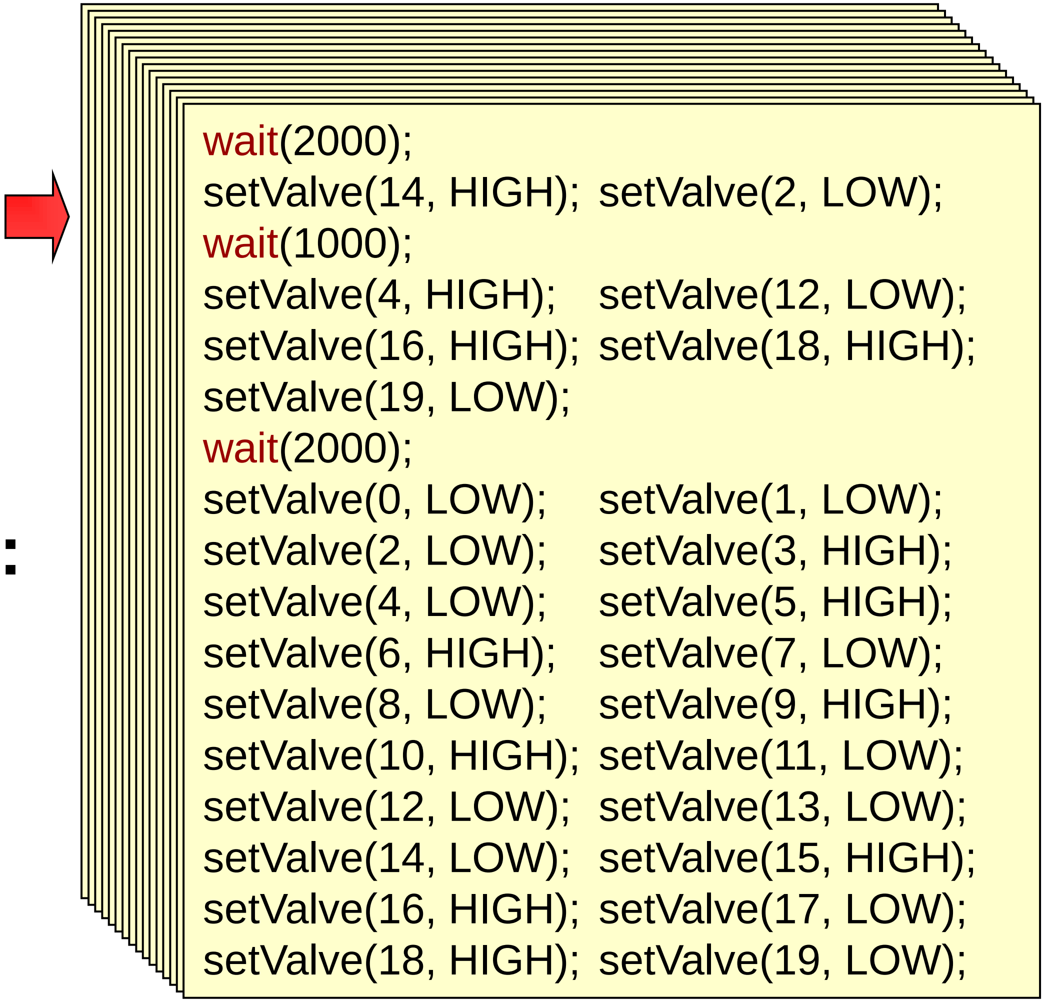

```
wait(2000);
setValve(14, HIGH); setValve(2, LOW);
wait(1000);
setValve(4, HIGH);   setValve(12, LOW);
setValve(16, HIGH); setValve(18, HIGH);
setValve(19, LOW);
wait(2000);
setValve(0, LOW);    setValve(1, LOW);
setValve(2, LOW);    setValve(3, HIGH);
setValve(4, LOW);    setValve(5, HIGH);
setValve(6, HIGH);   setValve(7, LOW);
setValve(8, LOW);    setValve(9, HIGH);
setValve(10, HIGH); setValve(11, LOW);
setValve(12, LOW);  setValve(13, LOW);
setValve(14, LOW);  setValve(15, HIGH);
setValve(16, HIGH); setValve(17, LOW);
setValve(18, HIGH); setValve(19, LOW);
```

- **Hidden from programmer:**
  - Location of fluids
  - Details of mixing, I/O
  - Logic of valve control
  - Timing of chip operations

Example: Plasmid DNA Extraction

- **Goal: extract DNA from bacterial cells for later analysis**

BioStream Code (102 lines)

```
…
next_step("");
t1 = measure_fluid(&bacterial_culture,600,ul,rxn_tube);
end_step();

next_step("");
t2=measure_and_add(&t1,&lysis_buffer,100,ul);
t1=mix(&t2,invert,4,6,NA,NA);
time_constraint(&t1,2,mins, nextstep);
end_step();

next_step("");
t2=measure_and_add(&t1,&neut_buffer,350,ul);
t1=mix(&t2,vortex,NA,NA,NA,NA);
…
```

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S15. V. Gaudin *et al.*, *Plant Physiol.* **122**, 1137 (2000).