



Ion University: Technology & Workflow



The world leader in serving science

Training Agenda

Ion University

- Overview of Ion Torrent™ Sequencing and Data Analysis

Hands-on training

- Template Preparation
 - Ion OneTouch™ 2 Instrument
 - Ion OneTouch™ ES
- Sequencing

Slides and Discussion

- Library Preparation
- Data Analysis
- Best Practices

Ion Torrent™ Technology

Ion Torrent™ Technology

Founded 2007 by Jonathan Rothberg

Pioneered next gen sequencing

Founded 454, CuraGen, Raindiance

Ion Torrent™ Technology by Life Technologies with offices in Connecticut and California

Global manufacturing sites

First PostLight™ sequencing technology



For Research Use Only. Not for use in diagnostic procedures.

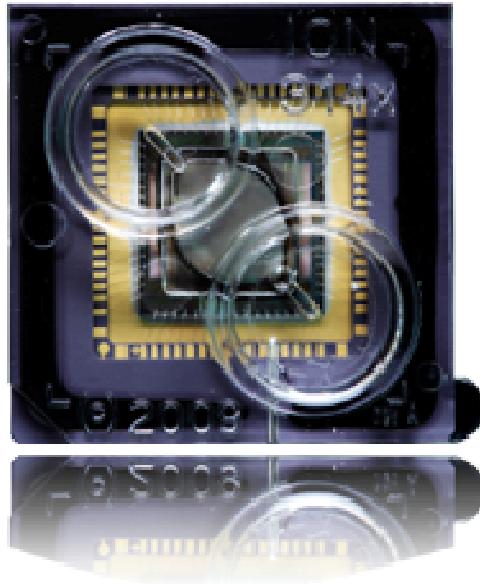
Personal Genome Machine® Sequencer & Chip



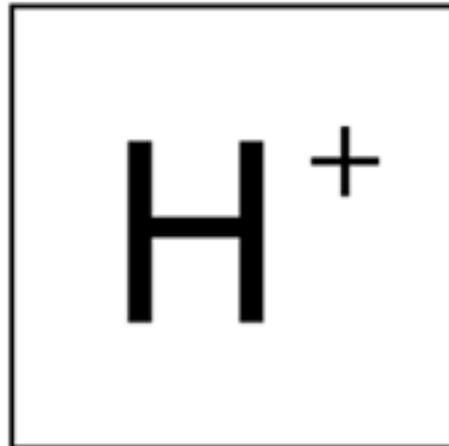
Low cost,
convenient, single
use device.



Easy, automatic fluid connections.
Match the size of the Ion chip to your
application.



Scalability

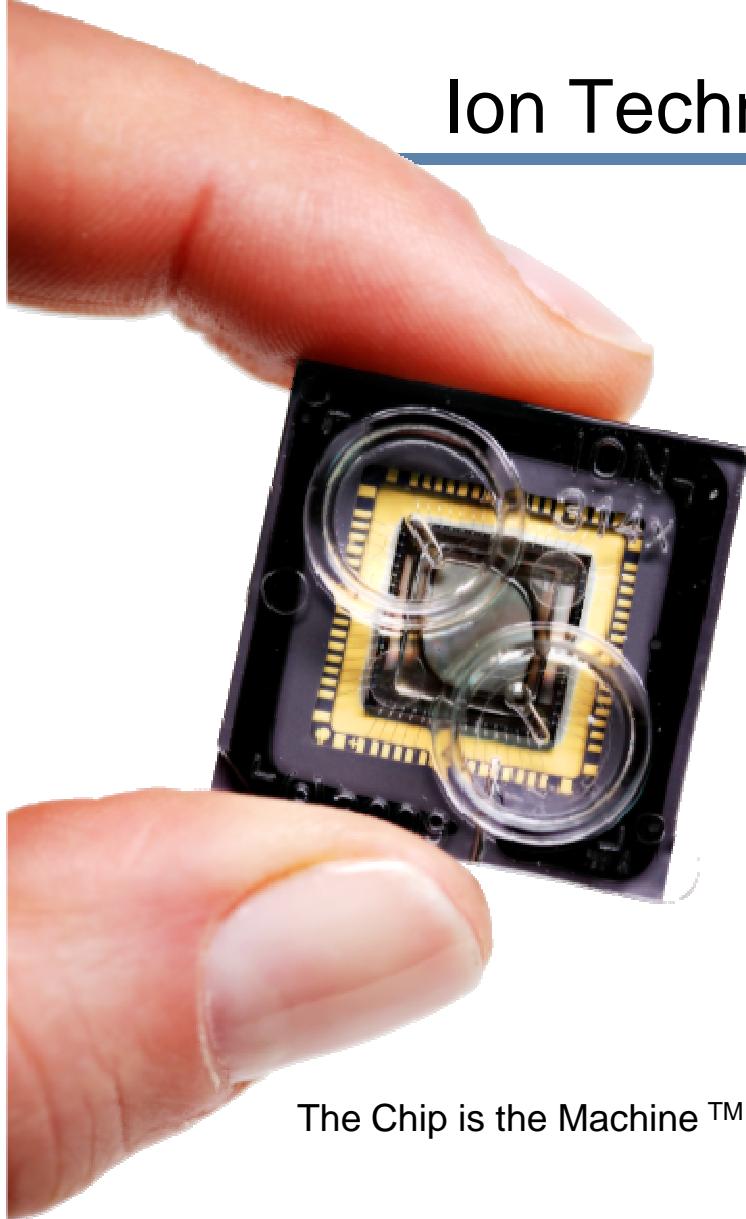


Simplicity



Speed

Ion Technology Core Principles



The Chip is the Machine™

Scalability

- CMOS technology
- 40 years of Moore's law

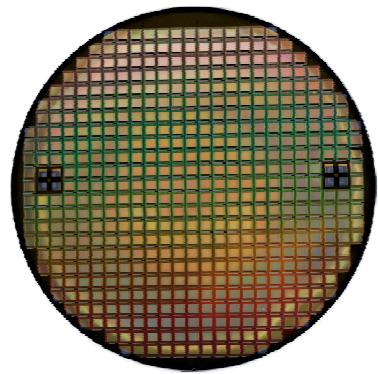
Simplicity

- Natural nucleotides
- No lasers
- No optics
- No camera
- No fluorescence
- No enzyme cascade

Speed

- Rapid detection of sequence extension

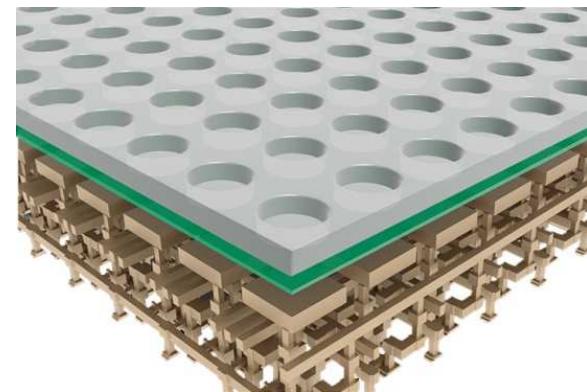
Scalability: Semiconductor Technology



Wafer

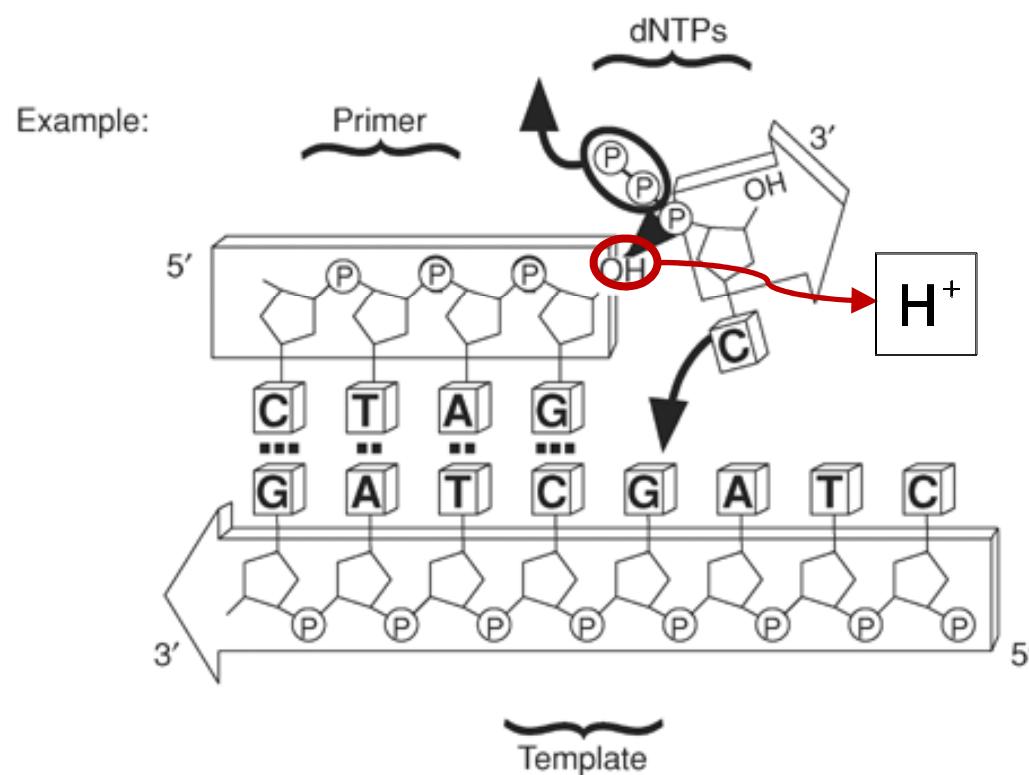


Chip

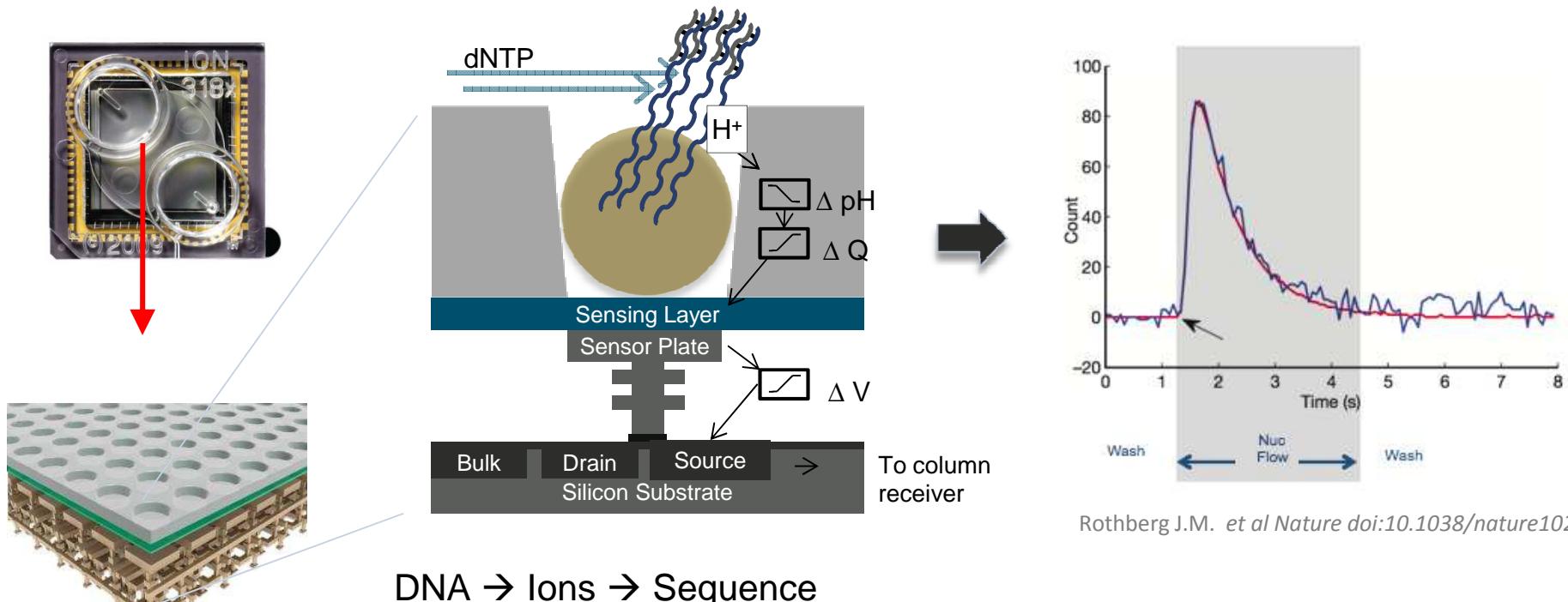


Chip Cross
Section

Simplicity: Natural Chemistry



Speed: Fast Direct Detection



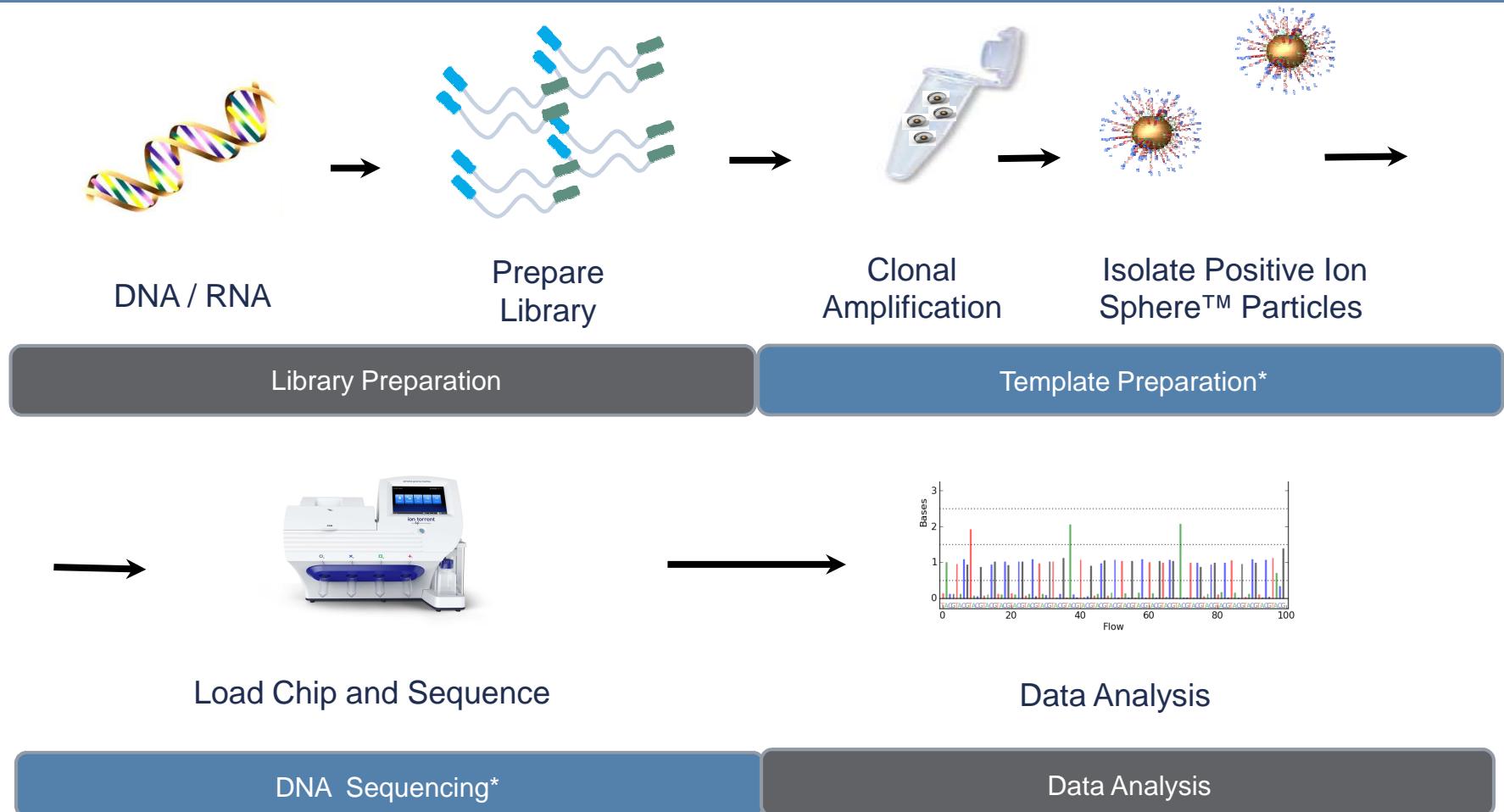
Rothberg J.M. et al *Nature* doi:10.1038/nature10242

DNA → Ions → Sequence

- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection

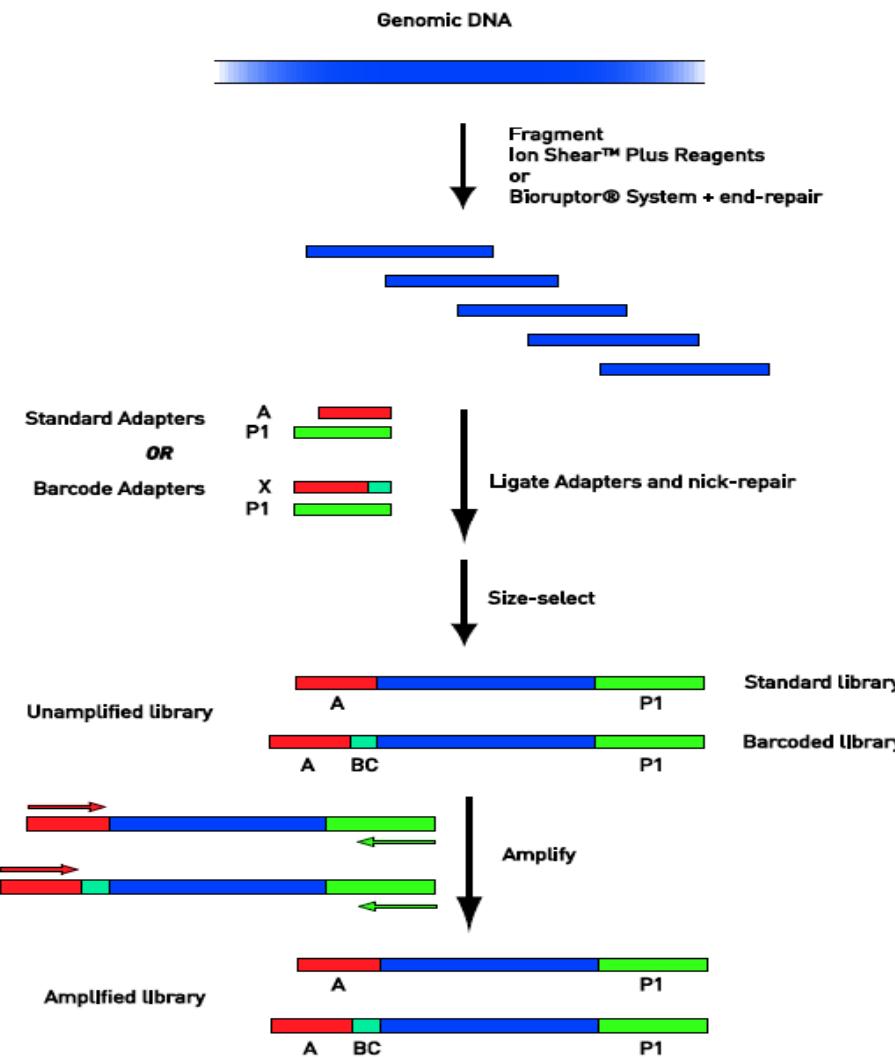
Ion Torrent™ Workflow: Library Prep

Ion Workflow Overview

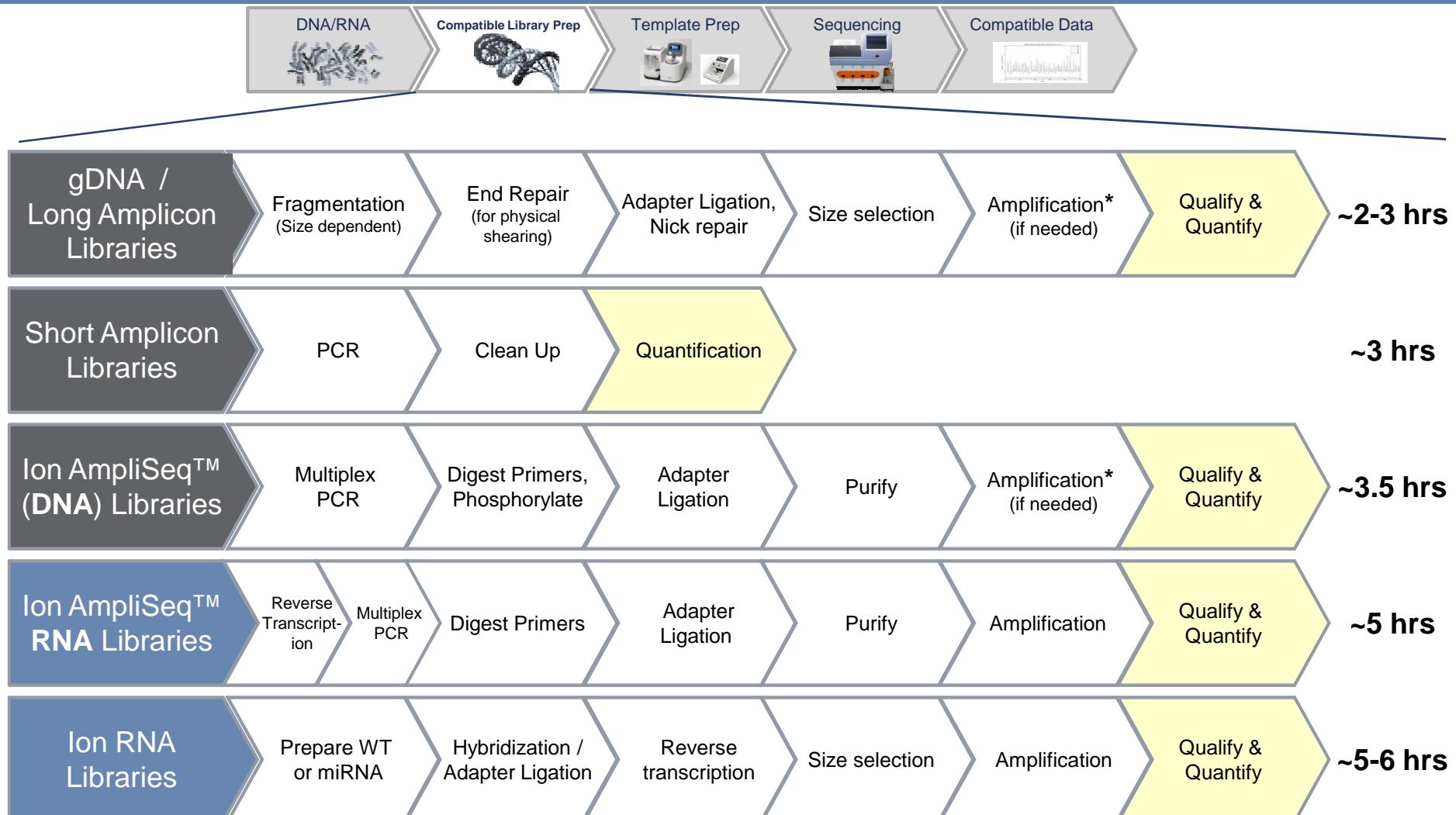


*Today's Course covers the sections highlighted in blue. Courses are available for sections highlighted in grey.

Ion Fragment Library Overview

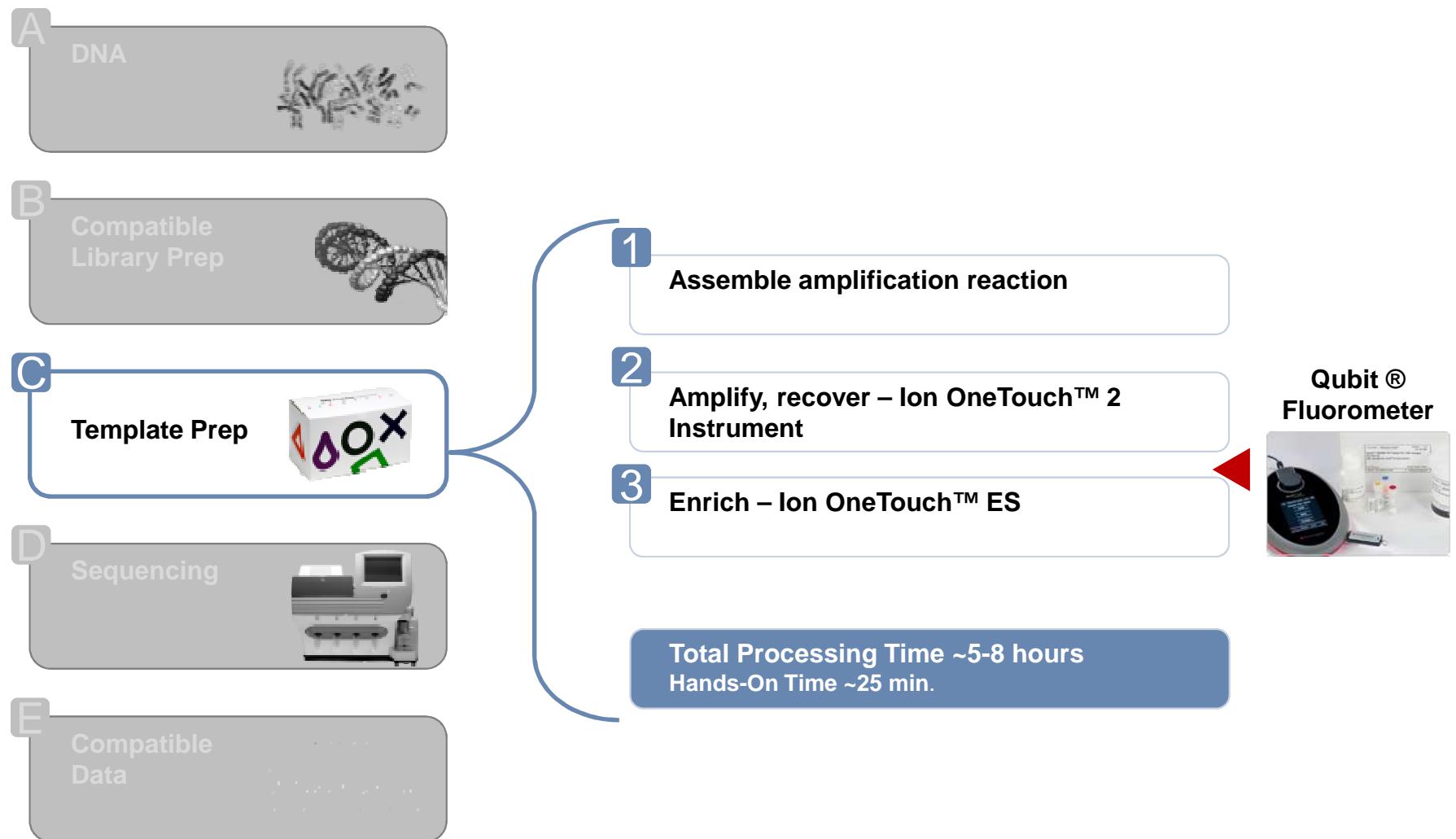


Ion Workflow – Library Prep

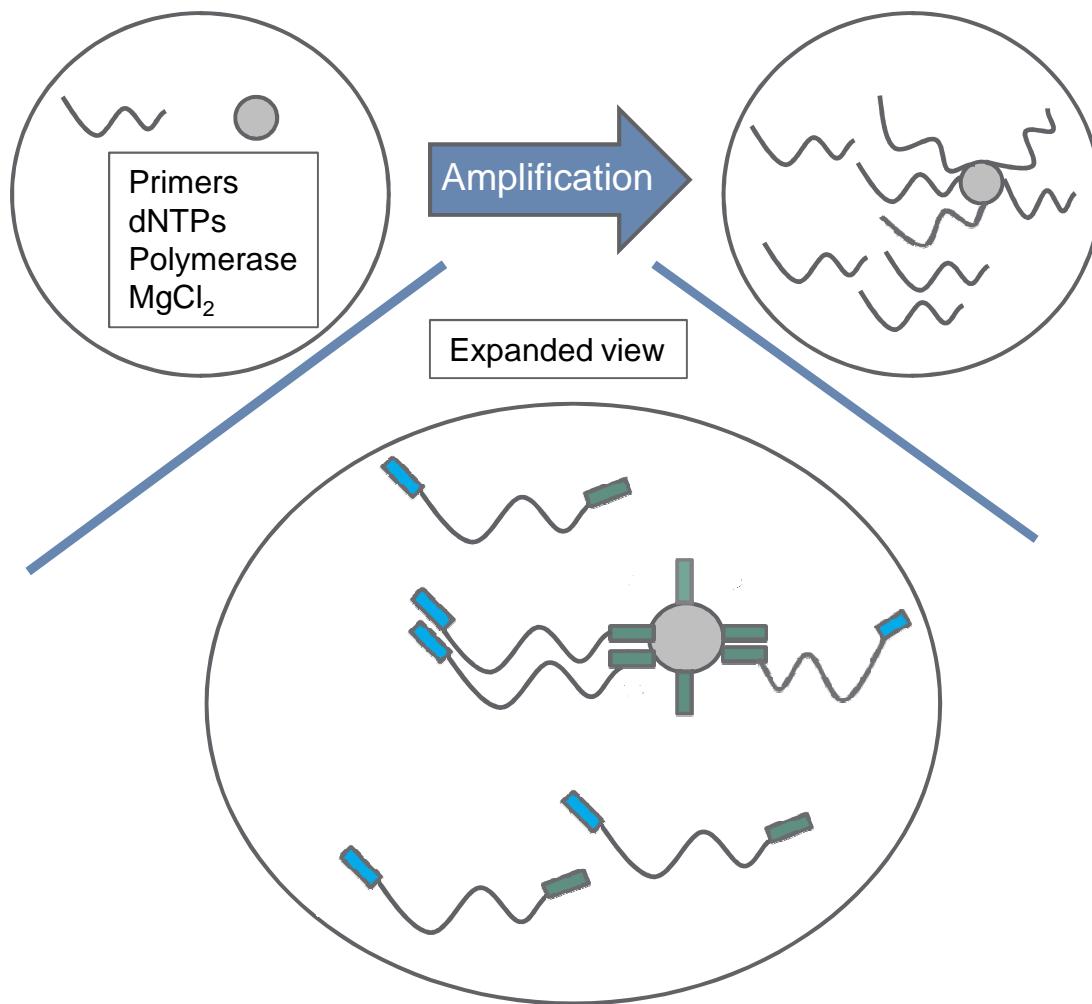


Ion Torrent™ Workflow: Template Prep

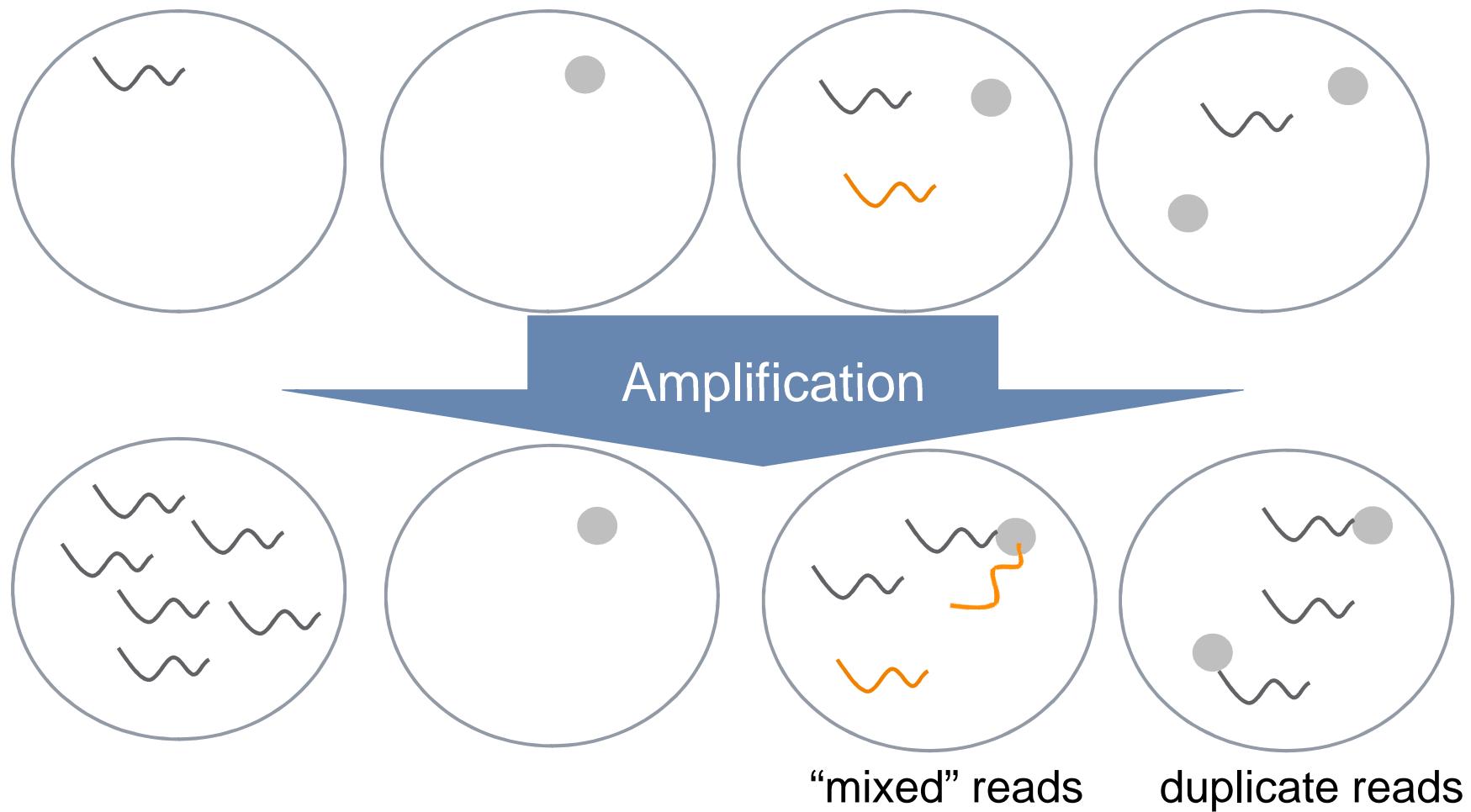
Ion Workflow – Template Prep



Ideal Clonal Amplification

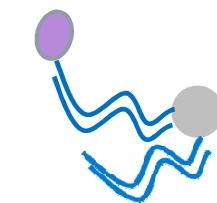


Non-Ideal Cases

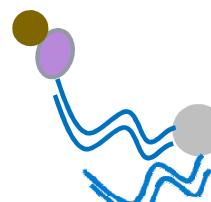


Ion Sphere™ Particle Enrichment

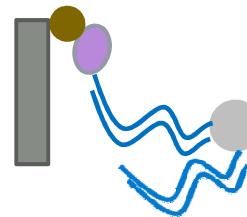
Post
Amplification



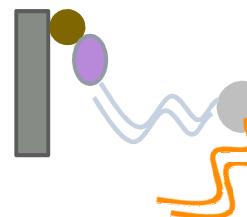
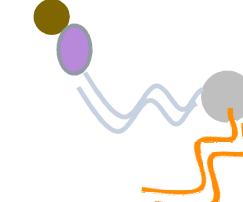
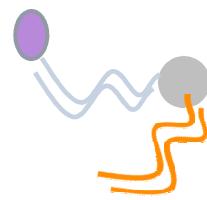
Add Magnetic
Streptavidin Bead



Immobilize to
Magnet and Wash



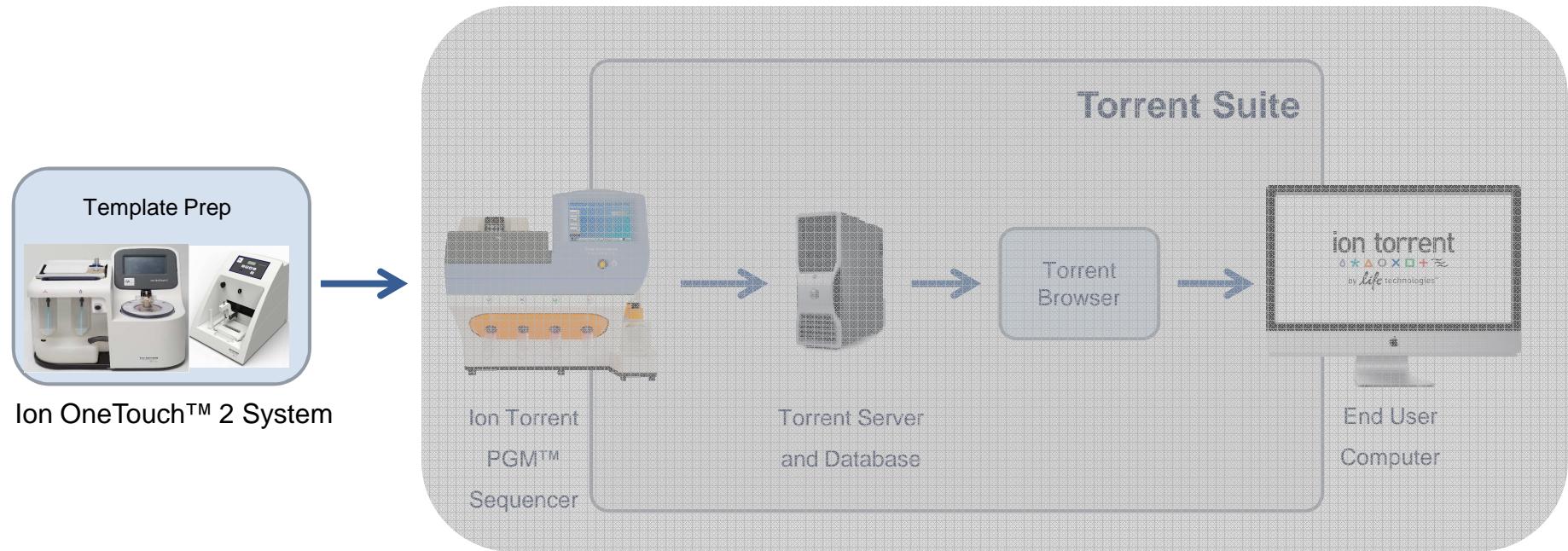
Denature ISP
with NaOH



*This species can be minimized through proper dilution.
Proper DNA to Ion Sphere™ Particle ratio is critical!

Ion Template Prep: Ion OneTouch™ 2 System

Template Prep: Ion OneTouch™ 2 System



Simple Workflow: Ion OneTouch™ 2 System

Ion OneTouch™ 2 Instrument

01
Set-Up Instrument



02
Set-Up Amp Reaction



03
Amplify:



Ion OneTouch™ ES

01
Set-Up ES



02
Retrieve Sample

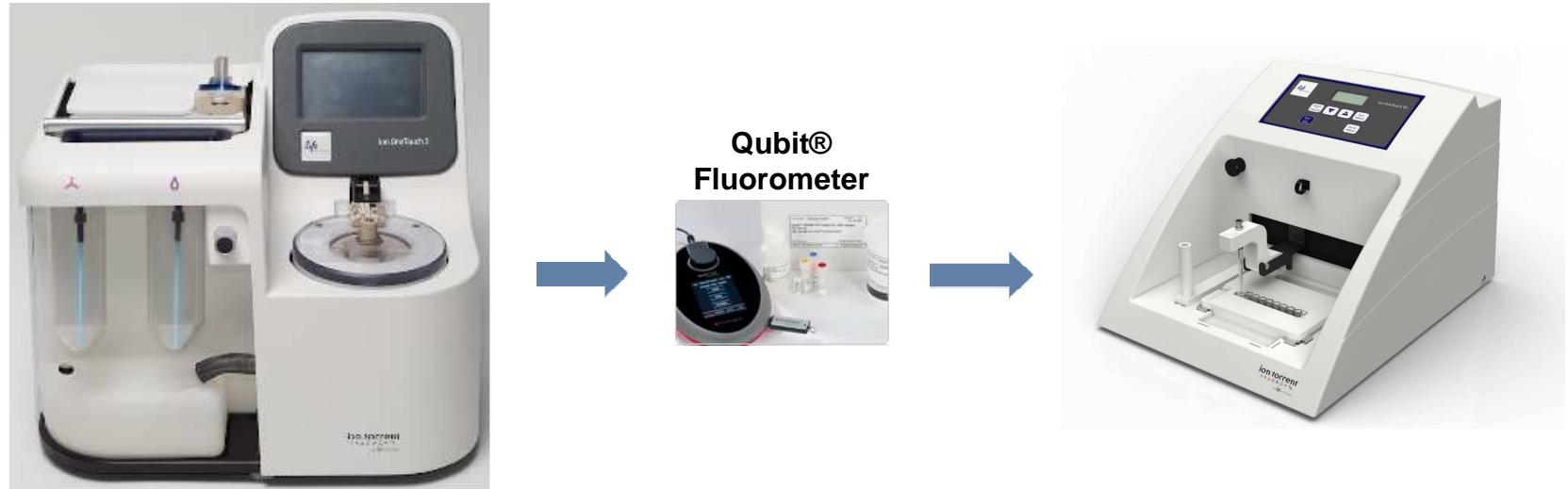


03
Enrich



Ion OneTouch™ 2 System

Ion OneTouch™2 Instrument & Enrichment System (ES)



- Easy-to-use, benchtop system that automates Ion's proven template prep protocol
- Minutes of hands-on time, ~5-8 hours total time
- Modular design enables template prep of samples **in parallel** with an Ion PGM™ Sequencer run and **quality control** of samples pre- and post- enrichment
- *Perform Ion's template prep and sequencing workflow in a single working day*

Ion OneTouch™ 2 Instrument Orientation



Minimal Site-Prep, Simple Installation

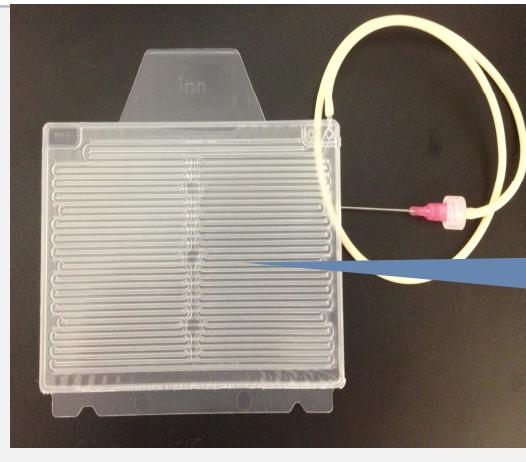
- Plug in electrical outlet
- Update firmware via USB drive or ethernet connection
- Installation includes one-time instrument initialization
- No longer need to be connected to Nitrogen gas

Ion OneTouch™ 2 Instrument Consumables

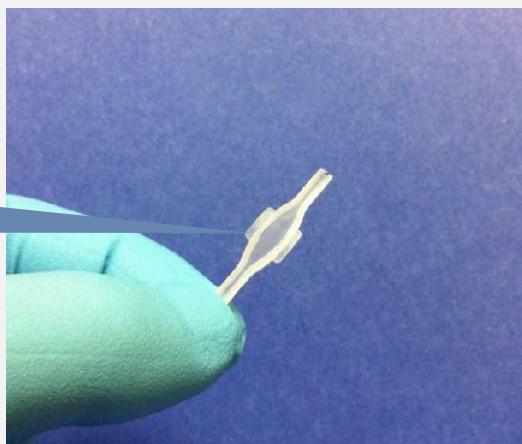
Ion
OneTouch™
Reaction Filter
Assembly



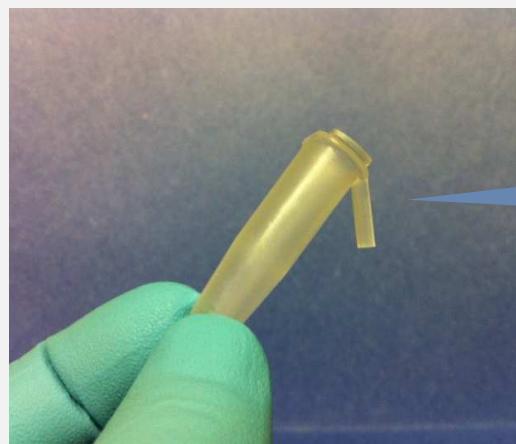
Amp Plate



Recovery
Router



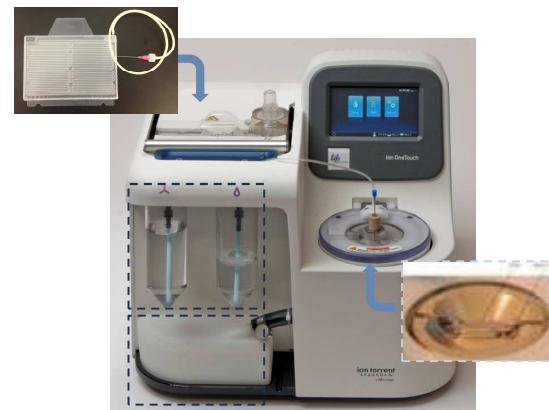
Recovery Tube



Ion OneTouch™ Instrument Workflow

1) Set-Up Instrument:

- Load amp plate
- Fill/load oil and recovery tubes
- Empty waste
- Prep centrifuge
- Thread disposable tubing through pinch valve



2) Set-Up Amplification Reaction:

- Combine library, amp mix, enzyme, and Ion Spheres
- Attach filter



3) Amplify:

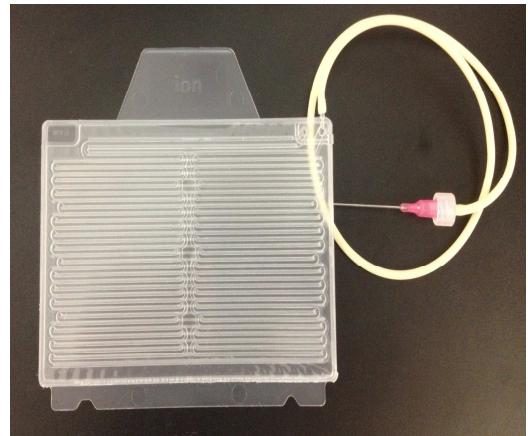
- Load Ion filter containing sample, amplification mix, and oil
- Initiate run from touch screen

What happens next?

- 1) Oil and Amp reaction mix are pushed into reaction filter to generate microscopic reaction vesicles.



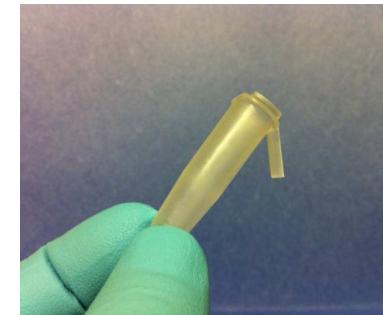
- 2) Vesicles enter amplification plate, and undergoes PCR.



- 3) Vesicles exit amp plate into centrifuge, where they are injected into recovery router, which routes vesicles into recovery tubes.

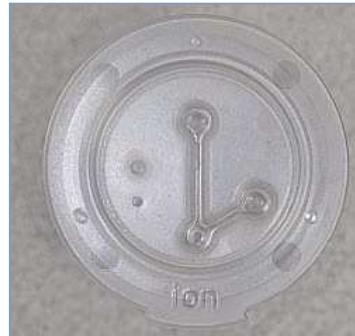


- 4) Vesicles break in Recovery solution, and ISPs are recovered through centripetal force. Oil is siphoned off top of recovery tube into waste.



Ion OneTouch™ Cleaning

*Perform cleaning procedure after **every** run!*



Included in template kit

Video: Maintaining Ion OneTouch™ Instrument
<http://ioncommunity.lifetechnologies.com/videos/1683>

Note: Expected volume output for Ion OneTouch™ 2 instrument is ~10 ml oil

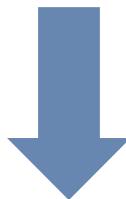
Ion OneTouch™ ES Orientation



Ion OneTouch™ ES Workflow

1) Set-Up ES:

- Load reagents into 8-well strip
- Position in ES
- Attach liquid handling tip



2) Retrieve Sample:

- Recover sample from Instrument
- Remove recovery solution
- Resuspend



3) Enrich:

- Load sample into strip
- Start enrichment run

What happens next?

ES runs through pre-programmed movements

- Adding MyOne™ beads to sample
- Washing
- Magnetizing
- Melting sample off the beads

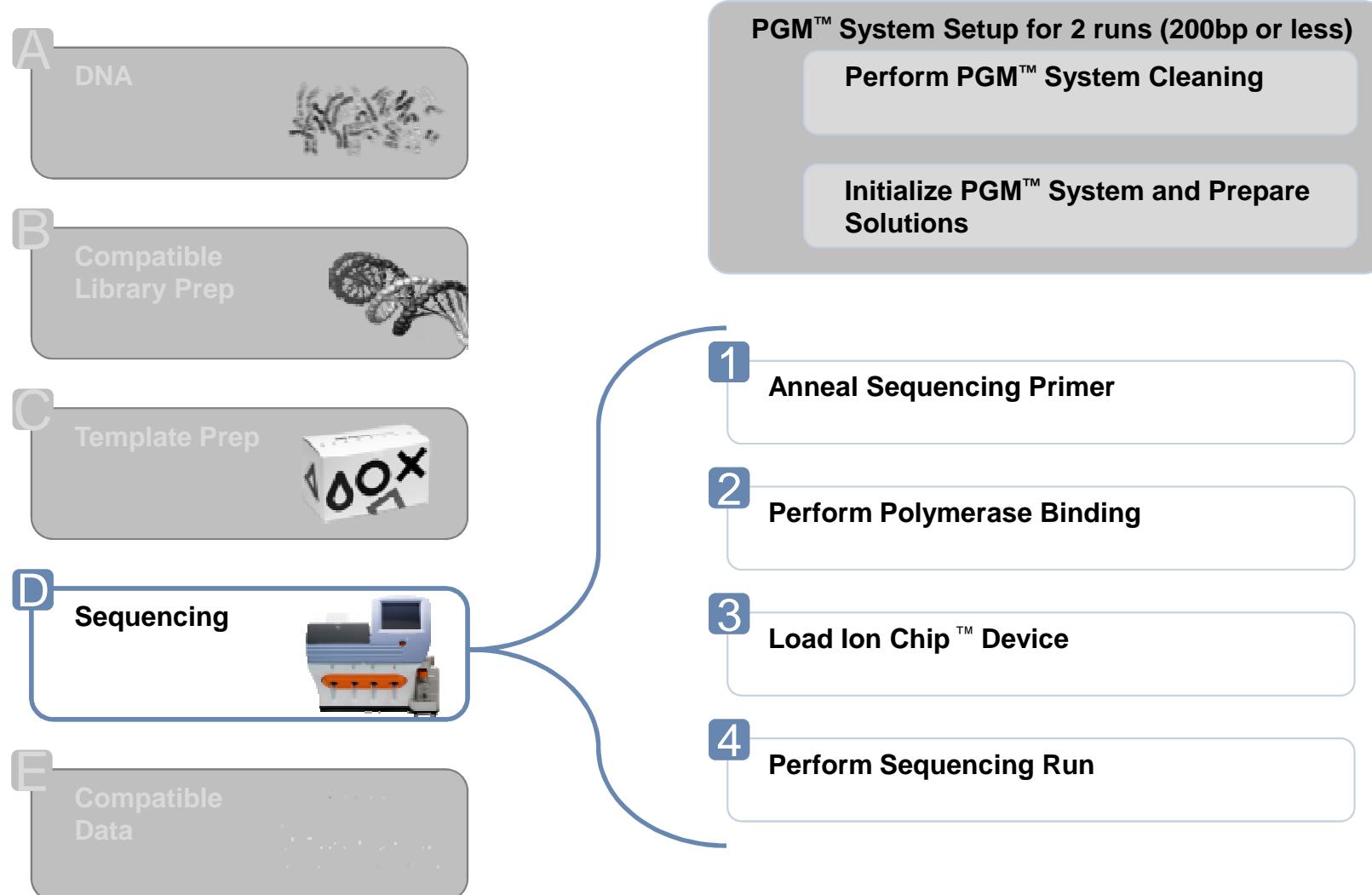
Magnet is on and off sample by having the robotic arm move 8-well strip close to or away from magnet



- 1) ISP sample
- 2) MyOne™ Beads
- 3) Wash Solution
- 4) Wash Solution
- 5) Wash Solution
- 6) Empty
- 7) Melt-Off Solution
- 8) Empty

Ion Torrent™ Workflow: Sequencing

Ion Workflow – PGM™ System Sequencing Run

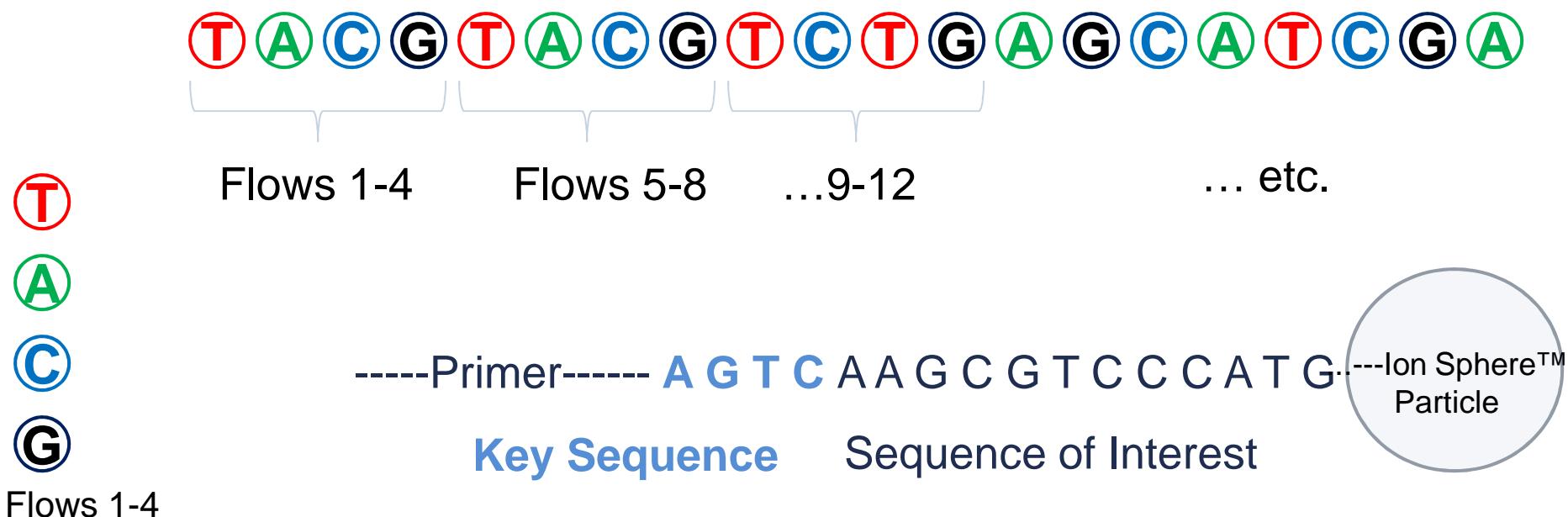


PGM™ System Orientation



Sequencing: Flows

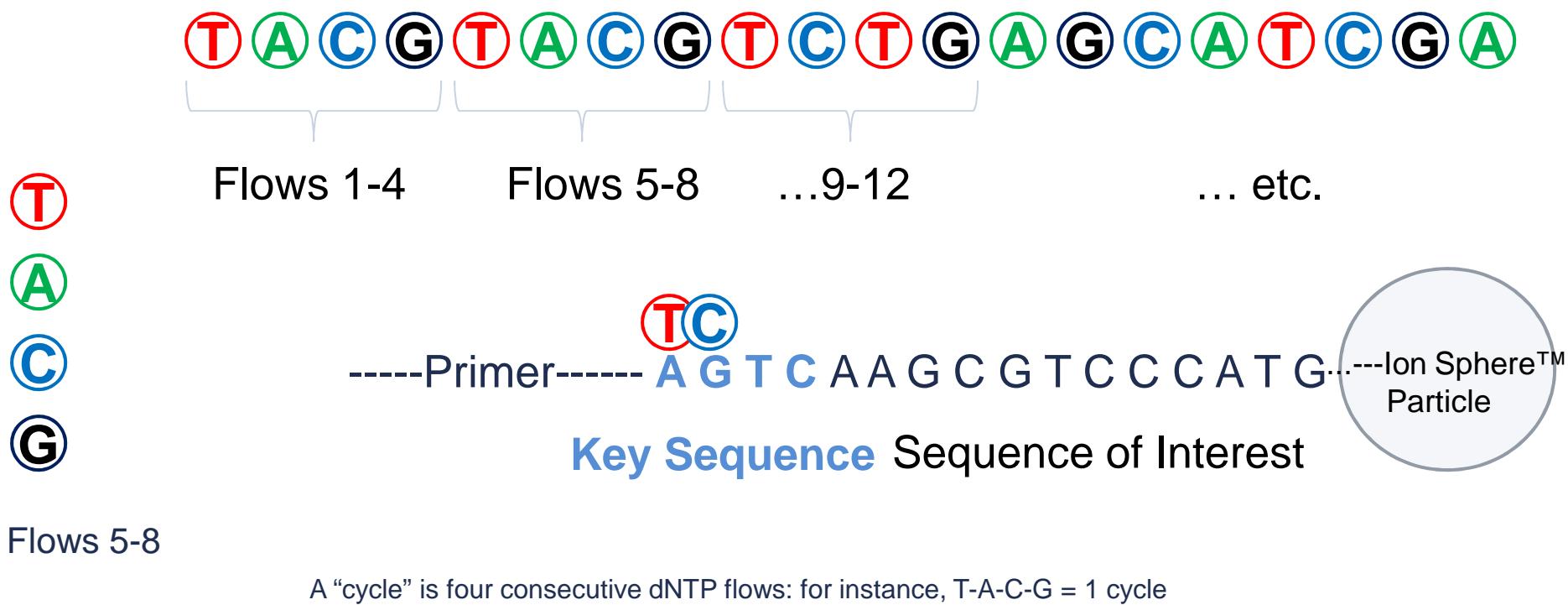
- A “flow” is the event of exposing the chip to one particular dNTP (T, A, C, or G), followed by a washing step
- The flow order repeats with pattern:
 - ‘TACGTACGTCTGAGCATCGATCGATGTACAGC’



A “cycle” is four consecutive dNTP flows: for instance, T-A-C-G = 1 cycle

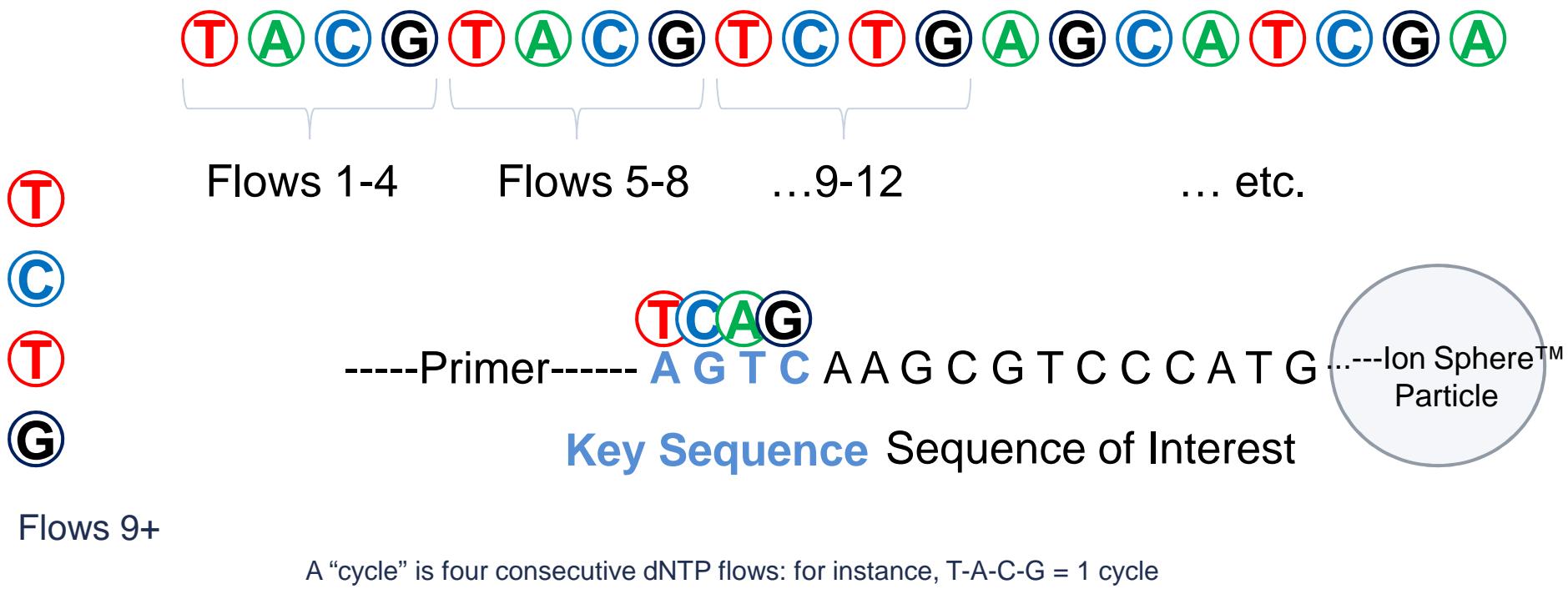
Sequencing: Flows

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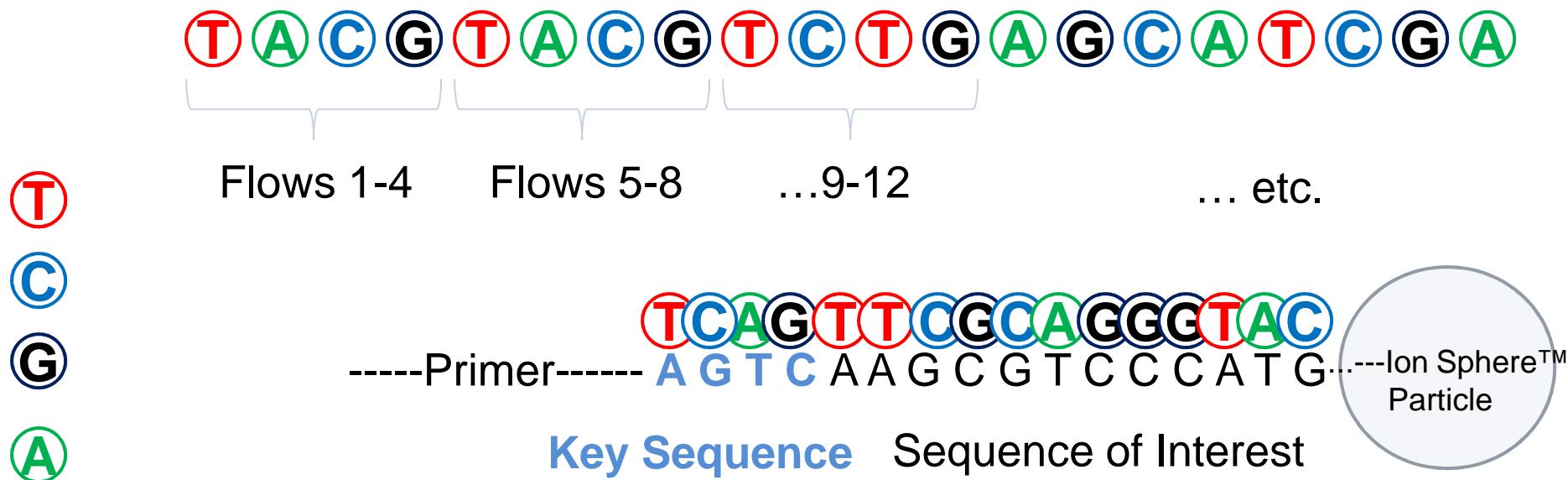
Sequencing: Flows

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Sequencing: Flows

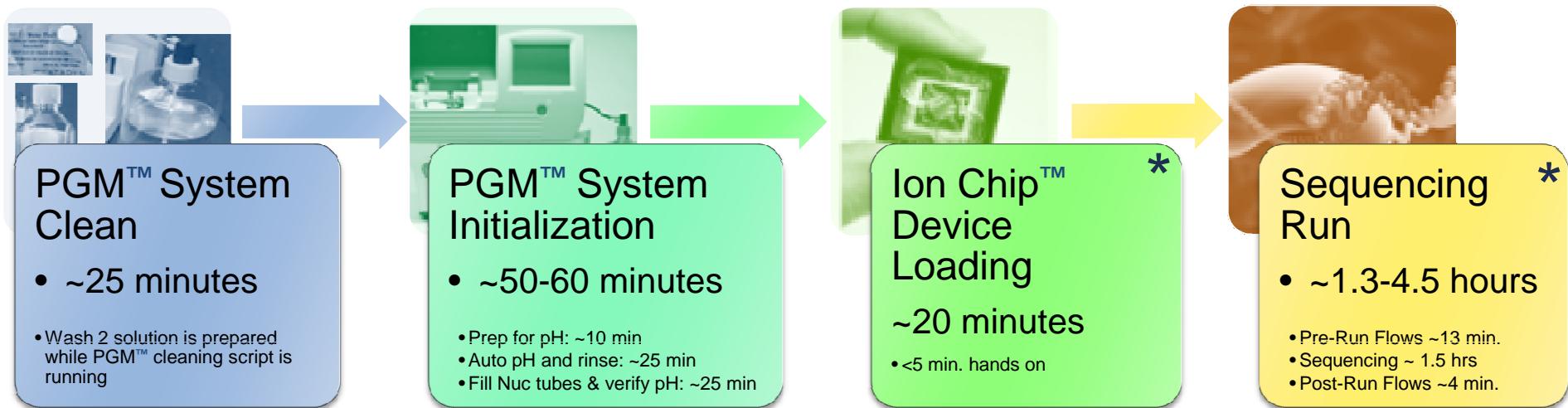
- A “flow” is the event of exposing the chip to one particular dNTP (T, A, C, or G), followed by a washing step
- The flow order repeats with pattern:
 - ‘TACGTACGTCTGAGCATCGATCGATGTACAGC’



And so on...

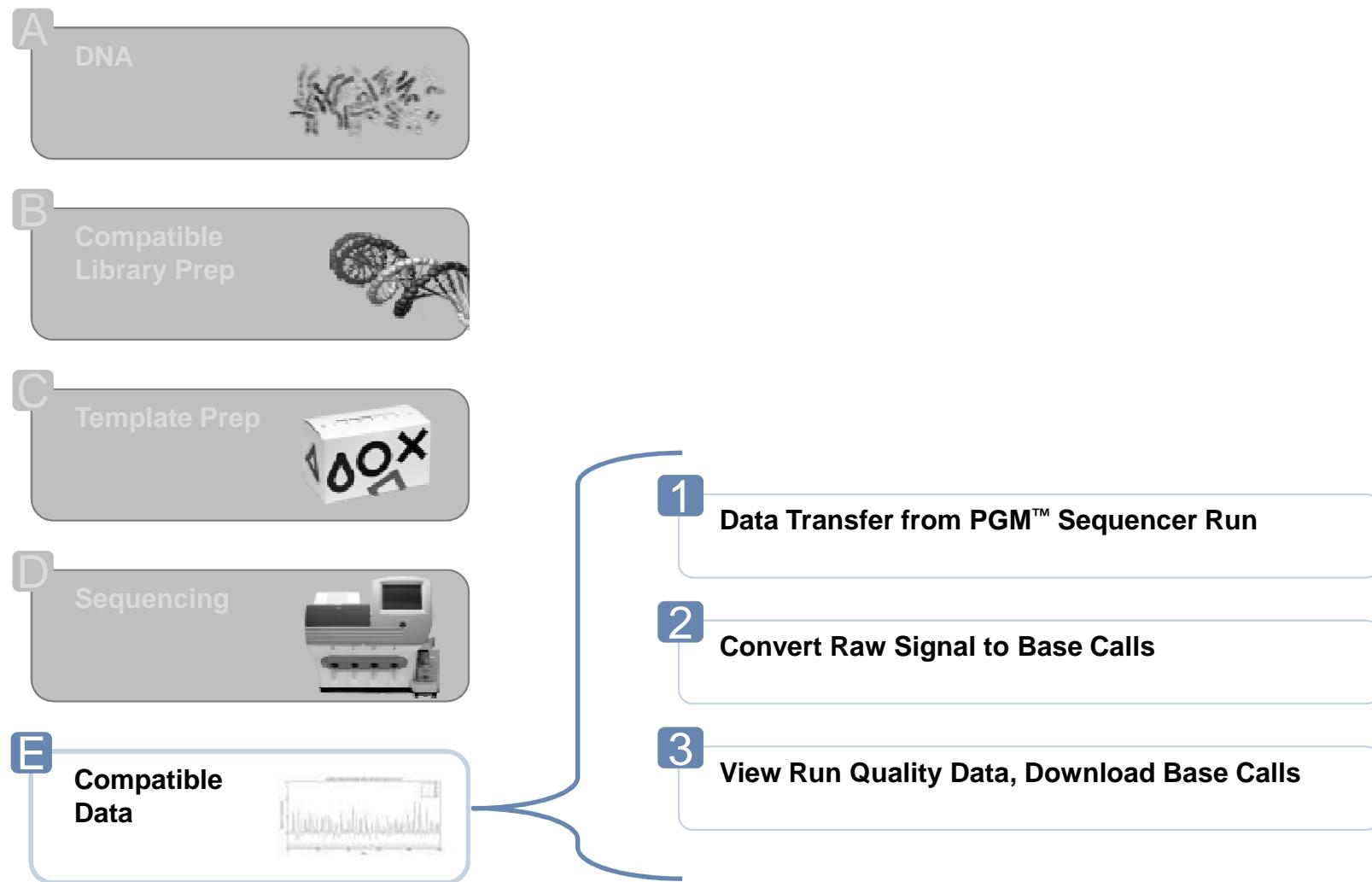
A “cycle” is four consecutive dNTP flows: for instance, T-A-C-G = 1 cycle

Sequencing Workflow: A Detailed Look



* Each *initialized* PGM™ Sequencer is capable of one or more sequencing runs. The number of experiments possible per “Init” depends on the number of flows selected (based on desired read length). To conduct subsequent sequencing experiments, repeat loading and sequencing steps once initial sequencing run is complete.

Analysis Workflow



Data Analysis Training Options

BioInformatics training	Time (hrs)
Ion Data Analysis Essentials (Life Technologies site)	14
Intro to Torrent Suite™ Software	3
Ion Variant Calling Data Analysis***	3
Ion AmpliSeq™ DNA Data Analysis	3
Ion AmpliSeq™ RNA Data Analysis	3
Ion Reporter™ Software Workflow	3
Variant Calling Analysis & Interpretation	2
RNA-Seq Data Analysis**	2
smRNA Data Analysis**	1
<i>De novo</i> Assembly*	1
Targeted Sequencing Introduction / Overview***	1
Linux Basics	3

*** DNA ** coming soon * small genome

In person two day course at a Life Technologies site.

Several Live instructor led interactive webinars.

Several self paced, recorded trainings.

Check out the Life Technologies training page to see all listings and course dates.

<https://learn.lifetechnologies.com/semiconductorsequencing>

Ion Torrent™ Products and Support

Required Materials Checklist

<http://ioncommunity.lifetechnologies.com/docs/DOC-1967>

Ion Torrent Products



Check for
newest
products!

01

Construct Library

- Ion AmpliSeq™ Designer (custom)
- Ion AmpliSeq™ DNA Ready-to-use Panels
- Ion AmpliSeq™ RNA Ready-to-use Panels
- Ion TargetSeq™ Custom Enrichment Kits
- Library Kits (RNA and DNA)
- Barcode Kits (1-16 ... 81-96)
- Control Kit
- Ion Library Equalizer™ Kit
- TaqMan® Library Quant Kit
- Ion Sphere Control Kit

+ ION KITS

02

Prepare Template

- Ion OneTouch™ 2**
- Ion PGM™ Template OT2 200 Kit
 - Ion PGM™ Template OT2 400 Kit

+ ION ONE-TOUCH™ SYSTEM

03

Run Sequence

- Ion 314™ Chip (8-pk)
- Ion 316™ Chip (4-pk, 8-pk)
- Ion 318™ Chip (4-pk, 8-pk)
- Ion 314™v2 Chip (8-pk)
- Ion 316™v2 Chip (4-pk, 8-pk)
- Ion 318™v2 Chip (4-pk, 8-pk)

Ion OneTouch™ 2

- Ion PGM™ Sequencing 200 Kit v2
- Ion PGM™ Sequencing 400 Kit

+ ION PGM™ SEQUENCER

04

Analyze Data

- Torrent Server
- Torrent Suite Software
- Bioinformatics Services
- Torrent Browser Plugin Store
- Ion Reporter™ software

+ TORRENT SERVER

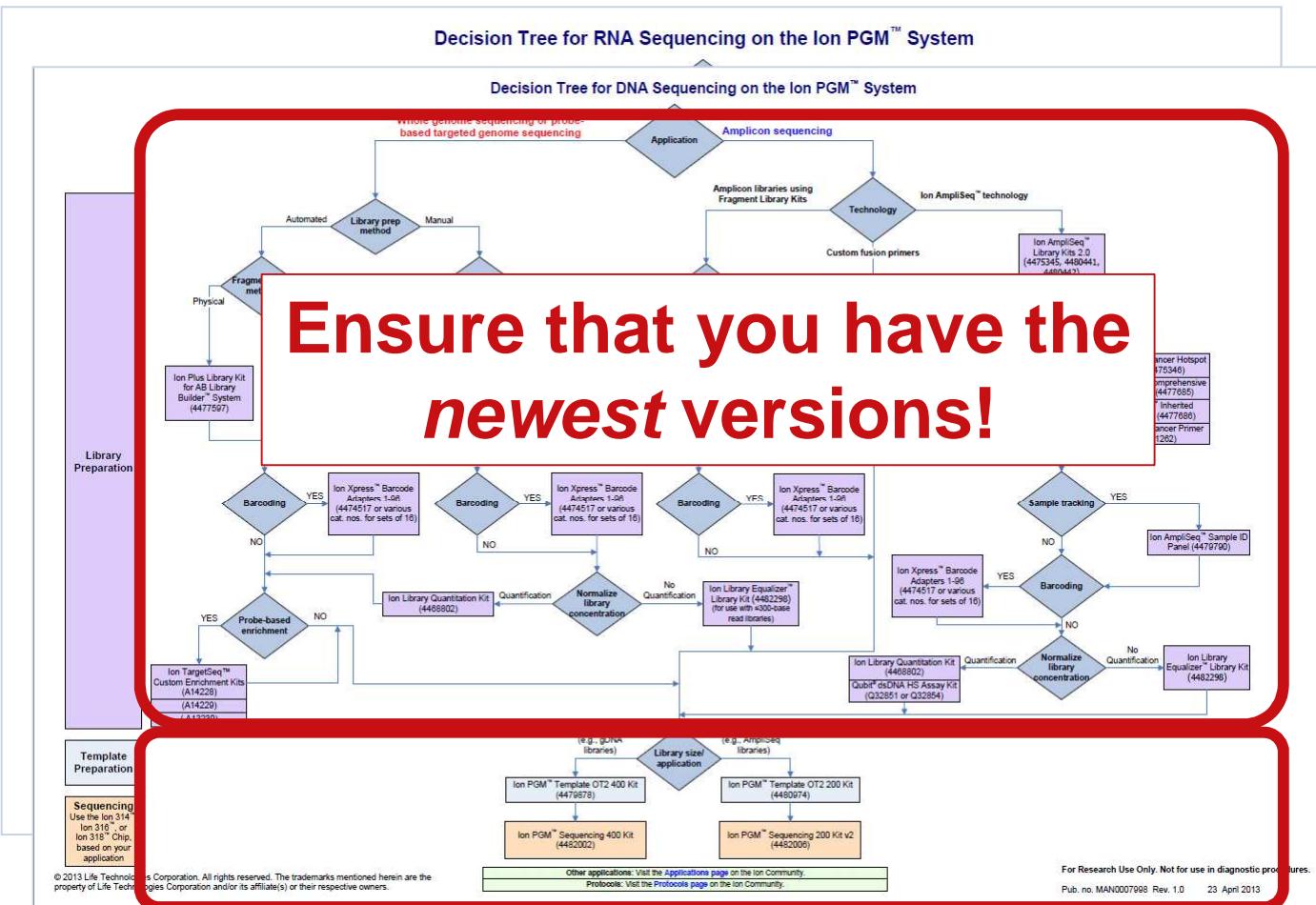
Ion Kit Compatibility for PGM™ System

Ion OneTouch™ System version	Controls	Template kit	Sequencing kit
Ion OneTouch™ 2 System 	<p>Ion PGM™ Controls Kit (4480449)</p> <p>Ion PGM™ Controls v2* (4482010)</p>	<p>Ion PGM™ Template OT2 200 Kit (4480974)</p> <p>Ion PGM™ Template OT2 400 Kit* (4479878)</p>	<p>Ion PGM™ Sequencing 200 Kit v2 (4482006)</p> <p>Ion PGM™ Sequencing 400 Kit* (4472002)</p>

* Use with Ion Chip Kit v2

** Check kit compatibility with instrument software version

Ion Kit Guide: Ion PGM™ Kit Decision Trees



Select application, library prep method & match kit

Select read length, match template prep kit & sequencing kits

<http://ioncommunity.lifetechnologies.com/docs/DOC-1804>

Ion Community

ion torrent by life technologies®

Ion Community

Search Ion Torrent Community

Applications Datasets Protocols Products Programs & Events

Experience One Community. A World of Possibilities. SEPT 13-14 / 2012 Hotel Sofitel, San Francisco Bay Register Now >

INTRO TO ion torrent by life technologies® Learn more about the Ion Torrent workflow, protocols, register for upcoming webinars and view recorded webinars.

Ask For Help Get Help

UPCOMING EVENTS

- 07 RNA-Seq Analysis Webinar August 07 (Online)
- 09 ECCB 2012 September 03-12 (Basel, Switzerland)
- 13 Ion World 2012 September 13-14 (San Francisco, CA)

RUN RECOGNITION

Ion 318™ League - Q20 Bases:

- 1 mholder 1,163,375,776
- 2 mholder 1,105,816,385

If you are looking for something from the old site, here is a quick reference guide to where some of the spaces are now located. If there are more you would like to add to this list, let us know.

- PGM Users : Products > PGM
- PGM User Discussions : Products > PGM > Content
- Torrent Dev : Products > Torrent Suite
- Sending /Viewing a private message : Click on your user name in the upper right-hand corner, then click on the Private Message tab

Best Regards,

INSTRUMENTS

- Ion OneTouch™ System
- Ion PGM™ Sequencer
- Ion Proton™ Sequencer
- Torrent Server

ION SOFTWARE

- Ion AmpliSeq Designer™
- Ion Reporter™
- Torrent Browser Plugin Store™
- Torrent Suite™

ION PARTNERS

- Avadis NGS
- BioTeam
- DNAStar
- Partek
- SoftGenetics
- Other Solutions

Protocols

Construct Library

Prepare Template

Run Sequence

Type to filter by text

Title

Do longer incubations of Sequencing Polymerase prior to loading PGM chip improve data output?? Scott Herke 0 0 8 194 August 9, 2012 3:29 PM

Lowest possible DNA input? wijfjes 0 0 2 46 August 9, 2012 2:55 PM

Necessary Number of Replicates for 'Controls' & 'Samples' in Ion qPCR Quantitation assay? Scott Herke 0 1 7 101 August 9, 2012 2:49 PM

Multiplex PCR amplicon results in 86% polyclonal ISP underbch 0 0 7 152 August 9, 2012 12:48 PM

Pariposer issues? gaglajm 0 0 23 200 August 9, 2012 10:55 AM

Discussions Forum

<http://ioncommunity.lifetechnologies.com/welcome>

Product Updates

The screenshot shows a web browser interface for the "Ion Community" section of the "ion torrent by life technologies" website. The top navigation bar includes links for Activity, Communications, Actions, Browse, Create, History, Bookmarks, and a welcome message for "Amy Tam". The main content area displays a group page for "ion torrent". The group is identified as a "private group" where the moderator must approve content or participation. A red callout box contains the text: "IMPORTANT: Join group ‘Ion Torrent Updates’ during training". Another red callout box below it contains the text: "Also sign up for automatic email notifications after joining group!". The footer of the page includes copyright information: "© 2012 Life Technologies Corporation. All Rights Reserved".

IMPORTANT:
Join group
“Ion Torrent Updates”
during training

Also sign up for automatic
email notifications after joining
group!

<http://ioncommunity.lifetechnologies.com/groups/updates>

Demonstrated Applications on Ion Community

Protocol Types

Protocols posted in the Ion Community are designated as one of three types:

- **Life Technologies Ion Supported Protocol** - Protocols fully supported by Ion Torrent.
- **Life Technologies Ion Demonstrated Protocol** – Protocols have been successfully demonstrated by Ion Torrent research and development, but not formally validated. There are no technical specifications for Ion demonstrated protocols. Users assume all risk when utilizing Ion demonstrated protocols and recognize that support for Ion demonstrated protocols occurs through community discussion. All customers are encouraged to discuss and contribute via the community.
- **Customer Demonstrated Protocol** - Protocols have been successfully demonstrated in customer's laboratory. Ion Torrent does not provide support for customer demonstrated protocols. Users assume all risk when utilizing customer demonstrated protocols and recognize that support for customer demonstrated protocols occurs through community discussion. All customers are encouraged to discuss and contribute via the community.

http://ioncommunity.lifetechnologies.com/community/products/pgm/user_guides_and_bulletins

Demonstrated Applications on Ion Community

Life Technologies 3rd Party Supported Protocol	
Thermo Scientific MuSeek Library Preparation Kit for Ion Torrent™ For MuSeek library preparation technical support, please contact Thermo Scientific: <ul style="list-style-type: none">• web site: www.thermoscientific.com• support email in North America: ts.molbio@thermofisher.com• support email in Europe: ts.molbio.eu@thermofisher.com	
Life Technologies Ion Demonstrated Protocols*	
Ion AmpliSeq 2.0 Library Preparation on the Tecan Freedom EVO 150	
Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan® RNase P Detection Reagents Kit	
Life Technologies Ion Demonstrated Protocols *	
Ion ChIP-Seq Library Preparation	
Ion Mate-Paired Library Preparation (Life Technologies Demonstrated Protocol)	
Customer Demonstrated Protocols *	
Size Selection of gDNA Fragment Libraries using Gel Electrophoresis	

Ion Trainings

Ion Torrent Community → Programs & Events → Training

WELCOME TO ION TORRENT TRAINING!

Welcome to the Ion Community Training Page! We recommend you start with the new Training Decision Tree to determine what training path is best for you: [Ion Torrent Training Decision Tree](#)

ION INTRODUCTORY TRAINING: FOR NEW USERS

Self-paced Training
 [Ion Torrent Workflow Tutorials](#)
 [Introduction to Ion Torrent Informatics](#)
 [How to get started with Torrent Suite Software](#)

Instructor-led Training
 PGM™ System Training [US/CAN, register >](#) [EU, register >](#)
NEW! Ion Proton™ System Training [US/CAN, register >](#) [EU, register >](#)
Ion Data Analysis Essentials [US/CAN, register >](#)
Introduction to Torrent Suite Software Web Course [US/CAN, register >](#) [EU, register >](#)

ION AMPLISEQ™ TRAINING

Self-paced Training
 [Ion AmpliSeq™ ready-to-use panels](#)
[Ion AmpliSeq™ ready-to-use panels Webinar \(June 2012\), view](#)
 [Ion AmpliSeq™ Custom Panels](#)
 [Ion AmpliSeq Designer Training Video Series](#)

Instructor-led Training
 Ion AmpliSeq™ Library Preparation Training [US/CAN, register >](#) [EU, register >](#)
Ion AmpliSeq™ Data Analysis Web Course [US/CAN, register >](#) [EU, register >](#)
Ion Reporter™ Software Workflows Web Course [US/CAN, register >](#) [EU, register >](#)
NEW! Ion Reporter™ Software Web Session [US/CAN, register >](#) [EU, register >](#)

ION RNA-SEQ TRAINING

Self-paced Training
 [Proton Transcriptome Library Preparation \(Proton Only\)](#)
 [Proton Transcriptome Data Analysis Workflow \(Proton Only\)](#)

Ion RNA-Seq Webinar Series
Total Transcriptome Analysis on the Ion Torrent™ Platform (June 2013), [view](#)
RNA Sequencing for Biomarker Discovery (June 2013), [view](#)
Performing transcriptome Sequencing on the Ion Proton System (June 2013), [view](#)
RNA Sequencing from cells to next-gen seq by Ambion, (June 2012), [view](#)
Finding the biology in your RNA-Seq data with Partek software, (July 2012), [view](#)
End-to-end RNA-Seq analysis in Avadis NGS, (Aug 2012), [view](#)

Instructor-led Training
 [Ion RNA Library Preparation Training](#) [US/CAN, register >](#) [EU, register >](#)
 [Ion AmpliSeq™ RNA Data Analysis Web Course](#) [US/CAN, register >](#)

ION WHOLE GENOME AND TARGETSEQ TRAINING

GETTING STARTED
 [Training](#)
 [Ion Training Decision Tree](#)
 [Ion Training for New Users](#)
 [Ion AmpliSeq™ Training](#)
 [Ion RNA-Seq Training](#)
 [Whole Genome & TargetSeq Training](#)
 [Instructor-led Training](#)
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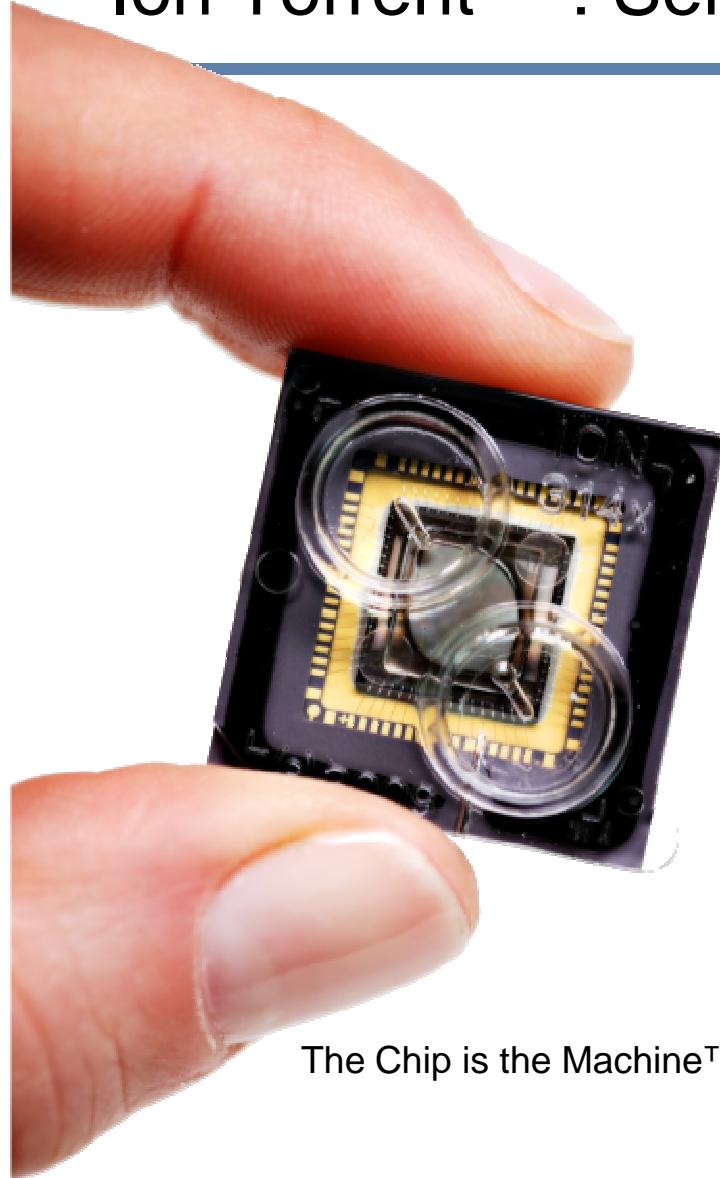
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