

Evaluation of the Promega Maxwell® CSC and the Maxwell® CSC DNA FFPE Kit.

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1. Introduction

Personalized medicine for oncology patients is increasingly based upon mutation detection in cancerous tissues, typically stored as Formalin Fixed Paraffin Embedded (FFPE) tissues. An important component of the workflow needed for biomarker discovery is a DNA extraction method that produces high quality DNA, suitable for high resolution downstream analysis, including next generation sequencing. FFPE samples are difficult samples for molecular labs as the fixation process causes DNA fragmentation and protein-DNA cross linking. Frequently the tissues also contain inhibitors and/or quenching substances that must be removed in the extraction process.

In this research study, we provide a side-by-side evaluation of the Promega Maxwell CSC DNA FFPE automated extraction and the Roche Life Sciences MagNaPure method for isolating DNA from FFPE tissues of various tumor types.

2. Methods

The Maxwell CSC³ instrument and Promega Maxwell CSC DNAFFPE Kit⁴ were used for automated extractions for comparison with the Roche MagNaPure FFPE Tissue DNA Isolation Kit.

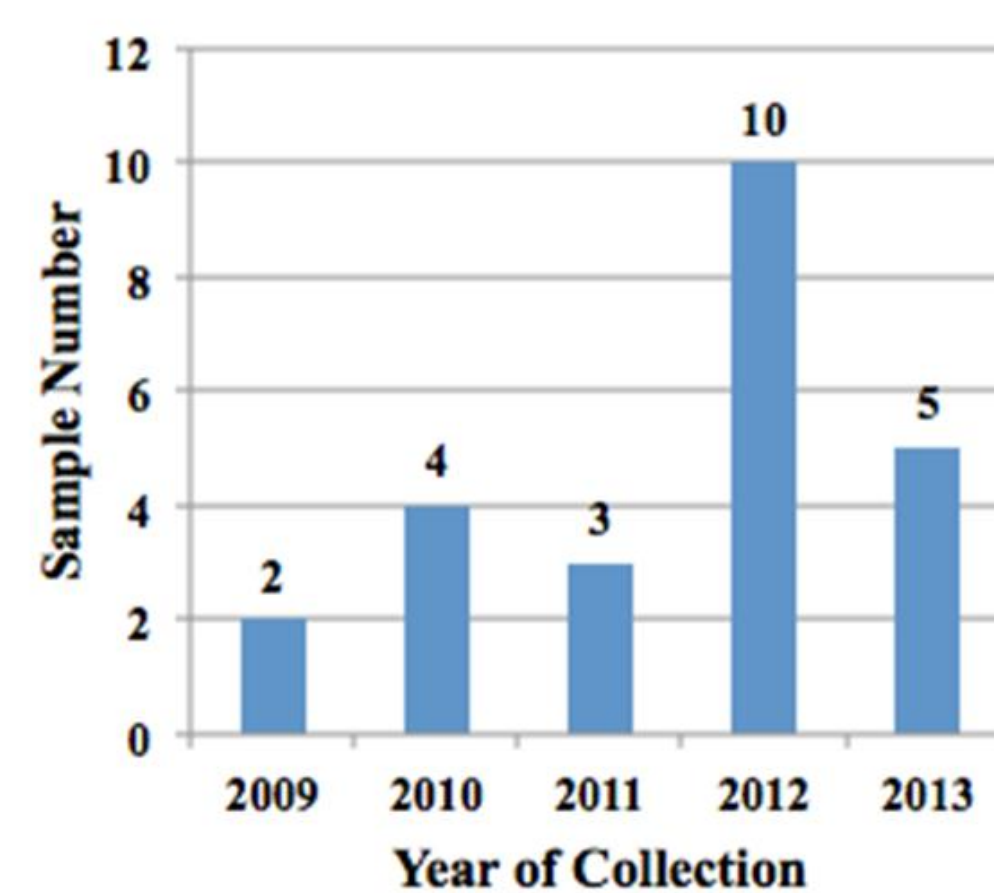
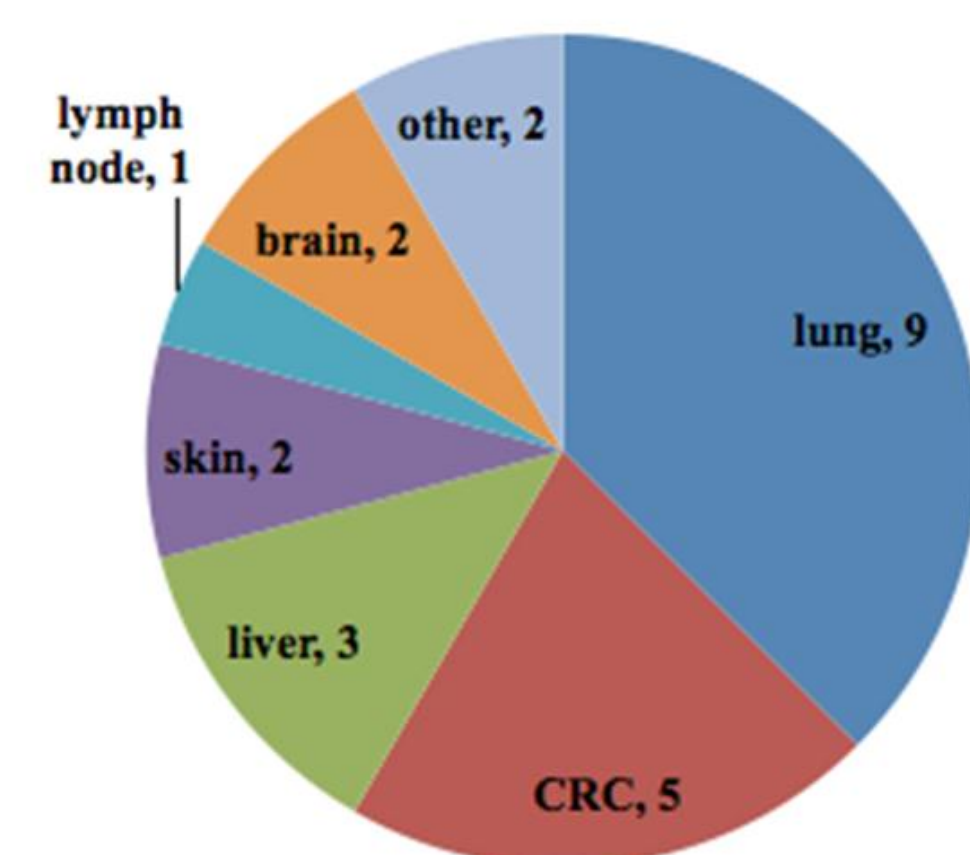
Equivalent single 5uM sections from 24 FFPE samples (Lung 9, Colorectal Cancer (CRC) 5, Liver 3, Skin 2, Lymph nodes 1, Brain 2, Other 2) were extracted via both methods following manufacturer's protocols.

Yields of DNA were assessed via fluorescent dye by the Promega Quantus Fluorometer⁵ and / or Life Technologies Qubit Fluorometer.

Sample libraries were prepared and sequenced on the Ion Torrent PGM, using the Ion AmpliSeq™ Cancer Hotspot Panel v2 (RUO) from Life Technologies.

3. FFPE Tissue Types Studied

FFPE Tissue Distribution and Date of Collection



4. Maxwell CSC DNA FFPE Extraction Process

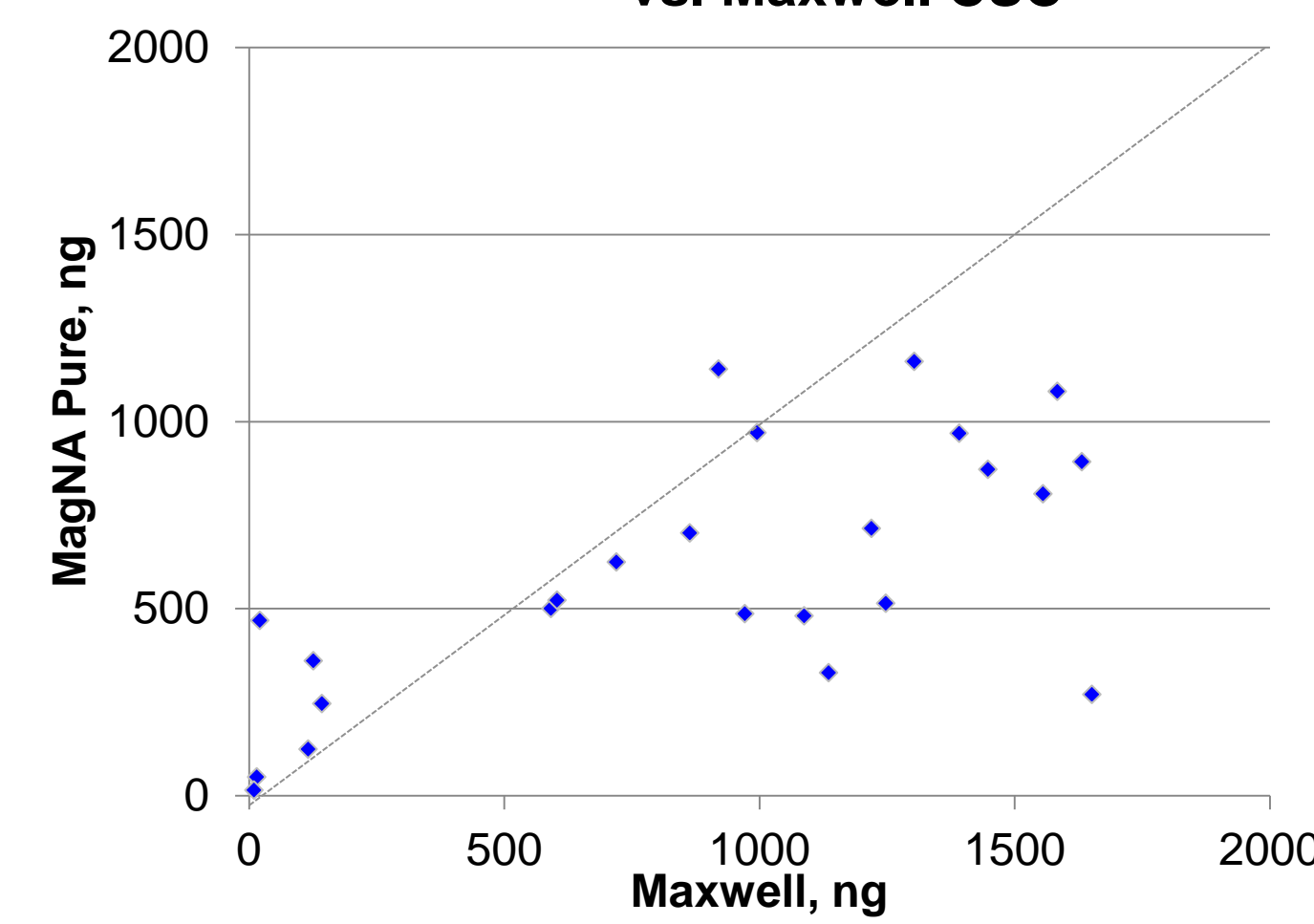


Promega Maxwell® CSC Maxwell® CSC DNA FFPE Kit

Step	Description																
1	Scrape entire paraffin sample into individual tube. Spin full speed briefly in microfuge to bring material down.																
2	Add 300 µl of mineral oil to each sample. Vortex 10 seconds to mix.																
3	Incubate at 80°C for 2 minutes. Vortex to mix. Prepare a master mix of the Lysis buffer, Proteinase K and blue dye as shown below.																
4	<table border="1"> <thead> <tr> <th>Reagent</th> <th>Amount/reaction</th> <th>Reactions (n+2)</th> <th>Total</th> </tr> </thead> <tbody> <tr> <td>Lysis Buffer</td> <td>224ul</td> <td>18</td> <td>4032ul</td> </tr> <tr> <td>Proteinase K</td> <td>25ul</td> <td>18</td> <td>450ul</td> </tr> <tr> <td>Blue Dye</td> <td>1ul</td> <td>18</td> <td>18ul</td> </tr> </tbody> </table>	Reagent	Amount/reaction	Reactions (n+2)	Total	Lysis Buffer	224ul	18	4032ul	Proteinase K	25ul	18	450ul	Blue Dye	1ul	18	18ul
Reagent	Amount/reaction	Reactions (n+2)	Total														
Lysis Buffer	224ul	18	4032ul														
Proteinase K	25ul	18	450ul														
Blue Dye	1ul	18	18ul														
5	Add 250ul of master mix to each sample tube, and vortex for 5 seconds.																
6	Centrifuge at 10,000 x g for 20 seconds to separate layers. Gently mix aqueous phase with pipet to break up pellet if present.																
7	Incubate at 56°C for 30 min																
8	Incubate at 80°C for 4 hours																
9	Allow the sample to cool to room temperature. (~5 minutes) Add 10 µl of RNase A directly to the lysed sample in the lower phase. Pipet to mix.																
10	Incubate at room temp for 5 minutes																
11	Spin sample full speed 2min. Add sample to cartridge, leaving pellet if present.																
12	Run Method																

4. Quantitation post extraction

Total Yield Comparison – MagNa Pure vs. Maxwell CSC

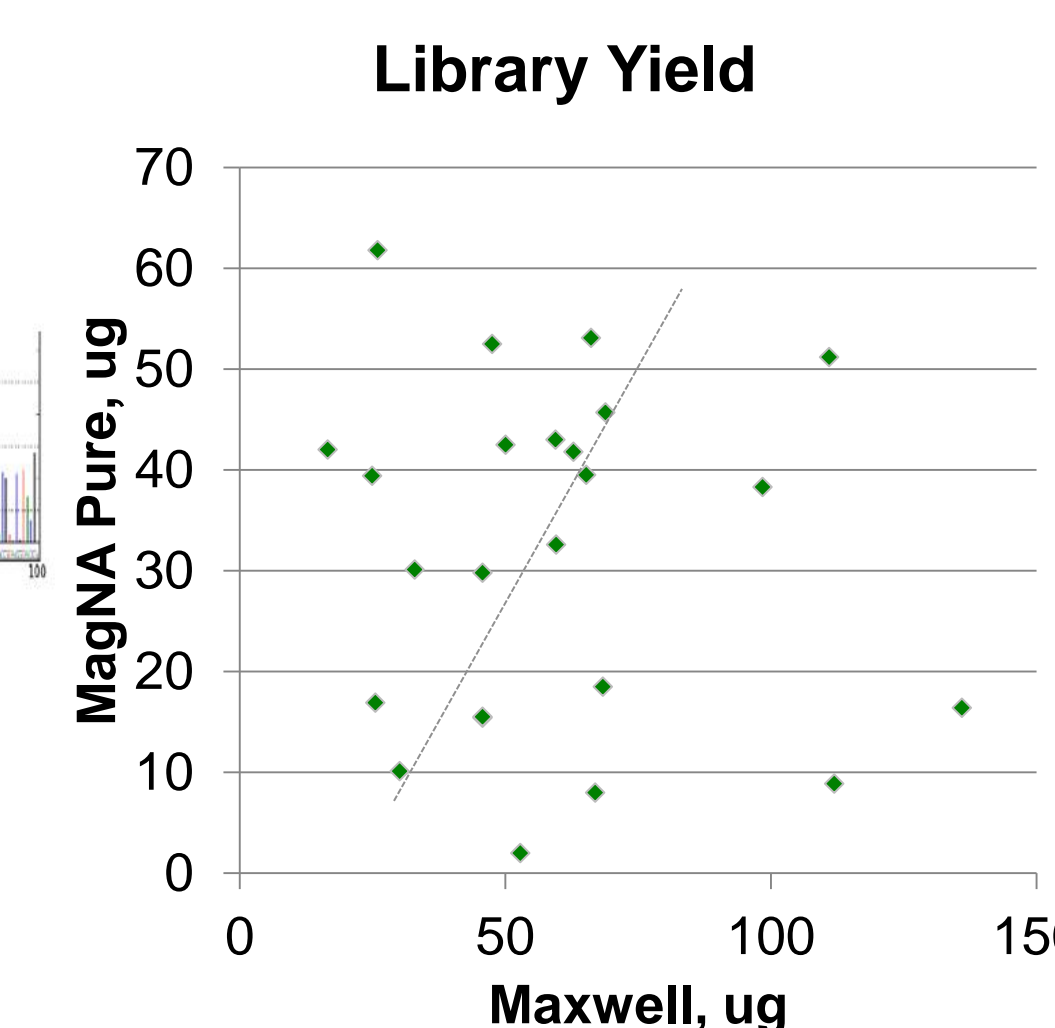
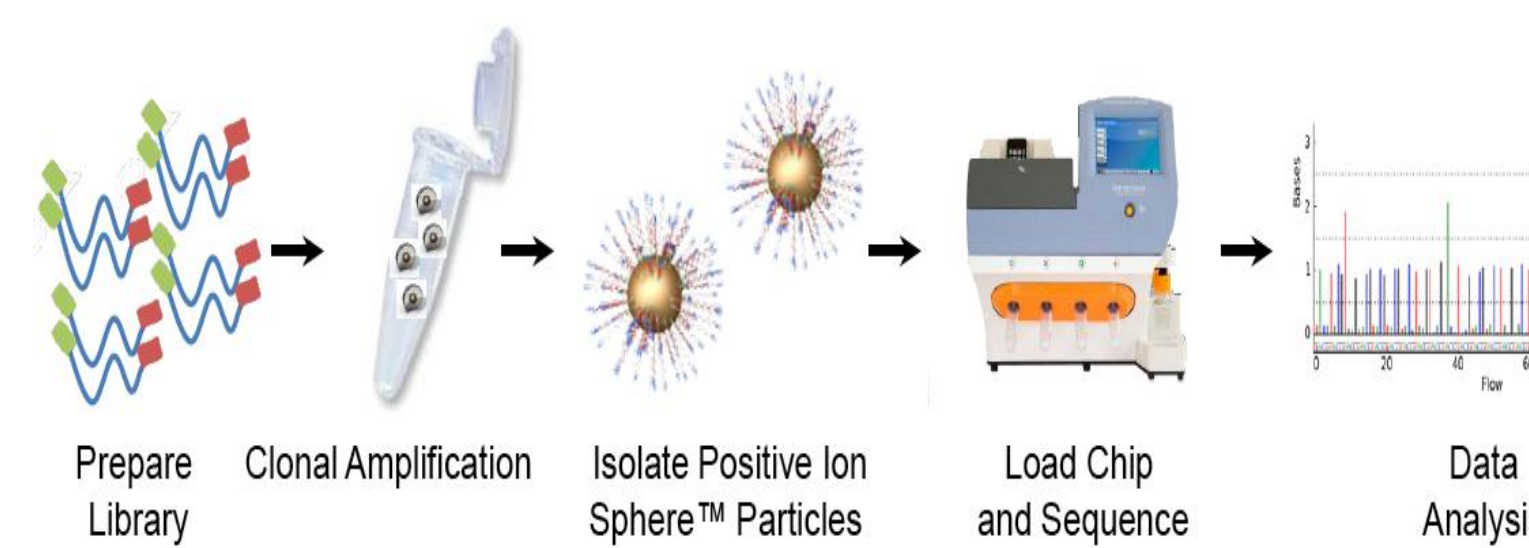


Promega Maxwell® CSC yields more DNA from the same input of FFPE tissue

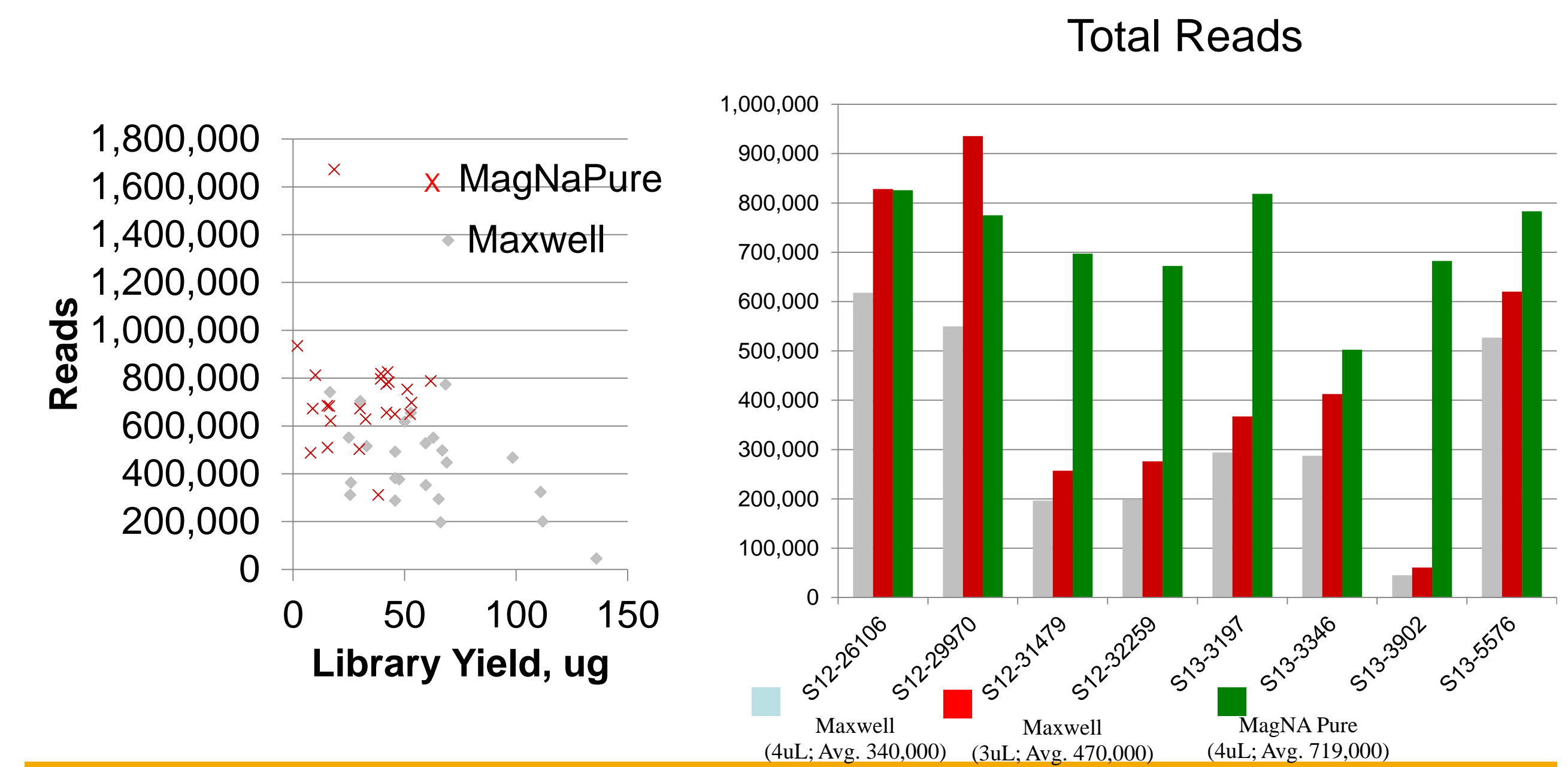


Promega Quantus® Fluorometer and QuantiFluor® Dyes⁵

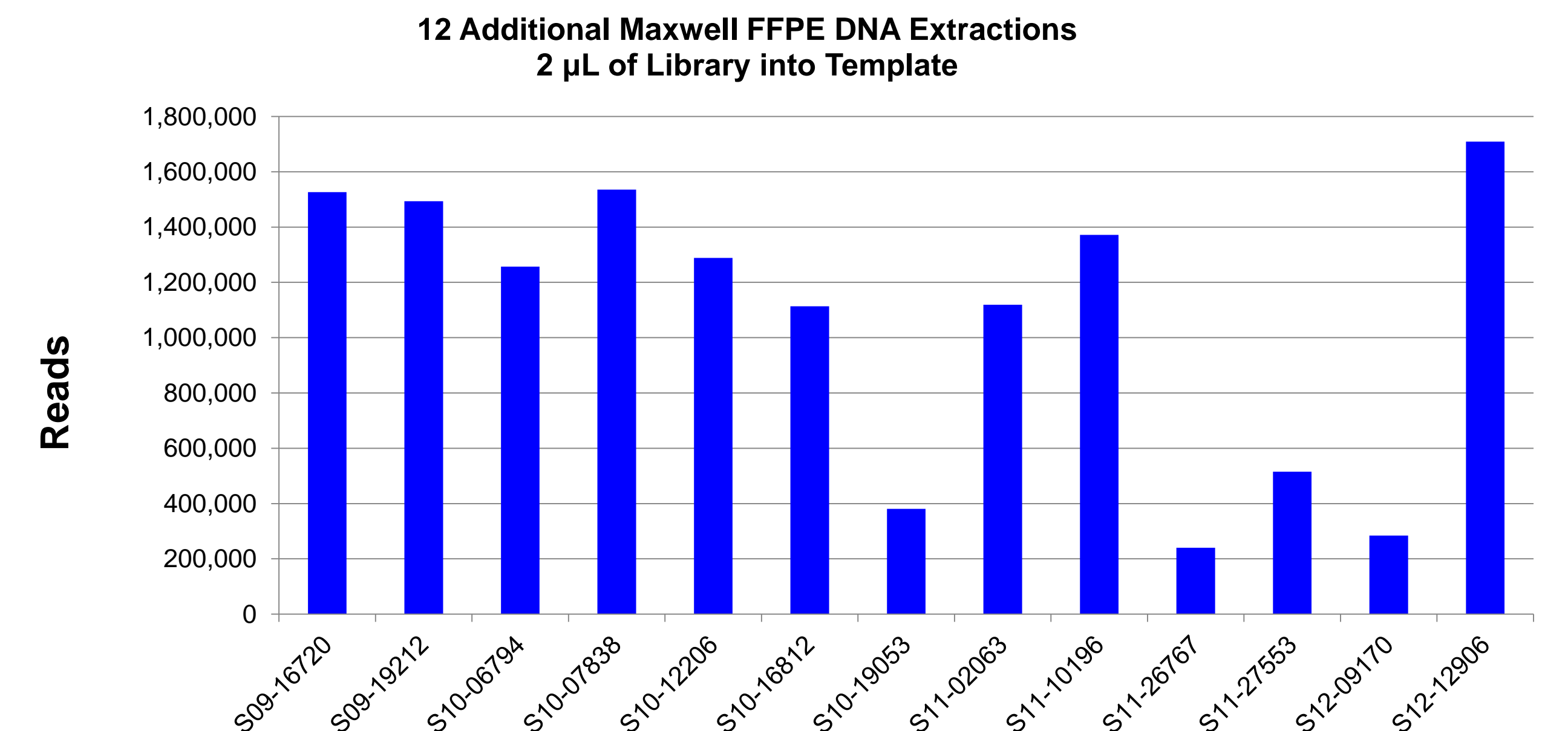
6. Ion Torrent PGM⁵ Workflow



7. Reads per Sample vs Library Yield 4uL of Library



8. Reads per sample 2uL of Maxwell® extracted FFPE Library



9. Conclusions

- Maxwell® CSC and CSC DNA FFPE Kit:
- is efficient and easy to use for FFPE DNA extraction
 - provides greater yield of DNA per each 5uM tissue section

Maxwell® CSC FFPE extracted DNA:

- Compatible with multiplex PCR (library prep), emulsion PCR (template PCR) and semiconductor DNA sequencing (Ion AmpliSeq from Life Technologies)

- Provides greater yield of library (optimized template) when compared to MagNa Pure

³ For In Vitro Diagnostic Use
⁴ For In Vitro Diagnostic Use. The Maxwell FFPE DNA Kit is only intended for use as an in vitro diagnostic medical device with FFPE tissue samples collected from breast, lung or colon.
⁵ For Research Use Only. Not for use in diagnostic procedures.