Automated Electrophoresis Applications for Synthetic Biology

Melissa Huang Liu, Ph.D.

Product Manager, Electrophoresis

Agilent Technologies

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Rev. 1 [December 17, 2015]

Outline

2100 Bioanalyzer & 4200 TapeStation Systems

Automated Electrophoresis in Genome Editing

Automated Electrophoresis in Functional Genomics

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Electrophoresis Product Portfolio

2100 Bioanalyzer System – Electrophoresis in microchannels

- *Analysis of biomolecules: DNA, RNA, Proteins and Cells*
- *RIN for user-independent RNA QC*

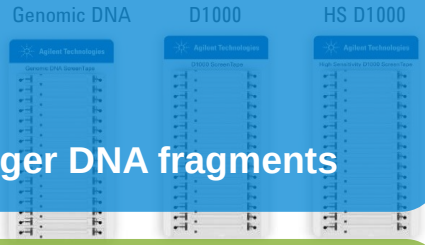
4200 TapeStation System - ScreenTape Technology

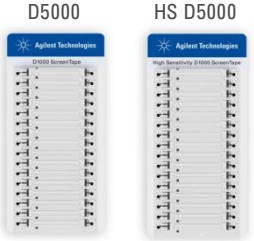
- *fully scalable from 1-96 samples*
- *96-well plate walkaway*
- *RIN^{e} for user-independent RNA QC*
- *DIN for user-independednt gDNA QC*

Assay Portfolio

DNA assays

- Sizing, Quantitation
- gDNA integrity (DIN)
- PCR products, digests, larger DNA fragments





RNA assays

- Sizing, Quantitation
- total RNA, mRNA
- Purity & integrity determination (RIN; RIN^e)

Protein assays

- Sizing, Quantitation
- cell lysates, column fractions, purified proteins, antibodies etc

Flow Cytometry Assay

- Two color detection
- Analysis of protein expression in cells

Slab gel analysis - a bottleneck in Protein & DNA analysis

- Manual process
- Difficult to automate
- Slow
- Not accurate enough
- Bad reproducibility
- No direct comparison

Automated Electrophoresis has the potential to address this

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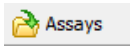
Overview run workflow

- Prepare gel/dye mix

- Prime Chip
- Prepare Chip

- Select assay



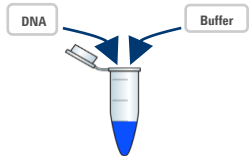
- Run

0 ——— 40 min

0

60 min

- Prepare samples



- Load Tips and Samples

- Automatic Assay selection
- Run

0 ——— 20 min

1 

= ~12 samples

in 30-40 minutes

1 

= 1-16 samples

in 10-20 minutes

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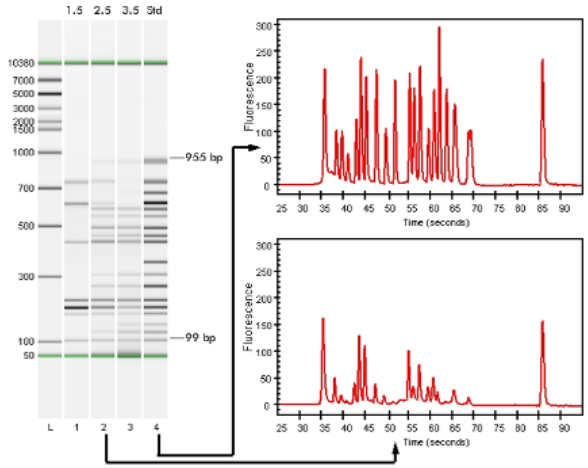
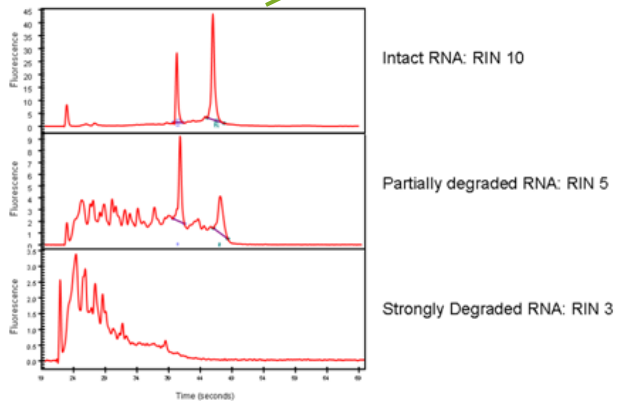
DNA& RNA Applications in Genome Editing

PCR products | gDNA integrity | Restriction Digest Analysis | Quality Control in NGS | Total RNA integrity | Quality Control of Oligos



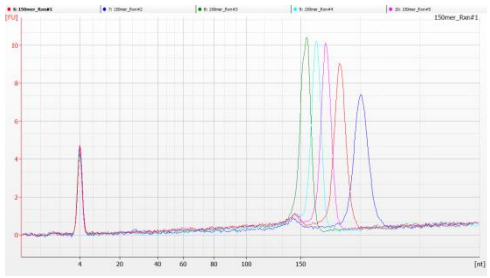


Intact RNA: RIN 10

Partially degraded RNA: RIN 5

Strongly Degraded RNA: RIN 3

Oligo tailing reaction



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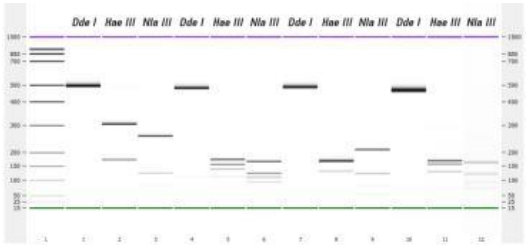
Examples of Electrophoresis in SynBio workflows

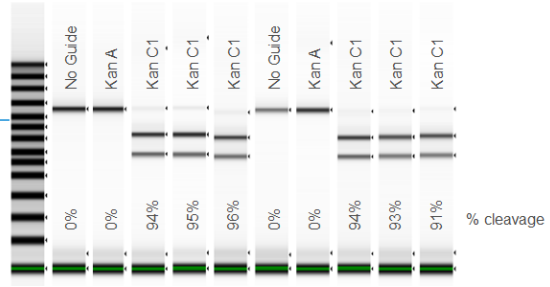
Vector Assembly

- ❑ Starting material QC: Gene of interest after PCR amplification
- ❑ Verifying vector composition with restriction analysis
- ❑ Vector analysis by NGS (including multiple QC steps)



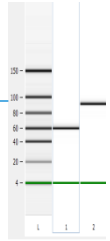
CRISPR/ Cas9

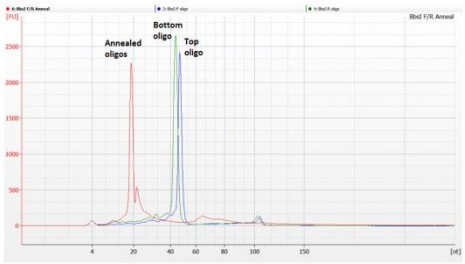
- ❑ DNA target after PCR amplification
- ❑ Cleavage efficiency of digestion with Cas9 Nuclease (Detection and Quantitation)



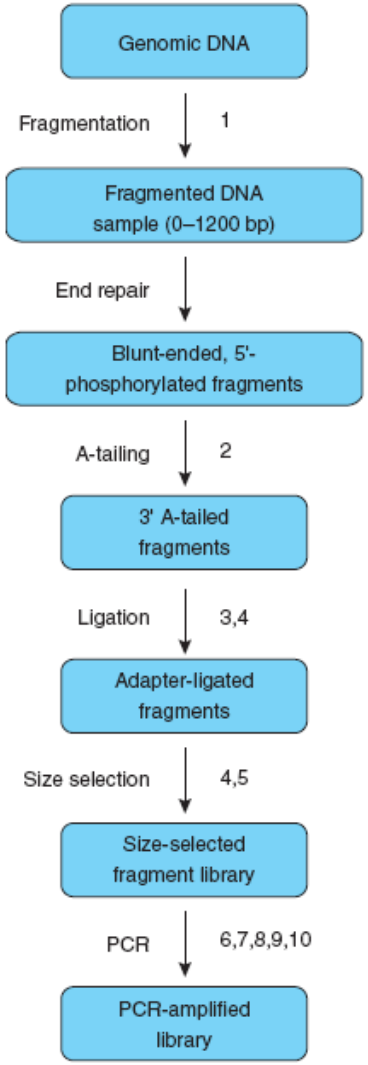
guide RNA synthesis

- ❑ Starting material QC: oligo templates
- ❑ Monitoring oligo annealing reactions
- ❑ Quantitation and QC of gRNA product





Example of Electrophoresis in a complex workflow: NGS Quality Control



Integrity of starting material

Size distribution after fragmentation

Size distribution of adapter-ligated fragment library

Quality control and quantification after PCR

Adapted from Quail et al (2008) Nature Methods)

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RNA Applications in Functional Genomics

Gene Expression

Transcriptome Profiling

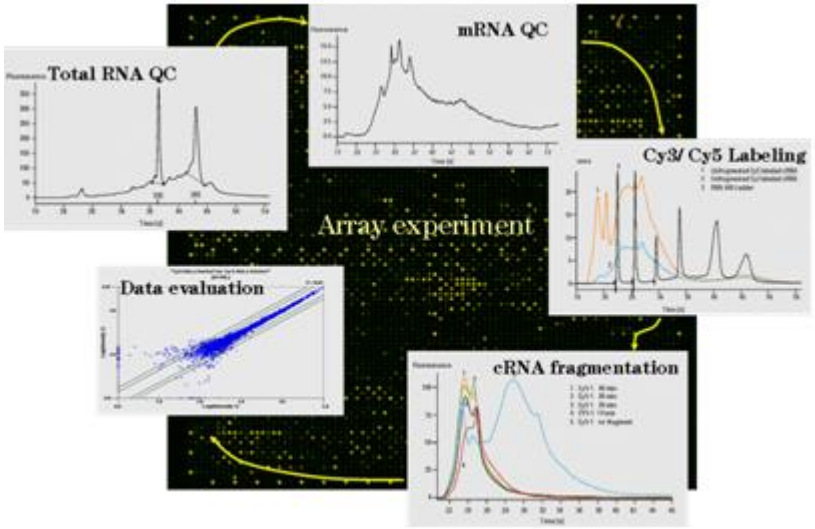
RNA QC for microarrays

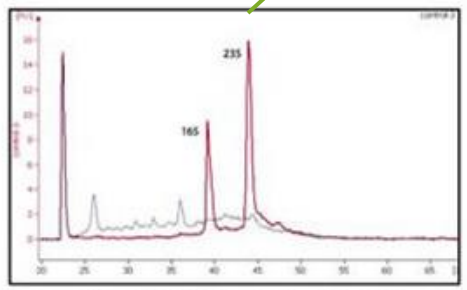
RNA QC for qPCR

RNA QC for NGS

smallRNA QC

mRNA extraction





C. Bacteria: Total RNA (red) and RiboMinus™ RNA (blue) isolated from *E. coli*

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Protein Applications in Functional Genomics

Monitor Protein Purification

Protein Expression in cell lysates

Protein quantitation

Protein QC prior to MS analysis

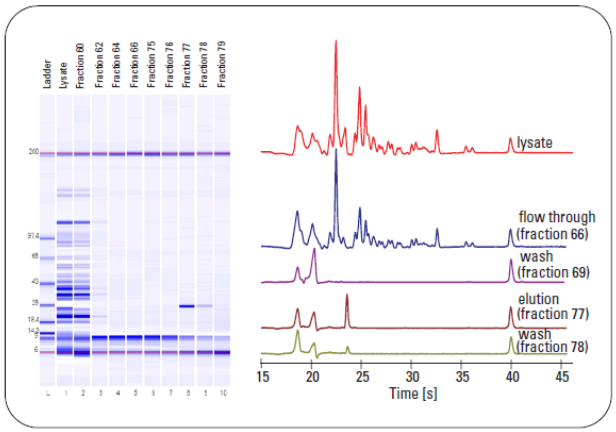
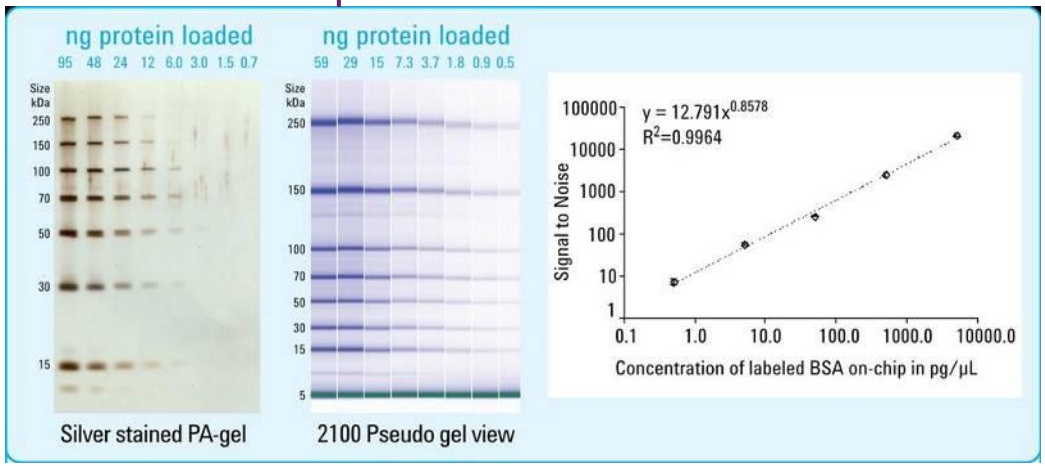


Figure 4
Analysis with the Protein 200 assay showing the gel-like image and the corresponding electropherograms.



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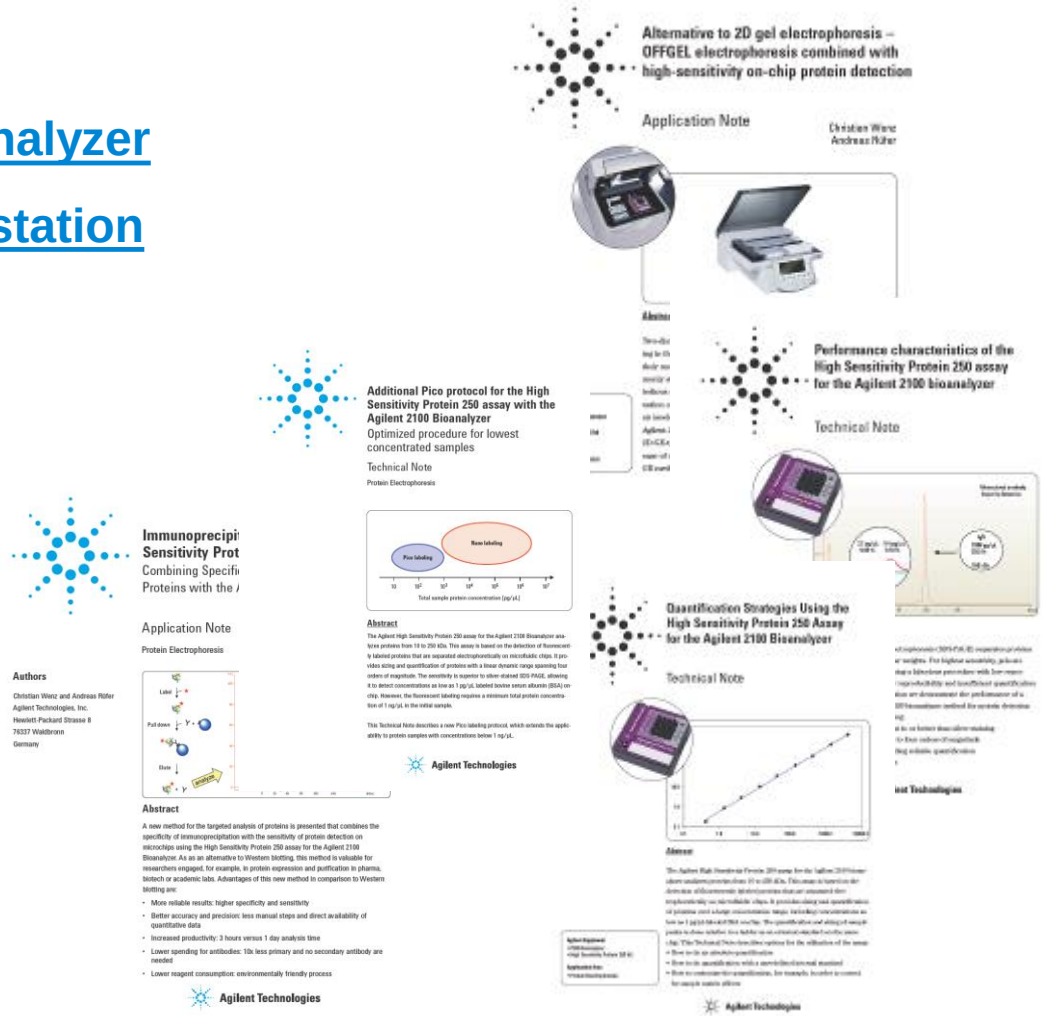

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