Comparative Genome and Transcriptome Analysis of Small Cell Numbers

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Director Scientific Applications, QIAGEN R&D, Hilden, Germany
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Overview

- Product portfolio
- Single cell genomics
- Single cell transcriptomics
- Comparative genome and transcriptome analysis
**REPLI-g® single-cell portfolio overview**

- **Single cell**
  - Multiple cells
  - Tissue
  - Blood
  - gDNA
  - RNA

- **Single-cell DNA sequencing** (1–1000 cells)
- **Single-cell RNA sequencing** (1–1000 cells)
- **Single-cell DNA analysis** (1–1000 cells)
- **Single-cell RNA analysis** (1–1000 cells)
- **Comparative analysis of DNA and RNA** (25–1000 cells)

- **REPLI-g Single Cell DNA Library Kit**
- **REPLI-g Single Cell RNA Library Kit**
- **REPLI-g Single Cell Kit**
- **REPLI-g WTA Single Cell Kit**
- **REPLI-g Cell WGA & WTA Kit**

- **NGS library**
- **Amplified WTA- DNA or WGA-DNA**

- **NGS**
- **Microarray**
- **qPCR**

**Sample to Insight**

**Comparative Genome and Transcriptome Analysis of Small Cell Numbers**
REPLI-g single-cell portfolio overview

Sample to Insight

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- **REPLI-g Single Cell Kit**

- **REPLI-g Cell WGA & WTA Kit**
REPLI-g single-cell portfolio overview

Single cell
- DNA Sequencing (1 – 1000 cells)
- Single Cell DNA Library Kit

Multiple cells
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Tissue
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Comparative analysis of DNA and RNA (25–1000 cells)
- REPLI-g Cell WGA & WTA Kit

Amplified WTA- DNA or WGA-DNA
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Comparative Genome and Transcriptome Analysis

REPLI-g Single Cell Kit
Comparative Genome and Transcriptome Analysis

Single cell genomics with REPLI-g Single Cell Kit

- 2–5 cells
- 10–25 femtograms DNA

WGA by REPLI-g Single Cell

Library Q
- QIAGEN

Library I
- Other vendor

MiSeq® sequencing

>98% of all reads are mapped to the genome after scWGA
I need an editable version of this file as the y-axis needs correcting

Devika Mathur - QIAGEN, 9/10/2015
Comparative Genome and Transcriptome Analysis

Single cell genomics with REPLI-g Single Cell Kit

<table>
<thead>
<tr>
<th></th>
<th>REPLI-g Single Cell Kit (1 cell)</th>
<th>REPLI-g Single Cell Kit (1000 cells)</th>
<th>gDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error rate</td>
<td>0.43%</td>
<td>0.22%</td>
<td>0.35%</td>
</tr>
<tr>
<td>Chimeras</td>
<td>0.08%</td>
<td>0.06%</td>
<td>0.11%</td>
</tr>
<tr>
<td>Indel rate</td>
<td>0.05%</td>
<td>0.02%</td>
<td>0.01%</td>
</tr>
</tbody>
</table>


No increase in error rates, chimeras or InDel rate by scWGA

Compared to our current method, the REPLI-g Single Cell Kit greatly reduced the amplification bias and delivered more uniform whole genome amplification (comparable to non-amplified genomic DNA) of single lymphocytes, making it possible to detect SNPs, CNVs, and SVs simultaneously. No significant differences were observed for next-generation sequencing parameters, such as the mean mapping quality, read mapping ratio, and read duplication ratio when compared to the high-quality results obtained using the REPLI-g Mini Kit.

Luting Song,
Staff Scientist, Oncology Research, Beijing Genome Institute (BGI), China
Comparative Genome and Transcriptome Analysis of Small Cell Numbers

Single Cell transcriptomics with REPLI-g WTA Single Cell Kit

- NGS workflow
- Single cells
- Whole genome amplification
- Library preparation
- Sequencing preparation
- NGS run
- Data alignment / analysis

High correlation of transcription patterns after REPLI-g Single Cell WTA reactions from low to high cell numbers

Central concept of phenotype realization

- The genome determines how the transcriptome will change upon changes within the environment

- Changes within the genome (e.g., mutation, CNV, SV) have an impact on:
  - How the transcriptome will change upon elicitors
  - Expression profile per se
Comparative Genome and Transcriptome Analysis

Requirements for comparative genome and transcriptome analysis

- The same sample is necessary for genome and transcriptome analysis
- Split of RNA and DNA reactions
- Equal processing steps with regards to
  - Reagents and enzymes
  - Temperature
  - Reaction times
  - Library or labeling processes
- Method is effective for **small samples** because of genomic changes from cell to cell (>WGA, WTA)
Comparative Genome and Transcriptome Analysis

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Comparative Genome and Transcriptome Analysis

REPLI-g Cell WGA & WTA: the process

Cells (25–1000) → Cell lysis

- gDNA removal
- WTA → WTA
- WGA → WGA
- cDNA synthesis
- Ligation
- Amplification
- Fragmentation
- Library prep
- Sequencing

- WGA-ready rxn
- Ligation
- Amplification
- Fragmentation
- Library prep
- Sequencing
HeLa cells: Genome changes and the transcriptome

<table>
<thead>
<tr>
<th>HeLa cells</th>
<th>HUVEC cells</th>
</tr>
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<tbody>
<tr>
<td>Immortal cervical cancer cells</td>
<td>Primary endothelial cells of umbilical cord</td>
</tr>
<tr>
<td>Triploid genome in average</td>
<td>Diploid cells</td>
</tr>
<tr>
<td>Strong heteroploidity on various chromosomes</td>
<td></td>
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<tr>
<td>Diploid, triploid and tetraploid chromosome regions</td>
<td></td>
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</tbody>
</table>

What is the impact of chromosome number variation on gene expression?

Genome and transcriptome comparison of HeLa cells with HUVEC cells
Comparative Genome and Transcriptome Analysis

HeLa cells: Genome changes and the transcriptome

50 HeLa cells
- Cell lysis
- REPLI-g Cell WGA & WTA Kit
- WGA
- WTA
- GeneRead™ Library
- Library prep
- Sequencing
- MiSeq
- Library prep
- Sequencing
- Bioinformatics
- Genome
- Transcriptome

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Genome
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Determination of numerical chromosome changes

Sample to Insight
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HeLa cells: Genome changes and the transcriptome

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50 HUVEC cells

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- GeneRead Library
- MiSeq
- Library prep
- WGA
- WTA
- Sequencing
- Transcriptome
- Genome

What is the impact of chromosome number variation on gene expression?
HeLa vs HUVEC cells: Very similar distribution of biotypes
  - except: 3x higher LincRNA RPKM in HeLa cells compared to HUVEC cells
- Most reads mapped to coding regions
- <10% reads not mapped to human sequence
- Small RNAs (e.g., miRNA) are underrepresented
- Almost no rRNA detection: < 0.01%
Comparative Genome and Transcriptome Analysis

>20,000 transcripts detected after WTA

- Smart reproducibility after WTA of 25 cells
- HeLa and HUVEC differ in expression profile
- >20,000 transcripts of >9000 genes detected
Comparative Genome and Transcriptome Analysis

Expression profile of HeLa and HUVEC cells differ significantly

A

B

C

D

HeLa vs HUVEC: 25 cells

8516 (HeLa)

5811 (68.2%)

7645 (HUVEC)

R² = 0.84

R² = 0.82

R² = 0.67
Comparative Genome and Transcriptome Analysis

Relative number of differentially expressed genes per chromosome

- Factor >3: 0.29 genes/Mb in average
- Factor <0.3: 0.09 genes/Mb in average

Most chromosomes harbor more genes that are overexpressed in HeLa cells compared to HUVEC cells.
Comparative Genome and Transcriptome Analysis

Relative number of differentially expressed genes per chromosome

Chromosome 4, 14 and 18:
Balanced number of genes that are higher and lower expressed compared to HUVEC
Comparative Genome and Transcriptome Analysis

Expectation for gene expression of genes that are *diploid* in HeLa and HUVEC cells

- Some genes are overexpressed in HeLa cells compared to HUVEC cells
- Other genes are expressed at a lower level
- Number of overexpressed genes ~ number of genes at a lower level
Copy number of chromosome 4 and 8 in HeLa
Comparative Genome and Transcriptome Analysis

Gene expression in HeLa cells compared to HUVEC cells in diploid genome regions

**Chromosome 4**
- Genes HIGHER expressed in HeLa compared to HUVEC cells
- Genes LOWER expressed in HeLa compared to HUVEC cells

**Chromosome 8**
- Genes HIGHER expressed in HeLa compared to HUVEC cells
- Genes LOWER expressed in HeLa compared to HUVEC cells
Comparative Genome and Transcriptome Analysis

Gene expression in HeLa cells compared to HUVEC cells in polyploid regions

Over expressed genes in subchromosomal regions with increased copy number

HeLa cells: Same number genes that are higher or lower expressed in diploid genome regions

HeLa cells: Polyploid regions result in higher gene expression
Comparative Genome and Transcriptome Analysis

Differentially expressed in diploid chromosomes in HeLa and HUVEC cells

HeLa cells: Same number genes that are higher or lower expressed in diploid genome regions
Comparative Genome and Transcriptome Analysis

Differentially expressed in diploid chromosomes in HeLa and HUVEC cells

HeLa cells: Polyploid regions result in higher genes expression
Relative number of differentially expressed genes per chromosome

Most chromosomes:
- Higher number of genes that are higher expressed in HeLa compared to HUVEC
- Exceptions are chromosomes 4, 14 and 18
Summary

- Changes within the genome (e.g., mutation, CNV, SV) have an impact on gene regulation

- Deep understanding of gene regulation requires the analysis of the genome, as well as the transcriptome from the same cell sample
  - Cancer research
  - Disease
  - Aging

- REPLI-g Cell WGA & WTA Kit provides a suitable tool for comparative analysis of genome and transcriptome
  - High-quality nucleic acid amplification from small samples
  - Comparison of like with like
  - Highly suitable for NGS
  - Based on proofreading REPLI-g SensiPhi Polymerase,
  - No introduction of errors due to proofreading activity
  - High affinity for low amounts of DNA
  - Strong strand-displacement activity
  - Reagents free of amplifiable DNA
Thank you to all webinar participants!

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Q&A Session

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