Clinical practice guidelines recommend limited genotyping by next-generation sequencing (NGS) for advanced non-small cell lung cancer (NSCLC) to guide treatment. Yet, small biopsies and tumor-in-containing samples pose challenges to testing. The tables below, from laboratories across the world, show how limited many of these samples are. PCR-CGP is generally seen as a tissue-saving method given its ability to deliver multiple biomarker results with a single sample. It is important to understand that the sample size and content requirements are not equal for all NGS-based methods. Some NGS-based methods can test smaller samples and deliver results for more patients.

### Multicentric feasibility study, US

- **Tumor area**
  - ≤25 mm²: 88%
  - >25 mm² and ≤25 mm²: 12%
  - >25 mm²: 4%

- **Tumor content**
  - ≤20%: 68%
  - >20%: 32%

**Number of samples**: 21,722

### Cancer Genetics, Inc., New Jersey

- **Tumor area**
  - ≤25 mm²: 58%
  - >25 mm²: 42%

- **Tumor content**
  - ≤20%: 43%
  - >20%: 57%

**Number of samples**: 1,791

### Life Lab, California

- **Tumor area**
  - ≤25 mm²: 75%

- **Tumor content**
  - ≤20%: 41%
  - >20%: 59%

**Number of samples**: 627

### Sarah Cannon Molecular Diagnostic Laboratory, London

- **Tumor area**
  - ≤25 mm²: 32%

- **Tumor content**
  - ≤20%: 68%
  - >20%: 32%

**Number of samples**: 2,796

### Sample requirements can differ greatly from one test to the next

<table>
<thead>
<tr>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGS (PCR amplicon-based)</td>
<td>NGS (PCR amplicon-based)</td>
</tr>
<tr>
<td>10% minimum tumor content</td>
<td>10% minimum tumor content</td>
</tr>
<tr>
<td>30% optimum tumor content</td>
<td>30% optimum tumor content</td>
</tr>
<tr>
<td>≤1 mm² tumor area, ≤100 ng input required</td>
<td>≤0.1 mm² tumor area, ≤50 ng input required</td>
</tr>
<tr>
<td>≤1 mm² tumor area, ≤1,000 ng input required</td>
<td>≤0.01 mm² tumor area, ≤5 ng input required</td>
</tr>
</tbody>
</table>

NGS-based testing input requirements are typically expressed as a quantity of nucleic acid (in nanograms) and can differ significantly between different NGS-based tests. The figures above illustrate the practical implications of these different requirements in terms of tumor area and tumor content. The histograms below show how limited many of these samples are. While NGS is widely adopted for advanced non-small cell lung cancer (NSCLC), the data below from laboratories across the world, show how limited many of these samples are. PCR-CGP is generally seen as a tissue-saving method given its ability to deliver multiple biomarker results with a single sample. It is important to understand that the sample size and content requirements are not equal for all NGS-based methods. Some NGS-based methods can test smaller samples and deliver results for more patients.

### Potential impact of different sample requirements on patients

- **Method 1**
  - ≤25 mm²: 88%
  - >25 mm² and ≤25 mm²: 12%
  - >25 mm²: 4%

- **Method 2**
  - ≤0.1 mm²: 98%
  - >0.1 mm²: 2%

Only one of two patients would have enough sample to be tested by Method 1 based on hybrid capture NGS, while 98% of samples could be successfully tested with a PCR amplicon-based method.