Next up, capture-specific considerations for

Start by considering your targets

Targets inform sonication, size-selection, and sequencing protocol
Library preparation

- sticky-end (Illumina®)
- blunt-end (Torrent®, dDNA)
- transposase (Nextera®)
- ssDNA (aDNA, Swift Bio)

 Ultimately:

- DNA input to library – maximize
- Library amplification – moderate
Capture materials

- Libraries
- Baits (+ hybridization & wash kit)
- Streptavidin-coated magnetic beads
- Magnetic rack

Capture materials considerations

- MYcroarray
- Water bath or hybridization oven
- Thermal cycler (×2)
- Low-bind PCR tubes (Axygen™)
- recc: Heat block, multi-channel pipettor

Library input to capture – 100-500 ng (14 - 72 ng/µL in 7 µL)

Multiplexing – up to 2 µg total per rxn

Choosing time & temperature

- Majority of projects:
  - 65°C temperatures (hyb, bind, wash)
  - Overnight hybridization
- Very rare and/or degraded templates:
  - 55 to 65°C temperatures
  - 24-72 hour hybridizations

Multiplexing – careful with your index combos!
### Budgeting – DNA to enriched library

<table>
<thead>
<tr>
<th>Step</th>
<th>Materials</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA extraction</td>
<td>$10</td>
<td>0.5 days</td>
</tr>
<tr>
<td>sonication / size-selection</td>
<td>$10</td>
<td>1 day</td>
</tr>
<tr>
<td>library preparation</td>
<td>$50</td>
<td>1 day</td>
</tr>
<tr>
<td>capture – bait kit</td>
<td>$150</td>
<td>2 days</td>
</tr>
<tr>
<td>capture – magbeads</td>
<td>$10</td>
<td>-</td>
</tr>
<tr>
<td>capture – reamplification</td>
<td>$5</td>
<td>-</td>
</tr>
<tr>
<td>library quantification</td>
<td>$5</td>
<td>0.5 days</td>
</tr>
<tr>
<td>TOTAL</td>
<td>$240/sample</td>
<td>5 days (70 samples)</td>
</tr>
</tbody>
</table>

### How should I sequence?

**MYcroarray**

- Protocol: how long of reads?
  - Consider library insert size – point to long PE?
  - Consider target type – SNPs, flanks, or contigs?

**MYcroarray**

- Depth - three important numbers:
  - Size of target
  - Required coverage depth
  - Expected % on target

### How deep to sequence?

**MYcroarray**

- required reads =
  - target length
  + read length
  × X coverage desired
  + expected % on-target
  + expected % unique

### Measuring success

**MYcroarray**

- **Specificity** (meas: % reads on-target)

**MYcroarray**

- **Sensitivity** (meas: library complexity)

[Diagram showing Mapped reads and Unique mapped reads vs. Sequencing depth]
Project Design & Troubleshooting

Contact us!

http://crossroads.uni-koeln.de/images/neb.jpg

https://www.kapabiosystems.com/assets/logo.png

https://www.diagenode.com/img/product/reagents/diamag02.png

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http://www.alpaqua.com/Portals/0/Images/Magnet%20Plates/MP021-260x140.jpg

http://www.clker.com/clipart-epipendorf-tube.html