Emily Davenport - 2020-08-03

DNA Extraction & Library Prep

Microbiome Center Kick Start Workshop

Overview

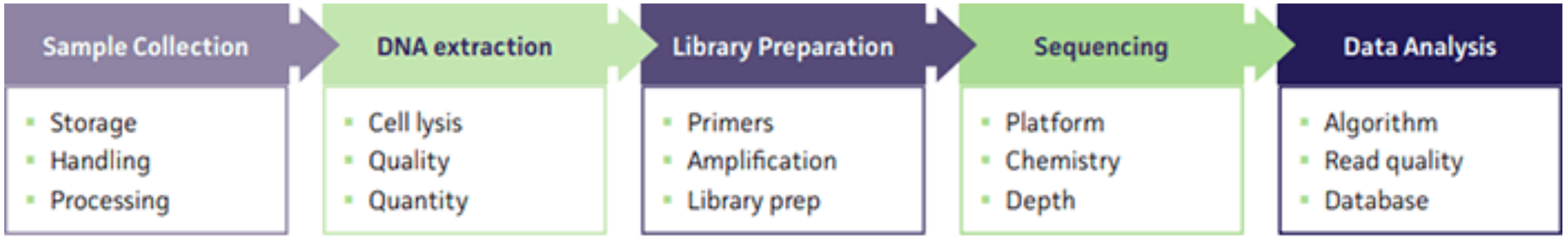

image: atcc.org/Microbiome

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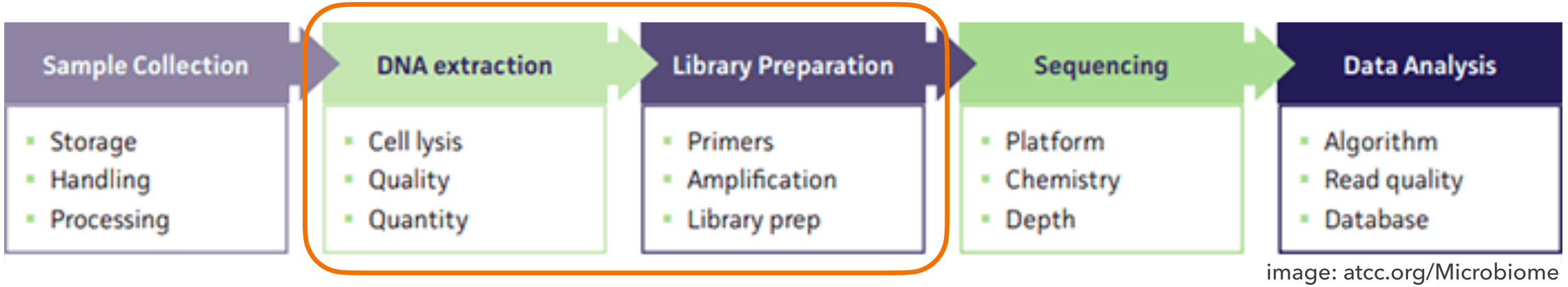

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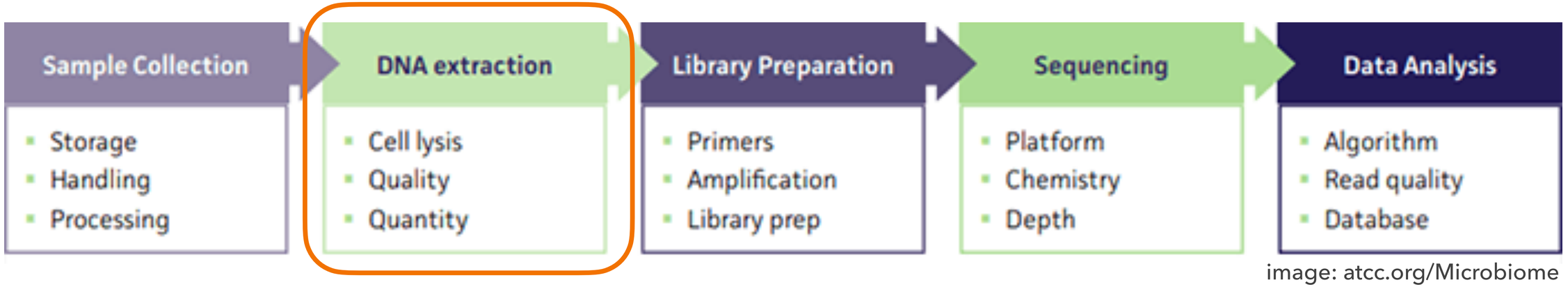

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DNA Extraction

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Getting purified DNA from our cells of interest.

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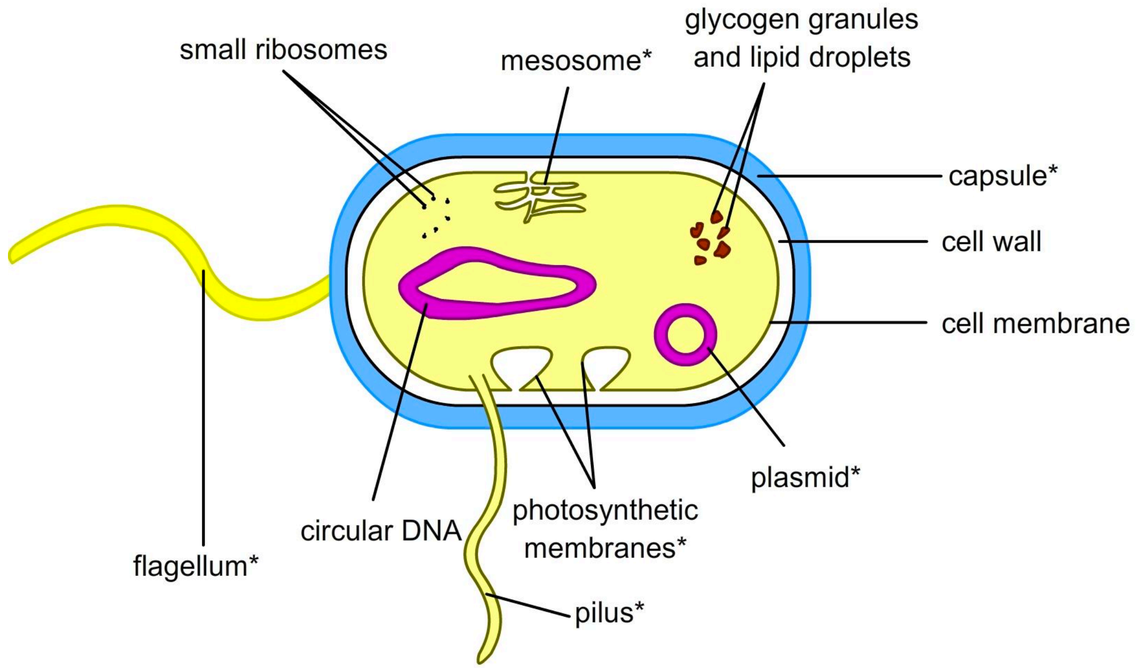

DNA Extraction

What is DNA extraction?

Steps:

1. Lyse Cells

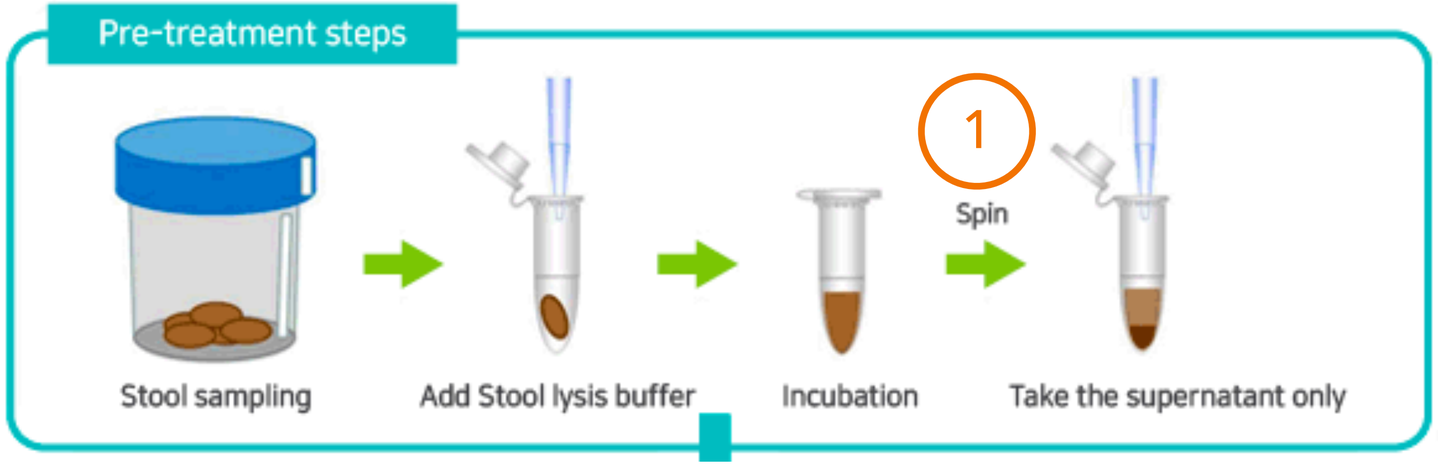

image: eng.bioneer.com

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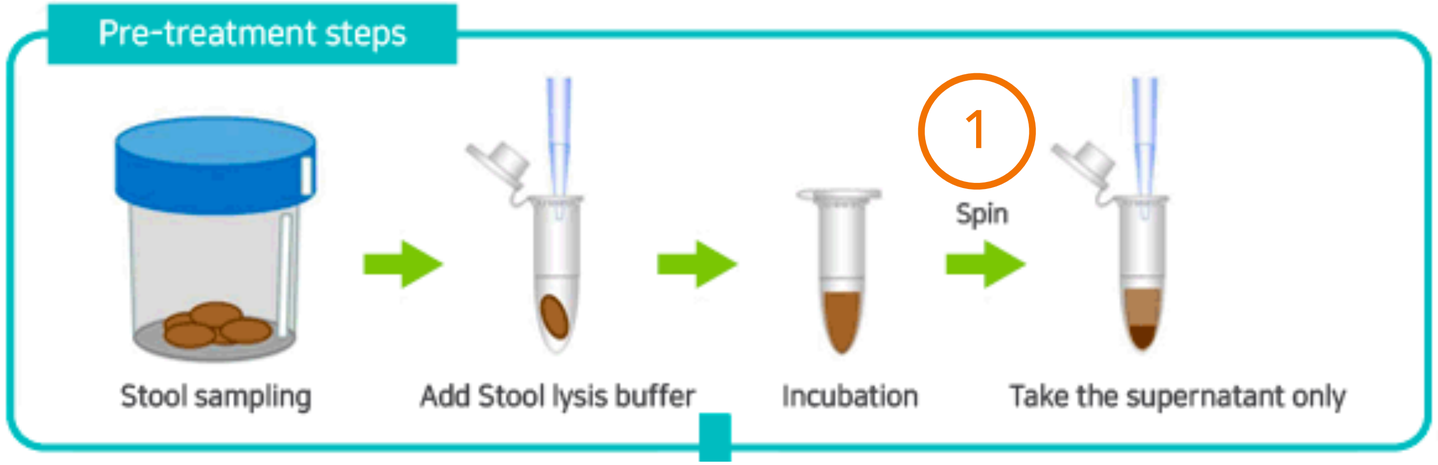

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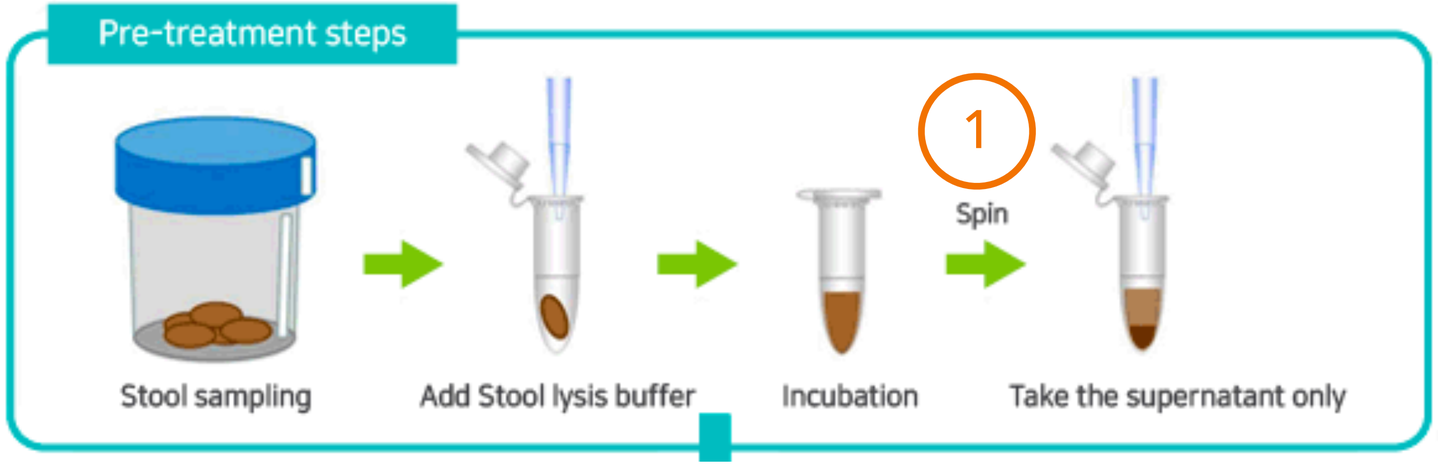

Considerations:

1. How aggressively to break open samples.

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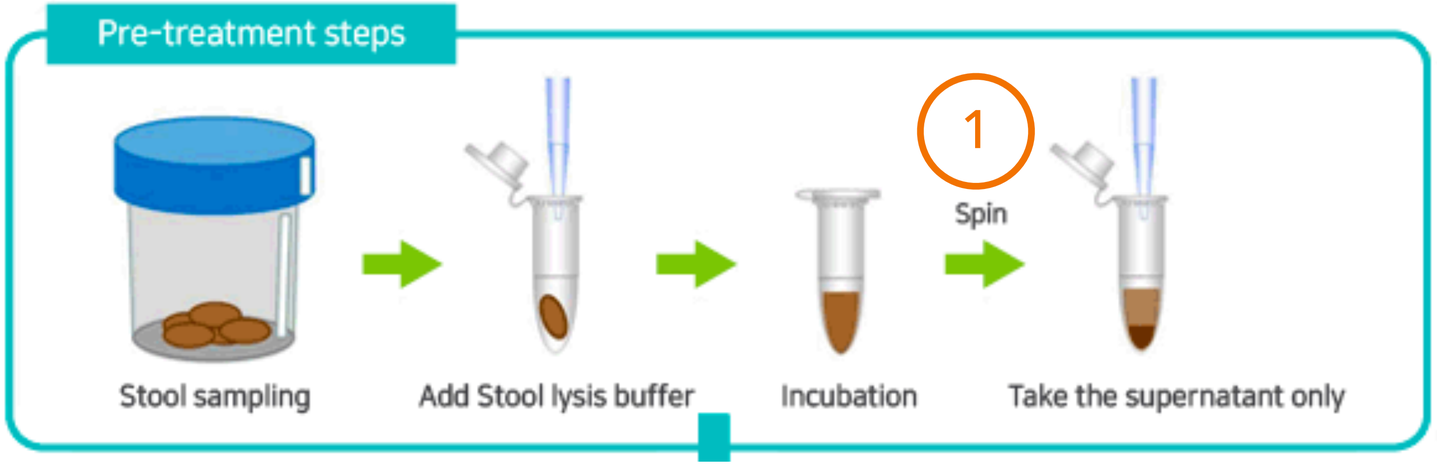

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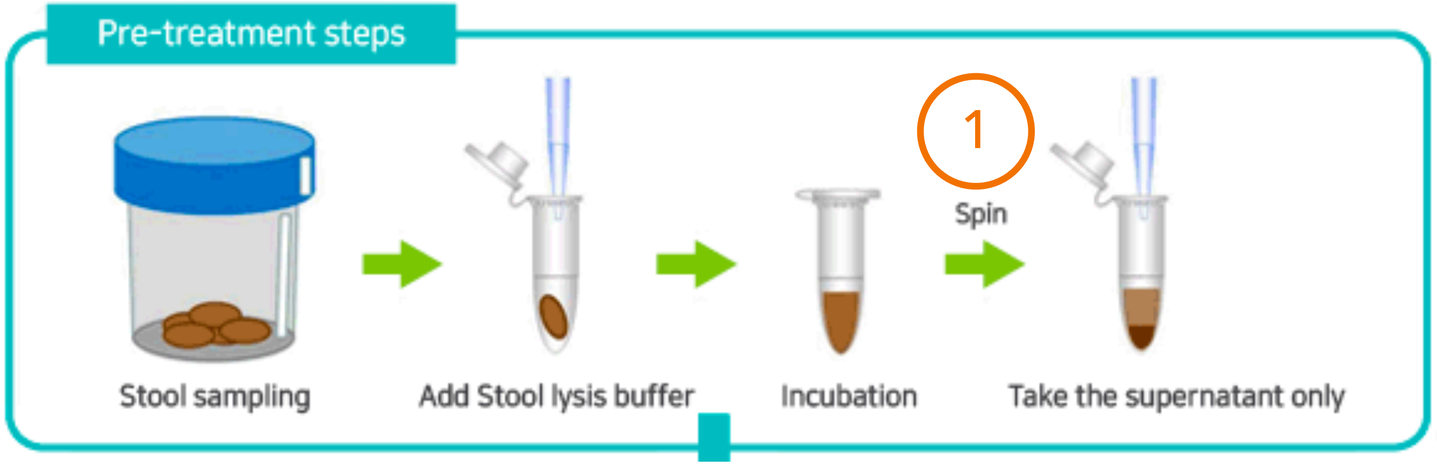

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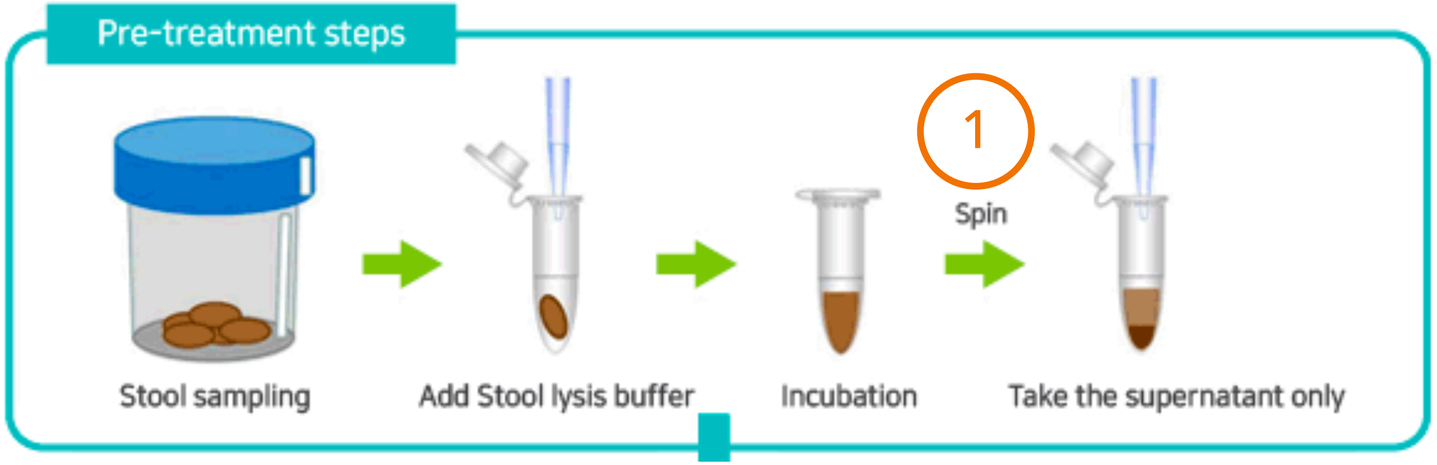

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Considerations:

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2. Probably want to bead-beat to ensure tough bacterial cell walls are broken.
3. Uniform sampling for DNA extraction: amount, location of sample, etc.
4. Spike in for assessing total abundance?

DNA Extraction

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Steps:

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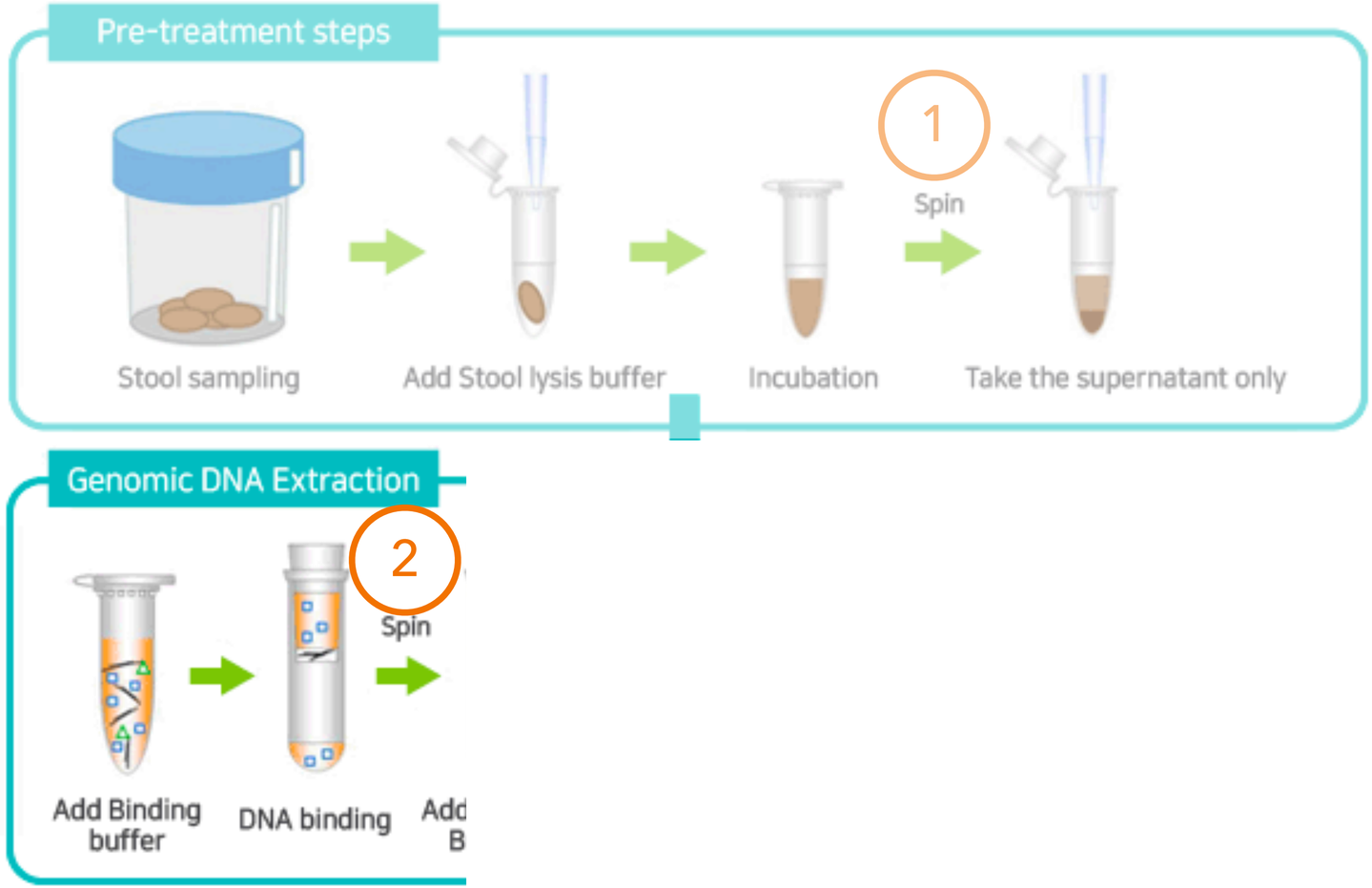
2. Separate nucleic acids from other molecules (proteins, etc.)



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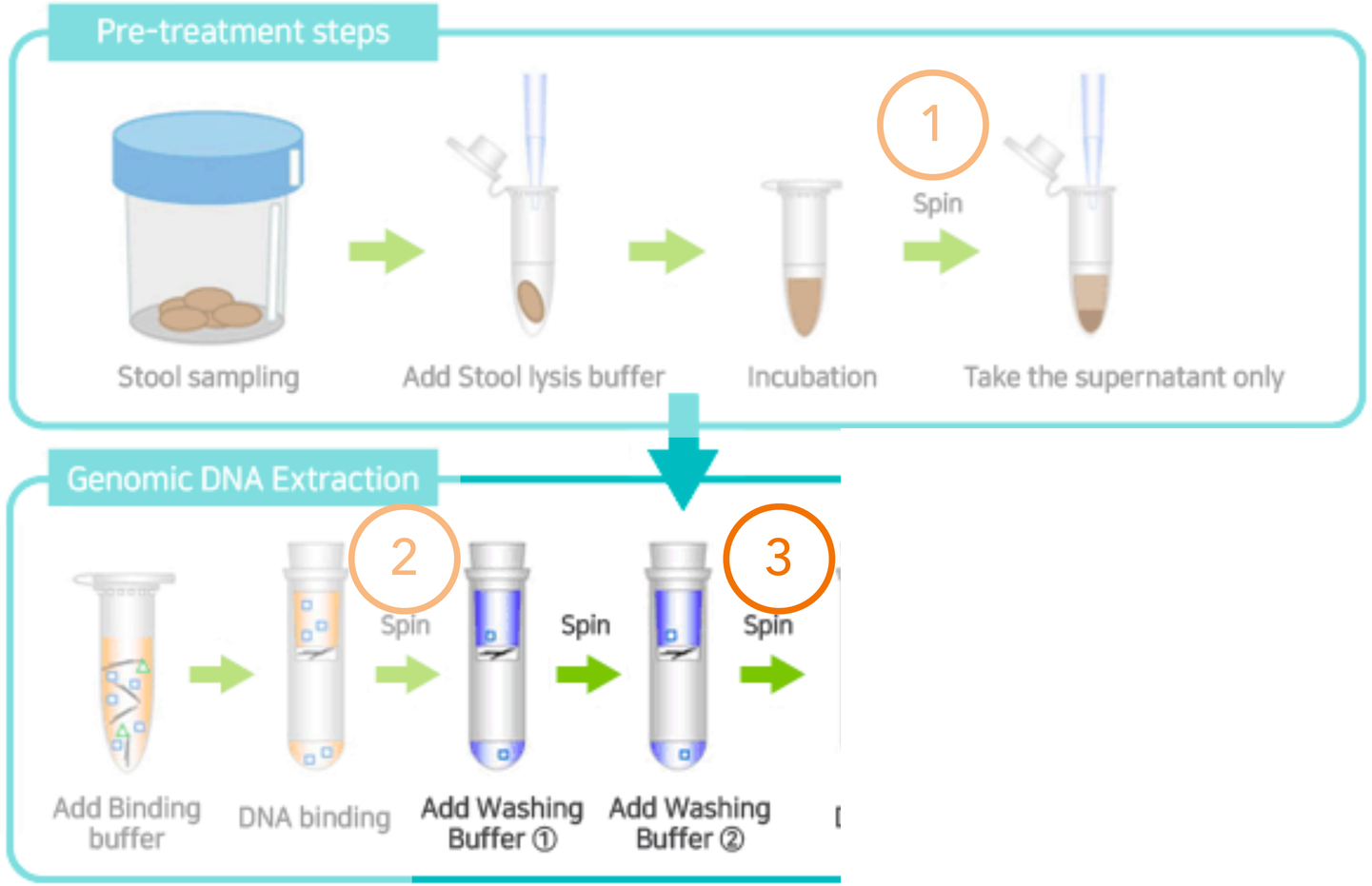

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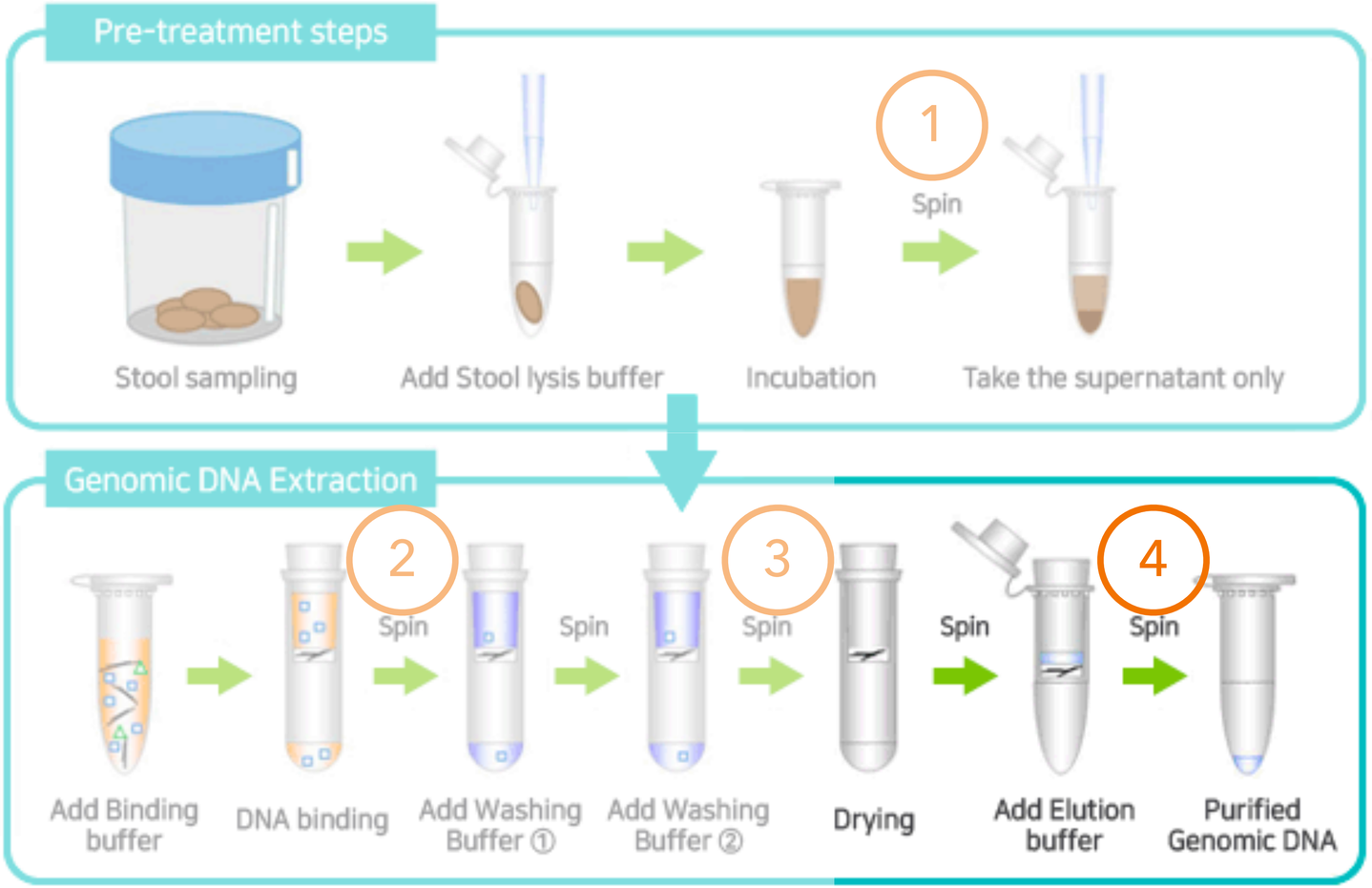

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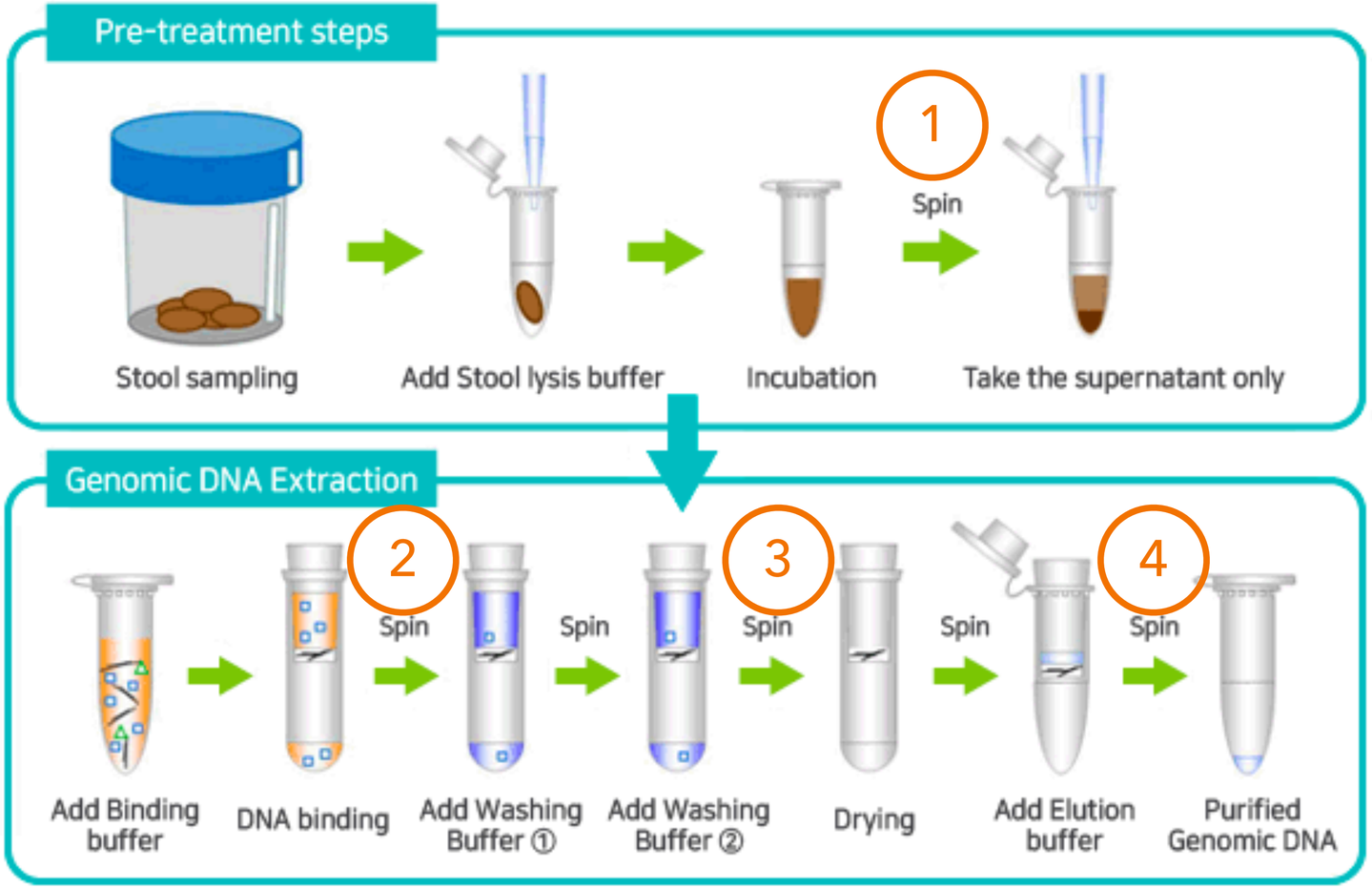

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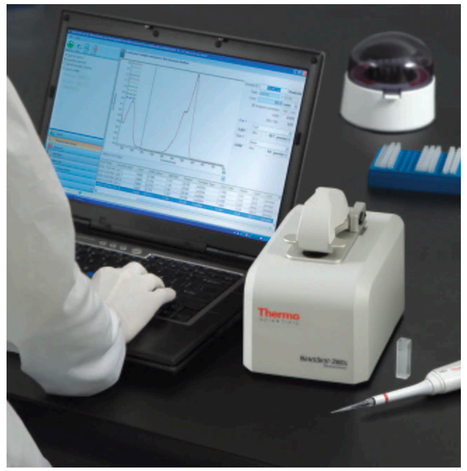
DNA Extraction

Quantification and quality control

less accurate, but faster:



nanodrop:

- Get ballpark estimates of amount of DNA*
- A260/280 ratio: should be 1.8 - 2.1. Lower = protein contamination
- A260/230 ratio: should be >1.8. Lower = presence of organic contaminants

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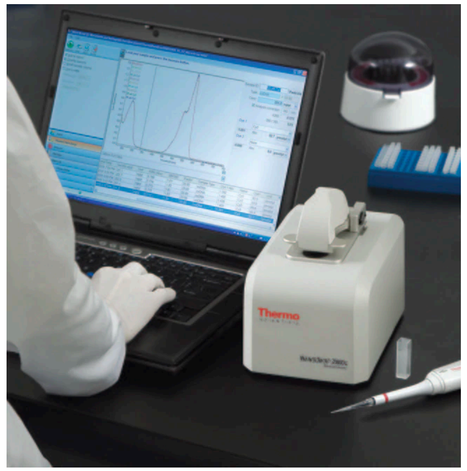

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qPCR

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5. How high throughput do you need to be?
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7. What controls should you consider, both positive and negative?
8. Are you cost-limited (*we all are!)?

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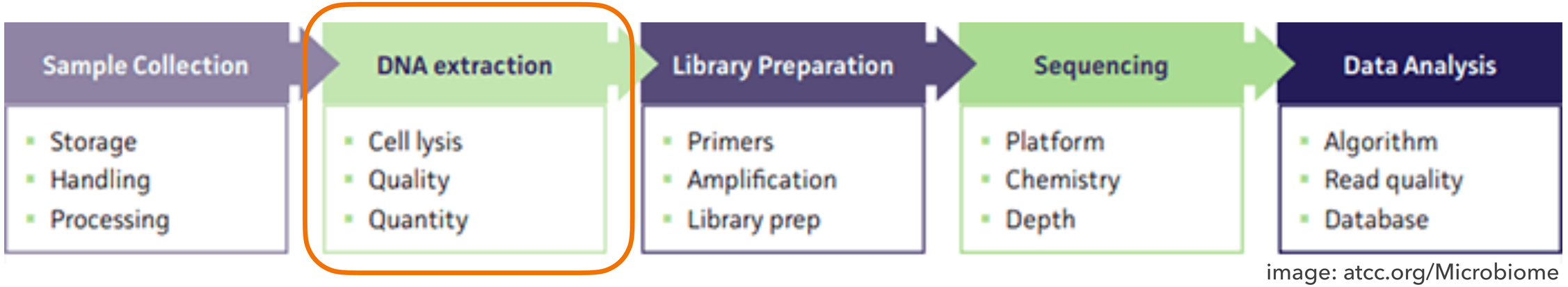

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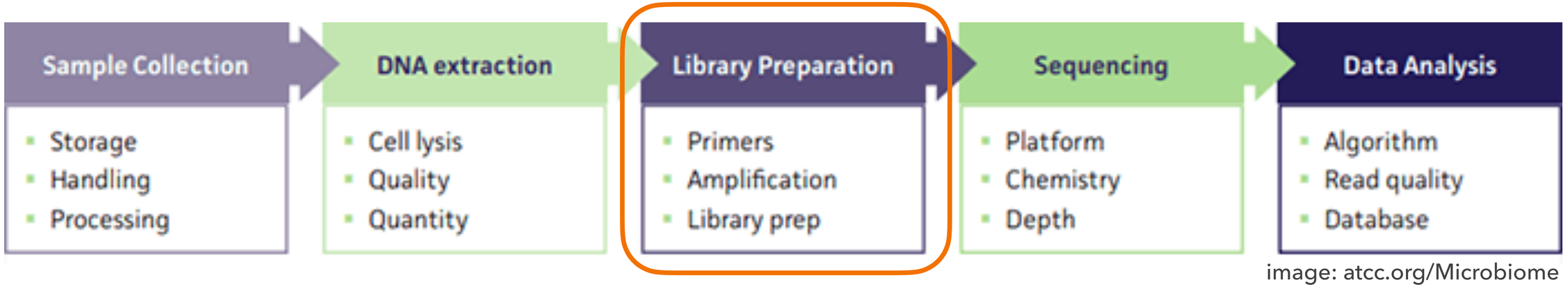

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Library Preparation

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The process of preparing genomic DNA to be sequenced via next-generation sequencing.

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The process of preparing genomic DNA to be sequenced via next-generation sequencing.

Key steps:

1. Need to make small fragments (<1000bp for Illumina)
2. Need to attach adapters that allow fragments to stick to flow cell
3. (Probably) need to attach barcodes so that multiple samples can be sequenced at the same time

Library Preparation

metagenomic libraries (Illumina sequencing - Nextera)

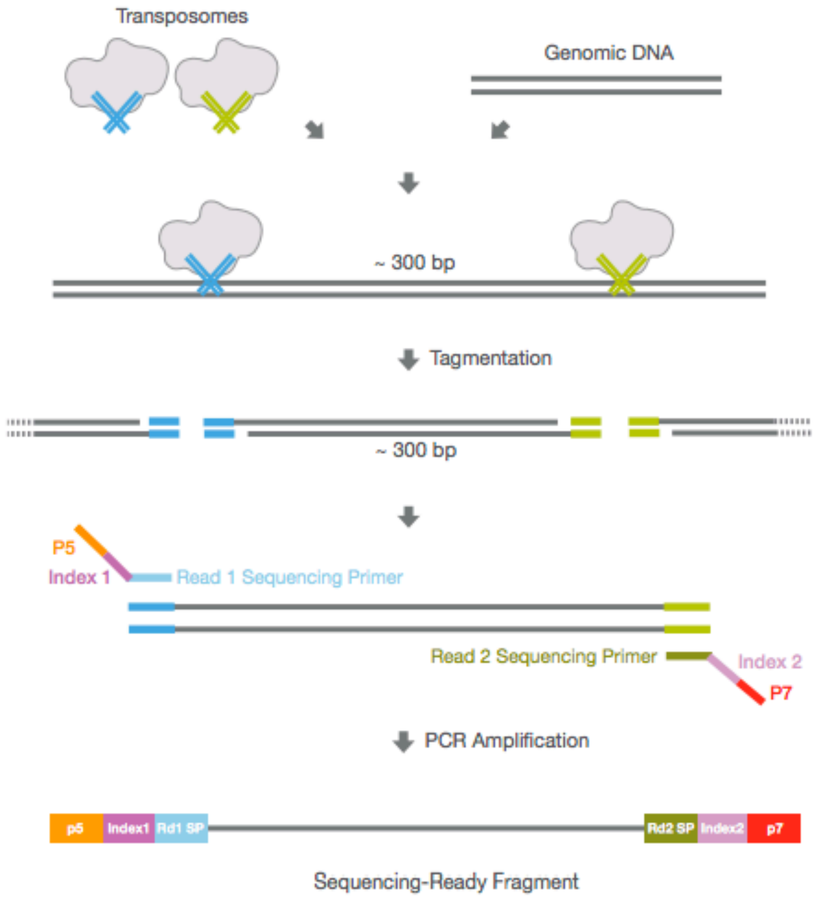

image: Illumina

Library Preparation

16S rRNA gene sequencing libraries (Illumina sequencing)

one step:

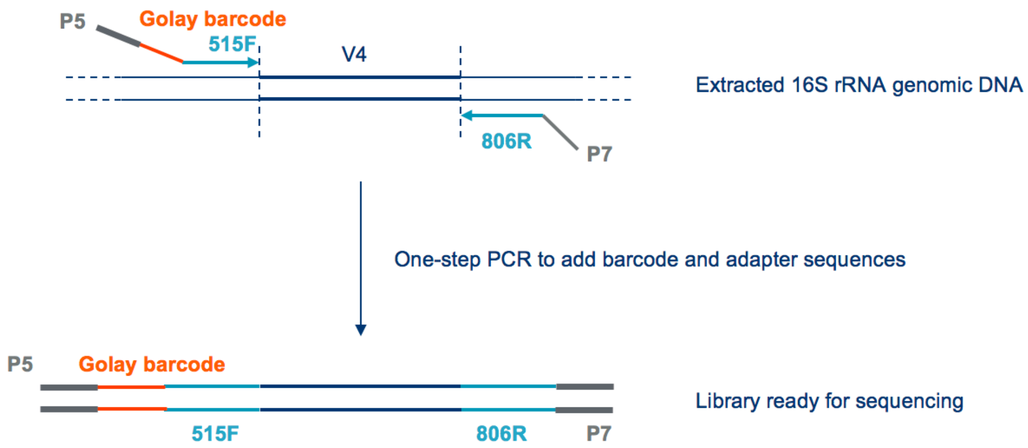

image: IDT.com

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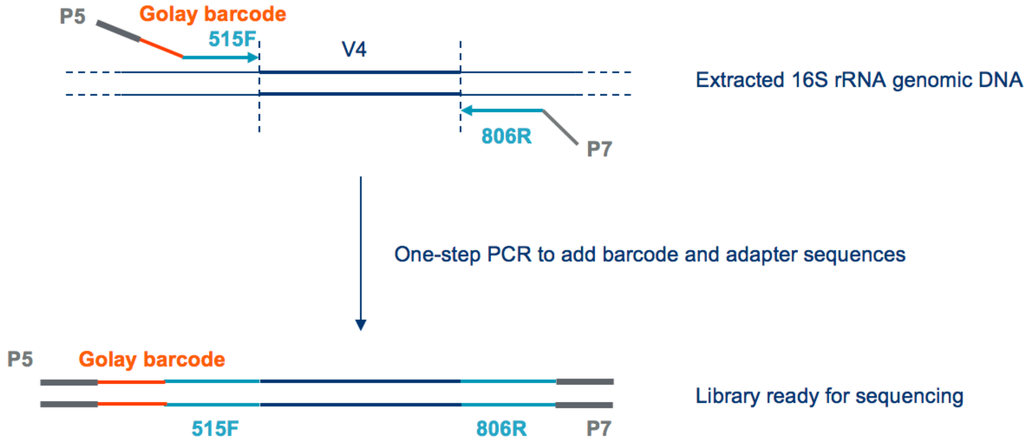

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two step:

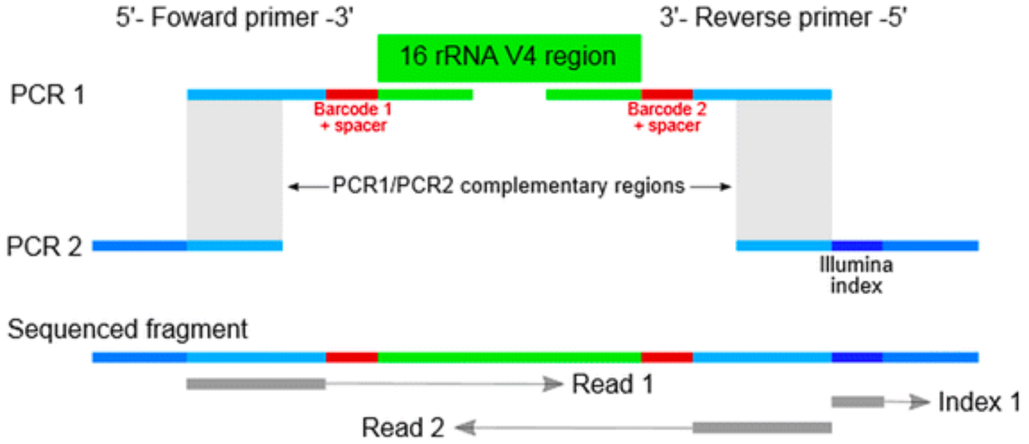

image: de Muinck et al. 2017

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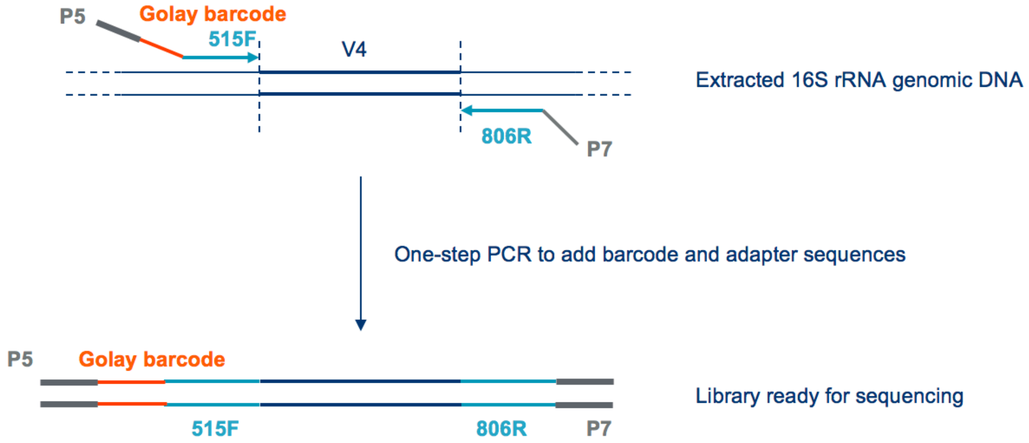
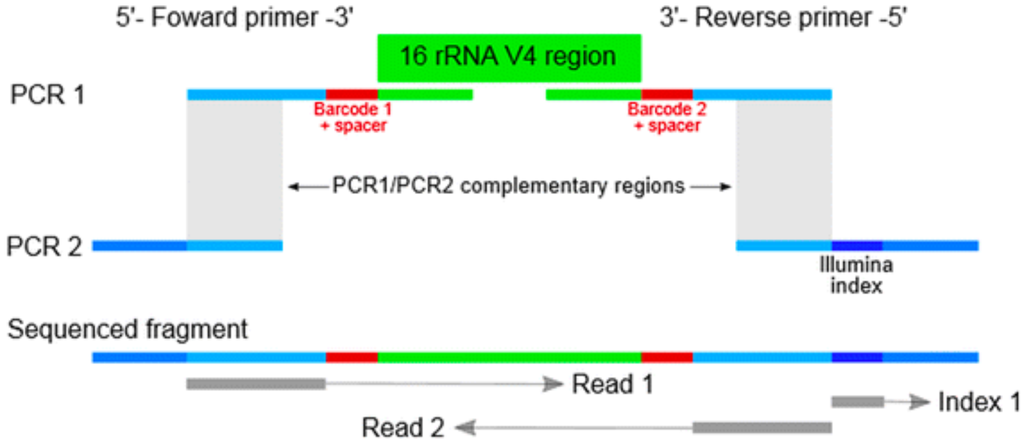


image: IDT.com

+ Faster, fewer steps
- Need to plan barcodes in advance
- Need to order many primers with barcodes in them

two step:



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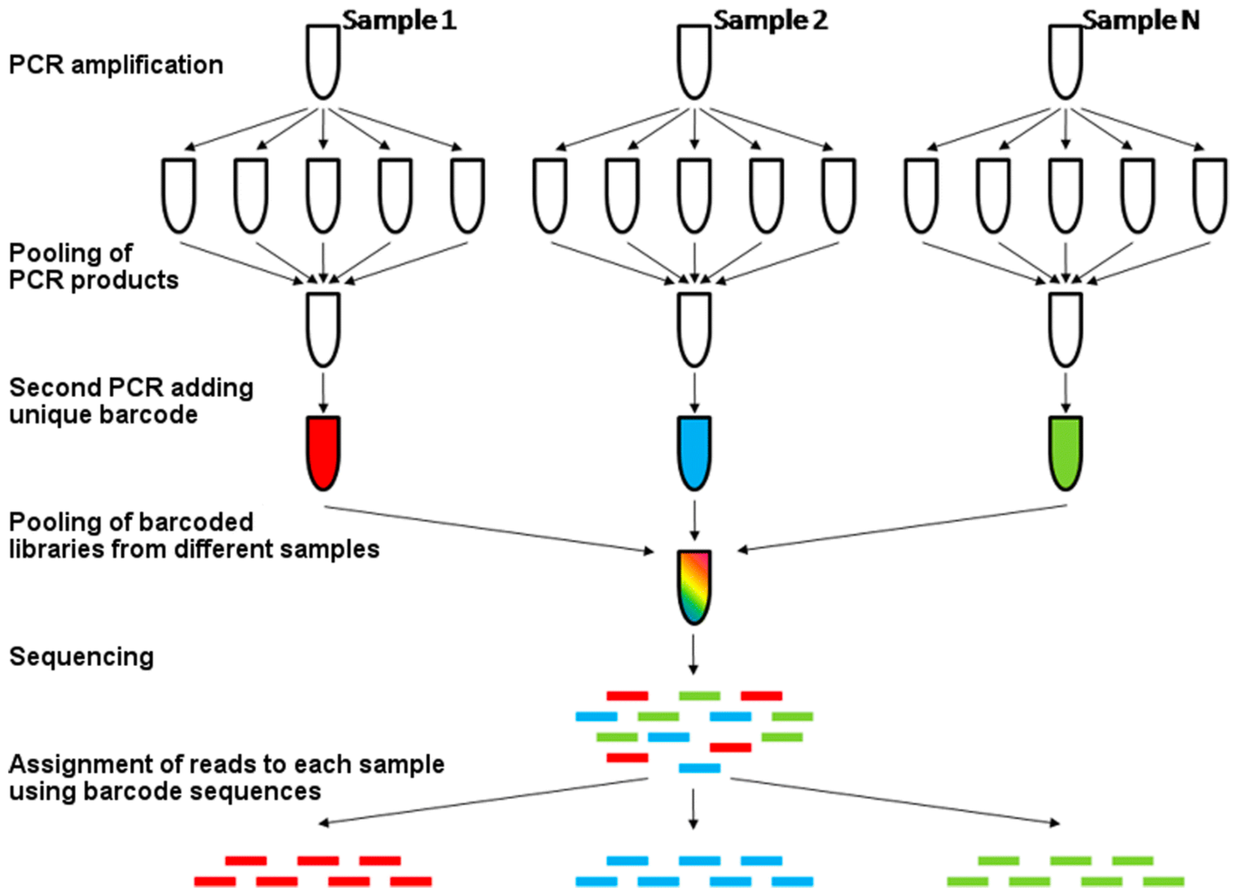
- Two steps
+ Do not need to plan barcodes in advance
+ What the Penn State Genomics Core Facility does

Library Preparation

Quantification and pooling



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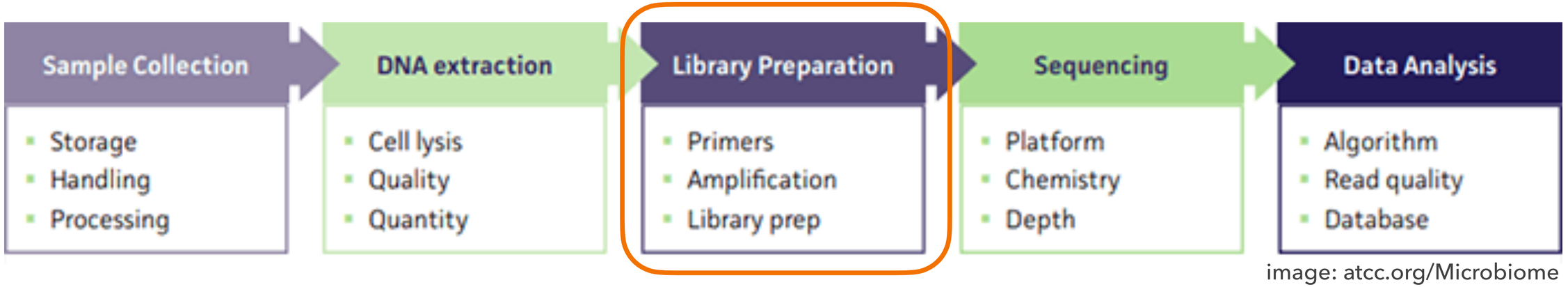

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