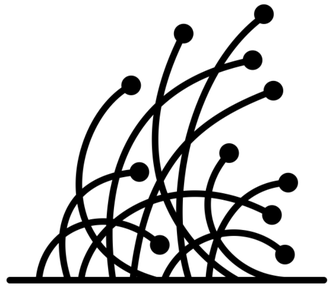


DNA extractions

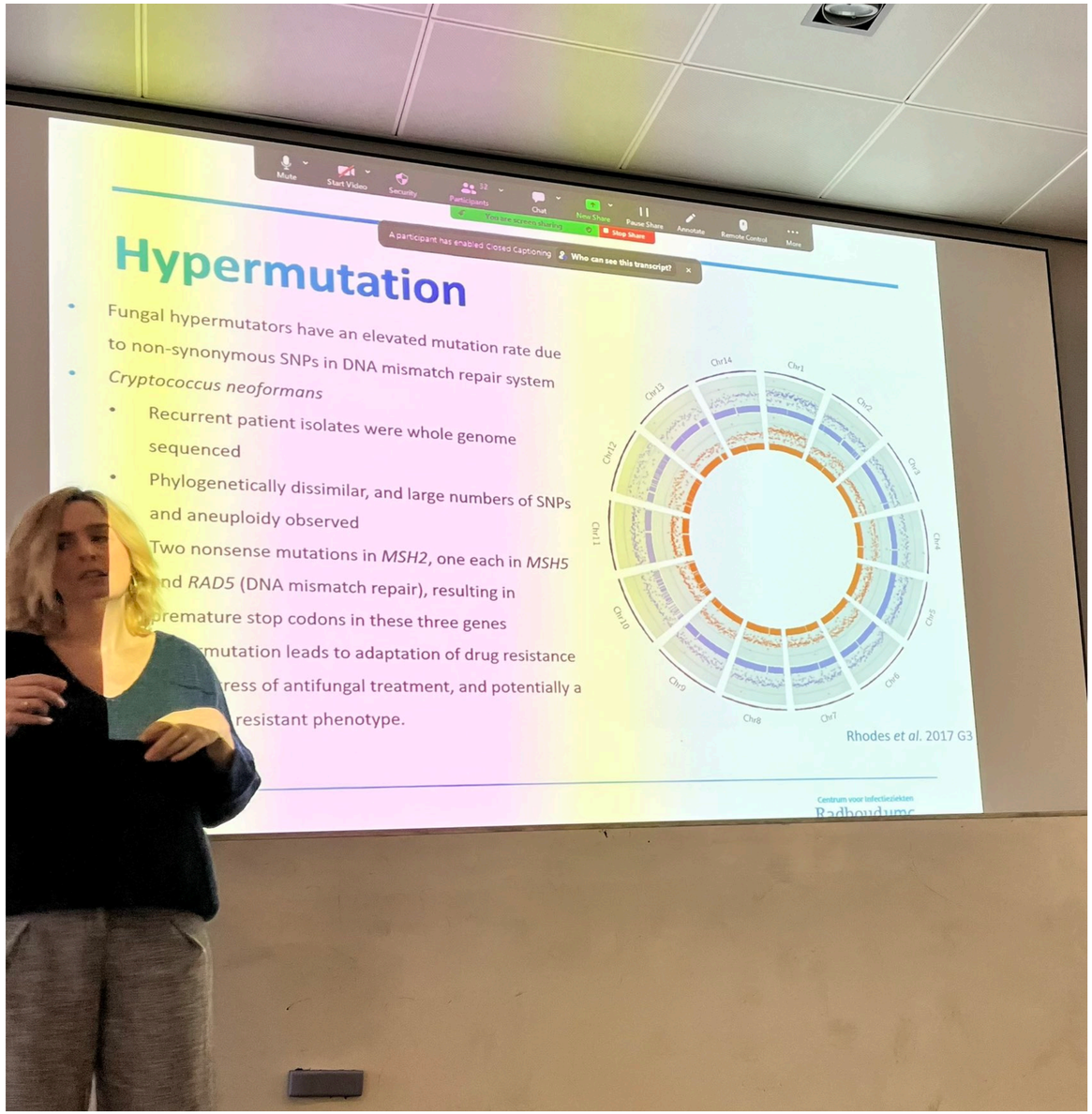
Considerations for fungi

Dr Johanna Rhodes

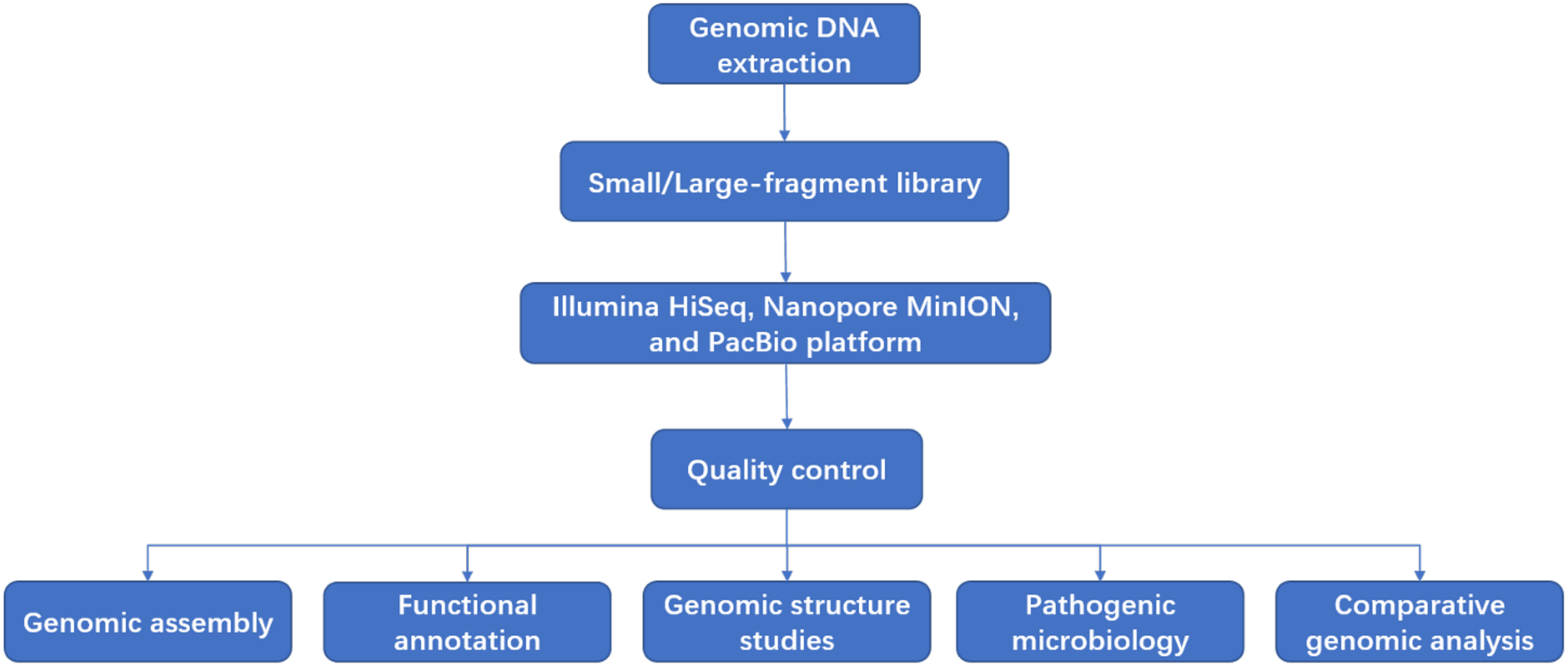
Learning outcomes

- **DNA extractions for NGS**
  - main steps and considerations for fungi
- **Applications**
- **Challenges and limitations**

Who am I?


Hypermutation
Fungal hypermutators have an elevated mutation rate due to non-synonymous SNPs in DNA mismatch repair system
Cryptococcus neoformans
Recurrent patient isolates were whole genome sequenced
Phylogenetically dissimilar, and large numbers of SNPs and aneuploidy observed
Two nonsense mutations in MSH2, one each in MSH5
nd RAD5 (DNA mismatch repair), resulting in
remature stop codons in these three genes
mutation leads to adaptation of drug resistance
ress of antifungal treatment, and potentially a
resistant phenotype.
Rhodes et al. 2017 G3
Centrum voor Infectieziekten
Radboudumc

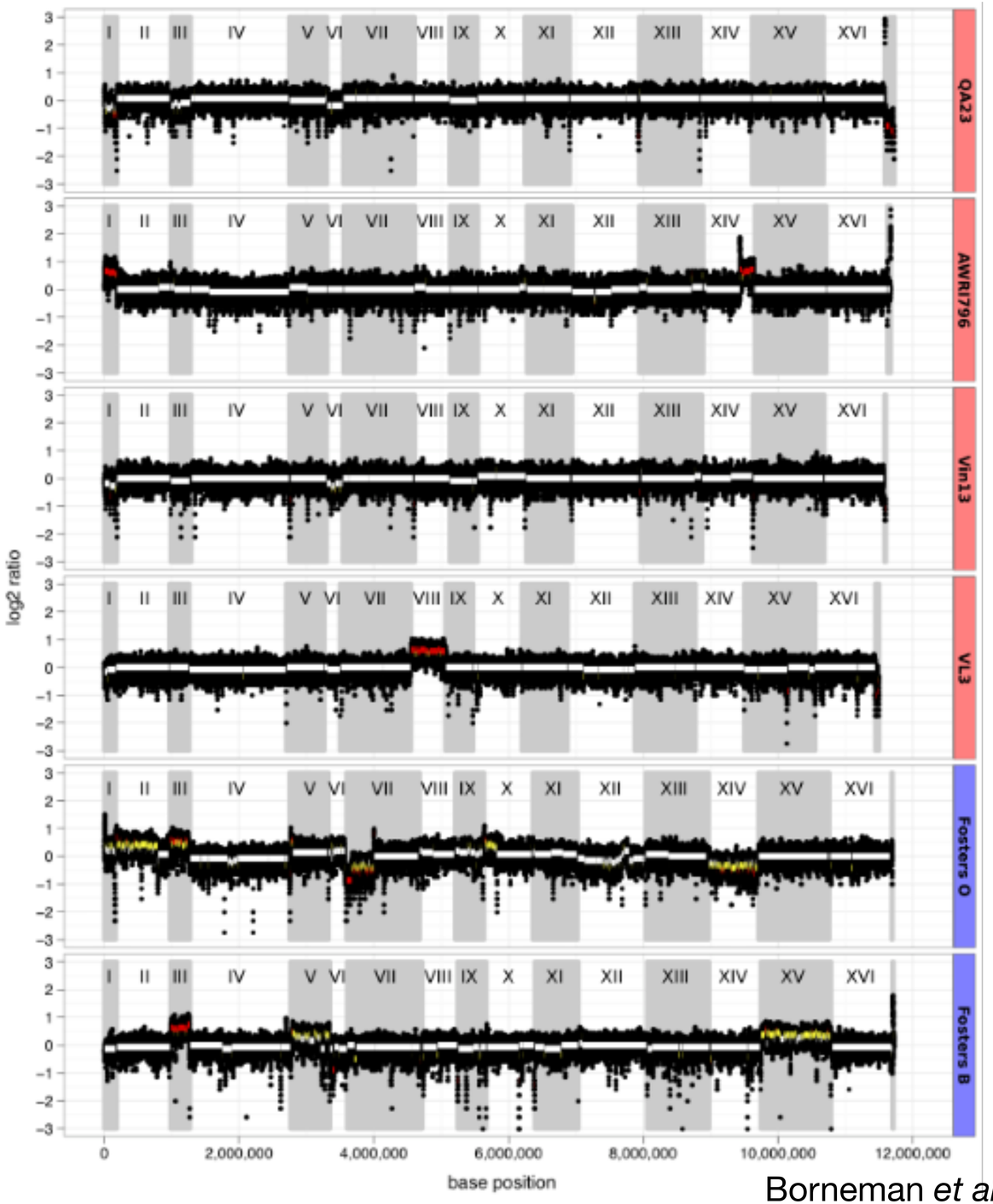
Genomics workflow



Fungal genomics complexities

Considerations

- >1000 fungal species have been genome sequenced
  - >130k bacterial species
- Genome sizes can range from ~2-180 million nucleotides
  - predicted proteomes 2-35 thousand proteins
- DNA extraction difficulties
  - Lysis buffer may not degrade cell wall containing ergosterol
    - beat beating not good for long read sequencing
- Genomes:
  - haploid, diploid, polyploid, aneuploidy



Borneman *et al.* 2011

DNA extractions

Considerations for fungi

- Pure or mixed culture, sample or ancient?
- Short or long read technology?
  - Sonication or bead-beating good to break cell wall -> fragmentation
  - Enzymatic digest -> less fragmentation
- Which life cycle stage?
- Personal recommendations (caveats - liquid culture best, and does depend on species….):
  - Illumina: Lucigen MasterPure DNA extraction plus bead beating
  - ONT/PacBio: in-house protocol for filamentous fungi, Epicentre with modifications for yeasts

DNA extractions

Considerations for fungi

- Pure or mixed culture, sample or ancient?
  - What is your question (research/diagnostic)?
    - Pure - liquid culture
    - Sample - host tissue —> dominate sequence reads
      - Extraction from BAL - High Pure PCR Template Prep kit (Roche)
    - Ancient - CTAB method

DNA extractions

Considerations for fungi

- Pure or mixed culture, sample or ancient?
- Short or long read technology?
  - Sonication or bead-beating good to break fungal ergosterol cell wall -> fragmentation
    - short read sequencing
  - Enzymatic digest -> less fragmentation
    - long read sequencing
      - Personnel recommendations for high-molecular weight DNA:
        - MasterPure with large liquid culture start and no bead beating, keep *very* cold throughout —> great for yeasts (see ‘Rhodes *et al.* Emerging Microbes & Infection’)
        - MasterPure extraction and QIAGEN Uneasy Blood & Tissue kit for clean up —> works for molds (see ‘Hemmings *et al.* Mycopathologia’)

DNA extractions

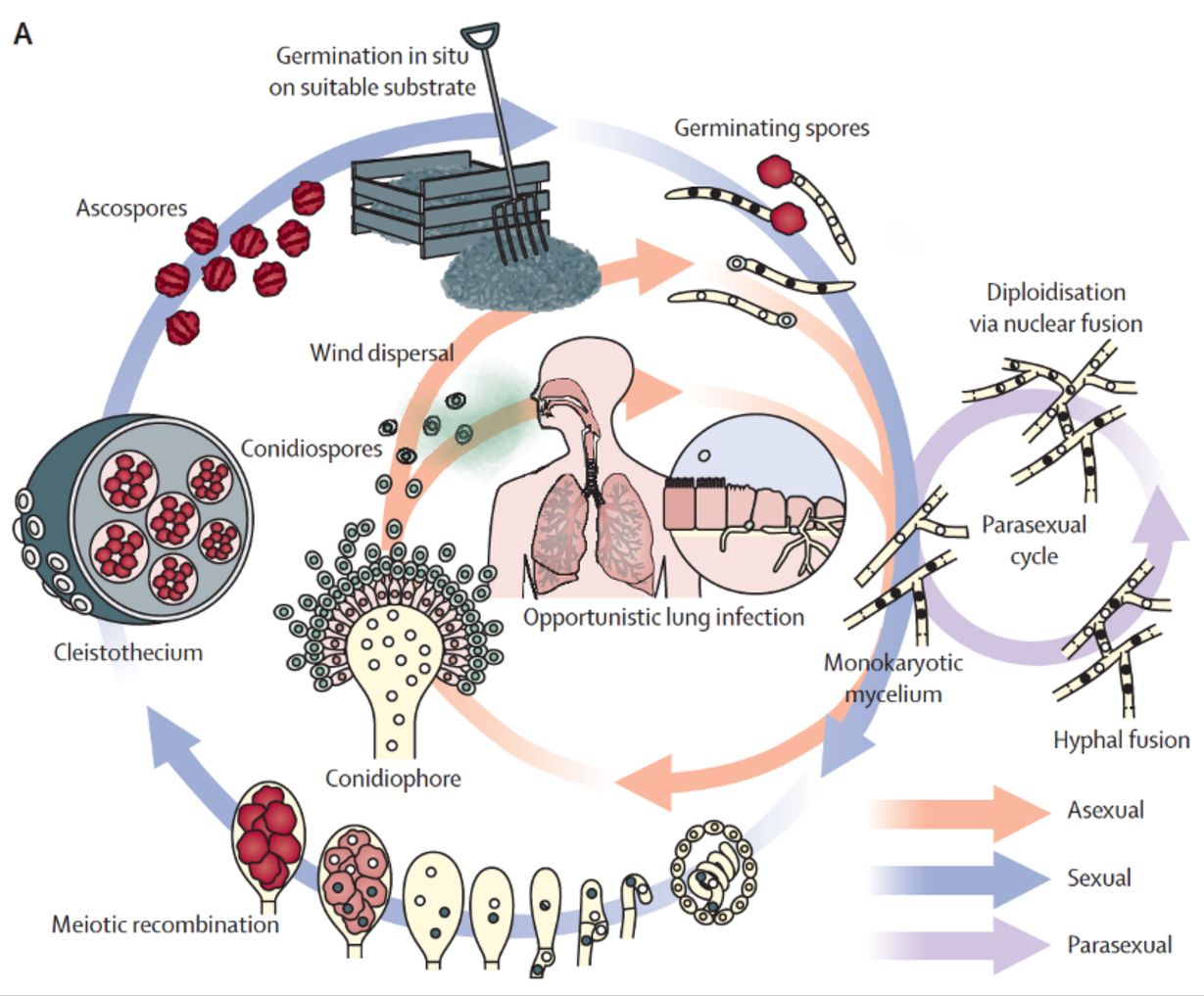
Considerations for fungi

- Pure or mixed culture, sample or ancient?
- Short or long read technology?
  - Sonication or bead-beating good to break cell wall -> fragmentation
  - Enzymatic digest -> less fragmentation
- Which life cycle stage?
  - spores, hyphae…

DNA extractions

Considerations for fungi - life cycle

- No single extraction method optimal for all species/life stages
- Spores:
  - Thick cell walls, maybe coated, making them resistant to standard lysis —> bead beating, enzymatic treatments
  - Small spore size means less biomass = lower DNA yield
    - Also consider purification to remove contaminants from extracellular material

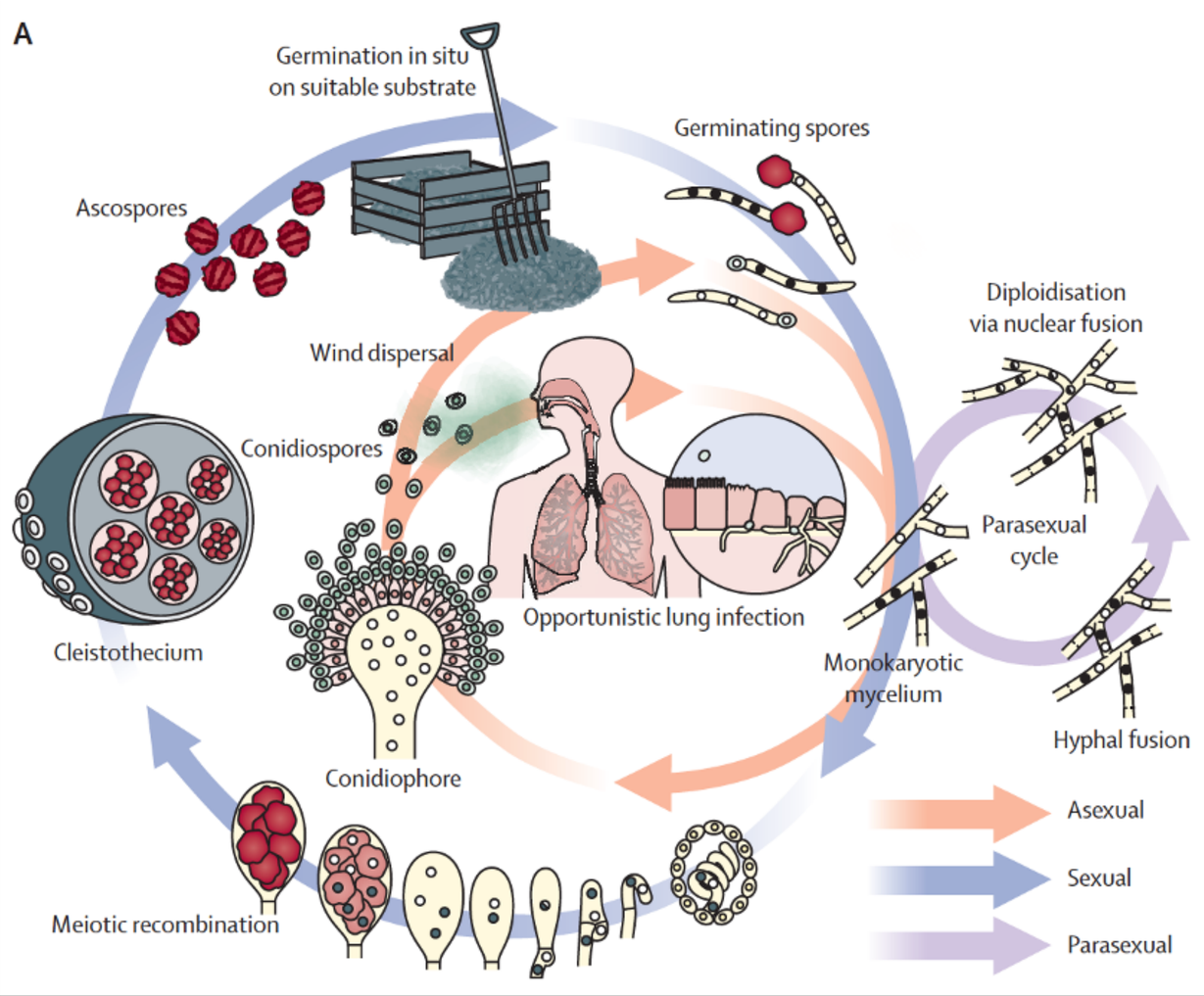


Zhang *et al.* 2021

DNA extractions

Considerations for fungi - life cycle

- No single extraction method optimal for all species/life stages
- Hyphae:
  - Filamentous, less thick cell walls but still a problem —> bead beating
  - DNA is usually of high yield and quality/easier to purify

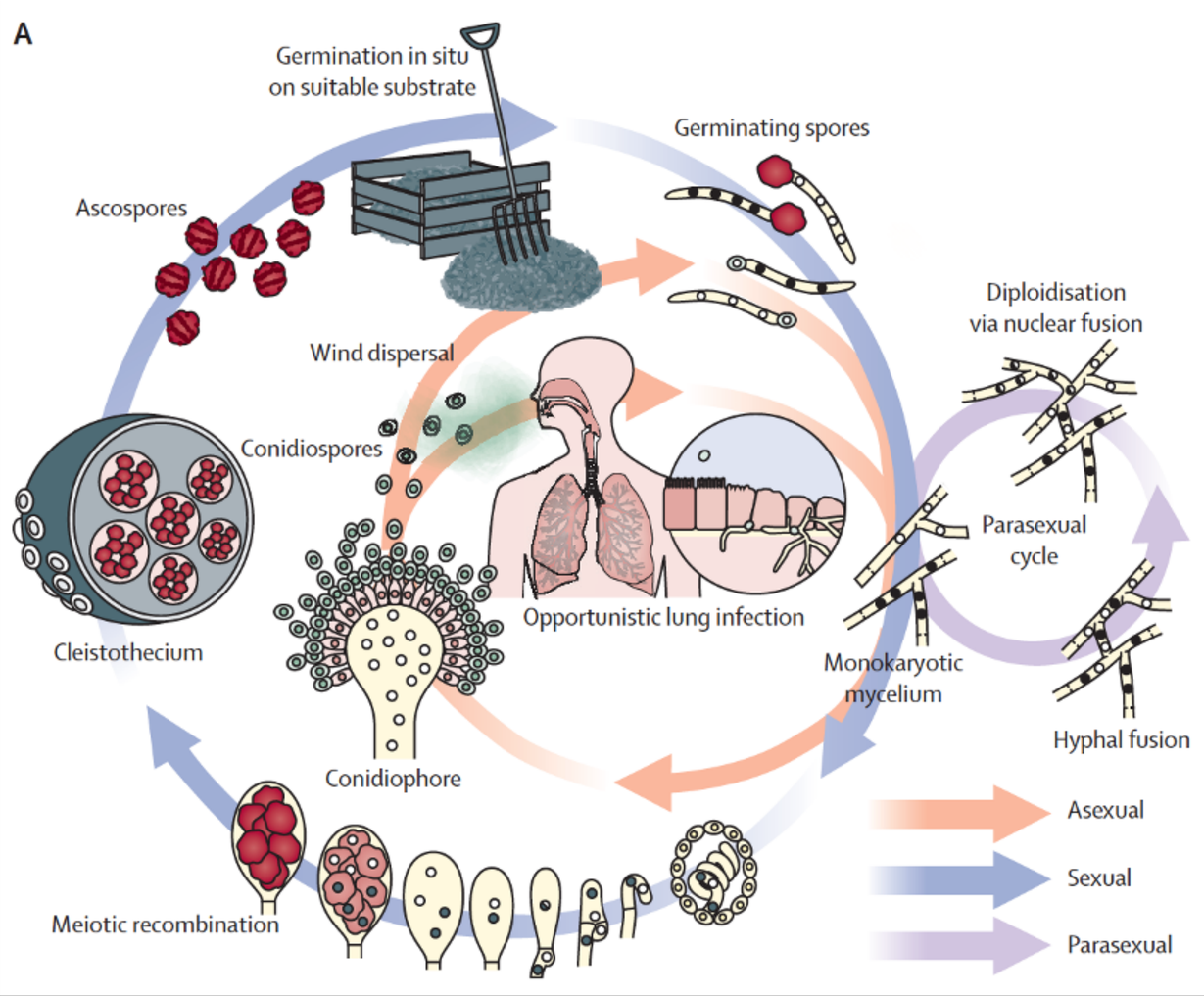


Zhang *et al.* 2021

DNA extractions

Considerations for fungi - life cycle

- No single extraction method optimal for all species/life stages
- Mycelium:
  - Dense network of hyphae containing extracellular proteins + polysaccharides which can inhibit extraction—> CTAB or phenol-chloroform
  - High biomass = good yield

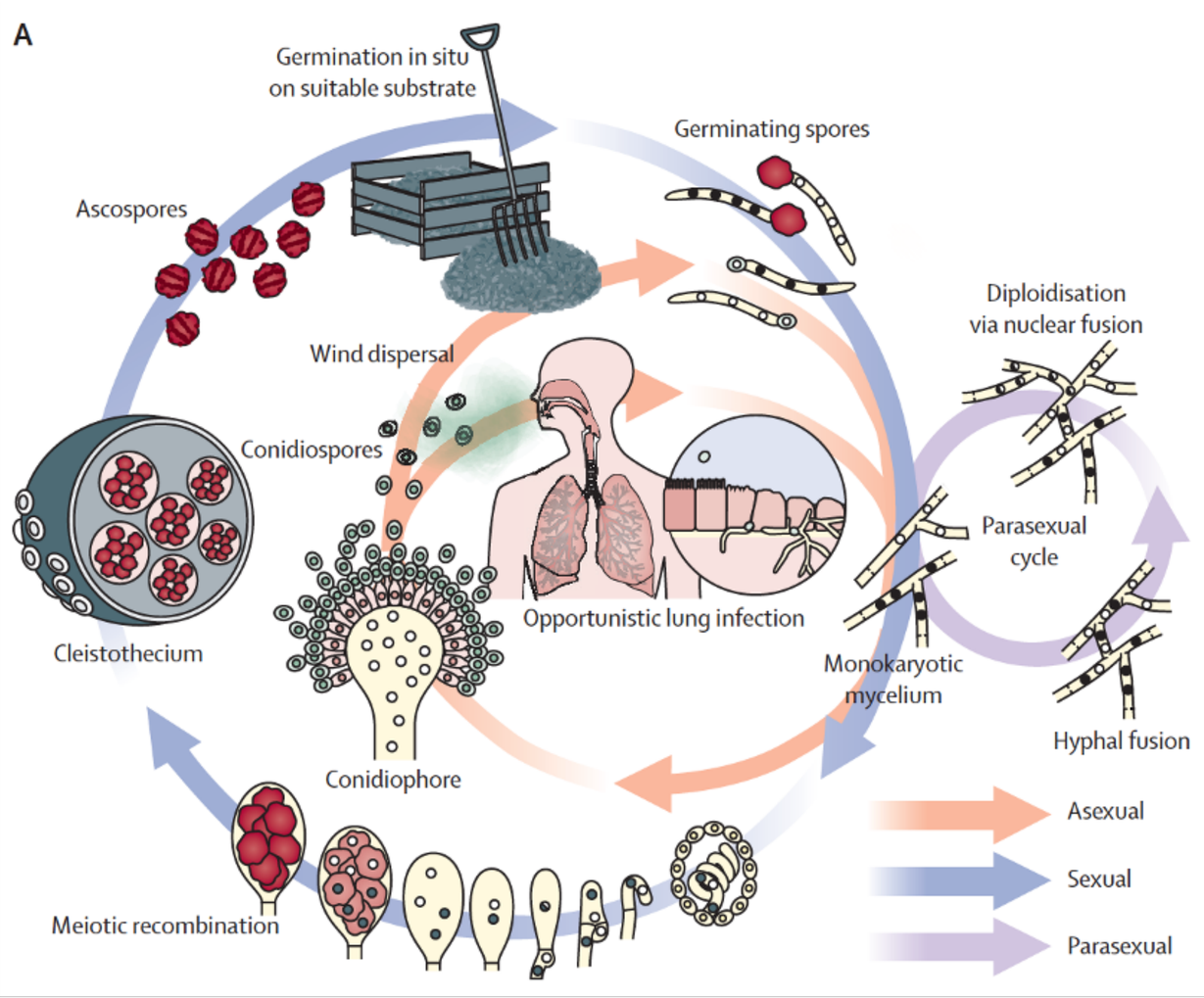


Zhang *et al.* 2021

DNA extractions

Considerations for fungi - life cycle

- No single extraction method optimal for all species/life stages
- Fruiting body
  - Complex, multicellular structure that produces spores.
  - Dense tissue needs extensive mechanical disruption
  - Needs extensive purification e.g. column purification
  - Large biomass = good yield



Zhang *et al.* 2021

DNA extractions

Considerations for fungi

- Other sequencing approaches:
  - amplicon-based on the MinION (or other ONT platform)
    - QIAGEN PowerSoil Pro kit
      - bead beating using FastPrep-24 5G (Thermo Fisher)

DNA extractions

Other considerations

- Cell wall composition varies across species, life stages and environmental conditions
- Secondary metabolites and pigments
  - can inhibit extractions, so important to perform purification step(s) to improve DNA quality
- DNA yield varies across species
  - Adjust initial sample based on expected/desired yield
- Biosafety requirements
- Storage
- Optimise buffers to improve DNA stability