Swiss TPH
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Diagnostics for malaria surveillance

- ❑ Parasite detection
- ❑ Quantification
- ❑ Genotyping

Programmatic decisions rely on surveillance data

populations / individuals
- potential sources of transmission
- at risk of infection

in different endemicity

most appropriate choice for delivery of interventions & evaluation

more rapid elimination

Standardized tools & metrics

- **understand infectious reservoir for malaria**
- **measure changes in malaria transmission**

Malaria infections

Symptomatic – **asymptomatic → submicroscopic**

↓

requires diagnostic sensitivity of 200 parasites/µL blood

↓

Microscopy and some RDTs

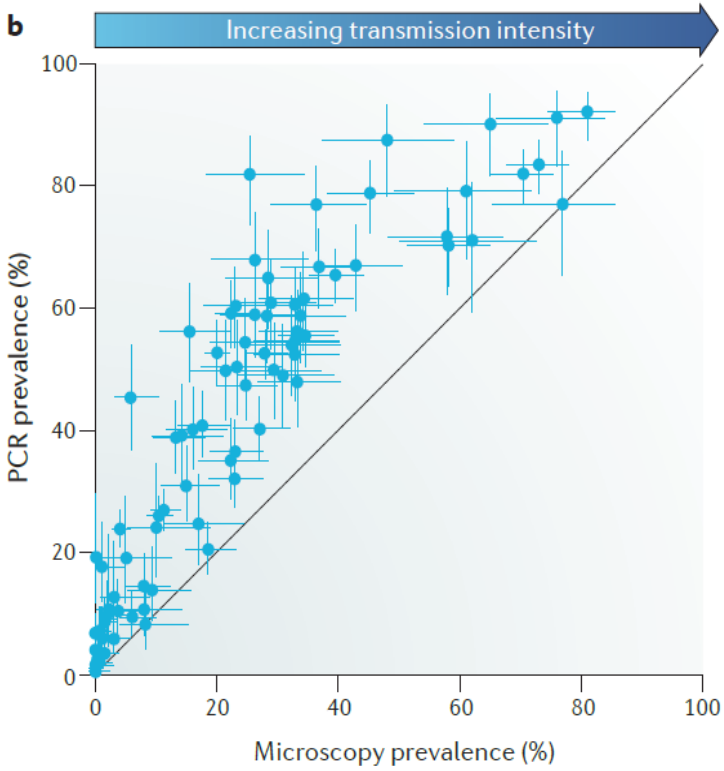
good LM & good RDT:
≈ 100 parasites/µL blood

Relevant for transmission?

All to be found?
All to be treated?
Proportional contribution to transmission?
Changes with levels of endemicity?

Malaria infections

Symptomatic – asymptomatic → submicroscopic



on average 50%

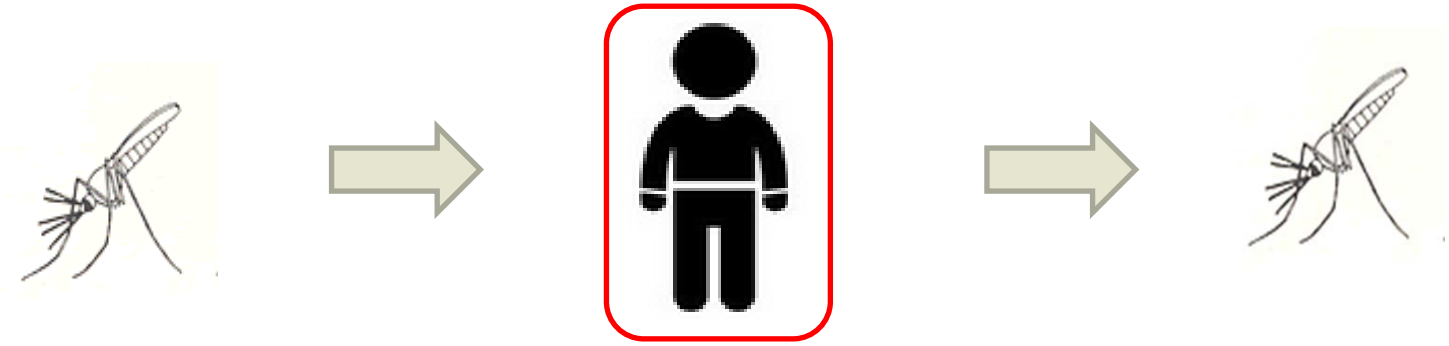
70-80% in low transmission
20% in high transmission

P. vivax problematic!

5-8 x lower mean densities by qPCR & LM compared to Pf in asymptomatic individuals

Bousema et al. Nature Rev. Microbiol 2014

The asymptomatic Reservoir of Infection



Parasite prevalence

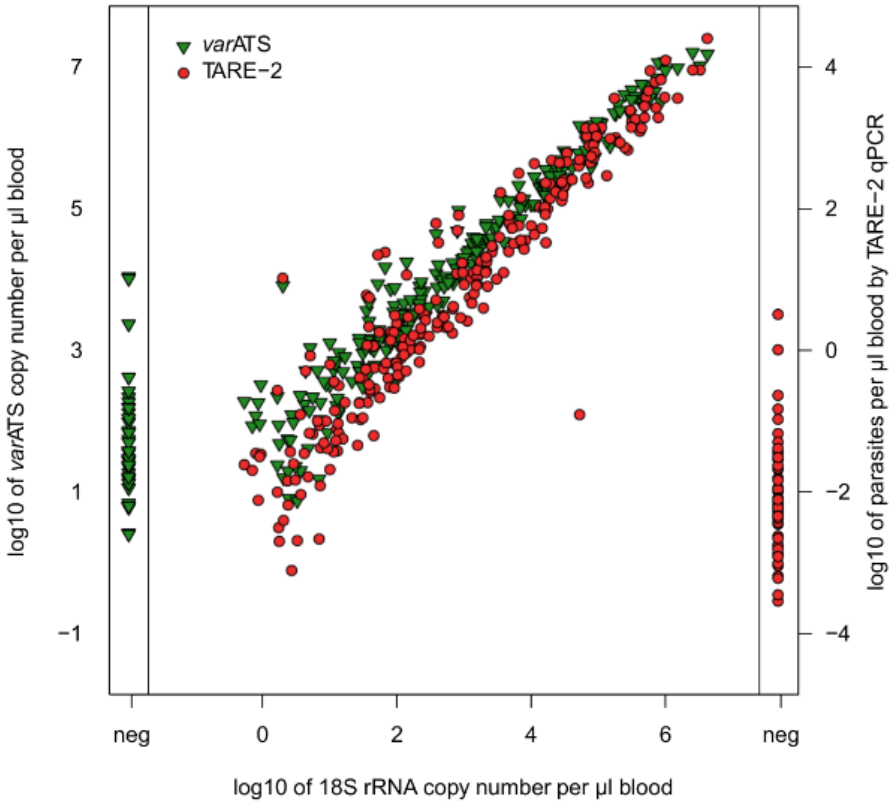
- simple field work
- cross-sectional surveys
- Correlates well with exposure / $_{mol}$FOI

→ optimize test sensitivity to explore submicroscopic reservoir

Development of ultra-sensitive DNA-based qPCR

- **TARE-2:** telomere-associated repetitive element 2
- 1.6 kb long blocks of 10-12 **135-bp repeat units** with slightly degenerate sequences
- approx. **250–280 copies / genome**

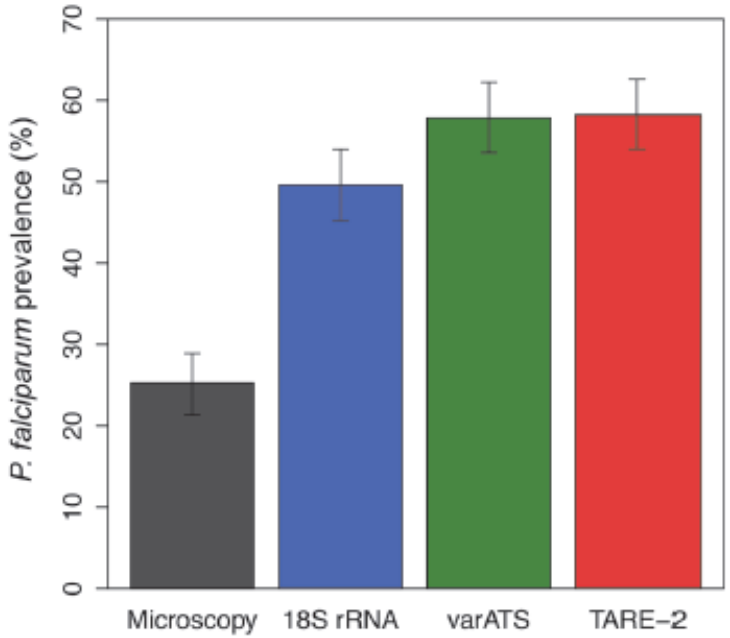
- ***var*-ATS:** acidic terminal segment (semi-conserved)
- 59 *var* genes in 3D7 genome



Good correlation between TARE2, ATS and standard 18S rRNA qPCR

→ useful for quantification of low densities

Implication for prevalence rate: **plus 16%**



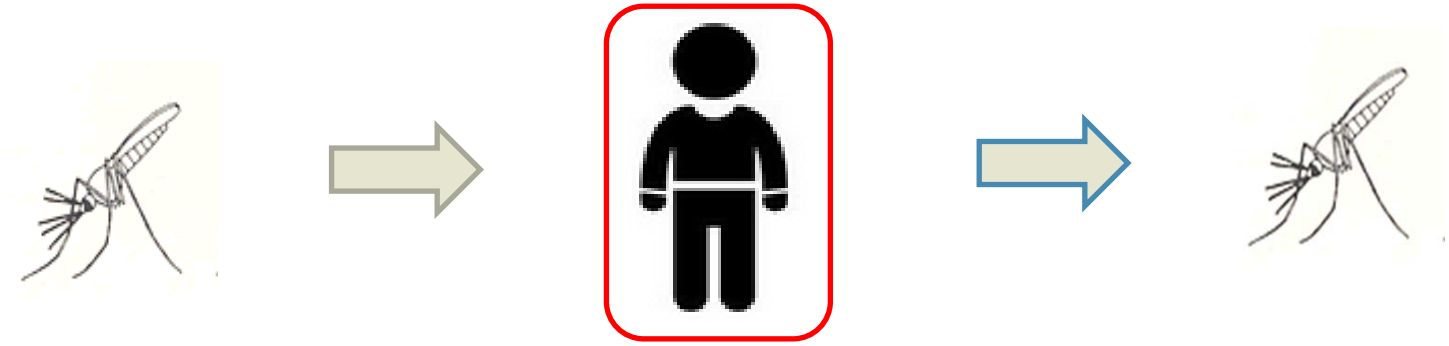
Major advantage of multi-copy PCR target:

Pooling of 10 blood samples without loss of sensitivity

***P. falciparum* prevalence**
in 498 individuals from Tanzania

Hofmann et al. PLOS Med 2015

The asymptomatic Reservoir of Infection

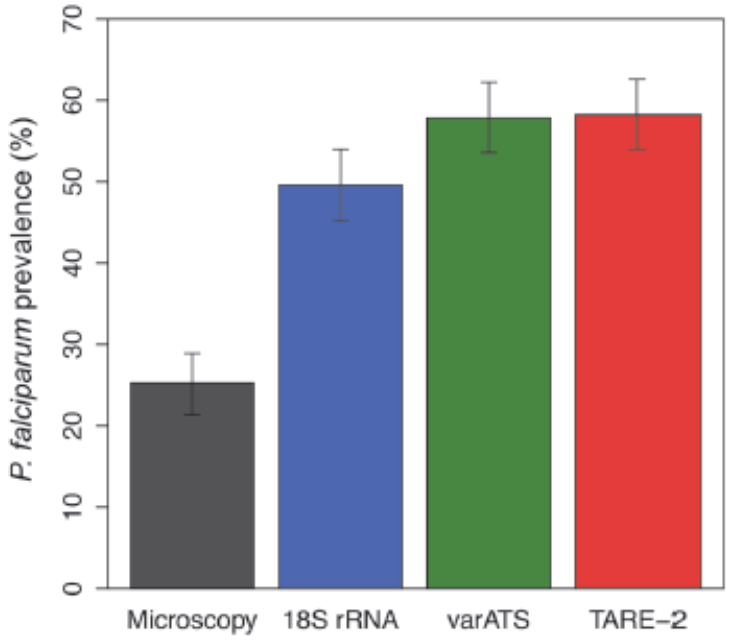


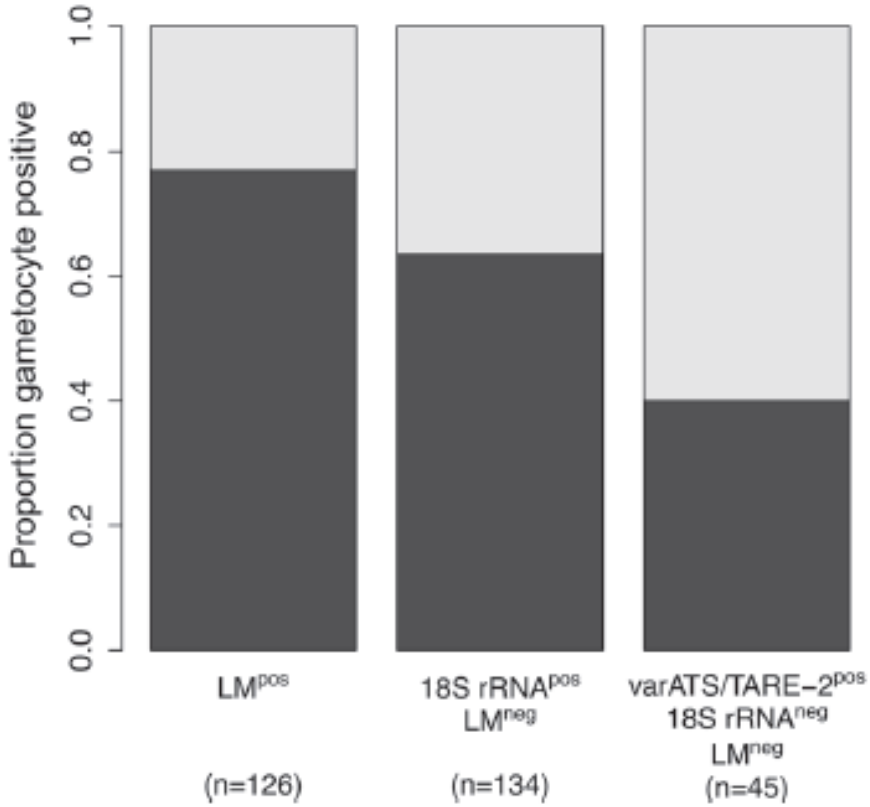
Parasite prevalence

Gametocyte prevalence

- Detected on transcript level by qRT-PCR or NASBA *(Pett et al. 2016 Mal J)*
- Most sensitive molec. marker: *pfs25* & *pvs25*
- RNA based → difficult sampling

Implication for prevalence rate: **plus 16%**

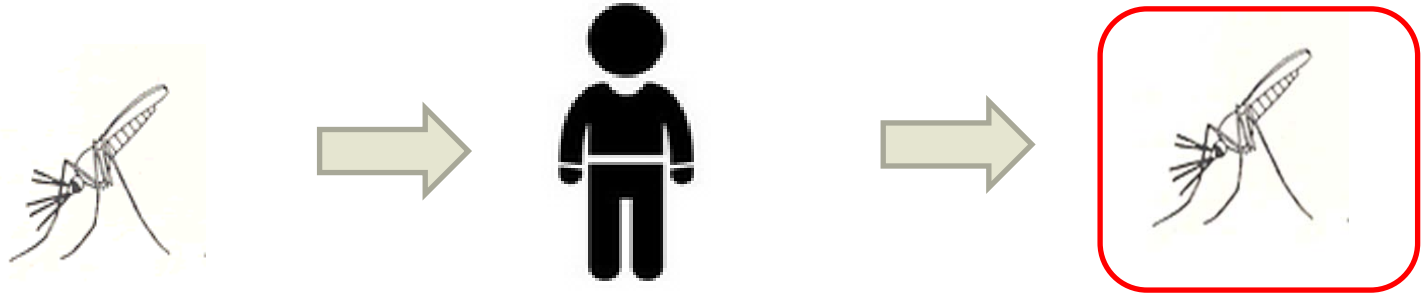


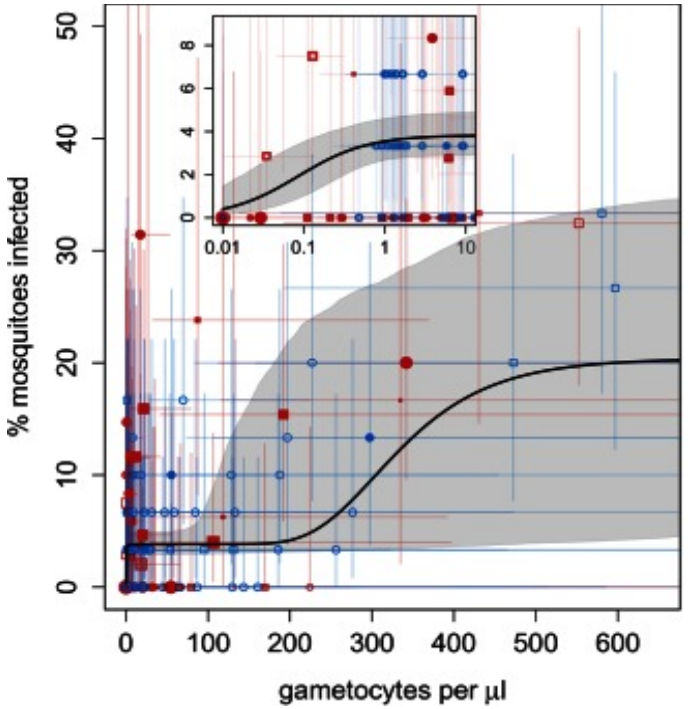


***P. falciparum* prevalence** in 498 individuals from Tanzania

Proportion of gametocyte carriers by pfs25 qRT-PCR

Hofmann et al. PLOS Med 2015





Parasite prevalence

Gametocyte density / prevalence

Infectivity to mosquitoes

Membrane feeding assays

More data needed

- Pf and Pv
- Different endemic sites

Landmark paper

Churcher et al. 2013 Elife

Do we need molecular assays for gametocytes?

Detection?

No

- All infections produce gametocytes at a steady rate *(Eksi et al.2012 PLOS Pathogen)*
- **Detection is a matter of blood volume**

Quantification?

Yes - for certain purposes

- Research: Describing the asymptomatic infective reservoir
- Monitoring of drug effects in clinical trials in comparator groups
- Vaccine effects on gametocytes in comparator groups

Current developments: increase sensitivity of assays

- **ultra-sensitive** qPCR (PNG, Brazil, Thailand)
  - **high copy number targets** for Pf and **Pv**
  - **qRT-PCR** targeting 18S ribosomal **RNA**

Manaos

Pv mtDNA	Pv 18S rRNA 0	Pv 18S rRNA 1
0	436	0
1	13	29

- increased blood volume **3 mL venous bleed** (in PNG; 4 malaria species)
  - white blood cell depletion → concentration of PCR template
  - magnetic enrichment of gametocytes
  - male / female gametocyte quantification
- evaluation of **iso-thermal** methods / suitable for **point-of-care tools**
  - LAMP (**L**oop-mediated isothermal **AMP**lification)
  - NASBA (**N**ucleic **A**cid **S**equence **B**ased **A**mplification) RNA-based

“Paper Machine” for POC Molecular Diagnostics

Photo American Chemical Society

Disposable device (5x10cm) intended for point-of-care use in resource-limited environments

Prototype tested 5 cells *E.coli* in 50 µL human serum

Device that integrates sample preparation and **loop-mediated isothermal amplification (LAMP; 1hr @ 65°C)** with end point detection using a hand-held UV source and camera phone

Connelly et al. 2015 Anal. Chem. 87 (15)

Elimination phase: Detection of malaria infections

Surveillance – response strategy

1 - 3 - 7

Day 1:
Diagnosis
Index case
Treatment
Digital
Reporting

Day 3:
Epidemiol.
Evaluation
at CDC

Day 7:
Response
in foci
Screen

↓

Focal Screen and Treat or **MSAT**

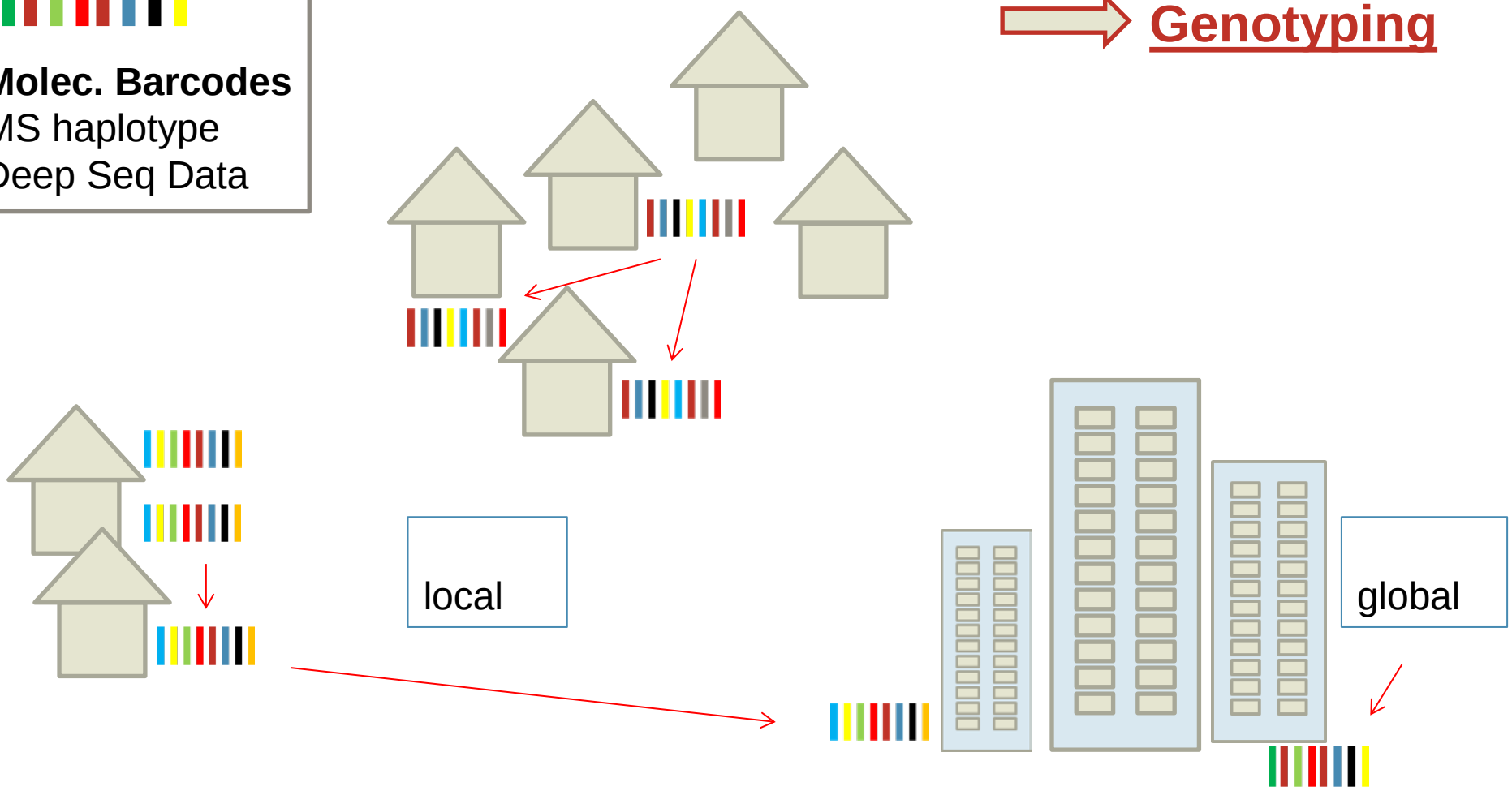
Example China Elimination until 2020

Weizhong Yang 2015 ECTMIH Conference

Elimination phase: Transmission network – routes of transmission

Molec. Barcodes
MS haplotype
Deep Seq Data

Genotyping

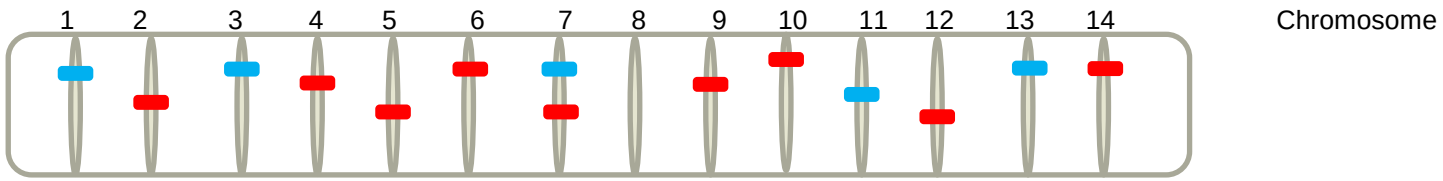


SNP-based genotyping methods

Barcoding

genome-wide single-nucleotide-polymorphisms are amplified by PCR

- ~20 SNPs, each detected by qPCR
- Barcodes for regional or global clone discrimination



1 *P. falciparum* genome = **14** chromosomes

Daniels et al. (2008)

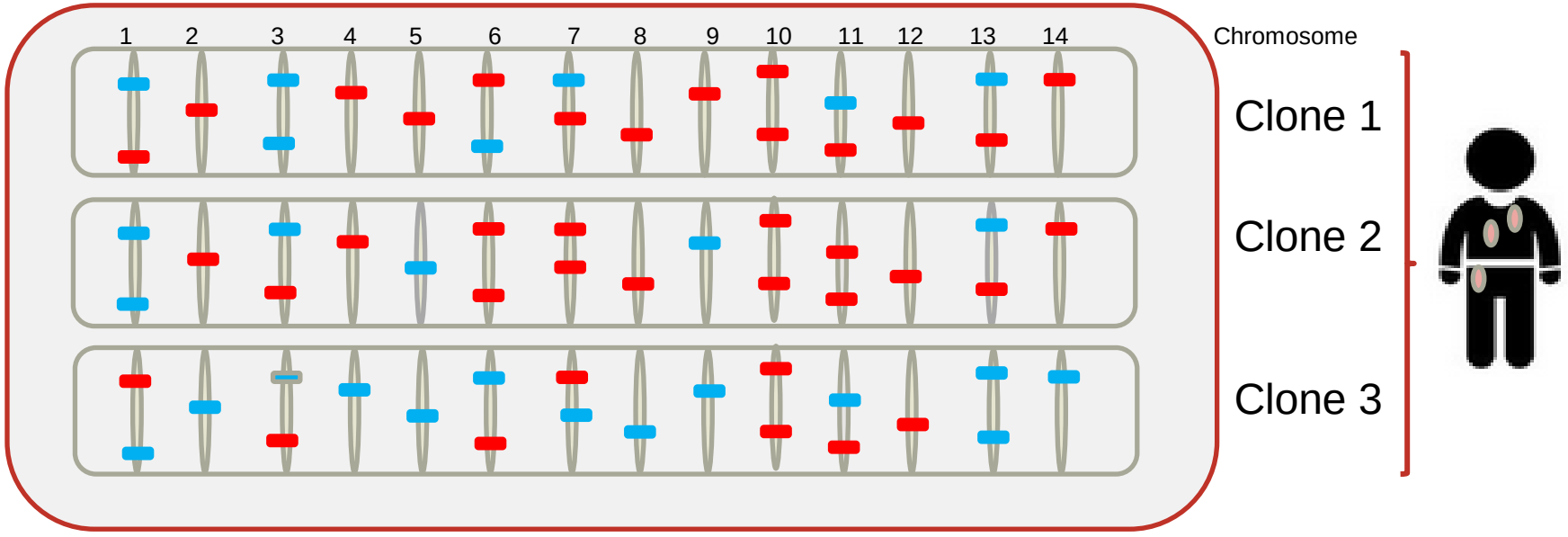
Alternative SNP-based genotyping methods

Barcoding

genome-wide single-nucleotide-polymorphisms are amplified by PCR

- ~20 SNPs each detected by qPCR
- Barcodes for regional or global clone discrimination

Work load !



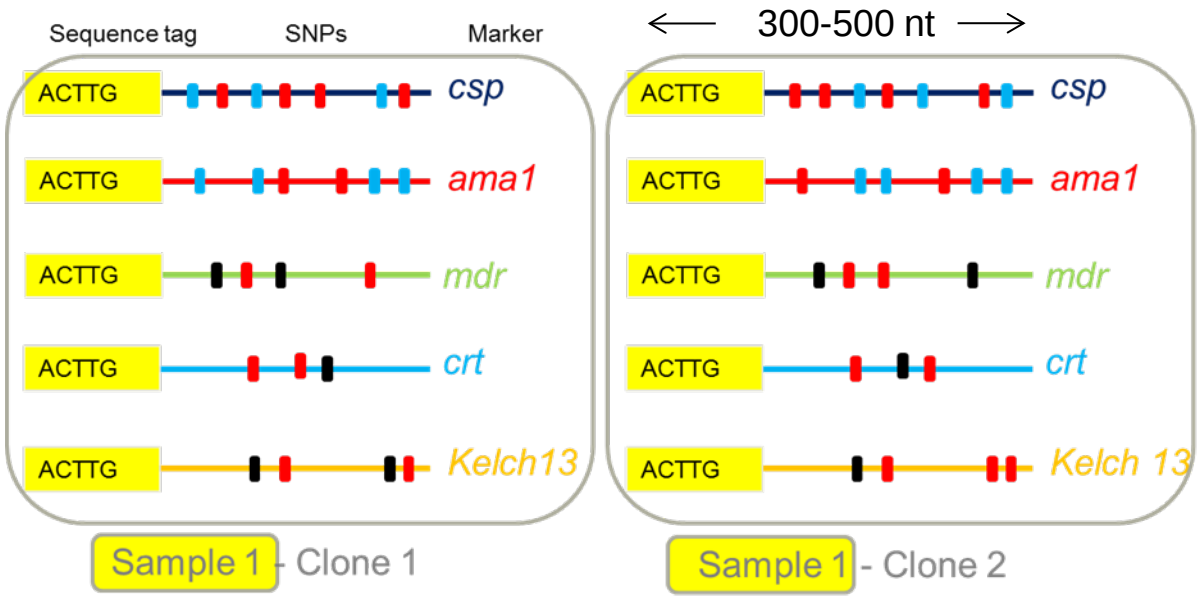
Problem to **reconstruct haplotype** in case of **multi-clone infections**

►►used primarily for **single clone** infections/low endemicity only

Amplicon “deep sequencing”

A. Lerch unpublished

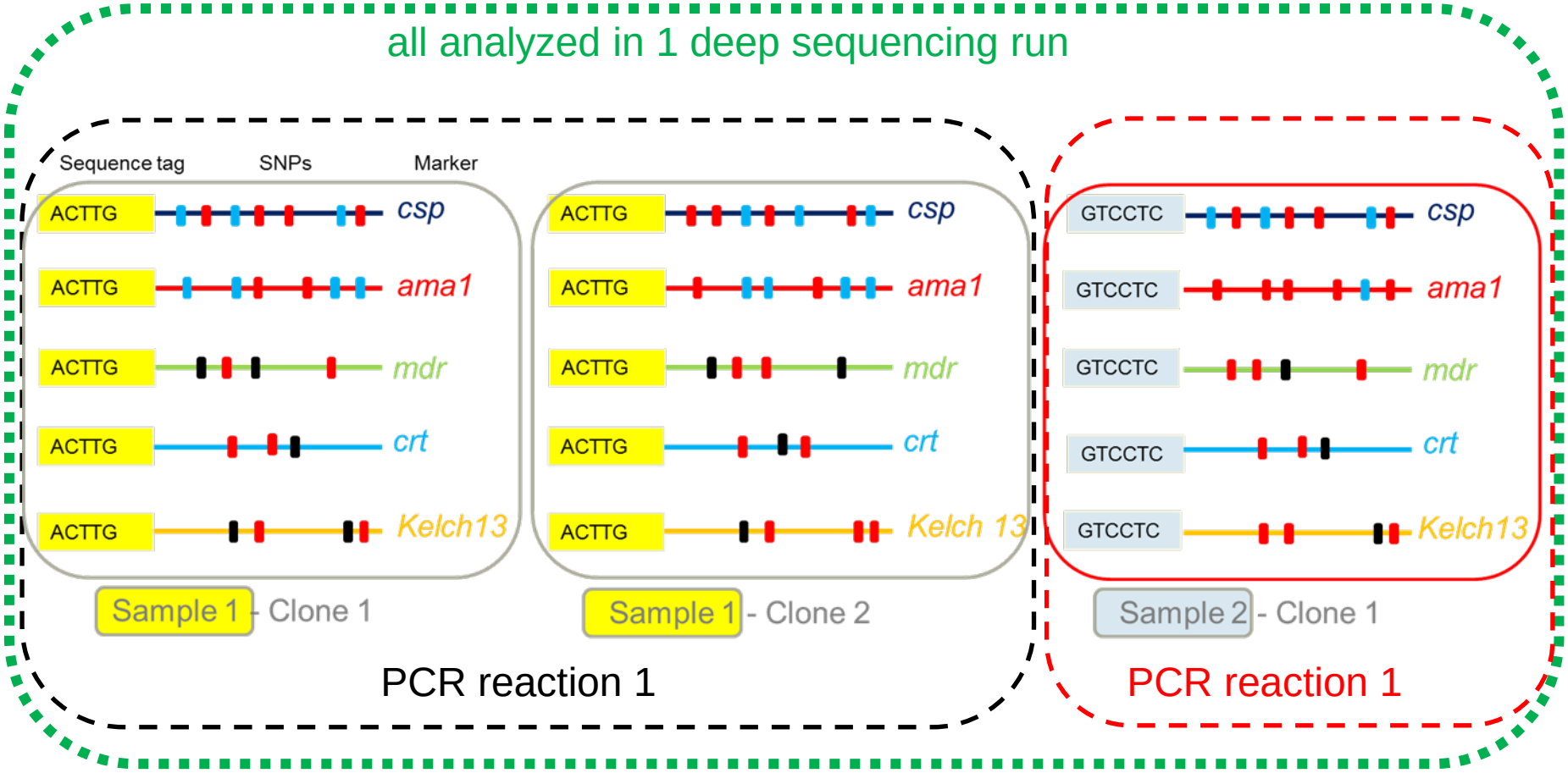
This blood sample 1 harbours 2 different parasite clones (= 2 genomes)



PCR reaction 1

Multiplex PCR with several sequence-tagged primer pairs

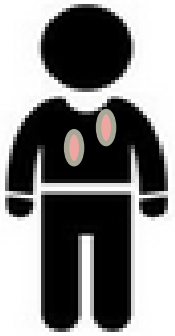
Amplicon “deep sequencing”



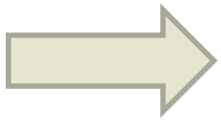
Example shows 2/384 blood samples with 5 markers/genome sequenced

Conclusion

human infectious reservoir



- ✓ Well described
- ✓ Low-density reservoir



Infectiousness to mosquitoes

Not enough knowledge on contribution of low-density infections
Needed: More hard work → MFAs

Needed

- Diagnostics Development for surveillance tools / POC
- Spatial and temporal heterogeneity of the infectious reservoir

Nov. 2016
Madang, PNG

Lincoln Timinao informing school children about **M**embrane **F**eeding **A**ssays

Information of school teachers at the IMR Entomology laboratory, Madang, PNG

IMR field team and Natalie Hofmann screening PNG schoolchildren November 2016

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