Drug resistant clones of the AS lineage

3. Clone information (clone by clone)

Please also consult the following files, as required

> "Drug resistant clones AS lineage – 1 outline, nomenclature, general info" and "Drug resistant clones AS lineage – 2 Published papers"

The following page-by-page notes for each clone include the origins, properties (inc phenotype and genotype) and the critical references of the various clones of the AS lineage

AS-sens

Name of clone (cloned unless otherwise stated)

AS-sens

Derived from

Parasites infecting a Thamnomys rutilans (thicket rat) trapped in Central African Republic (I. Landau, 1969) by growth and subsequent cloning. Other clones starting with A (eg AD, AQ etc) were cloned from the same animal), other clones BC, BW, CD, ER etc were cloned from other animals

By which drug

No drug selection involved

Drug selection protocol

n/a

Resistance phenotype

sensitive to all drugs used (PYR, CQ, MF, ART, ATN etc) except for sulfadoxine (SDX, natural resistance)

Genome re-sequencing

Illumina sequencing (36 base single-end read), reference AS genome database available (AS-WTSI)

Genotyping (relative to previous clone)

AS genome database available (AS-WTSI). Note that there were a small number of possible differences between Edinburgh Illumina sequencing of AS-sens and AS-WTSI sequences. These may be differences or sequencing errors in Edinburgh or AS-WTSI genome data. These have not been documented. Contact Martinelli (Sanger Institute) for more detail.

Genotyping (relative to AS-sens)

n/a

Genetic analysis

no

Critical References

Genome re-sequencing in Hunt *et al*. 2010, Martinelli *et al*. 2011, Borges *et al*. 2011, Modrzynska *et al*. 2012)

Notes

none

AS-PYR1

(alias AS-0CQ)

Name of clone (cloned unless otherwise stated)
AS-PYR1

Derived from
AS-sens

By which drug
Pyrimethamine

Drug selection protocol
4 day (50 mg/kg), single passage

Resistance phenotype
Resistant to PYR but with increased sensitivity to sulfadoxine (relative to AS-sens)

Genome re-sequencing
Not directly, but its genotype may be inferred by sequencing the mutated genes identified in AS-50S/P and AS-15MF, AS-30CQ and AS-ART etc.

Genotyping (relative to previous clone)
S106N *dhfr* chr07 (PCHAS_072830)
Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600
34 bp deletion chr07

Genotyping (relative to AS-sens)
S106N *dhfr* chr07 (PCHAS_072830)
Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600
34 bp deletion chr07

Genetic analysis
Culleton *et al*. 2005

Critical References
Culleton *et al*. 2005
Martinelli *et al*. 2011

Notes
PYR-R conferred by S106N *dhfr*. Strictly this was only formally confirmed by joining up Hayton *et al*. 2002, Culleton *et al*. 2011 who showed linkage on chr07 with Martinelli *et al*. 2011 who showed that there was only one mutation in this region of chr07

AS-50S/P

Name of clone (cloned unless otherwise stated)

AS-50S/P

Derived from

AS-PYR1

By which drug

Fansidar (i.e Pyrimethamine and Sulfadoxine combination)

Drug selection protocol

Gradual, many passages

Resistance phenotype

Resistant to PYR, and sulfadoxine (relative to AS-sens) and S/P combination

Genome re-sequencing

Illumina sequencing (50 base paired-end read), reference AS genome database available (AS-WTSI). See Martinelli *et al*. 2011

Identified mutations

Genotyping (relative to previous clone)

K392Q *mdr2* chr13 (multidrug resistance ABC transporter)

E109G PCHAS_020660 chr02

Genotyping (relative to AS-sens)

S106N *dhfr* chr07 (PCHAS_072830)

Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600

34 bp deletion chr07

K392Q *mdr2* chr13

E109G PCHAS_020660 chr02

Genetic analysis

Classical linkage analysis and QTL (Hayton *et al*. 2002)

Critical References

Hayton *et al*. 2002

Martinelli *et al*. 2011

Notes

none

AS-3CQ

Name of clone (cloned unless otherwise stated)

AS-3CQ

Derived from

AS-PYR1

By which drug

Chloroquine

Drug selection protocol

Gradual, many passages (Rosario 1976)

Resistance phenotype

Resistant to ~ 3 mg/kg CQ. Also PYR

Phenotyping

Carlton *et al*. 1998
Modrzynska *et al*. 2012

Genome re-sequencing

Not directly, but its genotype may be inferred by sequencing the mutated genes identified in AS-15MF, AS-30CQ and AS-ART etc.

Genotyping (relative to previous clone)

A173E *aat1* chr11 (amino acid transporter)
> 1 kb deletion chr05

Genotyping (relative to AS-sens)

S106N *dhfr* chr07 (PCHAS_072830)
Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600
34 bp deletion chr07
A173E *aat1* chr11 (amino acid transporter)
> 1 kb deletion chr05

Genetic analysis

Classical linkage analysis (and QTL) (Carlton *et al*. 1998)
Higher resolution linkage analysis (Hunt *et al*. 2004)
Progressive LGS analysis/Illumina quantitation (Modrzynska *et al*. 2012)

Critical References

Carlton *et al*. 1998
Hunt *et al*. 2004
Modrzynska *et al*. 2012

Notes

AS-15CQ

Name of line

Not cloned AS-15CQ

Derived from

AS-3CQ

By which drug

Chloroquine

Drug selection protocol

Gradual, many passages (Padua 1981)

Resistance phenotype

Resistant to ~ 15 mg/kg CQ. Also PYR

Phenotyping

Padua 1981, but phenotyping of uncloned line is largely meaningless

Genome re-sequencing

Not directly, but its genotype may be *partially* inferred by sequencing the mutated genes identified in AS-15MF, AS-30CQ and AS-ART etc.

Actually, since AS-15CQ is uncloned, it is clear that AS-15CQ is a (varying) population comprising parasites bearing different sets of mutations.

These mutations can also be recombined, since AS-15CQ has been passaged through mosquitoes. For example, different combinations of alternative alleles of two different genes (*ubp1*, chr02) and PCHAS_031370, chr03) appear in the lineage branches leading to AS-15MF, AS-ATN and AS-30CQ

Genotyping (relative to previous clone)

It is important to recognise that, because AS-15CQ is a line (not a clone) and furthermore has been passaged through mosquitoes, the mutations (alleles) below will be present in some (but likely not all of the parasites within this population). Furthermore, alternative alleles at different loci may have recombined in different (recombinant) parasites obtained during mosquito passage.

Ubp1 V2697F and V2728F (chr02, de-ubiquitinating enzyme)

T719N and I102del PCHAS_031370 (chr03, 12 TM transporter)

??Y162H PCHAS_101550 (chr10, ?voltage-gated postassium channel) – cannot be sure that this mutation

Genotyping (relative to AS-sens)

S106N *dhfr* chr07 (PCHAS_072830)

Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600

34 bp deletion chr07

A173E *aat1* chr11 (amino acid transporter)

> 1 kb deletion chr05

Ubp1 V2697F and V2728F (chr02, de-ubiquitinating enzyme)

T719N and I102del PCHAS_031370 (12 TM transporter)

??Chr10 kgvc

Genetic analysis

None

Critical References

Padua 1981

Modrzynska *et al*. 2012 (Additional file 1, section 3)

Notes

AS-30CQ

Name of clone (cloned unless otherwise stated)

AS-30CQ

Derived from

AS-3CQ via AS-15CQ (uncloned)

By which drug

Chloroquine – higher doses

Drug selection protocol

Gradual, many passages (Padua 1981)

Resistance phenotype

Resistant to ~ 30 mg/kg CQ. Also PYR
Artemisinin resistance phenotype 1 (100 mg/kg, 3 day)

Phenotyping

Padua 1981
Hunt *et al*. 2010 (artemisinin)
Modrzynska *et al*. 2012

Genome re-sequencing

Illumina genome re-sequencing (41 bp single-end)
Identified mutations
Described fully in combination of Hunt *et al*. 2010, Modrzynska *et al*. 2012

Genotyping (relative to previous clone)

Ubp1 V2728F (chr02, de-ubiquitinating enzyme)
T719N PCHAS_031370 (12 TM transporter)
Y162H PCHAS_101550 (voltage-gated potassium channel??)

Genotyping (relative to AS-sens)

S106N *dhfr* chr07 (PCHAS_072830)
Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600
34 bp deletion chr07
A173E *aat1* chr11 (amino acid transporter)
> 1 kb deletion chr05
Ubp1 V2728F (chr02, de-ubiquitinating enzyme)
T719N PCHAS_031370 (12 TM transporter)
Y162H PCHAS_101550 (voltage-gated potassium channel??)

Genetic analysis

Modrzynska *et al*. 2012

Critical References

Padua 1981
Hunt *et al*. 2010 (artemisinin)
Modrzynska *et al*. 2012

Notes

AS-ART

Name of clone (cloned unless otherwise stated)

AS-ART

Derived from

AS-3OCQ

By which drug

Artemisinin

Drug selection protocol

Gradual, many passages (Afonso *et al*. 2006)

Resistance phenotype

Poorly defined in Afonso *et al*. 2006
Artemisinin resistance phenotype 1 – 100 mg/kg 3 day
Artemisinin resistance phenotype 2 – 200 mg/kg 3 day and 5 day (large inoculum)

Phenotyping

Afonso *et al*. 2006
Hunt *et al*. 2010
Henriques *et al*. 2013

Genome re-sequencing

Illumina genome re-sequencing (41 bp single-end)
Identified mutations
Henriques *et al*. 2013

Genotyping (relative to previous clone)

AP2- µ-chain

Genotyping (relative to AS-sens)

S106N *dhfr* chr07 (PCHAS_072830)
Non-coding intergenic mutation chr14 (nt T936,945G) between genes PCHAS_142590 and PCHAS_142600
34 bp deletion chr07
A173E *aat1* chr11 (amino acid transporter)
> 1 kb deletion chr05
Ubp1 V2728F (chr02, de-ubiquitinating enzyme)
T719N PCHAS_031370 (12 TM transporter)
Y162H PCHAS_101550 (voltage-gated potassium channel??)
AP2- µ-chain

Genetic analysis

LGS-pyro Hunt *et al*. 2007
Modrzynska *et al*. 2012

Critical References

Hunt *et al.* 2007
Hunt *et al*. 2010
Modrzynska *et al*. 2012

Notes