

# Figure outline / packet

Measuring the depth and breadth of Fkh1-FHA-dependent chromatin structure and replication function at replication origins

2022-11-07

## Materials and methods

### SORT-seq

#### Cutluring and sorting

Followed protocol as outlined in [Batakou2020] with slight modifications. Briefly, 30 mL YPD cultures grown to 0.5 ODs/mL at 30°C. Harvested and fixed yeast in 70% EtOH, storing overnight at 4°C.

#### DNA extraction, library prep, and sequencing

#### Data processing and analysis

### FKKH1 motif mapping

### MNase-seq

### Benchwork

#### DNA extraction, library prep, and sequencing

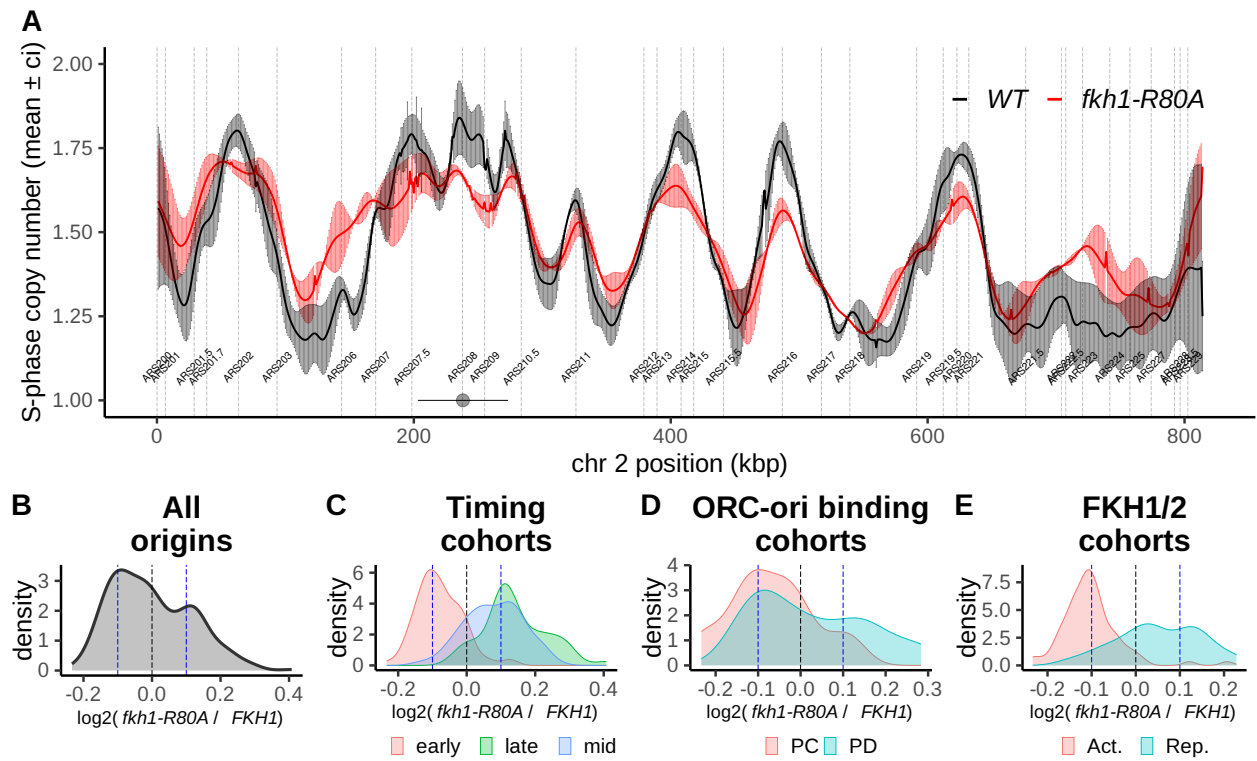
#### Data processing and analysis

## Results

**The loss of functional Fkh1FHA impacts replication at up to 25% of yeast origins.**

**Figure 1 (establishing the big Q and our SORT-seq experiment)**

**Take-home:** *Qualitatively and quantitatively, the loss of a functional Fkh1FHA changes replication dynamics at replication origins and perhaps even at termination events). Thus, more origins than the our original target PC cohort are affected by fkh1-R80A.*



### Supplemental 1 (all chromosomal scans)

**Take-home:** *In contrast to a previous study that measured replication through BrdU-chip (Knott et al, 2012), our study suggests that normal replication through yeast centromeres is dependent on a functional Fkh1FHA.*

## Supplemental 2 (all cen scans)

**Take-home:** *In contrast to the PC origins sensitive to fkh1-R80A in the NAR paper, fkh1-R80A-negative / FHA-SORT-dependent origins defined by the SORT-seq are characterized by a FKH1 match that overlaps the ORC binding site*

**Data Tk** May save for reviewers or as a “compromise” for resubmission

## Figure 5 (MNase-seq experiment validation)

**Take-home:** *We present data that suggest the Fkh1-FHA regulates chromatin structure as measured by MNase-seq experiments to such an extent that changes in MNase protection is evidenced for all confirmed origins and is G1-specific.*

- a) heats comparing confirmed to likely origins
- b) Quantification of heats
- c) Heats by region
- d) Quantification of heat regions.

## Supplemental 3

Comparison of experimental replicates for the two genotypes...

## Figure 6 (MNase-seq experiment with cohorts of interest)

**Take-home:** *As expected, Fkh1-FHA-dependent MNase protection is most evident in G1-phase and at FHA-sort-positive origins relative to FHA-sort-negative origins.*

As in figure 5, but with *fkh1-R80A*-SORT-negative and *fkh1-R80A*-SORT-positive.

## Supplemental 4

Comparison of experimental replicates for the two genotypes...

## Figure 7 (MNase-seq experiment with single locus plots & FKH1-matches)

Analysis Tk