# Mitochondrial nucleoids undergo remodeling in response to metabolic cues

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## Summary

**Mitochondrial DNA is organized as a nucleoprotein complex called the nucleoid. Its major protein components have been identified in different organisms, but it is yet unknown whether nucleoids undergo any form of remodeling. Using an in organello ChIP-on-chip assay, we demonstrate that the DNA-bending protein Abf2 binds to most of the mitochondrial genome with a preference for GC-rich gene sequences. Thus, Abf2 is a bona fide mitochondrial DNA-packaging protein in vivo. Nucleoids form a more open structure under respiring growth conditions in which the ratio of Abf2 to mitochondrial DNA is decreased. Bifunctional nucleoid proteins Hsp60 and Ilv5 are recruited to nucleoids during glucose repression and amino-acid starvation, respectively. Thus, mitochondrial nucleoids in yeast are dynamic structures that are remodeled in response to metabolic cues. A mutant form of Hsp60 that causes mtDNA instability has altered submitochondrial localization, which suggests that nucleoid remodeling is essential for the maintenance of mitochondrial genome.**

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## Introduction

Genomic DNA is organized as chromatin, a highly dynamic and intricate nucleoprotein structure. Histones are the major protein components of nuclear chromatin where, as octamers, they wrap and thus condense DNA in the form of nucleosomes. Nucleosome remodeling has emerged as a key strategy for the regulation of gene expression as well as for other chromosomal events, including DNA replication, recombination, repair and segregation (Ehrenhofer-Murray, 2004). Histones are extensively modified, undergoing different post-translational modifications, depending on their chromosomal location (Saha et al., 2006). As a result, chromatin remodeling is an essential process in eukaryotic cells, having a crucial role in growth, development and epigenetic events.

The mitochondrial genomes are packaged with proteins in structures, which, by analogy to bacterial chromosomes, are called mitochondrial DNA (mtDNA) nucleoids (Chen and Butow, 2005; Kucej and Butow, 2007; Malka et al., 2006). In contrast to nuclear chromatin, there is less information on the organization and dynamics of mtDNA nucleoids and how these properties might affect mtDNA transactions, including gene expression and inheritance. Initial studies in yeast (Chen et al., 2005; Kaufman et al., 2000; Miyakawa et al., 1987), human (Bogenhagen et al., 2008; Cheng et al., 2005; Garrido et al., 2003; Wang and Bogenhagen, 2006), and frog (Bogenhagen et al., 2003) revealed that, in addition to DNA transaction factors, high-mobility group (HMG)-like proteins in addition to a set of chaperones and metabolic enzymes are the major nucleoid components. Mitochondrial nucleoids lack histones. Instead, many mitochondrial genomes of different organisms are putatively packaged by HMG-like proteins, such as Abf2 in the budding yeast *Saccharomyces cerevisiae*, and Tfam in animal cells (Chen and Butow, 2005; Kaufman et al., 2007; Malka et al., 2006). These proteins are able to bend and wrap dsDNA in vitro (Brewer et al., 2003; Fisher et al., 1992; Friddle et al., 2004). Abf2 readily associates with DNA without sequence specificity, although it has a decreased affinity for simple polyA sequences (Diffley and Stillman, 1992). This feature causes phased binding, which was found to occur in vitro on replication origins and promoter sequences (Diffley and Stillman, 1991; Fisher et al., 1992). The mtDNA in *abf2* mutants is less protected and exhibits increased sensitivity to nuclease attack (Newman et al., 1996) and oxidative stress (O'Rourke et al., 2002). Nucleoids contain an assortment of proteins with no obvious role in mtDNA transactions. These 'unexpected' nucleoid proteins, such as aconitase and Ilv5 in yeast, have turned out to be bifunctional with distinct metabolic activities and, as nucleoid proteins, novel activities with respect to their functions in the maintenance of mtDNA (Bateman et al., 2002b; Chen et al., 2007; Chen et al., 2005; Kaufman et al., 2003; Shadel, 2005; Zelenaya-Troitskaya et al., 1995).

Here, we demonstrate by chromatin immunoprecipitation (ChIP) that yeast Abf2 is a bona fide mtDNA-packaging protein. Owing to changes in mtDNA copy number, the ratio of Abf2 to mtDNA varies under different growth conditions, which is accompanied by variable sensitivity of mtDNA to micrococcal nuclease. In addition, other nucleoid proteins, such as Hsp60 and Ilv5, are recruited to nucleoids during glucose repression and amino-acid starvation, respectively. We propose that these protein localization dynamics constitute a mitochondrial nucleoid remodeling. Finally, the nucleoid remodeling seems to be essential for the maintenance of mtDNA, because some mutant forms of nucleoid proteins that destabilize mtDNA, such as the Hsp60 A144V mutant and the previously

reported Ilv5 W327R mutant (Bateman et al., 2002a), have perturbed submitochondrial localization.

## Results

### Mapping the Abf2-mtDNA interactions

Abf2 is considered to be the major mtDNA-packaging protein in yeast, but no comprehensive analysis of Abf2 interaction with mtDNA in situ is available. Therefore, we examined the binding of Abf2 and mtDNA by means of chromatin immunoprecipitation experiments, in which proteins associated with mtDNA were crosslinked to DNA or to each other by formaldehyde treatment of isolated mitochondria (supplementary material Fig. S1). A strain expressing the fusion protein Abf2-13Myc was constructed for this purpose. Expression of the fusion protein was verified by western blotting with an anti-Myc antibody (supplementary material Fig. S2). We did not observe any mtDNA instability in the constructed strain, indicating that Abf2-13Myc is functional (data not shown). After formaldehyde treatment, the purified mitochondria were lysed with a non-ionic detergent, sheared by sonication to obtain ~500-bp DNA fragments, and nucleoid complexes were immunoprecipitated using an anti-Myc antibody. The coimmunoprecipitated DNA was first used as a probe for Southern blot hybridization (Fig. 1A). ChIP DNA was labeled without amplification and hybridized to blots of total mtDNA digested with *Dra*I. We found that the hybridization profile obtained with the ChIP DNA was similar to that obtained when CsCl-purified total mtDNA was used as a probe, indicating that Abf2p binds most regions of mtDNA. The difference in signal intensities between the strains expressing Abf2-13Myc and the untagged Abf2 demonstrates that ChIP with Abf2 was very efficient. As a result, almost all immunoprecipitated DNA from the Myc-tagged strain represented the DNA material that was specifically bound by Abf2. These observations strongly support the role of Abf2 as a major mtDNA-packaging protein.

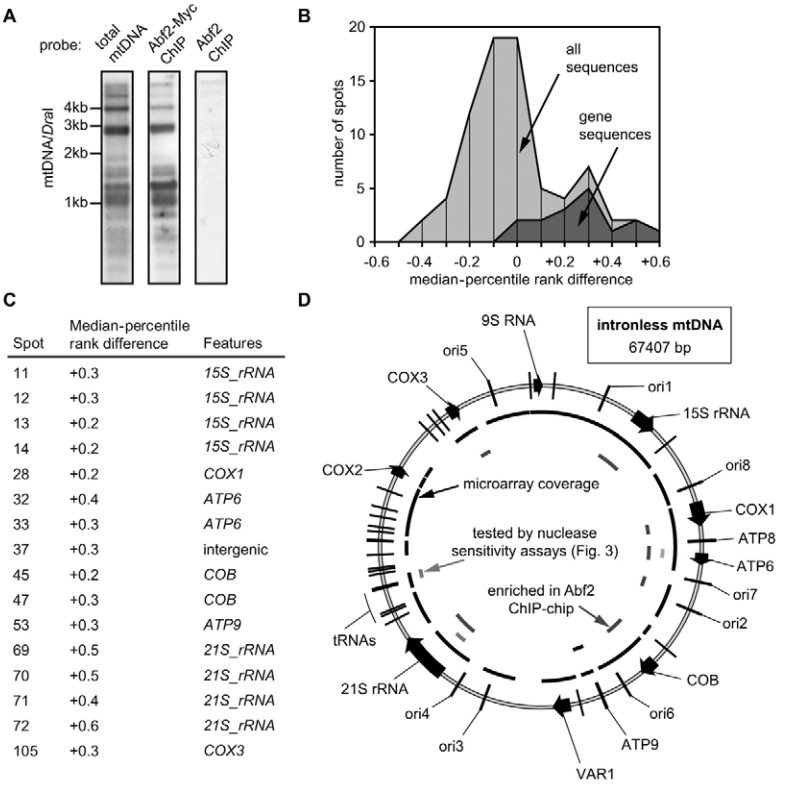


**Fig. 1.** Abf2 associates preferentially with complex GC-rich sequences. (A) Southern blot hybridizations of blots of mtDNA digested with *Dra*I with the following probes: CsCl-purified total mtDNA sheared by sonication (left panel), DNA coimmunoprecipitated with an anti-Myc antibody from the strain expressing Abf2-13Myc fusion protein (middle panel), and DNA coimmunoprecipitated with an anti-Myc antibody from the control strain expressing untagged Abf2 (right panel). (B) The median-percentile rank analysis of ChIP DNA microarray hybridizations (supplementary material Fig. S3). All data points were sorted from highest to lowest Cy5:Cy3 ratios and each data point was assigned a percentile rank. The median-percentile ranks of each mtDNA region from three Abf2-13Myc and four Abf2 ChIP experiments were compared by subtracting the control medians (Abf2) from the sample medians (Abf2-13Myc). The gray peak represents the median-percentile rank difference of all mtDNA regions examined with the microarrays. The dark-gray peak shows median-percentile ranks of all the complex GC-rich sequences in the dataset; a bias to higher median-percentile ranks is evident. Therefore, these sequences were enriched in Abf2-13Myc ChIP sample. (C) A list of all DNA spots consistently enriched in the Abf2-13Myc ChIP-chip experiments. (D) Circular map of the intronless mtDNA. The inner black markers identify sequences covered by mtDNA microarray analysis. The innermost gray markers identify the regions significantly enriched in the Abf2-13Myc ChIP-chip experiments.

To map the association of Abf2 with mtDNA in greater detail, we opted to examine DNA obtained from the ChIP experiments with DNA microarrays. A similar approach was successfully used to identify mtDNA-binding sites of Arg5 and Arg6 proteins in yeast (Hall et al., 2004). However, the authors of that study examined mostly mitochondrial gene sequences. We wanted to compare representatives of all features on the yeast mtDNA, including intergenic regions. Therefore, we prepared DNA microarrays of ~550-bp average length mtDNA fragments covering ~70% of a wild-type mitochondrial genome without introns, which we used for mitochondrial ChIP-chip (Fig. 1D). Amplified DNA samples from Abf2-13Myc and untagged Abf2 ChIP experiments were labeled with Cy5; CsCl-purified, ultrasonically sheared total mtDNA that was amplified by the same procedure, was used as a Cy3-labeled control (supplementary material Fig. S1). Microarray hybridization datasets of Abf2-13Myc experimental samples and Abf2 controls (supplementary material Fig. S3) were compared using a median-percentile rank method, as described previously (Buck and Lieb, 2004). When DNA spots with the same median-percentile ranks were grouped, a small subset of sequences appeared as a second peak (Fig. 1B). This group of sequences was represented almost exclusively by long, complex, GC rich sequences located in *COX1*, *COX3*, *COB*, *ATP6*, *ATP9*, *15S_rRNA* and *21S_rRNA* gene loci (Fig. 1C,D). Normally, only these enriched sequences would be considered to be bound by an analyzed protein. However, since the amount of crosslinked DNA was much higher in Abf2-13Myc samples compared with Abf2 controls (Fig. 1A), the correct interpretation is that Abf2 protein binds most of the mtDNA sequences with a relatively higher affinity for GC-rich gene regions.

### Abf2 protein-protein interactions in mitochondria

We performed ChIP-on-chip (ChIP-chip) assays with two additional mitochondrial nucleoid proteins, namely Aco1 in glycerol medium and Ilv5 in medium without amino acids. No specific DNA enrichment was observed in these experiments. The most probable cause is very inefficient crosslinking of the examined proteins to mtDNA. When an excessive treatment by formaldehyde was

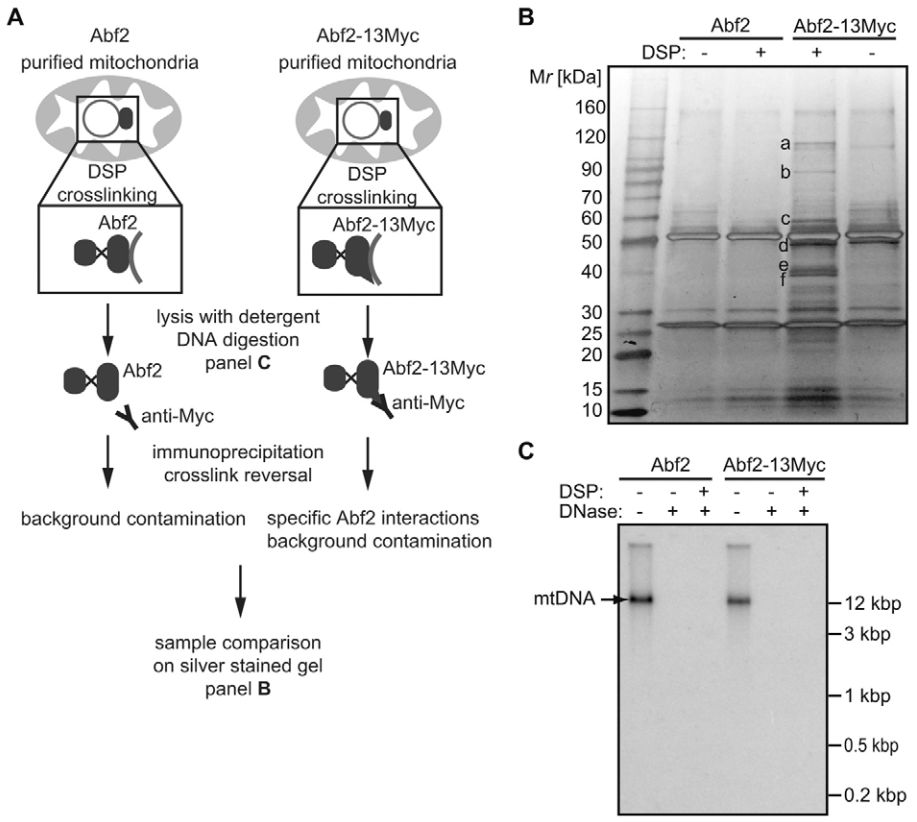


**Fig. 2.** Abf2 protein interactions in mitochondria. (A) Identification of mitochondrial Abf2-interacting proteins. Purified mitochondria from yeast strains expressing Abf2 and Abf2-13Myc were crosslinked with DSP. Mitochondrial lysates were incubated with DNaseI to remove mtDNA, and Abf2 protein was immunoprecipitated with anti-Myc antibody. Protein samples were compared on silver-stained gels. Protein bands that were unique to the Abf2-13Myc sample were further examined. (B) Silver-stained 4%-20% gradient SDS-PAGE gel of the protein samples immunoprecipitated with an anti-Myc antibody from the mitochondria isolated from MCC109 cells expressing the wild-type Abf2 protein and the Abf2-13Myc fusion protein. Where indicated, mitochondria were incubated with the protein crosslinker DSP prior to incubation with the antibody. a, Kgd1; b, Aco1; c, Ald4; d, Abf2-13Myc; e, Idh2; f, Idh1. (C) Southern blot confirming that the DNA was degraded after incubation with DNAseI in samples used for coimmunoprecipitation in B. DNA was isolated from ~5 μg of purified mitochondria and from an aliquot of DNAseI-treated lysate that contained ~50 μg of proteins. MtDNA was probed using a PCR-amplified region of the mitochondrial *COX2* gene.

applied, mitochondrial chromatin became resistant to DNA fragmentation and thus unusable for ChIP. It has been indeed observed that Aco1, for instance, exhibits only a weak binding to dsDNA (Chen et al., 2007). Also, it is conceivable that some nucleoid proteins are preferentially retained in proximity of mtDNA by protein-protein interactions with other DNA-binding proteins. We therefore asked whether such proteins can be coimmunoprecipitated with an mtDNA-binding protein, namely Abf2 (Fig. 2). Mitochondria isolated from yeast strains that express Abf2-13Myc and Abf2 as a control were crosslinked with dithiobis(succinimidylpropionate) (DSP) and lysed with a non-ionic detergent. The lysate was treated with DNaseI to prevent detection of protein interactions mediated by DNA (Fig. 2C). Abf2-13Myc protein was immunoprecipitated from the lysate using an anti-Myc antibody. The crosslinks were reversed and coimmunoprecipitated proteins were analyzed by gel electrophoresis (Fig. 2B). The examination of silver-stained bands, specific to the sample from the Abf2-13Myc expressing strain, revealed Aco1, Ald4, Idh1, Idh2 and Kgd1 as the major interacting proteins of Abf2. The proteins identified in this screen have been previously found in purified formaldehyde-fixed nucleoids (Kaufman et al., 2000). Thus, protein crosslinking with DSP further supports the localization of these proteins in nucleoids. Moreover, these proteins directly interact or are in proximity to the major nucleoid protein Abf2.

### Remodeling of mitochondrial nucleoids

Although the composition of mitochondrial nucleoids in yeast has been reasonably well-characterized, there is little information on whether nucleoids undergo structural or compositional remodeling. We therefore examined these issues in cells in which the respiration was repressed by glucose and activated by growth of cells on glycerol. To investigate whether nucleoid remodeling occurs under these conditions, we assessed the sensitivity of selected mtDNA sequences to micrococcal-nuclease digestion in toluene-permeabilized mitochondria (Newman et al., 1996). Nucleoids that are in a more compact structure or more covered with proteins should be more resistant to nuclease digestion, whereas nucleoids with a more open structure should be more sensitive to nuclease digestion. Accordingly, we compared three test regions of the mitochondrial genome – the *21S_rRNA* region 71, the *ATP6* region 33 and the intergenic DNA region 81 (supplementary material Table S1, Fig. 1D) – for their sensitivity to nuclease digestion. We found that these sequences were more resistant to nuclease attack in mitochondria isolated from glucose-grown cells than from glycerol-grown cells (Fig. 3A-C). Thus, the structure of mitochondrial nucleoids differs in these conditions and is more open in glycerol-grown cells compared with glucose-grown cells.

Our ChIP-chip experiments demonstrate that Abf2 binds most of the mtDNA sequences, consistent with it being an mtDNA packaging protein. Using nuclease sensitivity assays, it has been demonstrated previously that Abf2 is the major determinant of mtDNA protection from a nuclease (Newman et al., 1996). Thus, the nucleoid remodeling observed by micrococcal-nuclease-sensitivity assays could be accomplished by modulation of Abf2 levels, for example, by increasing the amount of Abf2 in glucose medium, or decreasing it in glycerol medium. However, expression of Abf2 was comparable in BY4741 cells grown in both types of medium (Fig. 3D). Notably, mtDNA copy number can vary too, depending on whether cells grow in glucose or on a non-fermentable carbon source (Goldthwaite et al., 1974), providing another means for nucleoid remodeling. Therefore, we measured mtDNA abundance in the BY4741 strain used in this study, and confirmed

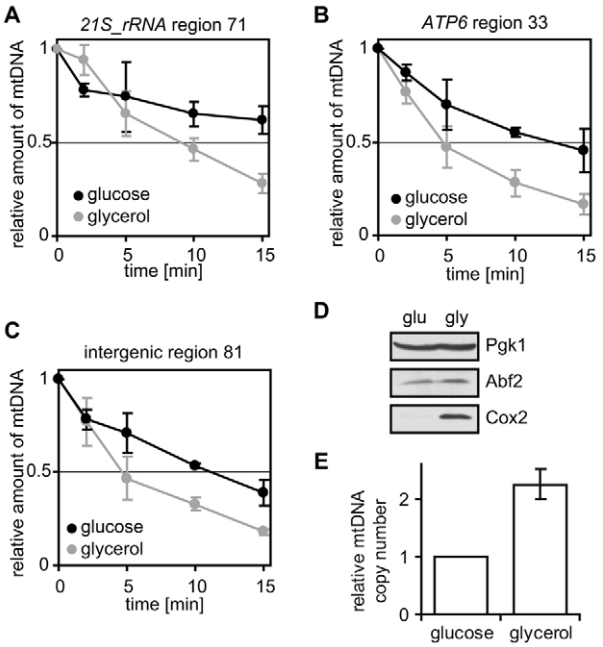


**Fig. 3.** Mitochondrial nucleoids undergo remodeling. (A-C) Quantification of micrococcal-nuclease-sensitivity assays of (A) *21S_rRNA* , (B) *ATP6* and (C) intergenic region 81 using ~1 mg of toluene-permeabilized mitochondria isolated from BY4741 cells grown in glucose-containing (●) and glycerol-containing (○) medium. Representative autoradiograms are shown in supplementary material Fig. S4. (D) Western blot of Pgk1, Abf2 and Cox2 proteins from cell extracts of BY4741 cells grown in YPD (glu) and YPG (gly) medium. Because expression of Pgk1 is not dependent on a carbon source (Roberts and Hudson, 2006), an anti-Pgk1 antibody was used to estimate the protein loading. Unlike Cox2 – the expression of which is much higher in glycerol than in glucose medium – Abf2 levels were comparable in both YPD and YPG. (E) Relative mtDNA copy number in BY4741 cells grown in YPD (glucose) and YPG (glycerol) medium assessed by Southern blot hybridizations using probes derived from the mitochondrial *21S_RNA* and nuclear *ACT1* genes. Error bars, average deviations.

that mtDNA copy number was ~twofold higher in glycerol-grown cells compared with glucose-grown cells (Fig. 3E). As a result, the Abf2 to mtDNA ratio in whole cells is decreased by a factor of two in glycerol medium. In agreement with this observation, nucleoids in glycerol-grown cells were less protected from nuclease digestion.

To examine whether the distribution of Abf2 to nucleoids varies under these different metabolic conditions, we assessed the submitochondrial localization of Abf2 by sedimentation of mitochondrial lysates in sucrose gradients. Purified mitochondria were lysed with a nonionic detergent and loaded onto sucrose gradients (20%-60%-80%). MtDNA was found in fractions near the bottom of the gradient (Fig. 4B), whereas the mitochondrial matrix marker protein Mdh1 remained near the top (Fig. 4A). We repeatedly observed that under our experimental conditions, Abf2 largely cofractionated with mtDNA in cells grown in both glucose and glycerol medium (Fig. 4A,B). We quantified the amount of mtDNA and Abf2 in the first seven fractions from the bottom, and confirmed that the average Abf2-to-mtDNA ratio in glycerol-grown cells reaches 59±21% of the ratio in glucose-grown cells. This reflects changes in the whole-cell ratio of Abf2 to mtDNA (Fig. 3D,E). In summary, because the level of expression and the pattern of distribution of Abf2 between nucleoids and the matrix does not vary – whereas the copy number of mtDNA does – the amount of Abf2 associated with mtDNA effectively decreases in mitochondrial nucleoids of glycerol-grown cells. This provides an explanation for a more-open structure of nucleoids detected by micrococcal nuclease.

### Recruitment of Hsp60 and Ilv5 to nucleoids

To investigate whether other mitochondrial nucleoid proteins are redistributed under different growth conditions, we assessed the localization of three bifunctional nucleoid proteins, aconitase (Aco1), Ilv5 and the 60-kDa heat shock protein Hsp60, by using sucrose gradients (Fig. 4B). As expected, the majority of these proteins cofractionated with the mitochondrial matrix marker protein Mdh1. However, Aco1, Ilv5 and Hsp60, but not Mdh1, exhibited a dual distribution in the sucrose gradients. A smaller fraction of these proteins was found to cosediment with mtDNA. Using this rapid and sensitive assay, we found that various nucleoid proteins behaved differently depending on whether mitochondrial lysates were prepared from glucose- or glycerol-grown cells (Fig. 4A). For example, we repeatedly found a several-fold increase in the amount of Hsp60 in nucleoids from glucose- versus glycerol-grown cells. By contrast, the distribution of Aco1 and Ilv5 in nucleoids was comparable under both conditions.

We have previously shown that Ilv5 is required for parsing mtDNA into a greater number of nucleoids during amino-acid starvation (MacAlpine et al., 2000). Therefore, we compared the distribution of Ilv5 and that of other nucleoid proteins in sucrose gradients of mitochondrial extracts from cells grown in minimal medium with no amino acids, or with isoleucine, leucine, valine as a control (Fig. 4C,D). We repeatedly found that there was more Ilv5 present in mtDNA-containing fractions of mitochondria that were isolated from cells that had been starved for amino acids compared with those isolated from control cells. At the same time, the distribution of other proteins to nucleoids was unchanged (or slightly lower, which could be attributed to decreased expression levels of these proteins under conditions of amino-acid starvation). These results suggest underlying functional differences for these nucleoid proteins that are reflected in their different responses to growth conditions. Together, these experiments demonstrate that mitochondrial nucleoids undergo specific compositional remodeling in response to various metabolic cues.

Mutant forms of bifunctional Hsp60 and Ilv5 proteins that cause mtDNA instability despite their ‘conventional’ functions being preserved have been characterized (Bateman et al., 2002b; Kaufman et al., 2003). Molecular functions of Hsp60 and Ilv5 proteins relating to the maintenance of mtDNA are not yet understood, but it has been demonstrated that, in certain *ilv5* mutants, which exhibit an mtDNA-instability phenotype, the submitochondrial localization of Ilv5 is altered; namely, aggregates of mutant Ilv5 protein are formed in the mitochondrial matrix (Bateman et al., 2002a). We investigated whether the A144V mutant form of Hsp60, which is unable to maintain mtDNA, also has impaired submitochondrial localization. We compared the distribution of Hsp60 A144V with the wild-type protein in sucrose gradients of mitochondrial lysates (Fig. 5). The levels of the A144V protein that we found associated with mtDNA were substantially elevated compared with that of the wild-type protein both in glucose and glycerol growth media. Thus, Hsp60 mutant protein, which causes defects in mtDNA metabolism, exhibits an altered recruitment to nucleoids.

## Discussion

Our results show that mtDNA nucleoids are not static structures but are subject to remodeling, which is linked to metabolic cues

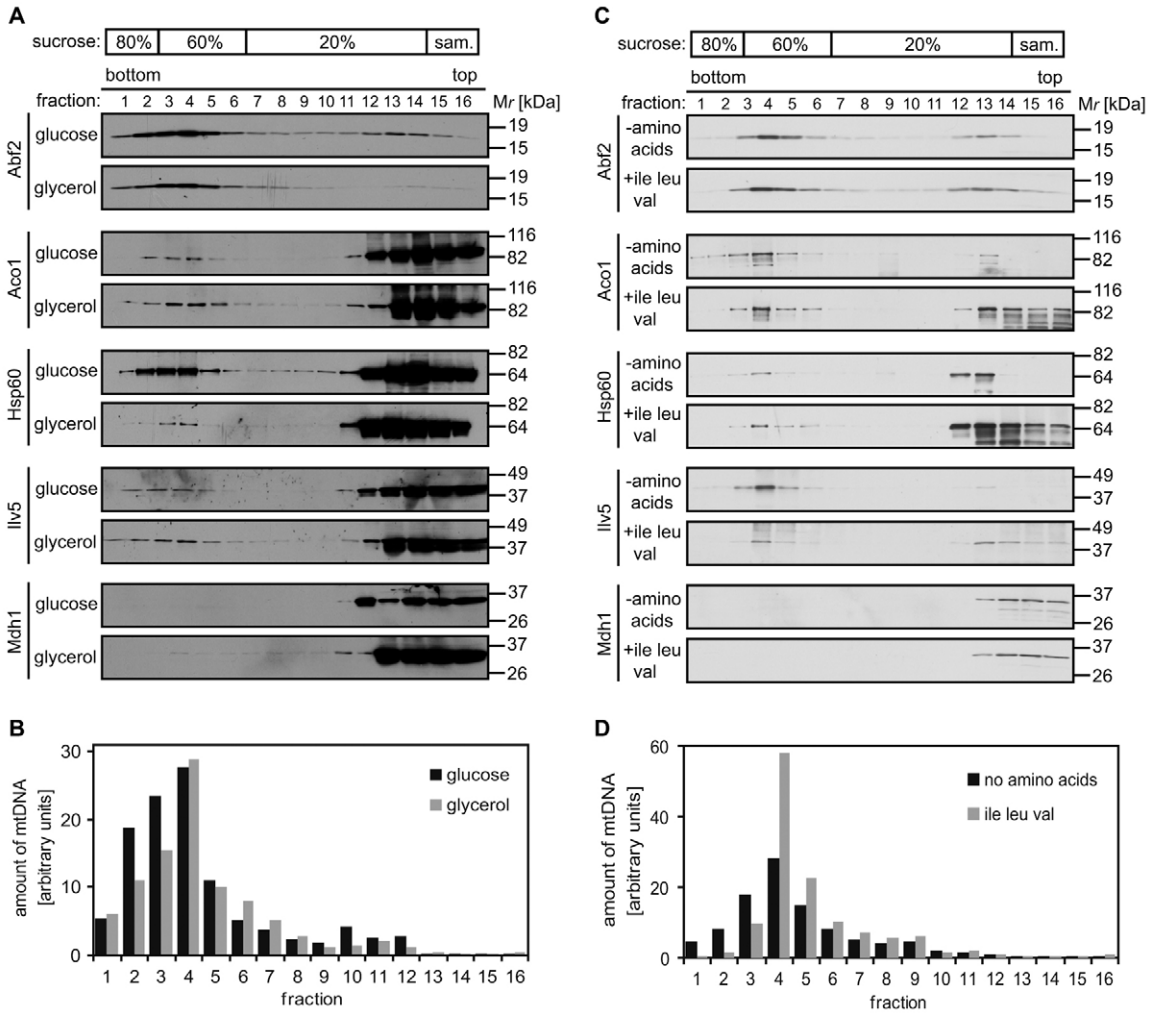


**Fig. 4.** Distribution of nucleoid proteins in sucrose gradients. Sucrose gradients of an equal amount of lysed purified mitochondria (~1 mg of proteins) isolated from BY4741 cells grown in glucose and glycerol containing medium (A,B) and MCC109 cells grown in SD medium with isoleucine, leucine, valine or no amino acids (C,D) were collected in 16 fractions. Every fraction was probed for the presence of proteins Abf2, Mdh1, Hsp60, Aco1, Ilv5 (A,C) and mtDNA (B,D). Mdh1 is a matrix protein, which represents the control for the total lysis of mitochondria and the presence of matrix contamination. The combined occurrence of Abf2 and mtDNA indicates gradient fractions, which contain nucleoids. (A) Hsp60 is the only protein increased in fractions containing nucleoids, when cells are grown in glucose medium compared with glycerol medium. (C) Ilv5 is the only protein increased in fractions containing nucleoids, when cells are grown in medium without amino acids compared with medium containing isoleucine, leucine and valine.

such as glucose repression or amino-acid starvation (Fig. 6). We propose that mitochondrial nucleoids are in a more-open state under conditions that are characterized by a decreased Abf2-to-mtDNA ratio, and are more compact when this ratio is increased. This scenario is supported by several of our observations.

First, using mitochondrial ChIP-chip assay, we demonstrated that Abf2 binds most of the mitochondrial genome and is, therefore, a bona fide mtDNA-packaging protein (Fig. 1). Thus, our study supports the hypothesis (that originated from previous in vitro studies (Brewer et al., 2003; Friddle et al., 2004), that the abundance of Abf2 in nucleoids determines the degree of compaction and the overall structure of nucleoids. In addition, our ChIP-chip experiments revealed a preferential binding of Abf2 to complex GC-rich sequences in vivo. In essence, the yeast mtDNA consists of gene sequences, which are the only complex GC-rich features on the genome, plus AT-rich spacers that are often interrupted by short GC clusters. Thus, the Abf2 distribution on the mitochondrial genome might reflect the inability of Abf2 to bind oligoA sequences, a fact that has been demonstrated previously in vitro (Diffley and Stillman, 1992). However, it is conceivable that the observed binding pattern represents an intentional recruitment of Abf2 to gene sequences to ensure better protection, packaging or other regulatory functions.

Second, we show that, because of a twofold increase in mtDNA copy number and stable expression of Abf2, the average whole-cell ratio of Abf2-to-mtDNA is decreased in cells grown in glycerol-containing medium compared with cells grown in medium containing glucose (Fig. 3D,E). Notably, the distribution of Abf2 between nucleoids and the matrix remains similar both in glucose-enriched and glycerol-enriched medium under our experimental conditions (Fig. 4A). Therefore, we conclude that, in transcriptionally active nucleoids, there is effectively less Abf2 bound to each mtDNA molecule. Our comparison of nuclease sensitivity of nucleoids in mitochondria isolated from glucose- and glycerol-grown cells supports this hypothesis, because glucose nucleoids are more resistant to micrococcal nuclease than glycerol

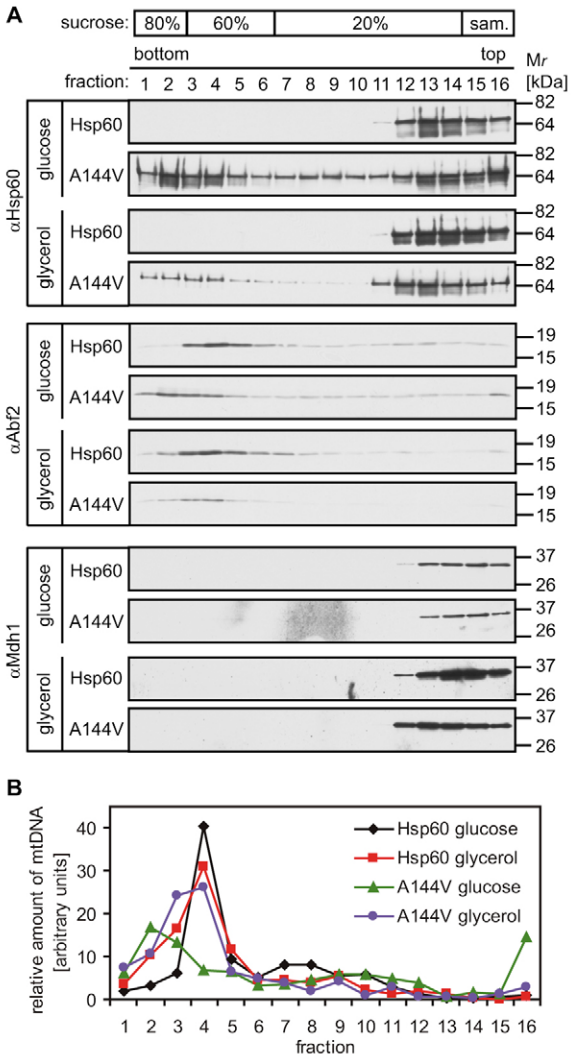


**Fig. 5.** Distribution of Hsp60 A144V mutant protein in sucrose gradients. Sucrose gradients of an equal amount of lysed purified mitochondria (~1 mg of proteins) from W303-1B cells expressing the wild-type Hsp60 protein or the Hsp60 A144V mutant grown in glucose and glycerol-containing medium were collected in 16 fractions. The gradient fractions were probed for the presence of proteins Hsp60, Abf2, Mdh1, (A) and mtDNA (B). Mdh1 is a matrix protein, which represents the control for the total lysis of mitochondria and the presence of matrix contamination. The combined occurrence of Abf2 and mtDNA indicates gradient fractions that contain nucleoids. An increased presence of Hsp60 A144V mutant protein in fractions containing nucleoids is visible. A much longer exposure time is required to detect the wild-type Hsp60 protein associated with nucleoids (compare with Fig. 4A).

nucleoids (Fig. 3A-C). The functional significance of this remodeling still remains unknown. It is conceivable that Abf2 regulates the access of other proteins to mtDNA. To test this, we examined a mitochondrial RNA polymerase, Rpo41, but found that it is predominantly associated with nucleoids, both in glucose- and glycerol-containing medium (M.K., unpublished data). Although the access of Rpo41 to mtDNA does not seem to be regulated by Abf2 there might be other DNA-binding factors that are affected by the interaction of Abf2 with mtDNA.

Although Abf2 is the major mtDNA-binding protein, other nucleoid proteins are also likely to contribute to the overall nucleoid structure and DNA protection under various growth conditions. Indeed, we show that the distribution of some nucleoid proteins is connected to metabolic cues; Hsp60 and Ilv5 are recruited to nucleoids during glucose repression and amino-acid starvation, respectively (Fig. 4). It is known that Ilv5 is required to parse DNA into greater number of nucleoids in medium lacking amino acids (MacAlpine et al., 2000), however, the role for Hsp60 during glucose repression is less clear. Hsp60 has been proposed to function in nucleoid division (Kaufman et al., 2003). It is conceivable that the recruitment of Hsp60 to nucleoids in glucose-grown cells reflects an increased requirement for this protein in order to ensure the reliable propagation of mitochondrial nucleoids under conditions in which loss of mtDNA is not lethal.

Mutants of Ilv5 that exhibit an mtDNA-instability phenotype have been previously studied (Bateman et al., 2002a). Some of these mutant Ilv5 proteins cannot perform their function in nucleoids because they form protein aggregates in the mitochondrial matrix. We thought that Hsp60 A144V mutant protein (Kaufman et al., 2003), which causes mtDNA instability, might be inadequately localized in the mitochondrial nucleoids as well. Surprisingly, we observed that Hsp60 A144V mutant proteins are associated with mtDNA in a great surplus (Fig. 5). Thus, the mtDNA-instability phenotype of the Hsp60 A144V mutant might be caused by this excessive presence of Hsp60 in the mitochondrial nucleoids. These two examples of an altered localization of nucleoid proteins suggest that correct recruitment of nucleoid factors and remodeling of mitochondrial nucleoprotein is essential for the maintenance of mtDNA under various metabolic conditions.

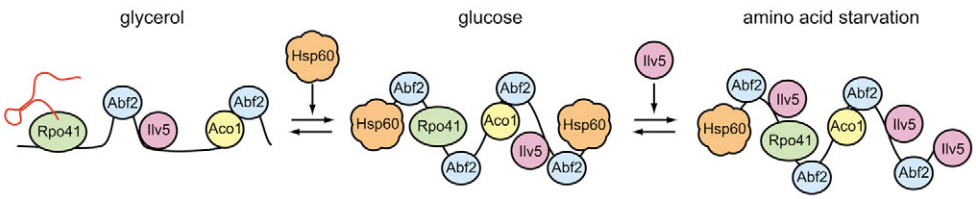


**Fig. 6.** A model of mitochondrial nucleoid remodeling. In repressed conditions (growth in glucose medium), mitochondrial nucleoids form a more compact conformation with an increased ratio of Abf2 to mtDNA. Hsp60 is recruited to nucleoids. In conditions that activate respiration (growth in glycerol medium), an increased mtDNA copy number causes a decrease in the ratio of Abf2 to mtDNA. Nucleoids form a more open structure. In cells starved of amino acids, Ilv5 is recruited to nucleoids.

## Materials and Methods

### Mitochondrial ChIP-chip assay

ChIP-chip assays were performed using the strain (MCC109) whose mtDNA lacks all introns. The *ABF2* gene was modified using homologous recombination, with a PCR-amplified 13Myc-kanMX6 cassette, so that the Abf2-13Myc fusion protein could be expressed from the *ABF2* genomic locus (Longtine et al., 1998). A wild-type untagged strain was used as a negative control throughout the ChIP-chip experiments. Mitochondria were isolated from cells grown in 2L YPG medium (1% yeast extract, 2% peptone, 3% glycerol) and purified on Histodenz™ (Sigma) gradients (Diekert et al., 2001). Approximately 2 mg of mitochondria were crosslinked with 1% formaldehyde for 2 hours at 4°C in 2 ml of 0.5M sucrose, 20 mM HEPES pH 7.4, 2 mM EDTA, 7 mM β-mercaptoethanol. After crosslinking, the mitochondria were lysed for 5 minutes at 4°C in 0.5% Nonidet P40 in the presence of protease inhibitor mix Complete™ (Roche) and 50 mM NaCl. The lysed mitochondria were sheared using ultrasound for 10×25 seconds with a Branson Sonifier 450 (output 20%, setting 3). An aliquot of the sheared DNA was purified and examined by agarose gel electrophoresis. The size of the resulting DNA fragments ranged from about 400 bp to 2 kbp. Prior to immunoprecipitation the samples were precleared with Protein G agarose (Roche). The extracts were incubated overnight at 4°C with ~2 μg of mouse antibodies against the Myc epitope (Roche). The immunocomplexes were precipitated with Protein G agarose (Roche) as specified by the manufacturer, except that 2 mM EDTA was present during all washing steps. The crosslinked DNA was released from the beads by incubating the samples at 65°C overnight in 1% SDS, 50 mM Tris-HCl pH 8.0, and 2 mM EDTA. The DNA samples were purified with Qiaquick® PCR-purification kit and amplified using a linear amplification method (Liu et al., 2003). Briefly, the DNA was dephosphorylated and dT-tailed with terminal transferase. T7 RNA polymerase promoters were incorporated into the extremities of DNA fragments and RNA was synthesized in vitro using the MEGAscript® T7 kit (Ambion). Approximately 0.5-1 μg of synthesized RNA was labeled using the AminoAllyl cDNA-labeling kit (Ambion). Cy5 mono NHS ester (Amersham) was used to label the cDNA sample from Abf2p-13Myc and the untagged strains. Cy3 mono NHS ester was conjugated with cDNA amplified identically from CsCl-purified mtDNA from the intronless MCC109 strain. The Cy5 and Cy3 labeled samples were mixed and used for hybridizations with microarrays. Custom microarrays were printed with PCR-amplified regions of intronless mtDNA by the University of Texas Southwestern Medical Center Microarray Core Facility (supplementary material Table S1). Since *S. cerevisiae* mtDNA contains many variable regions, some sequences were amplified from mtDNA of the FY1679 strain, which has been used to determine the complete mtDNA sequence (Foury et al., 1998). For ori5 and *VAR1* regions, HS40 that contained the entire ori5 region, and *VAR1* petite mtDNAs were used, respectively. In total, about 70% of the genome was covered with representatives of all features: ORFs, rRNAs, tRNA genes, promoters, oris, GC clusters, and AT rich spacer regions. Sequences of all oligonucleotides used for PCR reactions can be found in supplementary material Table S1. The ChIP-chip experiments were repeated three times with the Abf2-13Myc expressing strain and four times with the wild-type strain (supplementary material Fig. S3). The datasets were analyzed by a median-percentile ranking method as previously described (Buck and Lieb, 2004).

### Nuclease-sensitivity assay

Nuclease sensitivity of mitochondrial nucleoids in organello was assessed as previously described with modifications (Newman et al., 1996). Mitochondria were isolated from 3 l YPD medium (1% yeast extract, 2% peptone, 5% glucose) or 2 l YPG cultures of the strain BY4741 and were further purified on Histodenz™ (Sigma) gradients. Approximately 1 mg of mitochondria was treated with 1% toluene for 15 min at 22°C in 1 ml of 0.5 M sucrose, 20 mM Tris-HCl pH 7.4, 2 mM EDTA. The permeable mitochondria were collected by centrifugation and treated with 0.2 U/ml micrococcal nuclease (USB) at 30°C in 0.5 M sucrose, 20 mM Tris-HCl pH 7.4, 2 mM EDTA and 4 mM $CaCl_2$. Without the addition of micrococcal nuclease, mtDNA was stable for at least 60 min in the presence of 4 mM $CaCl_2$ (supplementary material Fig. S4A). The reactions were stopped with 10 mM EGTA pH 8.4. To isolate the DNA, mitochondria were treated for 2 hours at 65°C with 0.2 mg/ml proteinase K in the presence of 1% SDS. The DNA was extracted with phenol and precipitated with isopropanol in the presence of 5 μg of linear acrylamide (Ambion). The DNA was digested with *Hae*III, separated in agarose gels and transferred to nylon membranes. The blots were hybridized at 62°C in 6×SSC, 0.5% SDS and 5×Denhardt solution with various probes PCR-amplified from mtDNA (*21S_rRNA*/71, *ATP6*/33, product 81, supplementary material Table S1) labeled with [α-$^{32}$P]dATP (Perkin Elmer) using the Random Primed DNA Labeling Kit (Roche). Blots were washed twice for 30 minutes with 6×SSC, 0.5% SDS at 30°C and twice for 30 minutes with 0.1×SSC, 0.5% SDS at 62°C. The signal intensities were quantified using a phosphorImager and ImageQuant software (Molecular Dynamics).

### Submitochondrial fractionation

The mitochondria were isolated from 3-l YPD (5% glucose) and 2-l YPG cultures of the BY4741 strain grown at 30°C, 3-l YPD (5% glucose) and 2-l YPG cultures of W303-1B Δ*hsp60::HIS3* [pRS415-*HSP60*] and W303-1B Δ*hsp60::HIS3* [pRS415-*hsp60-A144V*] (Kaufman et al., 2003) grown at 26°C, and 3-l cultures of the MCC109 strain grown in SD (5% glucose) with or without the addition of 0.5 g/l isoleucine, leucine, and valine. The gradient-purified mitochondria (1 mg/ml) were lysed in 0.5% Nonidet P40 in 0.5 M sucrose, 20 mM Tris-HCl pH 7.4, 2 mM EDTA, 50 mM NaCl, 7 mM β-mercaptoethanol, and 1×Complete™ (Roche) for 5 minutes on ice. The same amount of lysed mitochondria (~1 mg of mitochondrial proteins) was loaded on top of a stepwise gradient composed of 4 ml of 20%, 2 ml of 60%, and 1 ml of 80% sucrose solution in 20 mM Tris-HCl pH 7.4, 2 mM EDTA, 50 mM NaCl, and 7 mM β-mercaptoethanol. After centrifugation at 110,000 ***g*** for 70 minutes at 4°C, ~0.5-ml fractions were collected from the bottom of the tubes. The fractions were analyzed for the presence of mtDNA by Southern dot blot hybridizations as follows: 50 μl from each fraction were treated overnight with 20 μg proteinase K (USB) and denatured in 0.1 M NaOH for 20 min at 37°C. The DNA was neutralized in 6×SSC and loaded onto nylon membranes using a multi-well manifold blotter (Bio-Rad). The membranes were hybridized at 62°C in 6×SSC, 0.5% SDS, 5×Denhardt solution with *ATP6*/33 PCR product, labeled with αP$^{32}$-dATP (Perkin Elmer) using the Random Primed DNA Labeling Kit (Roche). The dot blots were washed twice for 30 minutes with 6×SSC, 0.5% SDS at 30°C and twice for 30 minutes with 0.1×SSC, 0.5% SDS at 62°C. For protein analysis, 12-μl aliquots from each fraction were separated in 4%-20% gradient polyacrylamide gels (Bio-Rad), blotted to nitrocellulose membranes, and probed with rabbit polyclonal antibodies against Abf2, Ilv5, Hsp60, Aco1, and Mdh1 and with goat anti-rabbit secondary antibodies conjugated with horseradish peroxidase (Bio-Rad). The immunocomplexes were detected by chemiluminescence with ECL™ reagents (Amersham). To minimize chemiluminescence variations, membranes were always processed at the same time in one solution and exposed side by side on a single film. Also, a single nitrocellulose membrane, not parallel membranes, was used to detect all proteins. All gradients and western blots were repeated, yielding almost identical results.

### Mitochondrial DNA copy number

The BY4741 cells were grown in 100 ml of YPD and YPG medium. The total DNA was isolated by phenol/glass bead treatment. MtDNA copy number was measured by hybridizations of blots containing *Hae*III-digested total cellular DNA. The membranes were hybridized with *21S_rRNA* probe 71 and *ACT1* probe 117 (supplementary material Table S1). Hybridization, washing and detection of DNA blots was carried out as described elsewhere in Materials and Methods. Band intensities were quantified using a phosphorImager (Molecular Dynamics) and ImageQuant 5.0 software (Molecular Dynamics).

### Abf2 protein interactions

Pure mitochondria (~1 mg) from YPG-grown MCC109 and MCC109 Abf2-13Myc strains were suspended in 0.6 M sorbitol, 40 mM HEPES-KOH pH 7.4 to obtain a total volume of 1 ml. Mitochondria were incubated for 2 hours on ice with or without 1 mM DSP. To stop the crosslinking reaction, 2.5 M glycine was added to the final concentration of 100 mM. Mitochondria were isolated by centrifugation at 4°C, 20,000 ***g*** for 30 minutes, resuspended in 0.5 M sucrose, 50 mM Tris-HCl pH 7.4, 0.05 M NaCl, 0.5% Nonidet P40, 1×Complete™, and incubated on ice for 15 minutes. To digest the mtDNA, 100U of DNaseI and $MgCl_2$ to a concentration of 25 mM was added and the suspension was incubated at 30°C for 1 hour. The insoluble material was removed by centrifugation at 20,000 ***g*** for 10 minutes. About 15 μg of anti-Myc antibody was added and the samples were incubated on a rocking platform at 4°C for 2 hours, followed by incubation with a 100 μl of Protein G-Agarose (Roche) at 4°C for 16 hours. The agarose beads were washed twice in 50 mM Tris-HCl pH 7.4, 0.5 M NaCl, 1% Nonidet P40, 1×Complete™, twice in 50 mM Tris-HCl pH 7.4, 0.5 M NaCl, 0.1% Nonidet P40, and once in 50 mM Tris-HCl pH 7.4, 0.1% Nonidet P40. To reverse the crosslinks, the beads were suspended in ~160 μl of 60 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.25% Bromophenol Blue and incubated at 92°C for 15 minutes. The proteins separated by SDS-PAGE visualized using silver staining. Bands of interest were cut out and analyzed by the Protein Chemistry Technology Center of Southwestern Medical Center at Dallas. Briefly, the proteins were in-gel digested by modified porcine trypsin, the peptide extracts were injected into a nano-HPLC–MS-MS system, and mass spectrometric data were searched against the NCBI non-redundant protein sequence database.

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**Table S1**

| Spot | Name | Oligo sequence | Start | End | Length | Tm | Product (bp) | Printed on microarrays | Features |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F1 | AGATAGAAGCCAAAAGGTCAGG | 732 | 753 | 22 | 54.5 | | | |
| | R1 | GAAAGTCTCTGCTGAAAGTTAAATGG | 1522 | 1497 | 26 | 54.4 | 791 | yes | tRNA pro |
| 2 | F2 | CCATTTAACTTTCAGCAGAGACTTTC | 1497 | 1522 | 26 | 54.4 | | | |
| | R2 | TAAAGGTGTGAACCCCGAAAGG | 1906 | 1886 | 21 | 55.0 | 410 | yes | |
| 3 | F3 | CTTTCGGGGTTCACACCTTTA | 1886 | 1906 | 21 | 55.0 | | | |
| | R3 | GAAATAAAATGAGATGAGATGAATTGGGG | 2358 | 2330 | 29 | 54.7 | 473 | yes | |
| 4 | F4 | CCCCAATTCATCTCATCTCATTTTATTTC | 2330 | 2358 | 29 | 54.7 | | | |
| | R4 | TTAAATTAATACTCCTTCGGGGTCC | 2894 | 2870 | 25 | 54.4 | 565 | yes | |
| 5 | F5 | GGACCCCGAAGGAGTATTAATTTAA | 2870 | 2894 | 25 | 54.4 | | | |
| | R5 | CGTACCGATTCCCTACTTATTTATTATACAT | 3525 | 3495 | 31 | 54.5 | 656 | yes | |
| 6 | F6 | ATGTATAATAAATAAGTAGGGAATCGGTACG | 3495 | 3525 | 31 | 54.5 | | | |
| | R6 | GGACCCCCACAATAGAATAGAA | 4024 | 4003 | 22 | 53.9 | 530 | yes | ORF6 |
| 7 | F7 | TTCTATTCTATTGTGGGGGTCC | 4003 | 4024 | 22 | 53.9 | | | |
| | R7 | GGAACCCCGTAAGGAGAAATA | 4552 | 4532 | 21 | 53.9 | 550 | yes | ORF6, ORF7, ori1 |
| 8 | F8 | TATTTCTCCTTACGGGGTTCC | 4532 | 4552 | 21 | 53.9 | | | |
| | R8 | AACCTTTAAGACTATAACTTGCCATTAG | 4966 | 4939 | 28 | 53.3 | 435 | yes | |
| 9 | F9 | CTAATGGCAAGTTATAGTCTTAAAGGTT | 4939 | 4966 | 28 | 53.3 | | | |
| | R9 | CCCCCTTTTCGAAATTACAATTATAATTAGT | 5483 | 5453 | 31 | 54.3 | 545 | yes | |
| 10 | F10 | ACTAATTATAATTGTAATTTCGAAAAGGGGG | 5453 | 5483 | 31 | 54.3 | | | |
| | R10 | GAACCGAACCCCTTTTTAAGAAG | 6152 | 6130 | 23 | 54.1 | 700 | yes | |
| 11 | F11 | CTTCTTAAAAAGGGGTTCGGTTC | 6130 | 6152 | 23 | 54.1 | | | |
| | R11 | GTATGATTCGCATGTGTCATGT | 6619 | 6598 | 22 | 53.3 | 490 | yes | 15S rRNA |
| 12 | F12 | ACATGACACATGCGAATCATAC | 6598 | 6619 | 22 | 53.3 | | | |
| | R12 | CTGGCACAAATATTAGTCAGGAC | 7180 | 7158 | 23 | 53.5 | 583 | yes | 15S rRNA |
| 13 | F13 | GTCCTGACTAATATTTGTGCCAG | 7158 | 7180 | 23 | 53.5 | | | |
| | R13 | CGAACTAAAGACAACAATGTAACGC | 7664 | 7640 | 25 | 54.8 | 507 | yes | 15S rRNA |
| 14 | F14 | GCGTTACATTGTTGTCTTTAGTTCG | 7640 | 7664 | 25 | 54.8 | | | |
| | R14 | GTTCCCCTACGGTAACTGTATTTC | 8157 | 8134 | 24 | 55.0 | 518 | yes | 15S rRNA |
| 15 | F15 | GAAATACAGTTACCGTAGGGGAAC | 8134 | 8157 | 24 | 55.0 | | | |
| | R15 | TGAATAATAGTCCGGCCGAAG | 8677 | 8657 | 21 | 54.4 | 544 | yes | 15S rRNA |
| 16 | F16 | CTTCGGCCGGACTATTATTCA | 8657 | 8677 | 21 | 54.4 | | | |
| | R16 | ATACATTCTCCTTTCGGGGTTC | 9080 | 9059 | 22 | 54.5 | 424 | yes | |
| 17 | F17 | GAACCCCGAAAGGAGAATGTAT | 9059 | 9080 | 22 | 54.5 | | | |
| | R17 | TCAAGGATAAAGAGATTCGAACTCC | 9445 | 9421 | 25 | 54.3 | 387 | yes | tRNA trp1 |
| 18 | F18 | GGAGTTCGAATCTCTTTATCCTTGA | 9421 | 9445 | 25 | 54.3 | | | |
| | R18 | GTCCTCAACGGAGGAAAAAGA | 9958 | 9938 | 21 | 54.7 | 538 | yes | tRNA trp1 |
| 19 | F19 | TCTTTTTCCTCCGTTGAGGAC | 9938 | 9958 | 21 | 54.7 | | | |
| | R19 | CGAGTCTTTACCTTTAATAGGTAGGAA | 10834 | 10808 | 27 | 53.9 | 897 | no | |
| 20 | F20 | TTCCTACCTATTAAAGGTAAAGACTCG | 10808 | 10834 | 27 | 53.9 | | | |
| | R20 | GTTTAATAATACTCCTTTCGGGGTTC | 11099 | 11074 | 26 | 53.7 | 292 | yes | |
| 21 | F21 | GAACCCCGAAAGGAGTATTATTAAAC | 11074 | 11099 | 26 | 53.7 | | | |
| | R21 | CATAACCTCATATAGAGTGAATACTTTATATATCA | 11797 | 11763 | 35 | 53.0 | 724 | yes | ORF8 |
| 22 | F22 | TGATATATAAAGTATTCACTCTATATGAGGTTATG | 11763 | 11797 | 35 | 53.0 | | | |
| | R22 | CCCCGGAAGGAGAAATATAAAAG | 12280 | 12258 | 23 | 53.1 | 518 | yes | ORF8 |
| 23 | F23 | CTTTTATATTTCTCCTTCCGGGG | 12258 | 12280 | 23 | 53.1 | | | |
| | R23 | GTATTAAATATATAAGTCCCGGTTTCTTACG | 12832 | 12802 | 31 | 53.8 | 575 | yes | ori8 |
| 24 | F24 | CGTAAGAAACCGGGACTTATATATTTAATAC | 12802 | 12832 | 31 | 53.8 | | | |
| | R24 | CTCATTACAAAATACCAACTAAACCGAAC | 13312 | 13284 | 29 | 54.8 | 511 | yes | |
| 25 | F25 | GTTCGGTTTAGTTGGTATTTTGTAATGAG | 13284 | 13312 | 29 | 54.8 | | | |
| | R25 | CTGCAATATCTTTTGCATTTGTTGAAT | 13863 | 13837 | 27 | 53.7 | 580 | no | cox1 |
| 26 | F26 | ATTCAACAAATGCAAAAGATATTGCAG | 13837 | 13863 | 27 | 53.7 | | | |
| | R26 | TGCATTGTCATACCATTTGTTCTC | 14356 | 14333 | 24 | 53.9 | 520 | yes | cox1 |
| 27 | F27 | GAGAACAAATGGTATGACAATGCA | 14333 | 14356 | 24 | 53.9 | | | |
| | R27 | AATTGCATATAACATAGGTAGTGCTAATC | 14843 | 14815 | 29 | 53.5 | 511 | yes | cox1 |
| 28 | F28 | GATTAGCACTACCTATGTTATATGCAATT | 14815 | 14843 | 29 | 53.5 | | | |
| | R28 | GATAAGATTAGATTCTACAAAATCAGGTGC | 15326 | 15297 | 30 | 54.2 | 512 | yes | cox1 |
| 29 | F29 | GCACCTGATTTTGTAGAATCTAATCTTATC | 15297 | 15326 | 30 | 54.2 | | | |
| | R29 | CTCCTTTCGGGGTTCACTATAA | 16056 | 16035 | 22 | 54.0 | 760 | yes | cox1 |
| 30 | F30 | TTATAGTGAACCCCGAAAGGAG | 16035 | 16056 | 22 | 54.0 | | | |
| | R30 | ATCTTAAGATCATAGGTAAAAAGAATTGTGAG | 16498 | 16467 | 32 | 53.9 | 464 | yes | atp8 |
| 31 | F31 | CTCACAATTCTTTTTACCTATGATCTTAAGAT | 16467 | 16498 | 32 | 53.9 | | | |
| | R31 | TTATTACTCCTCCTTTGGGGTC | 16916 | 16895 | 22 | 53.8 | 450 | yes | atp8 |
| 32 | F32 | GACCCCAAAGGAGGAGTAATAA | 16895 | 16916 | 22 | 53.8 | | | |
| | R32 | CTTCTTGTGAAATTAATCATCTTGAACC | 17427 | 17400 | 28 | 53.2 | 533 | yes | atp6 |
| 33 | F33 | GGTTCAAGATGATTAATTTCACAAGAAG | 17400 | 17427 | 28 | 53.2 | | | |
| | R33 | CATATCCCTGAATGATACCAATAGC | 17934 | 17910 | 25 | 53.2 | 535 | yes | atp6 |
| 34 | F34 | GCTATTGGTATCATTCAGGGATATG | 17910 | 17934 | 25 | 53.2 | | | |
| | R34 | GGCCCCGGAACTATATGATATAA | 18573 | 18551 | 23 | 53.7 | 664 | yes | atp6 |
| 35 | F35 | TTATATCATATAGTTCCGGGGCC | 18551 | 18573 | 23 | 53.7 | | | |
| | R35 | ACGGGTAATGTTTACCCGTATTAC | 19669 | 19646 | 24 | 54.6 | 1119 | no | ori7, ORF5 |
| 36 | F36 | GTAATACGGGTAAACATTACCCGT | 19646 | 19669 | 24 | 54.6 | | | |
| | R36 | AAATTCCACCTTCAGCGTAGT | 20027 | 20007 | 21 | 54.3 | 382 | no | ORF5 |
| 37 | F37 | ACTACGCTGAAGGTGGAATTT | 20007 | 20027 | 21 | 54.3 | | | |
| | R37 | ACACTGGTGAATAACACAATAATGTTC | 20550 | 20524 | 27 | 54.1 | 544 | yes | |
| 38 | F38 | GAACATTATTGTGTTATTCACCAGTGT | 20524 | 20550 | 27 | 54.1 | | | |
| | R38 | GAAATTAATATCTCCTTACTCTTTCGGAG | 21394 | 21366 | 29 | 53.4 | 871 | yes | ori2 |
| 39 | F39 | CTCCGAAAGAGTAAGGAGATATTAATTTC | 21366 | 21394 | 29 | 53.4 | | | |
| | R39 | AATTAGTTCCGGAACCCAAAAAG | 21857 | 21835 | 23 | 53.7 | 492 | yes | |
| 40 | F40 | CTTTTTGGGTTCCGGAACTAATT | 21835 | 21857 | 23 | 53.7 | | | |
| | R40 | GTCCCTCACTCCTTTCTATAAAAAGT | 22501 | 22476 | 26 | 54.4 | 667 | yes | |
| 41 | F41 | ACTTTTTATAGAAAGGAGTGAGGGAC | 22476 | 22501 | 26 | 54.4 | | | |
| | R41 | TTTATACTCCTTCGGGGTTCG | 22976 | 22956 | 21 | 54.1 | 501 | no | |
| 42 | F42 | CGAACCCCGAAGGAGTATAAA | 22956 | 22976 | 21 | 54.1 | | | |
| | R42 | TTATCTTATTCCTCCTTTCGGGG | 23708 | 23686 | 23 | 54.0 | 753 | no | |
| 43 | F43 | CCCCGAAAGGAGGAATAAGATAA | 23686 | 23708 | 23 | 54.0 | | | |
| | R43 | CCTTAATCGGAATCGAACCGAT | 24162 | 24141 | 22 | 54.7 | 477 | yes | tRNA glu |
| 44 | F44 | ATCGGTTCGATTCCGATTAAGG | 24141 | 24162 | 22 | 54.7 | | | |
| | R44 | GTCATTGGTCCCGTGGATAATA | 24909 | 24888 | 22 | 54.3 | 769 | no | tRNA glu |
| 45 | F45 | TATTATCCACGGGACCAATGAC | 24888 | 24909 | 22 | 54.3 | | | |
| | R45 | TGCATAGCCATAAAAATACCTGTTAC | 25418 | 25393 | 26 | 53.6 | 531 | yes | COB |
| 46 | F46 | GTAACAGGTATTTTTATGGCTATGCA | 25393 | 25418 | 26 | 53.6 | | | |
| | R46 | TGGAATTCTATCTAAATTACCTGTAATACCT | 25920 | 25890 | 31 | 53.9 | 528 | yes | COB |
| 47 | F47 | AGGTATTACAGGTAATTTAGATAGAATTCCA | 25890 | 25920 | 31 | 53.9 | | | |
| | R47 | ACATTTTCAATAGTAGAGATAACAGGTACA | 26387 | 26358 | 30 | 54.1 | 498 | yes | COB |
| 48 | F48 | TGTACCTGTTATCTCTACTATTGAAAATGT | 26358 | 26387 | 30 | 54.1 | | | |
| | R48 | TAATTAATAAATCTCCTTGCGGGGT | 26803 | 26779 | 25 | 54.3 | 446 | yes | COB |
| 49 | F49 | ACCCCGCAAGGAGATTTATTAATTA | 26779 | 26803 | 25 | 54.3 | | | |
| | R49 | AATAAATAGGGTGAGTAGGACGC | 27307 | 27285 | 23 | 54.1 | 529 | yes | |
| 50 | F50 | GCGTCCTACTCACCCTATTTATT | 27285 | 27307 | 23 | 54.1 | | | |
| | R50 | CCGGTCGAAGGAGATAAGTAAT | 28209 | 28188 | 22 | 53.7 | 925 | yes | ori6 |
| 51 | F51 | ATTACTTATCTCCTTCGACCGG | 28188 | 28209 | 22 | 53.7 | | | |
| | R51 | TTCTCCTTTCGGGGAACTATATAAAT | 28798 | 28773 | 26 | 53.6 | 611 | yes | |
| 52 | F52 | ATTTATATAGTTCCCCGAAAGGAGAA | 28773 | 28798 | 26 | 53.6 | | | |
| | R52 | TTGTTGAGATACCTGCTCCAAT | 29542 | 29521 | 22 | 54.0 | 770 | no | ATP9 |
| 53 | F53 | ATTGGAGCAGGTATCTCAACAA | 29521 | 29542 | 22 | 54.0 | | | |
| | R53 | CCCGAAAGGAGATGTTCACTA | 30256 | 30236 | 21 | 54.0 | 736 | yes | ATP9 |
| 54 | F54 | TAGTGAACATCTCCTTTCGGG | 30236 | 30256 | 21 | 54.0 | 765 | no | tRNA ser |

**Table S1**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R54 | CACACTTTAAACCACTCAGTCAAC | 31000 | 30977 | 24 | 54.2 | | | |
| 55 | F55 | GTTGACTGAGTGGTTTAAAGTGTG | 30977 | 31000 | 24 | 54.2 | | | |
| | R55 | GCCCGCGGGGTTTATATTATTA | 31869 | 31848 | 22 | 54.9 | 893 | no | tRNA ser, VAR1 |
| 56 | F56 | TAATAATATAAACCCCGCGGGC | 31848 | 31869 | 22 | 54.9 | | | |
| | R56 | ACTAGTTCTACCATTATTATTACTTAATCTACCT | 32700 | 32667 | 34 | 53.8 | 853 | no | VAR1 |
| 57 | F57 | AGGTAGATTAAGTAATAATAATGGTAGAACTAGT | 32667 | 32700 | 34 | 53.8 | | | |
| | R57 | CCGAAGGAGTTTGGTTAAAGAAGA | 33498 | 33475 | 24 | 54.9 | 832 | no | VAR1 |
| 58 | F58 | TCTTCTTTAACCAAACTCCTTCGG | 33475 | 33498 | 24 | 54.9 | | | |
| | R58 | TCCGAACGAAATCCATAACCC | 34071 | 34051 | 21 | 54.9 | 597 | no | ORF9, ORF10 |
| 59 | F59 | GGGTTATGGATTTCGTTCGGA | 34051 | 34071 | 21 | 54.9 | | | |
| | R59 | AAATGATAAACAAGAAGCTATCTGGG | 35070 | 35045 | 26 | 53.4 | 1020 | no | ORF10 |
| 60 | F60 | CCCAGATAGCTTCTTGTTTATCATTT | 35045 | 35070 | 26 | 53.4 | | | |
| | R60 | TAGGTGTGAATCTCAAAAGGAGT | 35902 | 35880 | 23 | 53.6 | 858 | no | |
| 61 | F61 | ACTCCTTTTGAGATTCACACCTA | 35880 | 35902 | 23 | 53.6 | | | |
| | R61 | GGTCCCTCACTCCTTCTTAAT | 36502 | 36482 | 21 | 53.6 | 623 | yes | |
| 62 | F62 | ATTAAGAAGGAGTGAGGGACC | 36482 | 36502 | 21 | 53.6 | | | |
| | R62 | AAACATTACCAGTTGTTCACAGG | 37062 | 37040 | 23 | 53.6 | 581 | yes | |
| 63 | F63 | CCTGTGAACAACTGGTAATGTTT | 37040 | 37062 | 23 | 53.6 | | | |
| | R63 | GGACCAAAACCTCTAATGGAGT | 37765 | 37744 | 22 | 54.5 | 726 | yes | ori3 |
| 64 | F64 | ACTCCATTAGAGGTTTTGGTCC | 37744 | 37765 | 22 | 54.5 | | | |
| | R64 | GGTCCCTCACTCCTTCTTTAA | 38282 | 38262 | 21 | 53.8 | 539 | yes | |
| 65 | F65 | TTAAAGAAGGAGTGAGGGACC | 38262 | 38282 | 21 | 53.8 | | | |
| | R65 | TCATATTATATTGGGACCAAGTCGG | 38958 | 38934 | 25 | 54.3 | 697 | no | |
| 66 | F66 | CCGACTTGGTCCCAATATAATATGA | 38934 | 38958 | 25 | 54.3 | | | |
| | R66 | GAACCCCGAAAGGAGAAAGTATA | 39815 | 39793 | 23 | 54.1 | 882 | no | ori4 |
| 67 | F67 | TATACTTTCTCCTTTCGGGGTTC | 39793 | 39815 | 23 | 54.1 | | | |
| | R67 | TCGCCTCCAATGTGAACTATAT | 40292 | 40271 | 22 | 53.6 | 500 | yes | |
| 68 | F68 | ATATAGTTCACATTGGAGGCGA | 40271 | 40292 | 22 | 53.6 | | | |
| | R68 | GTTACTTAGATGTTTCAGTTCACTTAGT | 41028 | 41001 | 28 | 53.2 | 758 | yes | 21S rRNA |
| 69 | F69 | ACTAAGTGAACTGAAACATCTAAGTAAC | 41001 | 41028 | 28 | 53.2 | | | |
| | R69 | GGCTATACTTACATATATTTCATAGAAAACCA | 41508 | 41477 | 32 | 53.5 | 508 | yes | 21S rRNA |
| 70 | F70 | TGGTTTTCTATGAAATATATGTAAGTATAGCC | 41477 | 41508 | 32 | 53.5 | | | |
| | R70 | CGTTACTCATGTCAGCATTCTC | 42083 | 42062 | 22 | 53.5 | 607 | yes | 21S rRNA |
| 71 | F71 | GAGAATGCTGACATGAGTAACG | 42062 | 42083 | 22 | 53.5 | | | |
| | R71 | CCTCATAATAAGGCTGCTATTTACCTA | 42600 | 42574 | 27 | 54.2 | 539 | yes | 21S rRNA |
| 72 | F72 | TAGGTAAATAGCAGCCTTATTATGAGG | 42574 | 42600 | 27 | 54.2 | | | |
| | R72 | GGACTTATTCAGATACTTTTGCTGAT | 43091 | 43066 | 26 | 53.2 | 518 | yes | 21S rRNA |
| 73 | F73 | ATCAGCAAAAGTATCTGAATAAGTCC | 43066 | 43091 | 26 | 53.2 | | | |
| | R73 | AGAGGAAATGTTGAGTCGACA | 43569 | 43549 | 21 | 53.7 | 504 | no | 21S rRNA |
| 74 | F74 | TGTCGACTCAACATTTCCTCT | 43549 | 43569 | 21 | 53.7 | | | |
| | R74 | ATTTATAAAGGTGTGAACCAATCCC | 44238 | 44214 | 25 | 53.5 | 690 | no | 21S rRNA |
| 75 | F75 | GGGATTGGTTCACACCTTTATAAAT | 44214 | 44238 | 25 | 53.5 | | | |
| | R75 | GGGGGGTGAAGATATAAAGATTATAAAGT | 45031 | 45003 | 29 | 54.5 | 818 | yes | |
| 76 | F76 | ACTTTATAATCTTTATATCTTCACCCCCC | 45003 | 45031 | 29 | 54.5 | | | |
| | R76 | CCAAACCTTTCGATTACAAAACGA | 45540 | 45517 | 24 | 54.1 | 538 | yes | tRNA thr2 |
| 77 | F77 | TCGTTTTGTAATCGAAAGGTTTGG | 45517 | 45540 | 24 | 54.1 | | | |
| | R77 | AAGAGATGAAGAGAATCGAACTCT | 46115 | 46092 | 24 | 53.3 | 599 | yes | tRNA thr2, tRNA cys |
| 78 | F78 | AGAGTTCGATTCTCTTCATCTCTT | 46092 | 46115 | 24 | 53.3 | | | |
| | R78 | GGAGTCCGATCGAAAGAGATAA | 46684 | 46663 | 22 | 53.7 | 593 | yes | tRNA cys, tRNA his |
| 79 | F79 | TTATCTCTTTCGATCGGACTCC | 46663 | 46684 | 22 | 53.7 | | | |
| | R79 | TTTAAGTCGAACTCCTTCGGG | 47370 | 47350 | 21 | 54.4 | 708 | no | |
| 80 | F80 | CCCGAAGGAGTTCGACTTAAA | 47350 | 47370 | 21 | 54.4 | | | |
| | R80 | TCGAACAAACAAACTCCAAACTC | 47895 | 47873 | 23 | 53.9 | 546 | yes | tRNA leu, tRNA gln |
| 81 | F81 | GAGTTTGGAGTTTGTTTGTTCGA | 47873 | 47895 | 23 | 53.9 | | | |
| | R81 | AGTCCCCAAAGGAGTATTAACA | 48329 | 48308 | 22 | 53.3 | 457 | yes | tRNA gln |
| 82 | F82 | TGTTAATACTCCTTTGGGGACT | 48308 | 48329 | 22 | 53.3 | | | |
| | R82 | CCAAGCATGGGTTGCTTAAAA | 48740 | 48720 | 21 | 54.2 | 433 | no | tRNA lys |
| 83 | F83 | TTTTAAGCAACCCATGCTTGG | 48720 | 48740 | 21 | 54.2 | | | |
| | R83 | ATAGATAGCGAGAATCGAACTCG | 49166 | 49144 | 23 | 53.8 | 447 | no | tRNA lys, tRNA arg1, tRNA gly |
| 84 | F84 | CGAGTTCGATTCTCGCTATCTAT | 49144 | 49166 | 23 | 53.8 | | | |
| | R84 | TTTGCACTGACATCCTCCATT | 50007 | 49987 | 21 | 54.6 | 864 | no | tRNA gly, tRNA asp |
| 85 | F85 | AATGGAGGATGTCAGTGCAAA | 49987 | 50007 | 21 | 54.6 | | | |
| | R85 | ACTTAGCAAATAATCCACTTTACCTATAGT | 50869 | 50840 | 30 | 54.2 | 883 | yes | tRNA asp, tRNA ser2 |
| 86 | F86 | ACTATAGGTAAAGTGGATTATTTGCTAAGT | 50840 | 50869 | 30 | 54.2 | | | |
| | R86 | GTCGTTCTACCAAATTTAACTATAACCC | 51502 | 51475 | 28 | 54.0 | 663 | no | tRNA ser2, tRNA arg2, tRNA ala |
| 87 | F87 | GGGTTATAGTTAAATTTGGTAGAACGAC | 51475 | 51502 | 28 | 54.0 | | | |
| | R87 | TGAAACTAACAGGGATTGAACCT | 51862 | 51840 | 23 | 54.1 | 388 | yes | tRNA ala, tRNA ile |
| 88 | F88 | AGGTTCAATCCCTGTTAGTTTCA | 51840 | 51862 | 23 | 54.1 | | | |
| | R88 | CAACTACCAACATTGAAAATCCCTC | 52477 | 52453 | 25 | 54.4 | 638 | yes | tRNA ile, tRNA tyr |
| 89 | F89 | GAGGGATTTTCAATGTTGGTAGTTG | 52453 | 52477 | 25 | 54.4 | | | |
| | R89 | ATGCTTTAACCGATAAGCTATAAGGA | 53087 | 53062 | 26 | 54.0 | 635 | yes | tRNA tyr, tRNA asn |
| 90 | F90 | TCCTTATAGCTTATCGGTTAAAGCAT | 53062 | 53087 | 26 | 54.0 | | | |
| | R90 | AGAATACTTTTTATTCTCCTTCCGGA | 53654 | 53629 | 26 | 53.9 | 593 | yes | tRNA asn |
| 91 | F91 | TCCGGAAGGAGAATAAAAAGTATTCT | 53629 | 53654 | 26 | 53.9 | | | |
| | R91 | ACTTGTAGAAGGAATTGAACCTTACA | 54332 | 54307 | 26 | 54.2 | 704 | yes | tRNA met |
| 92 | F92 | TGTAAGGTTCAATTCCTTCTACAAGT | 54307 | 54332 | 26 | 54.2 | | | |
| | R92 | ATTTATCTCCTTCGGGGTTCC | 54685 | 54665 | 21 | 54.2 | 379 | yes | tRNA met |
| 93 | F93 | GGAACCCCGAAGGAGATAAAT | 54665 | 54685 | 21 | 54.2 | | | |
| | R93 | TACTAAAACTCCTTCGGTCCTC | 55146 | 55125 | 22 | 53.6 | 482 | yes | |
| 94 | F94 | GAGGACCGAAGGAGTTTTAGTA | 55125 | 55146 | 22 | 53.6 | | | |
| | R94 | TCAAATAACTTCAATAGTTTGTCCATGT | 55640 | 55613 | 28 | 53.6 | 516 | no | cox2 |
| 95 | F95 | ACATGGACAAACTATTGAAGTTATTTGA | 55613 | 55640 | 28 | 53.6 | | | |
| | R95 | AGGTAATGATACTGCTTCGATCTTA | 56111 | 56087 | 25 | 53.4 | 499 | yes | cox2 |
| 96 | F96 | TAAGATCGAAGCAGTATCATTACCT | 56087 | 56111 | 25 | 53.4 | | | |
| | R96 | TGTTTTAAGTAGTTCTTCAAAATTAGAAGGT | 56554 | 56524 | 31 | 53.8 | 468 | no | cox2 |
| 97 | F97 | ACCTTCTAATTTTGAAGAACTACTTAAAACA | 56524 | 56554 | 31 | 53.8 | | | |
| | R97 | TCATAATTGGAAATCAATTTCTTTACGACT | 57130 | 57101 | 30 | 54.0 | 607 | yes | |
| 98 | F98 | AGTCGTAAAGAAATTGATTTCCAATTATGA | 57101 | 57130 | 30 | 54.0 | | | |
| | R98 | AGTTATGGCTATAGCCATGAAGAA | 57766 | 57743 | 24 | 53.7 | 666 | no | |
| 99 | F99 | TTCTTCATGGCTATAGCCATAACT | 57743 | 57766 | 24 | 53.7 | | | |
| | R99 | CCAAAGAGGAGTGAATACCTATTAC | 58420 | 58395 | 26 | 54.1 | 678 | no | |
| 100 | F100 | TGTAATAGGTATTCACTCCTCTTTGG | 58395 | 58420 | 26 | 54.1 | | | |
| | R100 | CAATTTATCGCTTTACCACTAAGCT | 59091 | 59067 | 25 | 53.6 | 697 | no | tRNA phe |
| 101 | F101 | AGCTTAGTGGTAAAGCGATAAATTG | 59067 | 59091 | 25 | 53.6 | | | |
| | R101 | AGGTGCATATTATCTAAGTTCAAATCTTAG | 59756 | 59727 | 30 | 53.4 | 690 | no | tRNA phe, tRNA thr1 |
| 102 | F102 | CTAAGATTTGAACTTAGATAATATGCACCT | 59727 | 59756 | 30 | 53.4 | | | |
| | R102 | TTTAGGAAATATAGGGTTCGAACCTAT | 60235 | 60209 | 27 | 53.6 | 509 | no | tRNA thr1, tRNA val1 |
| 103 | F103 | ATAGGTTCGAACCCTATATTTCCTAAA | 60209 | 60235 | 27 | 53.6 | | | |
| | R103 | GATGTTGTTGATGTCTACTTCTTTCT | 60877 | 60852 | 26 | 53.2 | 669 | yes | tRNA val1, cox3 |
| 104 | F104 | AGAAAGAAGTAGACATCAACAACATC | 60852 | 60877 | 26 | 53.2 | | | |
| | R104 | CACCATCAGAGATAGTGAATGCA | 61438 | 61416 | 23 | 54.6 | 587 | yes | cox3 |
| 105 | F105 | TGCATTCACTATCTCTGATGGTG | 61416 | 61438 | 23 | 54.6 | | | |
| | R105 | CGGTGACCTCGAAGGAATATA | 61982 | 61962 | 21 | 53.6 | 567 | yes | cox3 |
| 106 | F106 | TATATTCCTTCGAGGTCACCG | 61962 | 61982 | 21 | 53.6 | | | |
| | R106 | GATAATTTATTTAATGAATACTTCTTTTGGGACT | 62652 | 62619 | 34 | 53.4 | 691 | no | |
| 107 | F107 | AGTCCCAAAAGAAGTATTCATTAAATAAATTATC | 62619 | 62652 | 34 | 53.4 | | | |
| | R107 | AGAAGATATATAATTATTTATTCTCCTTTCGGG | 63095 | 63063 | 33 | 53.4 | 477 | no | |
| 108 | F108 | CCCGAAAGGAGAATAAATAATTATATATCTTCT | 63063 | 63095 | 33 | 53.4 | 584 | yes | |

**Table S1**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R108 | GGTTCTAATAGATCTTCATTATATACCTTTATTAG | 63646 | 63612 | 35 | 52.3 | | | |
| 109 | F109 | CTAATAAAGGTATATAATGAAGATCTATTAGAACC | 63612 | 63646 | 35 | 52.3 | | | |
| | R109 | AAGAATAAAAAGGGATGCGGTTC | 64523 | 64501 | 23 | 53.8 | 912 | no | ori5 |
| 110 | F110 | GAACCGCATCCCTTTTTATTCTT | 64501 | 64523 | 23 | 53.8 | | | |
| | R110 | CTTTAAATAAAAAAAGGGGTTCGGTTC | 65042 | 65016 | 27 | 53.5 | 542 | yes | |
| 111 | F111 | GAACCGAACCCCTTTTTTTATTTAAAG | 65016 | 65042 | 27 | 53.5 | | | |
| | R111 | CTGAAAGCGGGGACTTATTATCT | 65540 | 65518 | 23 | 54.4 | 525 | yes | |
| 112 | F112 | AGATAATAAGTCCCCGCTTTCAG | 65518 | 65540 | 23 | 54.4 | | | |
| | R112 | TATCCTTTGGGGTTTTCCCTT | 66000 | 65980 | 21 | 54.0 | 483 | yes | |
| 113 | F113 | AAGGGAAAACCCCAAAGGATA | 65980 | 66000 | 21 | 54.0 | | | |
| | R113 | AGGAGTCCATCCTAAAGGAGA | 66380 | 66360 | 21 | 54.3 | 401 | yes | |
| 114 | F114 | TCTCCTTTAGGATGGACTCCT | 66360 | 66380 | 21 | 54.3 | | | |
| | R114 | AGCAATAATACGATTTGAACGTATATAATTAGG | 66734 | 66702 | 33 | 54.1 | 375 | no | tRNA f-met |
| 115 | F115 | CCTAATTATATACGTTCAAATCGTATTATTGCT | 66702 | 66734 | 33 | 54.1 | | | |
| | R115 | GGACTCCTTCTTAAAAAGGGGT | 67309 | 67288 | 22 | 54.5 | 608 | yes | tRNA f-met, 9S RNA |
| 116 | F116 | ACCCCTTTTTAAGAAGGAGTCC | 67288 | 67309 | 22 | 54.5 | | | |
| | R116 | CCTGACCTTTTGGCTTCTATCT | 753 | 732 | 22 | 54.5 | 873 | yes | 9S RNA, tRNA pro |
| 117 | F117B | CATTTCTTGTTCGAAGTCCAAGGCGAC | | | 26 | 60.5 | | | |
| | R117B | GGATGGATCCACTTGAGAGAGGATCAAG | | | 27 | 60.0 | 562 | yes | control ACT1 ORF internal |
| 118 | F118B | GCTTATGCGGCAAGGCAGTTTACTTG | | | 28 | 59.8 | | | |
| | R118B | CCTTCCTGCTTTCCAATGGTAACCGC | | | 26 | 60.7 | 463 | yes | control ChrIV non-coding |
| 119 | F119B | GGAAATATTCATGGCCGATAACGCGTTC | | | 26 | 62.0 | | | |
| | R119B | CTTTAACTCCAAATATGTGCTGGC | | | 28 | 59.9 | 444 | yes | control E coli pepN |
| 120 | F120B | CGTTTCCTGGAAGCGGAAGATTTTGATG | | | 28 | 60.0 | | | |
| | R120B | GATCCAGCTCTTCACCTTCAGCAGTCG | | | 27 | 62.4 | 550 | yes | control E coli deaD |