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Genome-wide Profiling of Transcription Factor-DNA Binding Interactions in *Candida albicans*: A Comprehensive CUT&RUN Method and Data Analysis Workflow

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Abstract

Regulatory transcription factors control many important biological processes, including cellular differentiation, responses to environmental perturbations and stresses, and host-pathogen interactions. Determining the genome-wide binding of regulatory transcription factors to DNA is essential to understanding the function of transcription factors in these often complex biological processes. Cleavage under targets and release using nuclease (CUT&RUN) is a modern method for genome-wide mapping of *in vivo* protein-DNA binding interactions that is an attractive alternative to the traditional and widely used chromatin immunoprecipitation followed by sequencing (ChIP-seq) method. CUT&RUN is amenable to a higher-throughput experimental setup and has a substantially higher dynamic range with lower per-sample sequencing costs than ChIP-seq. Here, a comprehensive CUT&RUN protocol and accompanying data analysis workflow tailored for genome-wide analysis of transcription factor-DNA binding interactions in the human fungal pathogen *Candida albicans* are described. This detailed protocol includes all necessary experimental procedures, from epitope tagging of transcription factor-coding genes to library preparation for sequencing; additionally, it includes a customized computational workflow for CUT&RUN data analysis.

Introduction

Candida albicans is a clinically relevant, polymorphic human fungal pathogen that exists in a variety of different modes of growth, such as the planktonic (free-floating) mode of growth and as communities of tightly adhered cells protected by an extracellular matrix, known as the biofilm mode of growth[1, 2, 3]. Similar to other developmental and cellular

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Disclosures

Clarissa J. Nobile is a cofounder of BioSynesis, Inc., a company developing diagnostics and therapeutics for biofilm infections.

processes, biofilm development is an important *C. albicans* virulence trait that is known to be controlled at the transcriptional level by regulatory transcription factors (TFs) that bind to DNA in a sequence-specific manner[4]. Recently, chromatin regulators and histone modifiers have also emerged as important regulators of *C. albicans* biofilm formation[5] and morphogenesis[6] by mediating DNA accessibility. To understand the complex biology of this important fungal pathogen, effective methods to determine the genome-wide localization of specific TFs during distinct developmental and cellular processes is valuable.

Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a widely used method to investigate protein-DNA interactions in *C. albicans*[5, 6] and has largely replaced the more classical chromatin immunoprecipitation followed by microarray (ChIP-chip)[9] method. Both ChIP-seq and ChIP-chip methods, however, require a large number of input cells[10], which can be a complicating factor when investigating TFs in the context of specific samples and modes of growth, such as biofilms collected from patients or animal models of infection. In addition, the chromatin immunoprecipitation (ChIP) assay often yields a significant amount of background signal throughout the genome, requiring a high level of enrichment for the target of interest to sufficiently separate signal from noise. While the ChIP-chip assay is largely outdated today, the sequencing depths necessary for ChIP-seq make this assay prohibitively expensive for many researchers, particularly those studying multiple TFs and/or chromatin-associated proteins.

Cleavage under targets and release using nuclease (CUT&RUN) is an attractive alternative to ChIP-seq. It was developed by the Henikoff lab in 2017 to circumvent the limitations of ChIP-seq and chromatin endogenous cleavage followed by sequencing ChEC-seq[11, 12], another method to identify protein-DNA interactions on a genome-wide level, while providing high-resolution, genome-wide mapping of TFs and chromatin-associated proteins[13]. CUT&RUN relies on the targeted digestion of chromatin within permeabilized nuclei using tethered micrococcal nucleases, followed by sequencing of the digested DNA fragments[9, 10]. As DNA fragments are specifically generated at the loci that are bound by a protein of interest, rather than being generated throughout the genome via random fragmentation as in ChIP assays, the CUT&RUN approach results in greatly reduced background signals and, thus, requires $1/10^{th}$ of the sequencing depth compared to ChIP-seq[11, 13, 14]. These improvements ultimately lead to significant reductions in sequencing costs and reductions in the total number of input cells needed as starting material for each sample.

Here, a robust CUT&RUN protocol is described that has been adapted and optimized for determining the genome-wide localization of TFs in *C. albicans* cells isolated from biofilms and planktonic cultures. A thorough data analysis pipeline is also presented, which enables the processing and analysis of the resulting sequence data and requires users to have minimal expertise in coding or bioinformatics. Briefly, this protocol describes epitope tagging of TF-coding genes, harvesting of biofilm and planktonic cells, isolation of intact permeabilized nuclei, incubation with primary antibodies against the specific protein or epitope-tagged protein of interest, tethering of the chimeric A/G-micrococcal nuclease (pAG-MNase) fusion proteins to the primary antibodies, genomic DNA recovery after chromatin digestion, and preparation of genomic DNA libraries for sequencing.

The experimental CUT&RUN protocol is followed by a purpose-built data analysis pipeline, which takes raw DNA sequencing reads in FASTQ format and implements all required processing steps to provide a complete list of significantly enriched loci bound by the TF of interest (targeted by the primary antibody). Note that multiple steps of the described library preparation protocol have been specifically adapted and optimized for CUT&RUN analysis of TFs (as opposed to nucleosomes). While the data presented in this manuscript were generated using TF-specific adaptations of a commercial CUT&RUN kit, these protocols have also been validated using individually sourced components (i.e., pAG-MNase enzyme and magnetic DNA purification beads) and in-house-prepared buffers, which can significantly reduce experimental cost. The comprehensive experimental and data analysis protocols are described in detail below in a step-by-step format. All reagents and critical equipment, as well as buffer and media recipes, are listed in the Table of Materials and Supplementary File 1, respectively.

Protocol

1. Epitope Tagging of *C. albicans* strains

1. Upload the gene of interest, along with its 1 kb upstream and downstream flanking sequences, from the *Candida* Genome Database to the primer design tool (see the Table of Materials). Design a guide RNA (gRNA) by highlighting 50 bp upstream and downstream from the stop codon, and click the **gRNA selection** tool on the right. Select **Design and Analyze Guides**. Use the Ca22 (*Candida albicans* SC5314 Assembly 22 (diploid)) genome and an NGG (SpCas9, 3' side) protospacer adjacent motif (PAM) for the guide parameters, and click **Finish**. Figure 1 describes the workflow for epitope tagging a *C. albicans* gene of interest with enhanced green fluorescent protein (eGFP).

    1. On the subsequent page, confirm the target region for gRNA design and press the green button. Sort gRNAs by the **On-Target Score**.

       NOTE: The primer design tool computes on-target and off-target scores to quantify the specificity of the gRNAs. An ideal guide has an on-target score of >60, an off-target score of ~33, and overlaps the stop codon. This enables high gRNA specificity while ablating gRNA targeting after GFP integration. A gRNA with an off-target score of ~50 indicates allelic variation; thus, only one allele will be recognized by the gRNA.

    2. Add the sequences (5'-CGTAAACTATTTTTAATTTG-3') and (5'-GTTTTAGAGCTAGAAATAGC-3') to the 5' and 3' ends, respectively, of the 20 bp gRNA target sequence, creating a 60 bp primer/oligonucleotide. Alternatively, copy the 20 bp sequence to the gRNA calculator supplied by Nguyen et al.[15]. Order the 60 bp custom gRNA oligonucleotide.

    3. Amplify the "universal A fragment" with 100 mM AHO1096 (5'-GACGGCACGGCCACGCGTTTAAACCGCC-3') and 100 mM

AHO1098 (5'-CAAATTAAAAATAGTTTACGCAAG-3') and the "unique B fragment" with the custom 60 bp gRNA oligonucleotide (100 mM)from step 1.1.2. and 100 mM AHO1097 (5'-CCCGCCAGGCGCTGGGGTTTAAACACCG-3') using pADH110 (plasmid repository ID# 90982) and pADH139 (plasmid repository ID# 90987), respectively, as template DNA. Use the PCR reaction and cycling conditions provided in Table 1.

NOTE: pADH139 is specific to strains that carry the heterologous *Candida maltosa LEU2* marker. If using a strain with a single copy of the *C. albicans LEU2* gene, substitute pADH119 (plasmid repository ID# 90985) in place of pADH139.

4. Confirm successful amplification by checking 5 µL of the PCR on a 1% agarose gel. Look for ~1 kb A and B fragment amplicons.

5. Mix 1 µL each of A and B fragments and stitch them together using the PCR reaction and cycling conditions provided in Table 2 to create a full-length C fragment.

6. Add 0.5 µL of 100 mM AHO1237 (5'-AGGTGATGCTGAAGCTATTGAAG-3') and 0.5 µL of 100 mM AHO1453 (5'-ATTTTAGTAACAGCTTCGACAATCG-3') to each PCR reaction, mix well by pipetting, and complete the cycling conditions listed in Table 3.

   NOTE: If using pADH119 in place of pADH139, substitute AHO1238 (5'-TGTATTTTGTTTTAAAATTTTAGTGACTGTTTC-3') in place of AHO1453.

7. Confirm proper stitching and amplification of the C fragment by checking 5 µL of the PCR on a 1% agarose gel. Look for a ~2 kb amplicon. Store the C fragment at −20 °C until ready for use.

   NOTE: If stitching and amplification results in multiple, nonspecific bands or smearing, perform a PCR cleanup of the A and B fragments and repeat from step 1.1.5.

2. Add the entire CTG-optimized monomeric eGFP with linker sequence (RIPLING)[16] (pCE1, plasmid repository ID# 174434) immediately upstream of the stop codon of the gene of interest using the primer design tool, creating a C-terminal translational fusion. Use this construct to design oligonucleotides for amplifying the donor DNA (dDNA) from pCE1.

   1. Design a forward oligonucleotide with 18–22 bp homology to the linker sequence and >50 bp homology to the 3' end of the open reading frame (ORF).

      NOTE: The 18–22 bp homology creates an annealing temperature for amplification between 55 °C and 58 °C. If the full-length

oligonucleotide forms primer dimers, adjust homology to the linker sequence/GFP or the genome accordingly.

2. Create a reverse oligonucleotide with 18–22 bp homology to the 3' end of GFP and >50 bp homology to the downstream noncoding sequence of the ORF to be tagged.

3. Order these oligonucleotides and amplify the dDNA using the provided touchdown PCR cycling conditions in Table 4.

3. Design two sets of colony PCR (cPCR) oligonucleotides for confirming the integration of GFP by amplifying across the flanking integration sites. First, select the forward dDNA oligonucleotide using the primer design tool and click the **Primer** button on the right.

1. Click **Create Primers | Wizard | Tm Param** and confirm that the algorithm is set to **SantaLucia 1998**. Click **Use Selection** to input the coordinates of the forward oligonucleotide designed in 1.2.1 as the target sequence.

2. Set the optimal primer temperature to 55 °C and the maximum amplicon size to 900 bp, and click the **Generate Primers** button at the top right.

3. Select the oligonucleotide pair with the lowest penalty score and confirm that the primers amplify across the 5' integration site. Ensure that the forward cPCR primer lies upstream of the forward dDNA primer sequence and the reverse cPCR primer fully within the eGFP tag or the linker sequence.

4. Repeat steps 1.3–1.3.3. with the reverse dDNA oligonucleotide to create the second set of cPCR oligonucleotides that amplify across the 3' integration site. Ensure that the forward cPCR primer lies entirely within the eGFP tag or the linker sequence and the reverse cPCR primer downstream of the reverse dDNA primer sequence.

5. Order these oligonucleotides.

4. Digest 2,500 ng of pADH140, which contains Cas9 (plasmid repository ID# 90988), with restriction enzyme for each gene to be GFP-tagged. Set the total volume of each digestion at 15 µL; adjust the volume of water accordingly based on the pADH140 plasmid concentration. Use the digestion conditions specified in Table 5. Store the digested plasmid at −20 °C until ready for use.

NOTE: If transforming a strain with a single copy of the *C. albicans LEU2* gene, instead of the heterologous *C. maltosa LEU2* marker, substitute pADH137 (plasmid repository ID# 90986) in place of pADH140.

5. Denature 12 µL of 10 mg/mL salmon sperm DNA for each gene that will be GFP-tagged at 99 °C for 10 min and rapidly cool to ≤4 °C. Store at −20 °C until ready for use.

6. Streak a *C. albicans LEU2* hemizygous nourseothricin-sensitive strain onto yeast peptone dextrose (YPD) plates and incubate at 30 °C for two days.

7. Select a single colony and transfer it to 4 mL of liquid YPD. Incubate for 12–16 h at 30 °C with shaking at 250 rpm.

8. Measure the optical density at 600 nm (OD_{600}) of the overnight (12–16 h) culture in a spectrophotometer using a disposable cuvette (1 mL, 1 cm path length).

9. Dilute the overnight culture into an Erlenmeyer flask to an OD_{600} of 0.1 in YPD. Account for 5 mL per reaction and include an additional 5 mL for checking the OD_{600} later.

   NOTE: The volume of the culture depends on the number of transformation reactions.

10. Incubate the diluted overnight culture in a shaking incubator at 30 °C with shaking at 250 rpm until it reaches an OD_{600} of 0.5–0.8.

11. Centrifuge at 4,000 × *g* at room temperature for 5 min; remove and discard the supernatant.

12. Resuspend the cell pellet in 1 mL of sterile water via gentle pipette mixing with filter tips and transfer to a sterile 1.5 mL microfuge tube.

13. Pellet the cells by centrifuging at 4,000 × *g* at room temperature for 1 min; remove and discard the supernatant. Resuspend in 1 mL of sterile water and repeat for a total of two washes.

14. Resuspend the pellet in $1/100^{th}$ of the volume used in step 1.10. For example, if 15 mL was used, resuspend the pellet in 150 µL of sterile water.

15. In a separate tube for each transformation reaction, mix 50 µL of C fragment, 50 µL of dDNA, 2,500 ng of restriction enzyme-digested pADH140, and 10 µL of denatured salmon sperm DNA.

16. Add 50 µL of the cell slurry from step 1.14 and mix by pipetting.

17. Make a stock of the plate mix (Supplementary File 1) for n + 1 transformations.

18. Add 1 mL of the plate mix to the cell/DNA mixture and mix by inverting 5 times.

    NOTE: Tap the bottoms of the tubes while inverting to dislodge any remaining liquid.

19. Place the mixture in an incubator at 30 °C overnight (12–16 h) without shaking.

20. Heat-shock the cells for 15 min at 44 °C in a water bath.

21. Centrifuge the 1.5 mL microfuge tubes at 5,000 × *g* at room temperature for 2 min.

22. Remove the PLATE mix by vacuum aspiration using sterile pipette tips, being careful to avoid disturbing the cell pellet.

23. Resuspend the cell pellet in 1 mL of YPD, pellet by centrifugation at 4,000 × *g* at room temperature for 1 min, and remove and discard the supernatant. Repeat for a second wash, resuspend the cell pellet in 1 mL of YPD, and transfer the suspension to a 10 mL round-bottom, disposable culture tube containing an additional 1 mL of YPD (2 mL final volume). Recover the cells at 30 °C with shaking at 250 rpm for 5 h.

24. Centrifuge the tubes at 4,000 × *g* at room temperature for 5 min; remove and discard the supernatant.

25. Resuspend the cell pellet in 100 µL of sterile water and plate on YPD supplemented with 200 µg/mL nourseothricin (NAT200). Incubate at 30 °C for 2–3 days.

26. Aliquot 100 µL of 20 mM NaOH into the wells of a 96-well PCR plate, with each well corresponding to an individual colony that grew on the NAT200 plates. Using a sterile toothpick or pipette tip, pick individual transformed colonies, patch them onto a new NAT200 plate, and swirl the remaining cells into a well with 20 mM NaOH. Repeat for the remaining colonies to create the cell lysate used as the DNA template for the cPCR reaction.

27. Seal the PCR plate and incubate for 10 min at 99 °C in a thermocycler with a heated lid.

28. Set up two cPCR reactions with the oligonucleotides designed in steps 1.3–1.3.5. Scale up the number of reactions as needed. Perform the PCR reaction with the cell lysate prepared in step 1.26 following cycling conditions and PCR reaction mixtures from Table 6. Run 10–20 µL from each well on a 1% agarose gel. Look for colonies with amplification of the two cPCR primer sets indicating properly incorporated GFP dDNA.

29. Restreak colonies that incorporated GFP on synthetic complete (SC) media lacking leucine. Incubate in a 30 °C incubator for 2–3 days. Pick individual colonies and patch onto YPD and YPD supplemented with 400 µg/mL nourseothricin (NAT400) plates. Identify colonies that fail to grow on NAT400 plates after 24 h as those that have successfully lost the CRISPR components.

30. Confirm that the GFP tag is retained by repeating steps 1.25–1.28 using cells from the YPD patch plate. If the correct bands are present, inoculate into 4 mL of YPD and grow overnight (12–16 h), as described in step 1.7.

31. Mix the overnight culture of the new GFP-tagged strain with filter-sterilized 50% glycerol in a 1:1 ratio in a sterile cryotube. Store at −80 °C and restreak onto YPD plates as needed.

NOTE: It is recommended to validate the GFP-tagged strains by confirming nuclear localization of the tagged TF via fluorescent microscopy and confirming a wild-type phenotype in an appropriate phenotypic assay.

2. Sample preparation of biofilm cultures

1. Streak *C. albicans* GFP-tagged strain(s) onto YPD agar plates and incubate at 30 °C for 2–3 days. Using a single isolated colony from the agar plate, inoculate into 4 mL of YPD liquid medium. Incubate at 30 °C with shaking overnight (12–16 h). Determine the OD_{600} of the overnight culture(s).

   NOTE: It is recommended to use three biological replicates per sample for the CUT&RUN experiments.

2. Inoculate a sterile 12-well untreated cell culture plate with the overnight culture to a final OD_{600} of 0.5 (equivalent to 2×10^7 cells/mL) in Roswell Park Memorial Institute (RPMI)-1640 medium to a final volume of 2 mL. Incubate for 90 min at 37 °C in a microplate incubator with shaking at 250 rpm.

   NOTE: It is recommended to use one 12-well cell culture plate per strain with one well uninoculated as a medium-alone contamination control. This protocol has been successfully applied using as little as $1/10^{th}$ of one 12-well cell culture plate well (or as few as 5 million cells). Using a higher number of cells increases total DNA yields, which typically results in high-quality sequencing libraries.

3. Remove unadhered cells by aspiration using sterile pipette tips attached via flexible plastic tubing to a vacuum trap apparatus. Wash the adhered cells once with 2 mL of sterile 1x phosphate-buffered saline (PBS). Add 2 mL of fresh RPMI-1640 medium to the wells and incubate for 24 h at 37 °C with shaking at 250 rpm.

   NOTE: Change pipette tips between wells of different strains and/or conditions. Do not scrape the bottom of the well with the tip while aspirating.

4. At the end of the 24 h incubation, collect and pool the liquid and biofilm material from each of the 11 inoculated wells into a single, sterile 50 mL conical tube. Repeat as necessary with independent pools if processing more than one strain or growth condition concurrently.

   NOTE: Scrape the bottoms and edges of each well with a pipette filter tip to dislodge cells that remain adhered to the surface. Use the pipette to homogenize the biofilms.

5. Pellet samples by centrifuging at 4,000 × *g* at room temperature for 5 min. Decant as much of the supernatant as possible, taking care to minimize disruption of the pellet. Snap-freeze the pellet in liquid nitrogen and store at −80 °C immediately after collection or continue directly to step 4 (isolation of nuclei).

3. Sample preparation of planktonic cultures

1. Streak *C. albicans* GFP-tagged strain(s) onto YPD agar plates and incubate at 30 °C for 2–3 days. Using a single isolated colony from the agar plate, inoculate into 4 mL of YPD liquid medium. Incubate at 30 °C with shaking overnight (12–16 h). Determine the OD_{600} of the overnight culture(s).

2. Back-dilute overnight cultures to OD_{600} of 0.1 in 50 mL of RPMI-1640 liquid medium and incubate at 30 °C with shaking at 225 rpm for 2–5 h until OD_{600} is between 0.5 and 0.8.

   NOTE: Cells should go through at least two doublings before being harvested. Conditions used for planktonic cultures can be adjusted as needed.

3. Pellet the samples by centrifuging at 4,000 × *g* at room temperature for 5 min. Decant as much of the supernatant as possible, taking care to minimize disruption of the pellet. Snap-freeze the pellet in liquid nitrogen and store at −80 °C immediately after collection or continue directly to step 4 (isolation of nuclei).

4. Isolation of nuclei

NOTE: On the day of the experiment, prepare fresh Ficoll Buffer, add 2-mercaptoethanol and protease inhibitor to aliquot(s) of the Resuspension Buffer, and add protease inhibitor to aliquot(s) of the SPC Buffer (see Supplementary File 1). To resuspend the pellets, gently pipette using either 200 µL or 1 mL pipette tips to avoid damaging the cells or nuclei. Before beginning the nuclei isolation, turn on the heat block to preheat it to 30 °C. All pipette tips and tubes for the remainder of this protocol should be certified DNA/RNA and DNase/RNase-free, and the use of filter tips is recommended for all subsequent pipetting steps.

1. Resuspend pellet(s) in 1 mL of room-temperature Resuspension Buffer and transfer to a sterile 1.5 mL microfuge tube. Pellet at 2,000 × *g* at room temperature for 2 min in a table-top centrifuge and remove the supernatant.

   NOTE: Remove the supernatant using either 200 µL or 1 mL pipette tip, taking care to minimize disruption of the pellets.

2. Resuspend the pellet(s) in 200 µL of room-temperature Resuspension Buffer. From the resuspended pellet, transfer a 5 µL aliquot into a new PCR tube and store it at 4 °C for use later.

   NOTE: This aliquot will be used as a control during a subsequent quality control step to evaluate the quality of the isolated nuclei.

3. Pellet at 2,000 × *g* for 2 min and remove and discard the supernatant using a pipette. Repeat the wash step twice using 200 µL of Resuspension Buffer.

4. Centrifuge at 2,000 × *g* at room temperature for 2 min and remove the supernatant. Add 300 µL of Resuspension Buffer and 10 µL of lyticase solution (50 mg/mL, see the Table of Materials). Incubate for 30 min at 30 °C in a heat block.

   NOTE: Alternatively, a water bath heated to 30 °C can also be used instead of a heat block. The spheroplasting conditions used here have been optimized to be effective for both yeast and hyphal cells of *C. albicans*. It is recommended to optimize the spheroplasting conditions when applying this protocol to *C. albicans* cells with mutations that impact cell wall integrity or other cellular

morphologies. During this 30 min incubation step, the user has the option to complete step 5 (Concanavalin A Bead Activation) ahead of time to save time.

1. CRITICAL STEP: After the 30 min incubation step, transfer a 5 µL aliquot into a new PCR tube. To the 5 µL of isolated nuclei and the 5 µL aliquot of intact cells stored at 4 °C from step 4.2, add 1 µL calcofluor white (a fluorescent cell wall dye) and 1 µL of SYTO 13 (a nucleic acid stain). Incubate at 30 °C in the dark for 30 min.

2. Visually inspect the integrity and purity of the isolated nuclei using a fluorescence microscope. Look for isolated nuclei that show prominently stained intact nuclei (using a 488–509 nm excitation filter) and ensure that there is no cell wall staining by the calcofluor white dye (using a 390–420 nm excitation filter). In the intact control cells, look for prominent cell wall staining by the calcofluor white dye (using a 390–420 nm excitation filter) and stained intact nuclei (using a 488–509 nm excitation filter).

5. Centrifuge at 2,000 × *g* at 4 °C for 5 min and remove the supernatant. Resuspend the pellet in 500 µL of ice-cold Resuspension Buffer using 1 mL filter tips by pipetting gently up and down 5 times. Centrifuge at 2,000 × *g* at 4 °C for 5 min, and remove the supernatant using a 1 mL pipette. Resuspend the pellet with 1 mL of freshly made ice-cold Ficoll Buffer.

   NOTE: Keep the samples and buffers on ice from this point forward.

6. Centrifuge the samples at 5,000 × *g* at 4 °C for 10 min and remove the supernatant. Resuspend the pellet in 500 µL of ice-cold SPC Buffer.

   NOTE: From this point onward, handle the nuclei extremely gently to avoid damaging them.

7. Centrifuge the samples at 5,000 × *g* at 4 °C for 10 min and remove as much of the supernatant as possible without disrupting the pellet. Place the tubes containing the pelleted nuclei on ice and proceed to step 5. If step was already completed ahead of time in step 4.4, proceed to step 6 or snap-freeze the pellets in liquid nitrogen and store them at −80 °C immediately after collection.

5. Concanavalin A bead activation

NOTE: This is a critical step. From this point forward, users have the option to continue with the protocol using a commercially available CUT&RUN kit or source key components individually and prepare buffers in-house. If using the commercial kit, all buffers and reagents used below are included in the kit unless otherwise noted. Individual catalog numbers for sourcing reagents independently are also provided in the Table of Materials. Chill all buffers on ice before use. Once step 5 is completed, it is recommended to proceed to step 6 immediately. Avoid multiple freeze-thawing of isolated nuclei as it is known to increase DNA damage and could lead to poor quality results.

1. Gently resuspend the concanavalin A (ConA) beads using a pipette. Transfer 22 µL of ConA bead suspension per sample to be processed in a single 1.5 mL microfuge tube. Place the tube on a magnetic rack until the bead slurry is clear; remove and discard the supernatant using a pipette.

   NOTE: When performing CUT&RUN for a total of 10 samples, for example, transfer 220 µL of the ConA bead suspension to a 1.5 mL microfuge tube.

2. Remove the tube containing the ConA beads from the magnetic rack and immediately add 200 µL of ice-cold Bead Activation Buffer and gently mix using a pipette. Place the tube on the magnetic rack until the bead slurry is clear; remove and discard the supernatant using a pipette. Repeat this step for a total of two washes.

3. Resuspend the beads in 22 µL of ice-cold Bead Activation Buffer per sample of nuclei to be processed. Keep the beads on ice until needed.

   NOTE: The throughput of the subsequent steps is dependent on the number and capacity of magnetic tube racks available. Processing 32 samples in two 16-well tube racks is a manageable number for most users of this protocol. However, higher throughput is possible for more experienced users, or if robotic liquid handling systems are available.

6. Binding nuclei to activated beads

NOTE: Chill all buffers on ice before use. All buffers supplemented with protease inhibitors should be prepared fresh on the day of the experiment. It is recommended to use 0.2 mL strip tubes in the subsequent steps.

1. Resuspend the pelleted nuclei from step 4 in 100 µL of ice-cold SPC Buffer and transfer to a new 8-tube 0.2 mL strip. Add 20 µL of the activated beads to each sample and gently pipette to mix. Incubate at room temperature for 10 min without agitation.

2. Place the tubes on the magnetic rack until the slurry is clear; remove and discard the supernatant using a pipette. Remove the tubes from the magnetic rack, and add 200 µL of ice-cold Wash Buffer to each sample. Resuspend the beads by gently pipetting up and down 5 times. Transfer 100 µL aliquots from each sample into a new 8-tube 0.2 mL strip.

   1. CRITICAL STEP: Divide each CUT&RUN sample into two separate aliquots. Use one of the aliquots for the negative control antibody (e.g., IgG negative control antibody) and the other for the target antibody against the protein of interest (e.g., anti-GFP antibody).

      NOTE: Both samples are required for the computational pipeline to accurately identify enrichment signals specific to the TF of interest. An additional control using anti-GFP antibodies with an untagged strain can also be performed. This control has shown results comparable to the use of IgG antibodies in a GFP-tagged strain. Therefore, for

simplicity, it is recommended to use the standard IgG control for all experiments.

7. Primary antibody binding

NOTE: pAG-MNase fusion protein binds well to rabbit, goat, donkey, guinea pig, and mouse IgG antibodies[17]. Generally, most commercial ChIP-seq-certified commercial antibodies are compatible with CUT&RUN procedures. The amount of primary antibody used depends on the efficiency of the antibody, and titration of the antibody (e.g., 1:50, 1:100, 1:200, and 1:400 final dilution) may be necessary if the antibody of interest has not been previously tested in ChIP or CUT&RUN experiments. Chill all buffers on ice prior to use. All buffers used for antibody binding steps should be prepared fresh on the day of the experiment.

1. Place the tubes on a magnetic rack and wait until the slurry is completely clear; remove and discard the supernatant using a pipette. Add 50 µL of the Antibody Buffer and gently mix by pipetting.
2. Add 3 µL of the anti-GFP polyclonal antibody (or 0.5 µg if using an untested antibody). Incubate the tubes on a nutating mixer at 4 °C for 2 h.

   NOTE: Some CUT&RUN protocols report increased yield by adding a secondary antibody prior to pAG-MNase addition[14]; however, no significant improvement was observed using this added step, and thus, it is not included in this protocol.
3. Briefly centrifuge the tubes at 100 × *g* at room temperature for 5 s, place the tubes on a magnetic rack, and once the slurry is clear, remove and discard the supernatant using a pipette. While the tubes containing the beads are still on the magnetic rack, add 200 µL of ice-cold Cell Permeabilization Buffer directly onto the beads. Remove and discard the supernatant using a pipette. Repeat for a total of two washes with the ice-cold Cell Permeabilization Buffer.
4. Add 50 µL of ice-cold Cell Permeabilization Buffer to each tube and gently mix by pipetting.

   NOTE: Beads are often aggregated at this point but can easily be dispersed by mixing gently using a 200 µL pipette.

8. Binding of pAG-MNase to antibody

1. Add 2.5 µL of the pAG-Mnase (20x stock) to each sample and gently mix by pipetting. Place the samples (slightly elevated at ~45° angle) on a nutator at 4 °C. Turn on the nutator and incubate the samples for 1 h.
2. Briefly centrifuge the strip tubes at 100 × *g* at room temperature for 5 s, place the tubes on a magnetic rack and, once the slurry is clear, remove and discard the supernatant using a pipette.

   NOTE: This step is critical. Carryover antibody remaining in the cap or sides of the tubes after this step will significantly increase the amount of background signal.

3. While the tubes containing the beads are still on the magnetic rack, add 200 µL of ice-cold Cell Permeabilization Buffer, allow the slurry to clear, and remove and discard the supernatant using a pipette. Repeat this step for a total of two washes with the Cell Permeabilization Buffer.

4. Add 100 µL of the ice-cold Cell Permeabilization Buffer to the samples and gently pipette up and down 5 times.

9. Targeted chromatin digestion and release

1. Incubate the tubes containing the sample(s) in a wet ice bath for 5 min. Add 3 µL of 100 mM $CaCl_2$ into each sample using a multichannel pipette. Gently pipette up and down 5 times, immediately return the tubes to the wet ice bath, and incubate for 30 min.

2. Add 66 µL of the Stop Buffer to each sample and gently vortex to mix. Incubate samples for 10 min at 37 °C in a dry bath.

   NOTE: It is recommended to add 1.5 pg of heterologous *E. coli* spike-in DNA per sample in the Stop Buffer. The addition of 1.5 pg of *E. coli* spike-in DNA results in 1,000–10,000 mapped spike-in reads for 1–10 million mapped experimental reads[14]. The spike-in DNA is used to calibrate the sequencing depth and is especially important for comparing samples in a series. The addition of spike-in *E. coli* is highly recommended but not essential. The commercial CUT&RUN kit includes *E. coli* spike-in DNA, but it can also be purchased separately.

3. Place the tubes on the magnetic rack and transfer 160 µL of the supernatant into a 1.5 mL microfuge tube. Transfer 80 µL of the sample into a new 2 mL microfuge tube and store at −20 °C in the event that a backup sample is needed. Proceed to step 10 with the 80 µL sample.

10. Cleanup of collected DNA samples

NOTE: Incubate DNA Purification Beads at room temperature for 30 min before use. Prechill 100% isopropanol on ice. When mixing the samples, pipette up and down 10 times.

1. Vortex DNA Purification Beads to homogenize the bead suspension. Add 50 µL (~0.6x sample volume) of the resuspended beads to each sample. Pipette-mix and incubate the samples on a nutator for 5 min at room temperature.

   NOTE: The ratio of DNA purification beads to sample used is critical. Using 0.6x volume of DNA Purification Bead solution relative to the sample allows the magnetic beads to bind to large DNA fragments released from damaged nuclei. CUT&RUN-enriched DNA fragments are much smaller than these large DNA fragments and are thus retained in the supernatant at this step.

2. Place the tubes on a magnetic rack and transfer 130 µL of the supernatant containing the DNA to a 0.2 mL 8-tube strip. Add an additional 30 µL of DNA Purification Beads to the sample(s) (the total volume is 160 µL).

3. Add 170 µL (~1x sample volume) of ice-cold 100% isopropanol, mix well by pipetting up and down 10 times, and incubate on ice for 10 min.

   NOTE: It is critical that 100% ice-cold isopropanol is used for this step for the DNA purification beads to efficiently capture the CUT&RUN-enriched small fragments.

4. Place the tubes on the magnetic rack, and once the slurry has cleared, carefully remove and discard the supernatant using a pipette.

5. While the tubes are on the magnetic rack, add 200 µL of freshly prepared, room-temperature 80% ethanol to the tubes and incubate at room temperature for 30 s. Carefully remove and discard the supernatant using a pipette. Repeat this step for a total of two washes with 80% ethanol.

6. Quickly spin the tubes at 100 × *g*, place the tubes back on the magnetic rack, and remove any residual ethanol using a pipette after the slurry has cleared. Air-dry the beads for 5 min while the tubes remain on the magnetic rack with the lid open.

   NOTE: Do not exceed 5 min of drying time as this can significantly reduce the final DNA yield.

7. Remove the tubes from the magnetic rack and elute the DNA from the beads by adding 17 µL of 0.1x Tris-EDTA (TE) at pH 8. Mix well and incubate the tubes for 5 min at room temperature.

8. Place the tubes on the magnetic rack until the slurry becomes clear. Once the slurry has cleared, carefully transfer 15 µL of the supernatant to a sterile 0.2 mL PCR tube.

9. Measure the concentration of the collected DNA using a fluorometer following the manufacturer's protocol.

   NOTE: Typically, the concentration of the collected DNA is ~1 ng/µL. Sometimes, the concentration of the collected DNA is too low to quantify using a fluorometer. This is not an indicator of a failed experiment. Proceed with the library preparation regardless of the concentration of the collected DNA.

10. Proceed to step 11 or store the samples at −20 °C until ready.

11. Library preparation for sequencing

NOTE: The following steps use a commercially available library prep kit. When performing steps using the Ligation Master Mix, minimize touching the tubes and always keep them on ice.

1. Using 0.1x TE at pH 8, bring up the total volume of the CUT&RUN DNA to 50 µL. Make a master mix of 3 µL of End Prep Enzyme Mix and 7 µL of End Prep Reaction Buffer per sample. Add 10 µL of master mix to the CUT&RUN DNA and mix thoroughly by pipetting up and down 5 times.

2. Perform a quick spin at 100 × g to collect all liquid from the sides of the tube. Place the tubes in a thermocycler with the heated lid set to ≥75 °C and run the cycling conditions in Table 7.

   NOTE: Depending on the starting input DNA concentrations collected from step 10, follow the required adapter dilution from Table 8.

3. Add 2.5 µL of Adapter per sample and mix thoroughly by pipetting up and down 10 times.

   NOTE: It is critical that the adapter is added to the sample and mixed thoroughly before the ligation master mix is added.

4. Make a master mix of 30 µL of Ligation Master Mix and 1 µL of Ligation Enhancer. Add 31 µL of the master mix to the sample(s). Mix thoroughly by pipetting up and down 10 times.

5. Incubate at 20 °C for 15 min in a thermocycler with the heated lid off.

   NOTE: It is critical that samples be kept on ice and transferred to the thermocycler only after the thermocycler has reached 20 °C.

6. Perform a quick spin at 100 × g to collect all liquid from the sides of the tube, add 3 µL of Uracil Excision Enzyme, and incubate the tubes in the thermocycler at 37 °C for 15 min with the heated lid set to ≥47 °C.

   NOTE: This is a safe stopping point; store the samples at −20 °C or continue directly to step 11.7. If continuing directly to step 11.7, incubate the DNA Purification Beads at room temperature for 30 min before use.

7. Add 154.4 µL (~1.6x sample volume) of the DNA Purification Beads to the Adapter Ligation reaction from step 11.6. Pipette-mix and incubate the samples for 5 min at room temperature.

8. Place the tubes on the magnetic rack and once the slurry has cleared, carefully remove and discard the supernatant using a pipette.

9. Add 200 µL of freshly prepared, room-temperature 80% ethanol to the tubes and incubate at room temperature for 30 s. Carefully remove and discard the supernatant using a pipette and repeat this step for a total of two washes with 80% ethanol.

10. Spin the tubes briefly at 100 × *g*. Place the tubes back on the magnetic rack and remove any residual ethanol using a pipette. Air dry the beads for 5 min while the tubes remain on the magnetic rack with the lid open.

    NOTE: Do not exceed 5 min of drying time as this can significantly reduce the final DNA yield.

11. Remove the tubes from the magnetic rack and elute the DNA from the beads by adding 17 µL of 0.1x TE at pH 8. Mix well and incubate for 5 min at room temperature.

12. Place the tubes on the magnetic rack until the slurry becomes clear. Once the slurry has cleared, carefully transfer 15 µL of the supernatant to a sterile 0.2 mL PCR tube.

13. Make a master mix of 25 µL of DNA Polymerase Master Mix and 5 µL of Universal Forward Library Amplification Primer (10 µM) per sample.

    NOTE: Prepare one extra sample of master mix to account for pipetting losses.

14. Add 30 µL of the master mix to the 15 µL of Adapter-ligated DNA sample. Add 5 µL of Reverse Uniquely Indexed Library Amplification Primer (10 µM) to each sample to bring the final volume to a total of 50 µL. Mix thoroughly by pipetting up and down 10 times. Perform the PCR cycling conditions in Table 9.

    NOTE: Incubate the DNA Purification Beads at room temperature for 30 min before use.

15. Vortex the DNA Purification Beads to resuspend. Add 35 µL (~0.7x sample volume) of the resuspended beads to the PCR-amplified DNA samples. Mix and incubate the samples on a nutator for 5 min at room temperature.

16. Place the tubes on the magnetic rack and once the slurry is clear, transfer the supernatant containing the DNA to a new 0.2 mL 8-well PCR strip tube.

17. Add 119 µL (~1.4x sample volume) of beads to the sample, and mix by pipetting up and down 5 times. Incubate the samples on a nutator for 5 min at room temperature.

18. Place the tubes on the magnetic rack and once the slurry has cleared, carefully remove and discard the supernatant using a pipette.

19. Add 200 µL of freshly prepared, room-temperature 80% ethanol to the tubes and incubate at room temperature for 30 s. Carefully remove and discard the supernatant using a pipette; repeat the step for a total of two washes with 80% ethanol.

20. Spin the tubes briefly at 100 × *g*. Place the tubes back on the magnetic rack and remove any residual ethanol using a pipette. Air-dry the beads for 5 min while the tubes remain on the magnetic rack with the lid open.

    NOTE: Do not exceed 5 min of drying time as this can significantly reduce the final DNA yield.

21. Remove the tubes from the magnetic rack and elute the DNA from the beads by adding 14 µL of 0.1x TE at pH 8. Mix well and incubate for 5 min at room temperature.

22. Place the tubes on the magnetic rack until the slurry becomes clear. Once the slurry has cleared, carefully transfer 13 µL of the supernatant to a sterile 0.2 mL PCR tube.

23. Prepare fresh 1x Tris-borate-EDTA (TBE) and insert premade, commercial 10% acrylamide TBE gel into the gel electrophoresis apparatus filled with 1x TBE.

24. In the first well, add 2 µL of Low-range DNA ladder. Mix 3 µL of 6x loading dye with 13 µL of the sample previously collected from step 11.22. Carefully add 15 µL into each well of the gel. Run the gel for 90 min at 70 V.

NOTE: It is recommended to leave one well in the gel empty between each sample, as this reduces the likelihood of sample cross contamination. Experienced users may find it appropriate to use all wells while carefully avoiding cross contamination, particularly when processing a large number of samples.

25. Remove the gel cast from the gel box. Open the gel cast per the manufacturer's instructions, gently remove the gel from the gel cast, and place it inside a gel holding tray containing 100 mL of 1x TBE.

NOTE: Make sure to gently remove the gel from the gel cast to avoid ripping the gel as it is thin and fragile. It is critical to prewet gloves and the gel with 1x TBE whenever handling the gel. The gel holding tray should be slightly larger than the size of the gel (approximately 0.5" on each side). The plastic lids provided with 96-well PCR-tube storage boxes are convenient holding trays for standard mini gel sizes.

26. Add 10 µL of the nucleic acid gel stain to the tray and gently swirl. Cover with foil to protect from light and incubate statically at room temperature for 10 min.

27. Rinse the gel twice with 100 mL of deionized tap water. Image the gel under blue light illumination using an amber filter cover (Figure 2).

NOTE: Successful libraries show a smear between 100 and 500 bp. There will also be a prominent ~125 adapter dimer band. The presence of adapter dimers is not an indicator of poor library quality. This amount of adapter dimers is unavoidable for CUT&RUN experiments performed on low-abundance TFs and is a consequence of the low amount of input material used to prepare these libraries. Do not use ultraviolet light, which can damage the DNA.

28. As shown in Figure 2, for each library, cut the gel slightly above the ~125 bp prominent adapter dimer band (making sure to avoid touching the adapter dimer band) and below the 400 bp ladder mark.

NOTE: It is critical to avoid the ~125 adapter dimer band. Even tiny amounts of adapter dimers will significantly reduce the library quality.

29. Puncture the bottom of a 0.65 mL tube using a 22 G needle and place the punctured tube inside a sterile 2 mL microfuge tube. Transfer the gel slice to the punctured tube inside the 2 mL microfuge tube.

30. Centrifuge the 2 mL microfuge tube containing the 0.65 mL punctured tube and sample at 10,000 × *g* at room temperature for 2 min to collect the gel slurry inside the 2 mL microfuge tube.

NOTE: The punctured tube should now be empty and can be discarded. If the punctured tube still has any gel remaining inside, place the punctured tube back

inside the 2 mL microfuge tube and centrifuge again at 10,000 × *g* at room temperature for an additional 2 min.

31. To the gel slurry inside the 2 mL microfuge tube, add 300 µL of ice-cold gel elution Buffer and mix on a nutator at room temperature for a minimum of 3 h or overnight (12–16 h).

32. Transfer all liquid and gel slurry to a 0.22 µm filter column. Centrifuge at 10,000 × *g* at room temperature for 1 min; the collected volume should be ~300 µL.

33. Add 450 µL (~1.5x sample volume) of DNA Purification Beads, incubate at room temperature for 5 min on a nutator, and then place the sample on the magnetic rack until the slurry is clear.

NOTE: Incubate the DNA Purification Beads at room temperature for 30 min before use.

34. Remove and discard 500 µL of the supernatant, making sure not to disrupt the beads.

35. Remove the sample from the magnetic rack and mix the beads by pipetting up and down 5 times. Transfer 200 µL of the sample into a new PCR strip tube.

36. Place the strip tube on the magnetic rack, and once the slurry has cleared, carefully remove and discard the supernatant using a pipette.

37. Add 200 µL of freshly prepared, room-temperature 80% ethanol to the tubes and incubate at room temperature for 30 s. Carefully remove and discard the supernatant using a pipette; repeat this step for a total of two washes with 80% ethanol.

38. Spin the tubes briefly at 100 × *g*. Place the tubes back on the magnetic rack and remove any residual ethanol using a pipette. Air-dry the beads for up to 5 min while the tubes remain on the magnetic rack with the lid open.

NOTE: Do not exceed 5 min of drying time as this can significantly reduce the final DNA yield.

39. Remove the tubes from the magnetic rack and elute the DNA from the beads by adding 17 µL of 0.1x TE at pH 8. Mix well and incubate the tubes for 5 min at room temperature.

40. Place the tubes on the magnetic rack until the slurry becomes clear. Once the slurry has cleared, carefully transfer 15 µL of the supernatant to a sterile 0.2 mL PCR tube.

41. Measure the final library quantity using the fluorometer; use this final library for sequencing.

NOTE: Up to 48 libraries can be pooled and sequenced together in a single lane using a sequencing platform that provides at least 300 million 40 bp or longer paired-end reads.

12. CUT&RUN sequence analysis

NOTE: This section presents the computational protocol used to analyze the CUT&RUN sequence data. The protocol begins with setting up the computational virtual environment and walks users through executing the commands on their local machine. This protocol will work on all computational resources, such as local machines, virtual cloud servers, and high-performance computing clusters. All CUT&RUN data presented in this paper can be accessed at NCBI GEO under accession number GSE193803.

1. Download the source code for the CUT&RUN analysis from https://github.com/akshayparopkari/cut_run_analysis.

   NOTE: The workflow will work best on a MacOS or Linux OS system. Windows users can run the workflow using GitBash (see the Table of Materials).

   1. Directly download the code from the GitHub page by clicking on the green **Code** button | **Download ZIP** option. Unzip the folder to a relevant location on the local machine.

2. Install Conda environment (see the Table of Materials) and run only once.

   NOTE: This workflow uses the Conda command line tool environment to install all required software and tools.

3. Once Conda is installed (run only once), create a virtual environment using the Supplementary File 2 provided with the following command:

   conda create –name <env> --file Supplementary_File_2.txt

4. Activate the virtual environment every time this workflow is to be executed using:

   conda activate <env>

5. Organize the input raw FASTQ files into a single folder, ideally one folder per CUT&RUN experiment.

13. Generation of the genome file for alignment

1. Generate the genome file for alignment (run only once for each genome file). Create a folder to save all *C. albicans* genome files, such as:

   mkdir ca_genome_files

14. Downloading *C. albicans* genome assembly 21

1. Download *C. albicans* genome assembly 21 from the *Candida* Genome Database using either wget or curl tools (see the Table of Materials)[18].

   NOTE: *C. albicans* assembly 21 was used here to compare CUT&RUN results with previously published ChIP-chip results, which were aligned to Assembly 21. Users can download other assembly versions and run similar commands to generate relevant genome files for their alignment needs.

15. Generate a Bowtie 2 index database (database name: ca21)

1. Use the following:

   bowtie2-build C_albicans_SC5314_A21_current_chromosomes.fasta.gz ca21

   bowtie2-inspect -s ca21

16. Run the CUT&RUN analysis pipeline

1. Read the help section to become familiar with the parameters of the pipeline.

   bash cut_n_run_pipeline.sh -h

17. Execute the cut_n_run_pipeline.sh file with relevant parameters

1. Execute the script:

   bash cut_n_run_pipeline.sh /path/to/input/folder 4 y y y y y > /path/to/output.log 2>&1

   NOTE: The relevant parameters with detailed descriptions on lines 19–36 of the code are described on the GitHub page https://github.com/akshayparopkari/cut_run_analysis/blob/main/cut_n_run_pipeline.sh.

18. Organize output files

1. Merge significant peaks from all replicates called by MACS2 located in /path/to/input/folder/peakcalling/macs2 using the BedTools merge function[19].

   Cat /path/to/input/folder/peakcalling/macs2/all_replicate_files sort -k1,1 -k2,2n | mergeBed -c 4,5,6,7,8,9 -o last,mean,first,mean,mean,mean > /path/to/merged_output.bed

   NOTE: For additional information and best practices on assessing overlapping peaks in replicate samples, please refer to Landt et al.[20] and Boyd et al.[21].

19. Remove matches to blocklisted genomic regions using the BedTools subtract function

1. Use the following: *subtractBed - a /path/to/merged_output.bed -b /path/to/* Supplementary_File_3.bed *-A > /path/to/merged_output_no_blocklist_hits.bed*

   NOTE: The blocklisted regions in the *C. albicans* genome are provided as a .bed file in Supplementary File 3. The list consists primarily of highly repetitive sequence elements and regions such as telomeric repeats and centromeres that commonly yield false-positive results in *C. albicans* CUT&RUN, ChIP-seq, and ChIP-chip datasets. Hence, it is recommended to remove the blocklisted regions. However, for certain protein targets, it may be inappropriate or undesirable to exclude these loci. Users can skip this step to retain signals contained within these blocklisted regions or create their own blocklisted regions.

20. Merge BigWig files from replicates using the UCSC bigWigMerge function[22]

1. Use the following: *bigWigMerge /path/to/input/folder/bigwig/all_final_bw_files /path/to/input/folder/bigwig/all_final_bdg_file*
2. Convert the BedGraph output from bigWigMerge to BigWig using the UCSC bedGraphToBigWig function. *bedGraphToBigWig /path/to/input/folder/bigwig/all_final_bdg_file /path/to/input/folder/bigwig/all_final_bw_file*

Representative Results

This robust CUT&RUN protocol was adapted and optimized for investigating the genome-wide localization of specific TFs in *C. albicans* biofilms and planktonic cultures (see Figure 2 for an overview of the experimental approach). A thorough data analysis pipeline is also included to facilitate analysis of the resulting CUT&RUN sequencing data and requires users to have minimal expertise in coding or bioinformatics (see Figure 3 for an overview of the analysis pipeline). Contrary to the ChIP-chip and ChIP-seq methods, CUT&RUN is carried out using intact, permeabilized nuclei prepared from a significantly reduced number of input cells, without formaldehyde crosslinking. Isolating intact nuclei from *C. albicans* spheroplasts is a critical step in the protocol. Efficient spheroplasting via the digestion of the *C. albicans* cell wall using lyticase can be challenging as the enzymatic digestion reaction conditions must be optimized for each cell type. Thus, to ensure a successful CUT&RUN experiment with high-quality sequencing results, an early quality control step is included, and the presence of intact nuclei is verified using a standard fluorescence microscope.

Cell wall digestion and nuclear integrity are regularly assessed by visualizing both control intact cells and isolated nuclei stained with fluorescent cell wall and nucleic acid strains. In contrast to the isolated intact nuclei, where cell wall staining is not observed, both nuclei and the cell walls are fluorescently labeled in the intact control cells (Figure 4A). Lastly, prior to sequencing, fragment size distribution of CUT&RUN libraries is evaluated using a capillary electrophoresis instrument. This quality control step is a reliable measure in assessing the quality of CUT&RUN libraries. As seen in the electrophoresis trace on the top panel of Figure 4B, successful libraries generated for experiments investigating TFs show high enrichment for fragments smaller than 280 bp. The electrophoresis trace in the bottom panel of Figure 4B represents results from an unsuccessful CUT&RUN experiment.

Here, the peak at ~2,000 bp arose mainly from undigested DNA released from nuclei extensively damaged or broken during the CUT&RUN experiment. Assessing the fragment size distribution of the final pooled libraries is also recommended to confirm the complete removal of contaminating adapter dimers (Figure 4C). Typically, 5–10 million paired-end reads (~40 base read length) per library provide sufficient sequencing depth for most TF CUT&RUN experiments in *C. albicans*. Alternative sequencing read lengths, either paired-end or single-end, can be used to sequence CUT&RUN libraries. For example, paired-end read lengths between 25 and 150 bp should not affect the quality of the results. Single-end reads greater than or equal to 150 bp should also work for TF CUT&RUN experiments. However, the accompanying data analysis pipeline must be modified to accommodate single-end reads.

This CUT&RUN protocol and accompanying data analysis pipeline were validated by investigating two TFs, Ndt80 and Efg1, which control *C. albicans* biofilm formation. As shown in Figure 4D, Ndt80 is bound at intergenic regions (highlighted by the black bars indicating significantly enriched Ndt80 ChIP-chip loci) upstream of the *EFG1* ORF. This intergenic region upstream of *EFG1* was previously shown to be highly enriched for Ndt80 binding during biofilm formation by ChIP-chip[4]. However, CUT&RUN experiments identified significantly more peaks within this region than ChIP-chip experiments (10 peaks vs 4 peaks). Ndt80 DNA binding motifs are enriched across all the Ndt80-bound loci identified by CUT&RUN (Supplementary Figure 1), indicating that the additional peaks identified by this methodology are likely bona fide Ndt80-bound sites. A systematic comparative analysis indicated that this presented CUT&RUN protocol successfully identified the majority of the previously known binding events for Ndt80 and Efg1 during biofilm formation[4] (Figure 5).

Furthermore, many new TF binding events that were not captured in the previously published ChIP-chip experiments were identified using CUT&RUN (Figure 5). Overall, both Ndt80 and Efg1 bound to loci overlapping with previously published ChIP-chip data and to loci identified only using the CUT&RUN method (overlaps between these CUT&RUN data and previously published ChIP-chip data for Ndt80 and Efg1 are summarized in the Venn diagrams in Figure 5). Furthermore, the fraction of reads within significantly called peaks (FRiP scores) were consistently higher in GFP-tagged samples than in their control IgG samples (see Supplementary Figure 2). In summary, these results show that the CUT&RUN protocol described here is a robust method optimized to investigate *C. albicans* TF-DNA binding interactions from low-abundance samples.

Discussion

This protocol presents a comprehensive experimental and computational pipeline for genome-wide localization of regulatory TFs in *C. albicans*. It is designed to be highly accessible to anyone with standard microbiology and molecular biology training. By leveraging the high dynamic range and low sample input requirements of the CUT&RUN assay and including optimizations for the localization of TF-DNA binding interactions in *C. albicans* biofilm and planktonic cultures, this protocol presents a powerful and low-cost alternative to traditional ChIP-seq approaches. Compared to ChIP-seq, CUT&RUN yields significantly higher sensitivity, with a higher proportion of sequencing reads mapped to bound peaks, is more amenable to high-throughput, requires substantially lower input cell numbers, does not require the use of toxic crosslinking agents, and requires tenfold fewer sequencing reads per sample to produce high-quality results[13, 14, 17, 23, 24]. To further reduce the per-sample cost of this protocol, buffer and media recipes are included along with a detailed reagent list to facilitate the in-house preparation of all necessary buffers and media, as well as the bulk sourcing of essential reagents. As *C. albicans* biofilm formation, phenotypic switching, and commensalism are all regulated by complex interwoven transcriptional networks[26], this robust, facile, and cost-effective CUT&RUN protocol provides a powerful new tool for understanding these and many other cellular processes in this important human fungal pathogen.

TFs are not as abundant as histones or other chromatin-associated proteins, creating a unique challenge for investigating TF-DNA binding interactions via CUT&RUN. To address this challenge, critical adjustments and optimizations were made to the standard CUT&RUN experimental protocol[13]. As most successful CUT&RUN experiments targeting TFs yield a small amount of DNA that is too dilute to quantify and is often enriched for fragments smaller than 150 bp[13, 23], the library preparation reaction conditions were also optimized to favor these smaller fragments[27]. Even with this optimization step, the resulting PCR-amplified libraries contain a significant proportion of adapter dimers, which are not completely removed using magnetic bead-based DNA size selection methods. To address this issue, a PAGE gel size-selection step was included to generate final sequencing-ready libraries that are largely devoid of adapter dimers. This is a critical step of the protocol, as removing adapter dimers while retaining the smaller TF-derived CUT&RUN fragments is essential for obtaining high-quality results.

Furthermore, the detailed computational pipeline filters the sequencing data to focus on the smaller reads derived from TF-DNA binding interactions in the CUT&RUN assay. Due to these TF-specific adjustments, this protocol is not recommended for profiling large chromatin-associated complexes such as nucleosomes. While it is theoretically possible to adapt the protocol for this purpose by following the standard library preparation protocol included with the commercially available library prep kit, the user would need to adjust the post sequencing size selection included in the computational pipeline. Specifically, in the size-filtering section in the code file cut_n_run_pipeline.sh, users would need to replace the current value of "14400" (120 bp * 120 bp) with the square of the desired fragment length to enable the analysis pipeline to analyze the sequencing results generated for other types of chromatin-DNA binding interactions.

Another key step in a successful CUT&RUN experiment includes choosing optimal post sequencing data analysis parameters. While most of the computational pipeline is designed to be standardized and applicable to the study of any regulatory TF of interest in *C. albicans*, there are two important considerations that the user should evaluate while running the pipeline. The first consideration is whether to include or remove duplicate reads from the sequencing data prior to the identification of bound target sites. As low-abundance TFs will typically yield sequencing data containing a significant percentage of reads that are derived from PCR duplication during the library amplification step, removing PCR duplicates can have a significantly negative impact on the results. However, with highly abundant TFs or chromatin-associated proteins, PCR duplicates typically represent a smaller portion of the total number of reads and are often removed to suppress background noise in the data. Ultimately, this decision to keep or remove PCR duplicates is dependent on the TF of interest and the depth of the sequencing data obtained. Thus, the pipeline automatically generates independent output files for data derived with or without PCR duplicate reads, so the user can decide which output files yield the best results for each experiment.

The second consideration is whether to identify and remove problematic loci that yield significant, yet highly variable, enrichment in both experimental (antibody against the protein of interest) and negative control (IgG) samples. The peak-calling algorithm uses MACS2 to identify significantly enriched loci in both the experimental and control samples

and excludes those that appear in both. While this approach typically eliminates most problematic loci, some occasionally appear as significant peaks in certain experiments, even though prior experience indicates that they are unlikely to be true positive sites of TF enrichment. Thus, an optional filtering step is provided to remove these problematic loci, referred to as "blocklisted" loci. The list of blocklisted loci primarily contains highly repetitive sequence elements and regions such as telomeric repeats and centromeres that have historically yielded false-positive results in previous genome-wide binding assays. This is a very conservative list of loci assigned as problematic with high confidence. However, each user should evaluate whether this filter is appropriate for their experiment(s) on a case-by-case basis. A potential alternative to the IgG negative control would be to perform CUT&RUN against a nuclear-localized protein that does not bind DNA. This approach has been shown to be an ideal control for ChIP experiments[28], and a similar approach is worth considering for CUT&RUN.

CUT&RUN has become a popular choice for investigating protein-DNA interactions in higher eukaryotes and the model yeast *Saccharomyces cerevisiae*. Here, it has been successfully adapted to investigate genome-wide TF-DNA binding interactions in the clinically relevant fungal pathogen *C. albicans*. This protocol provides detailed methods for all necessary experimental and computational procedures, from the engineering of strains that express epitope-tagged TFs, through to the computational analysis of the resulting CUT&RUN sequencing data. Overall, this protocol and the accompanying data analysis pipeline produce robust TF-DNA binding profiles, even when using complex multimorphic populations of cells isolated from low-abundance biofilm samples and provides superior data quality at a lower overall cost than ChIP-seq methodologies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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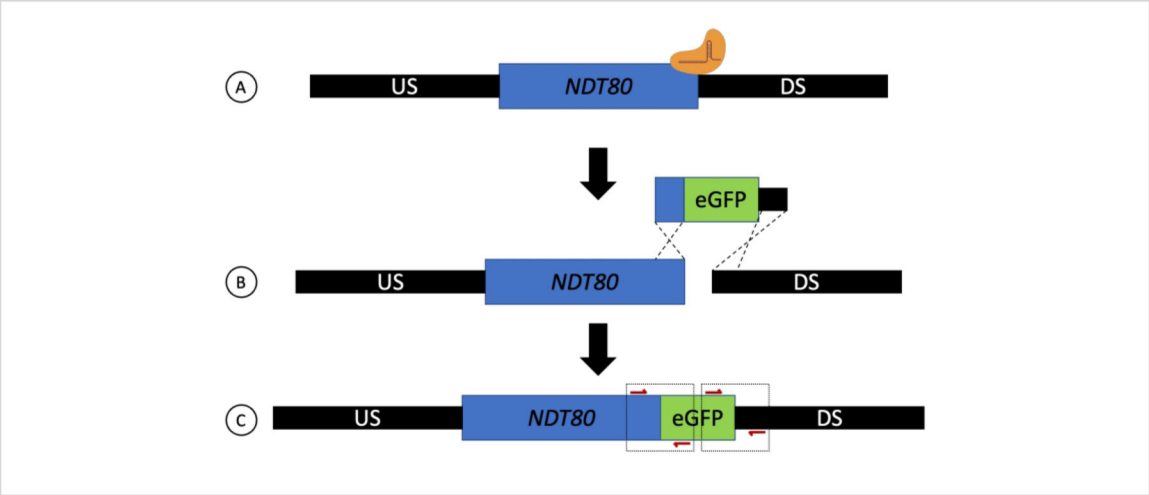

Figure 1: Epitope tagging of *C. albicans* TFs.

(**A**) Identify a TF gene of interest (*NDT80* in this case) and select a gRNA to target the cutting of Cas9 (represented by the orange shape) at the 3' end of the ORF. (**B**) *Candida* clade-optimized eGFP with >50 bp homology upstream and downstream from the cut site will provide the dDNA to repair the DSB. (**C**) Use colony PCR to screen individual colonies to confirm the intended integration. Primers are indicated by red arrows, and amplicons are denoted by dashed boxes. This figure was created using BioRender.com. Abbreviations: TF = transcription factor; gRNA = guide RNA; ORF = open reading frame; eGFP = enhanced green fluorescent protein; DSB = double-stranded break; US = upstream; DS = downstream.

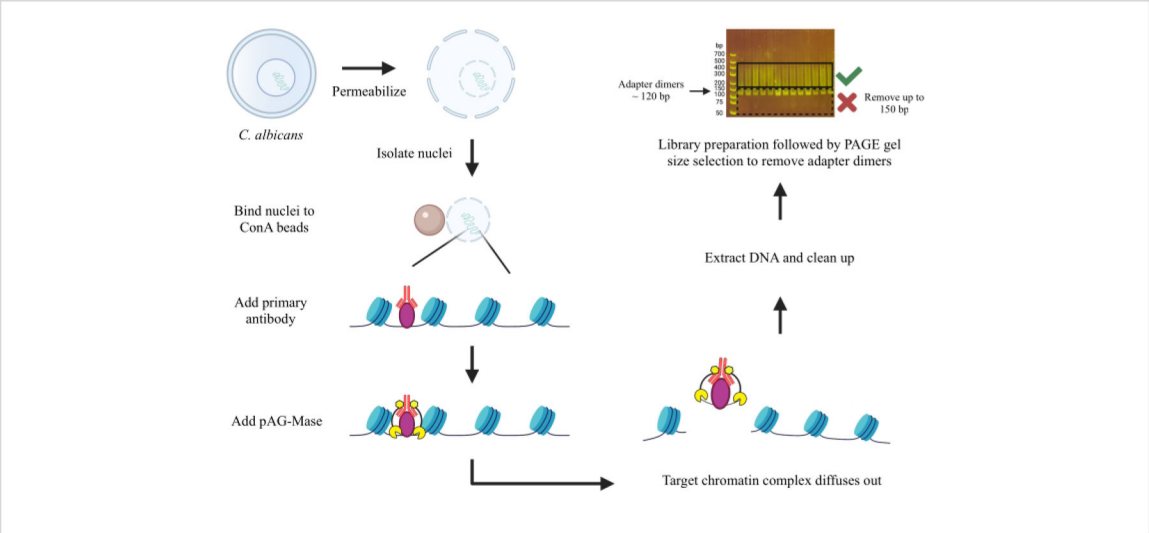

Figure 2: Schematic overview of the CUT&RUN experimental protocol.

C. albicans cells collected from biofilm or planktonic culture conditions are permeabilized to isolate intact nuclei. ConA beads are activated, and the intact nuclei are then bound to the activated ConA beads. The antibody of interest is added to the bead-bound nuclei and incubated at 4 °C. Next, pAG-MNase is added and allowed to bind to the target antibody. After the addition of $CaCl_2$, pAG-MNase is activated, and targeted chromatin digestion proceeds until the addition of the chelating reagent to inactivate pAG-MNase. The pAG-MNase-bound antibody complex is allowed to diffuse out of the permeabilized nuclei, and the resulting DNA is extracted and cleaned. Sequencing libraries are prepared from the CUT&RUN-enriched DNA fragments, and the resulting libraries are then run on a 10% PAGE gel to separate and remove contaminating adapter dimers prior to sequencing. This figure was created using BioRender.com. Abbreviations: CUT&RUN = cleavage under targets and release using nuclease; ConA = concanavalin A; PAGE = polyacrylamide gel electrophoresis.

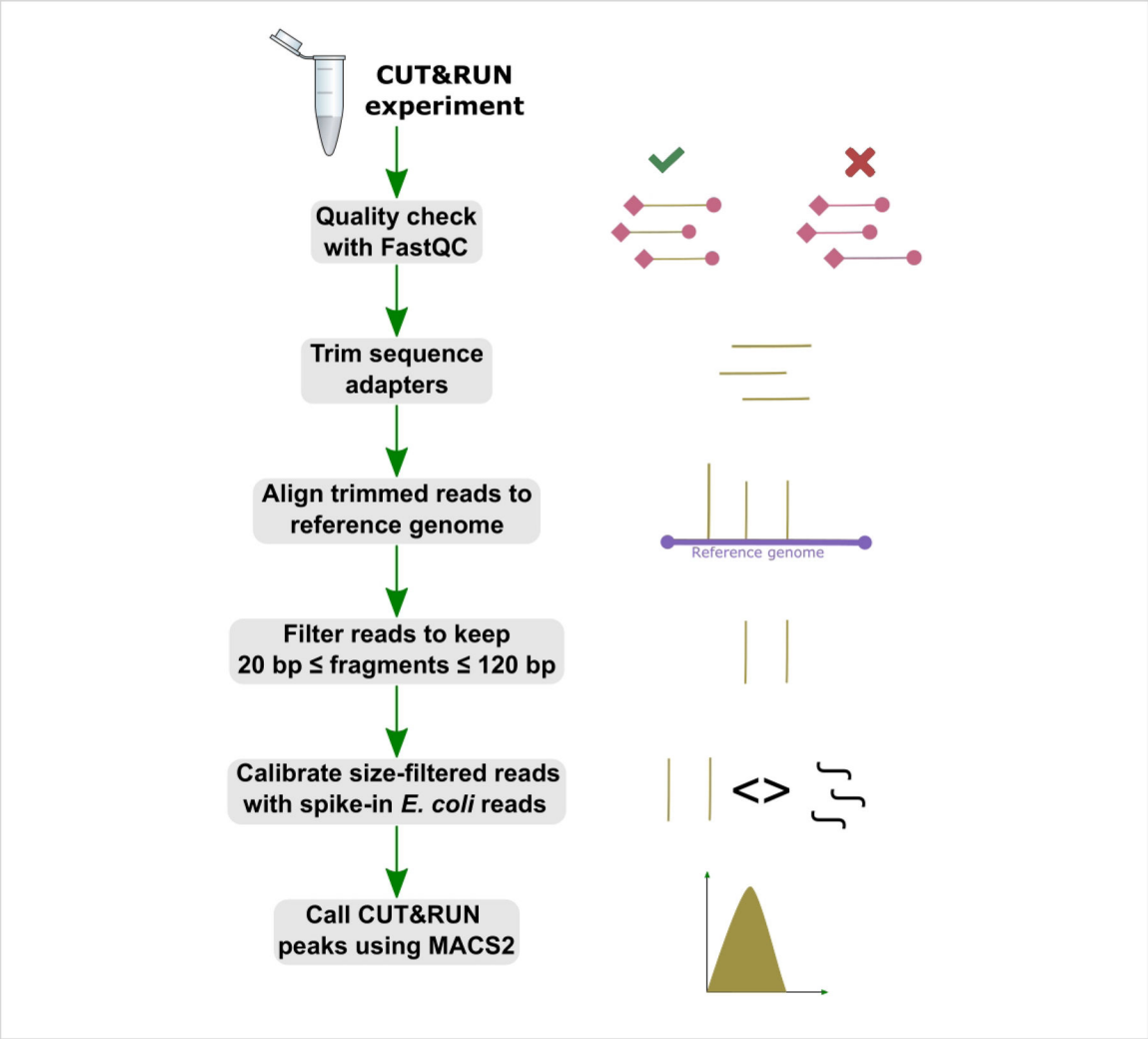

Figure 3: Schematic overview of the CUT&RUN data analysis pipeline.

The workflow starts by performing quality checks on the raw FASTQ files using FastQC, followed by trimming to remove sequencing adapters. The trimmed reads are then aligned to the reference genome, and the aligned reads are filtered based on their size to enrich for TF-sized binding signals (20 bp ≤ aligned read ≤ 120 bp). Size-selected reads are then calibrated against spike-in *Escherichia coli* reads, and calibrated reads are used to call peaks using MACS2. The <> symbol is a visual representation to indicate that *C. albicans* read counts were calibrated using *E. coli* read counts for each sample. Abbreviations: CUT&RUN = cleavage under targets and release using nuclease; TF = transcription factor.

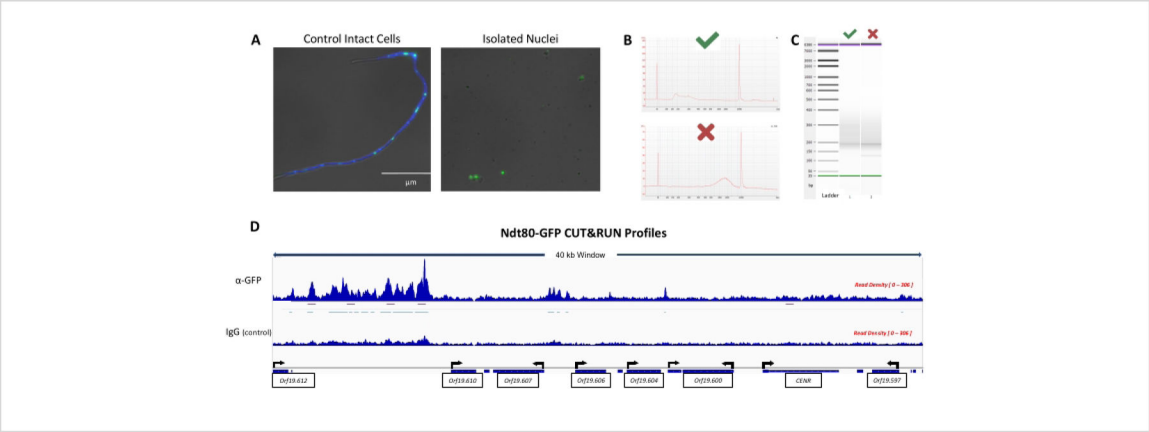

Figure 4: Quality control steps critical for successful CUT&RUN experiments.

(**A**) Cells are stained with fluorescent cell wall (blue) and nucleic acid (green) stains before and after nuclei isolation and visualized using a fluorescence microscope. (**B**) CUT&RUN TF libraries are analyzed using a capillary electrophoresis instrument. Successful CUT&RUN TF libraries (indicated by the green checkmark) are enriched for short fragments smaller than 200 bp. Suboptimal CUT&RUN TF libraries (indicated by the red "X") show enrichment for large DNA fragments. (**C**) 48 CUT&RUN libraries are pooled together and analyzed using a capillary electrophoresis instrument. High-quality, pooled libraries (indicated by the green checkmark) are free of adapter dimers (lane 1), while low-quality pooled libraries (indicated by the red "X") retain small amounts of adapter dimers (lane 2). (**D**) Representative IGV tracks from CUT&RUN datasets (including the Ndt80 IgG control, bottom track) showing significant enrichment for Ndt80 binding (top track) at the intergenic region upstream of the *EFG1* ORF (Orf19.610). The black bars represent significantly enriched Ndt80 binding peaks identified by previously published ChIP-chip experiments[4]. The blue bars represent significantly enriched Ndt80 binding peaks identified in the CUT&RUN experiments presented here. Scale bars = 50 μm (**A**). Abbreviations: CUT&RUN = cleavage under targets and release using nuclease; TF = transcription factor; GFP = green fluorescent protein; ORF = open reading frame.

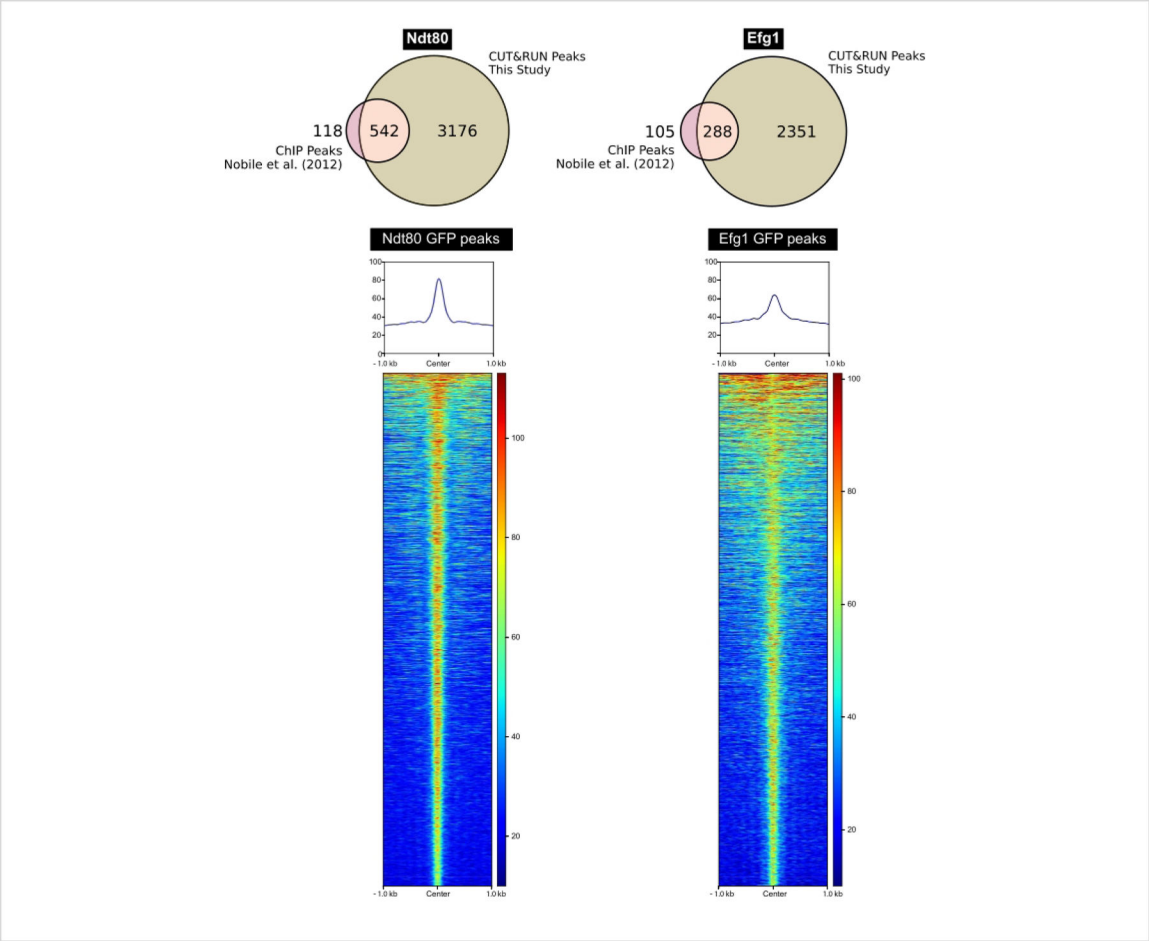

Figure 5: Evaluation of Ndt80 and Efg1 enriched peaks identified using the presented CUT&RUN protocol and data analysis pipeline on *Candida albicans* biofilms.

The Venn diagrams in the top row illustrate the degree of overlap between Ndt80 and Efg1 binding sites identified via CUT&RUN with previously published ChIP-chip data[4]. The CUT&RUN signals for all binding events for Ndt80 and Efg1 are shown in the bottom row as colored heatmaps (red = high peak signal; blue = low/no peak signal; colored bar indicates IgG signal subtracted from GFP signal); 1,000 bp regions upstream (−1.0 kb) and downstream (+1.0 kb) are displayed in the heatmaps. The signal intensity (i.e., enrichment) as a profile plot is shown above the heatmaps. Three biological replicates for Ndt80 and Efg1 were evaluated for visualizing the CUT&RUN enrichment. Abbreviations: ChIP = chromatin immunoprecipitation; CUT&RUN = cleavage under targets and release using nuclease; GFP = green fluorescent protein.

Table 1:

PCR reaction mix and PCR cycling conditions to amplify universal A and unique B fragments.

	Universal A Fragment	**Unique B Fragment**				
dH_2O	75.5 µL	75.5 µL		**Temp (°C)**	**Time**	**Cycles**
DNA Polymerase Buffer	20 µL	20 µL		98	30 s	1
10 mM dNTP	2 µL	2 µL		98	20 s	30
Foward Primer (100 µM)	0.5 µL (AHO1096)	0.5 µL unique gRNA		58	20 s	
Reverse Primer (100 µM)	0.5 µL (AHO1098)	0.5 µL (AHO1097)		72	30 s	
DNA (1 ng/µL)	1 µL pADH110	1 µL pADH139		72	15 s	1
DNA Polymerase	0.5 µL	0.5 µL		8	hold	1

Table 2:

PCR reaction mix and PCR cycling conditions to stitch A and B fragments to create the full-length C fragment.

C Fragment PCR Reaction			**Cycling Condition 1**		
dH_2O	74.5 µL		Temp (°C)	Time	Cycles
DNA Polymerase Buffer	20 µL		98	30 s	1
10 mM dNTPs	2 µL		98	10 s	5
Universal A Fragment	1 µL		58	20 s	
Unique B Fragment	1 µL		72	60 s	
DNA Polymerase	0.5 µL				

Table 3:

PCR cycling conditions to PCR amplify the full-length C fragment.

Cycling Condition 2		
Temp (°C)	Time	Cycles
98	30 s	1
98	10 s	30
66	20 s	
72	60 s	
72	30 s	1
8	hold	1

Table 4:

PCR reaction mix and PCR cycling conditions to amplify the donor DNA GFP tag.

GFP Amplification PCR Reaction			**Temp (°C)**	**Time**	**Cycles**	
dH_2O	37.25 µL		98	30 s	1	
DNA Polymerase Buffer	10 µL		98	10 s	10	Δ−1 °C / cycle
10 mM dNTPs	1 µL		65	20 s		
pCE1 (1 ng/µL)	1 µL		72	30 s		
Forward Primer (100 µM)	0.25 µL		98	10 s	25	
Reverse Primer (100 µM)	0.25 µL		57	20 s		
DNA Polymerase	0.25 µL		72	30 s		
			72	15 s	1	
			8	hold	1	

Table 5:

Plasmid digestion reaction mix and reaction conditions for the plasmid digestion reactions.

Plasmid Digestion Reaction				
dH_2O	Variable		**Temp (°C)**	**Time**
pADH140	Variable		37	1 h
Restriction Enzyme Buffer	1.5 μL		65	15 m
Restriction Enzyme	0.8 μL		8	hold

Table 6:

PCR reaction mix and PCR cycling conditions for colony PCR.

cPCR Reaction					
dH_2O	11.66 µL				
cPCR DNA Polymerase Buffer	2.2 µL		**Temp (°C)**	**Time**	**Cycles**
5M Betaine	4.4 µL		94	30 s	1
$MgCl_2$	0.44 µL		94	10 s	35
10 mM dNTP	0.44 µL		55	30 s	
cPCR DNA Polymerase	0.22 µL		72	1 min	
Forward Primer (100 µM)	0.22 µL		72	15 s	1
Reverse Primer (100 µM)	0.22 µL		8	hold	1
Lysate	2.2 µL				

Table 7:

PCR cycling conditions for the end repair step of the library in preparation for sequencing.

Temp (°C)	Time	Total Number of Cycles
20	30 min	1
50	60 min	1
4	Hold	1

Table 8:

Adapter dilution recommendations for the input DNA to prepare sequencing libraries.

Input DNA	Adapter Dilution	Working Adapter Concentration
101 ng-1 µg	No dilution	15 µM
5–100 ng	10-fold	1.5 µM
< 5 ng	25-fold	0.6 µM

Table 9:

PCR cycling conditions to PCR amplify the adapter-ligated DNA.

	Temp (°C)	Time	Total Number of Cycles
Initial Denaturation	98	45 s	1
Denaturation	98	15 s	14
Annealing/Extension	65	10 s	
Final Extension	65	5 min	1
Hold	4	Hold	1

Materials

Name	Company	Catalog Number	Comments
0.22 μm filter	Millipore Sigma	SLGPM33RS	
0.65 mL low-adhesion tubes	VWR	490003-190	
1 M $CaCl_2$	Fisher Scientific	50-152-341	
1 M PIPES	Fisher Scientific	AAJ61224AK	
12-well untreated cell culture plates	Corning	351143	
2-mercaptoethanol	Sigma-Aldrich	60-24-2	
2% Digitonin	Fisher Scientific	CHR103MI	
50 mL conical tubes	VWR	89039-658	
5x phusion HF buffer	Fisher Scientific	F530S	Item part of the Phusion high fidelity DNA polymerase; referred to in text as "DNA polymerase buffer"
Agar	Criterion	C5001	
Agencourt AMPure XP magnetic beads	Beckman Coulter	A63880	
Agilent Bioanalyzer	Agilent	G2939BA	Referred to in the text as "capillary electrophoresis instrument"; user-dependepent
Amplitube PCR reaction strips with attached caps, Simport Scientific	VWR	89133-910	
Bacto peptone	BD Biosciences	211677	
Benchling primer design tool	Benchling		https://www.benchling.com/molecular-biology/; Referred to in the text as "the primer design tool"
Betaine	Fisher Scientific	AAJ77507AB	
Calcofluor white stain	Sigma-Aldrich	18909-100ML-F	
Candida Genome Database			http://www.candidagenome.org/
Concanavalin A (ConA) conjugated paramagnetic beads	Polysciences	86057-3	
Conda software			https://docs.conda.io/en/latest/miniconda.html
curl tool			http://www.candidagenome.org/download/sequence/C_albicans_SC5314/Assembly21/current/C_albicans_SC5314_A21_current_chromosomes.fasta.gz
CUTANA ChIC/CUT&RUN kit	Epicypher	14-1048	Referred to in the text as "the CUT&RUN kit"
Deoxynucleotide (dNTP) solution mix (10 mM)	New England Biolabs	N0447S	
Dextrose (D-glucose)	Fisher Scientific	D163	
Difco D-mannitol	BD Biosciences	217020	
Disposable cuvettes	Fisher Scientific	14-955-127	
Disposable transfer pipets	Fisher Scientific	13-711-20	
DNA Gel Loading Dye (6x)	Fisher Scientific	R0611	
DreamTaq green DNA polymerase	Fisher Scientific	EP0713	Referred to in the text as "cPCR DNA polymerase"

Name	Company	Catalog Number	Comments
DreamTaq green DNA polymerase buffer	Fisher Scientific	EP0713	Item part of the DreamTaq green DNA polymerase; referred to in the text as "cPCR DNA polymerase buffer"
E. coli spike-in DNA	Epicypher	18-1401	
ELMI Microplate incubator	ELMI	TRMS-04	Referred to in the text as "microplate incubator"
End Prep Enzyme Mix			Item part of the NEBNext Ultra II DNA Library Prep kit
End Prep Reaction Buffer			Item part of the NEBNext Ultra II DNA Library Prep kit
Ethanol 200 proof	VWR	89125-170	
FastDigest MssI	Fisher Scientific	FD1344	Referred to in the text as "restriction enzyme"
FastDigest MssI Buffer	Fisher Scientific	FD1344	Item part of the FastDigest MssI kit; referred to in the text as "restriction enzyme buffer"
Ficoll 400	Fisher BioReagents	BP525-25	
Fluorescence microscope			User-dependent
Gel electrophoresis apparatus			User-dependent
GeneRuler low range DNA ladder	Fisher Scientific	FERSM1192	
GitBash workflow			https://gitforwindows.org/
GitHub source code			https://github.com/akshayparopkari/cut_run_analysis
HEPES-KOH pH 7.5	Boston BioProducts	BBH-75-K	
High-speed centrifuge			User-dependent
Isopropanol	Sigma-Aldrich	PX1830-4	
Lens paper	VWR	52846-001	
Ligation Enhancer			Item part of the NEBNext Ultra II DNA Library Prep kit
Lithium acetate dihydrate	MP Biomedicals	215525683	
Living Colors Full-Length GFP polyclonal antibody	Takara	632592	User-dependent
MACS2			https://pypi.org/project/MACS2/
Magnetic separation rack, 0.2 mL tubes	Epicypher	10-0008	
Magnetic separation rack, 1.5 mL tubes	Fisher Scientific	MR02	
MgCl2	Sigma-Aldrich	M8266	
Microcentrifuge tubes 1.5 mL	Fisher Scientific	05-408-129	
Microplate and cuvette spectrophotometer	BioTek	EPOCH2TC	Referred to in the text as "spectrophotometer"; user-dependent
MochiView			http://www.johnsonlab.ucsf.edu/mochiview-downloads
MOPS	Sigma-Aldrich	M3183	
NaCl	VWR	470302-522	
NaOH	Fisher Scientific	S318-500	
NCBI GEO			https://www.ncbi.nlm.nih.gov/geo/
NEBNext Adaptor for Illumina			Item part of the NEBNext Multiplex Oligos for Illumina (Index Primers Set 1); referred to in the text as "Adapter"

Name	Company	Catalog Number	Comments
NEBNext Index X Primer for Illumina			Item part of the NEBNext Multiplex Oligos for Illumina (Index Primers Set 1); referred to in the text as "Reverse Uniquely Indexed Library Amplification Primer"
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	New England Biolabs	E7335S	
NEBNext Ultra II DNA Library Prep kit	New England Biolabs	E7645S	Referred to in the text as "library prep kit"
NEBNext Universal PCR Primer for Illumina			Item part of the NEBNext Multiplex Oligos for Illumina (Index Primers Set 1); referred to in the text as "Universal Forward Library Amplification Primer"
Nourseothricin sulfate (NAT)	Goldbio	N-500-2	
Novex TBE Gels, 10%, 15 well	Fisher Scientific	EC62755BOX	
Nutating mixer	VWR	82007-202	
Nutrient broth	Criterion	C6471	
pADH110	Addgene	90982	Referred to in the text as "plasmid repository ID# 90982"
pADH119	Addgene	90985	Referred to in the text as "plasmid repository ID# 90985"
pADH137	Addgene	90986	Referred to in the text as "plasmid repository ID# 90986"
pADH139	Addgene	90987	Referred to in the text as "plasmid repository ID# 90987"
pADH140	Addgene	90988	Referred to in the text as "plasmid repository ID# 90988"
pAG-MNase	Epicypher	15-1016 or 15-1116	50 rxn or 250 rxn
pCE1	Addgene	174434	Referred to in the text as "plasmid repository ID# 174434"
Petri dishes with clear lid	Fisher Scientific	FB0875712	
Phusion high fidelity DNA polymerase	Fisher Scientific	F530S	Referred to in the text as "DNA polymerase"
Polyethylene glycol (PEG) 3350	VWR	10791-816	
Potassium phosphate monobasic	Fisher Scientific	P285-500	
Qubit 1x dsDNA HS assay kit	Invitrogen	Q33230	
Qubit fluorometer	Life Technologies	Q33216	Referred to in the text as "fluorometer"; user-dependent
Rabbit IgG negative control antibody	Epicypher	13-0042	
RNase A	Sigma-Aldrich	10109169001	
Roche Complete protease inhibitor (EDTA-free) tablets	Sigma-Aldrich	5056489001	
RPMI-1640	Sigma-Aldrich	R6504	
Shaking incubator	Eppendorf	M12820004	User-dependent
Sorbitol	Sigma-Aldrich	S1876-500G	
Spin-X centrifuge tube filters	Fisher Scientific	07-200-385	
Sterile inoculating loops	VWR	30002-094	

Name	Company	Catalog Number	Comments
SYBR Gold nucleic acid gel stain	Fisher Scientific	S11494	
SYTO 13 nucleic acid stain	Fisher Scientific	S7575	Referred to in the text as "nucleic acid gel stain"
Thermocycler			User-dependent
ThermoMixer C	Eppendorf	5382000023	
Tris (hydroxymethyl) aminomethane	Sigma-Aldrich	252859-100G	
Ultra II Ligation Master Mix			Item part of the NEBNext Ultra II DNA Library Prep kit; referred to in the text as "Ligation Master Mix"
Ultra II Q5 Master Mix			Item part of the NEBNext Ultra II DNA Library Prep kit; referred to in the text as "High Fidelity DNA Polymerase Master Mix "
UltraPure salmon sperm DNA solution	Invitrogen	15632011	
USER Enzyme			Item part of the NEBNext Ultra II DNA Library Prep kit; referred to in the text as "Uracil Excision Enzyme"
Vortex mixer	VWR	10153-834	
wget tool			http://www.candidagenome.org/download/sequence/C_albicans_SC5314/Assembly21/current/C_albicans_SC5314_A21_current_chromosomes.fasta.gz
Yeast extract	Criterion	C7341	
Zymolyase 100T (lyticase, yeast lytic enzyme)	Fisher Scientific	NC0439194	