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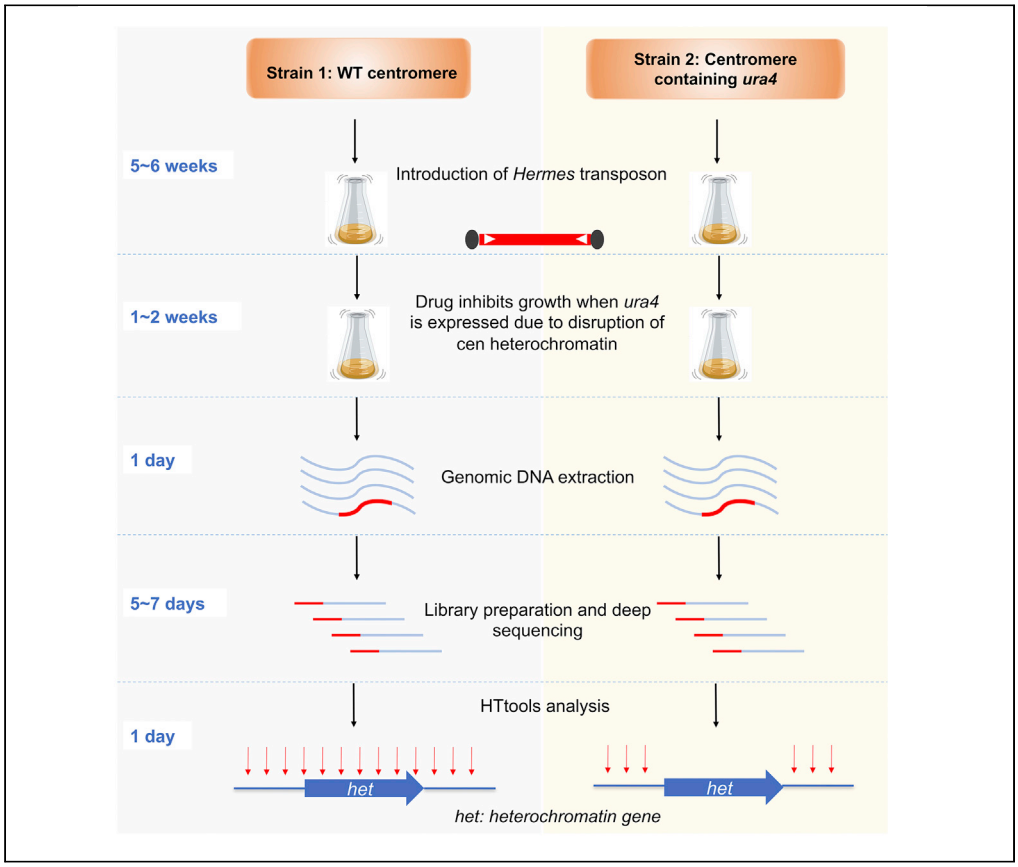

Transposon insertion sequencing (TIS) is a highly effective method used with bacteria to identify genes important for growth in any condition of interest. Previously, we adapted this method to identify essential genes of the yeast *Schizosaccharomyces pombe*. Here, we describe modifications used to identify genes necessary for the formation of centromeric heterochromatin.

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Highlights

Procedure to prepare strains with plasmids that produce *Hermes* transposition

Method to measure the accumulation of transposition events in liquid cultures

Selection strategy to identify genes important for heterochromatin formation

Library preparation and computational pipeline to analyze insertion site profiles

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A protocol for transposon insertion sequencing in *Schizosaccharomyces pombe* to identify factors that maintain heterochromatin

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SUMMARY

Transposon insertion sequencing (TIS) is a highly effective method used with bacteria to identify genes important for growth in any condition of interest. Previously, we adapted this method to identify essential genes of the yeast *Schizosaccharomyces pombe*. Here, we describe modifications used to identify genes necessary for the formation of centromeric heterochromatin.

For complete details on the use and execution of this protocol, please refer to Lee et al. (2020).

BEFORE YOU BEGIN

To understand centromere function and gene silencing it is critical to Identify regulators of heterochromatin formation. The limitation of previous screens for heterochromatin factors is that essential genes are mostly excluded. Here we described an approach to identify factors important for heterochromatin formation by transposon insertion sequencing (TIS). This protocol is made up of two distinct parts. The first is to generate cultures with *Hermes* integration. The *Hermes* transposon is introduced into both a wild-type (WT) strain and a strain containing *ura4* that is silenced in centromeric heterochromatin. Cultures with high integration frequency are passaged in 5-fluoroorotic acid (FOA) medium to select against insertion mutants that express *ura4* due to disruption of genes that contribute to the formation of centromere heterochromatin. The second part of this protocol is focused on library preparation for deep sequencing of integration profiles. Genomic DNA is fragmented and ligated to linkers. PCR amplification is conducted to capture fragments containing *Hermes* transposon termini and their adjacent flanks. After deep sequencing, the raw reads are processed and analyzed by HTtools, a software package designed to map *Hermes* integrations across the *S. pombe* genome. Genes with lower integration densities in the strain with *ura4* compared to the WT strain are candidates with a potential role in heterochromatin formation.

S. pombe media

Timing: 4–6 h

1. Make sure that there is enough liquid media and plates before you begin growth. Yeast extract with supplements (YES) plates are used to grow strains before introducing plasmids, while the

pombe glutamate medium (PMG) dropout media is to select for the plasmids (for additional details, please see (Sangesland et al., 2016)).

2. PMG dropout medium lacking leucine (PMG-L) is used to select for the *LEU2* gene on the transposase expression plasmid pHL2578. This synthetic medium provides high transposition activity.

   Note: When activating transposition vitamin B1 (thiamine) is excluded from media to induce *Hermes* transposase expression via the Rep81x-*nmt1* promoter. All measures to reduce even small amounts of contaminating B1 should be taken because trace levels of B1 inhibit expression. For this reason we use disposable plasticware.

3. Solid agar plates are made by combining an equal volume of 4% (w/v) Difco Bacto Agar to 2× concentrated liquid media. 1 L liquid media with 2% agar is enough to fill approximately forty 100 mm plates. The plates should be stored at 4°C and are good for at least 6 months.
4. Vitamin B1 is added to a final concentration of 10 μM to repress the promoter Rep81x-*nmt1*, 5-fluoroorotic acid (FOA) to a final concentration of 1 mg/mL, and antibiotic (G418) to a final concentration of 500 μg/mL corrected for purity.
5. Four days before the start of the experiment, streak the strains of interest on YES plates and incubate at 32° C.

   Note: all incubations are performed at 32° C and for liquid media set the shaker to 230 rpm.

YES and PMG media recipe

Media	Reagent	Amount/volume
YES media (per liter), store at 25°C	yeast extract	5 g
	glucose	30 g
	complete dropout powder [a]	2 g
PMG (per liter), store at 25°C	potassium hydrogen phthalate	3 g
	Na_2PO_4	2.2 g
	l-glutamic acid monosodium salt	3.75 g
	glucose	20 g
	50× salt stock [b]	20 mL
	1000× vitamin stock [c]	1 mL
	10,000× mineral stock [d]	0.1 mL
	dropout powder lacking uracil and leucine	2 g

[a]**Complete dropout powder:** 5 g adenine SO_4 and 2 g each of the following amino acids: alanine, arginine HCl, aspartic acid, asparagine H_2O, cysteine HCl·H_2O, glutamic acid, glutamine, glycine, histidine HCl·H_2O, isoleucine, leucine, lysine HCl, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, uracil, and valine.
[b]**50× salt stock (per liter):** 2 g Na_2SO_4 [14.1 mM], 50 g KCL [0.67 M], 0.735 g $CaCl_2$ $2H_2O$ [4.99 mM], and 52.5 g $MgCl_2$ $6H_2O$ [0.26 M]. Dissolve in deionized water and autoclave.
[c]**1000× vitamin stock (per liter):** 1 g pantothenic acid [4.20 mM], 10 g nicotinic acid [81.2 mM], 10 g inositol [55.5 mM], and 10 mg biotin [40.8 μM]. Dissolve in deionized water and filter sterilize.
[d]**10,000× mineral stock (per liter):** 5 g boric acid [80.9 mM], 4 g $MnSO_4$ [23.7 mM], 4 g $ZnSO_4$ ·$7H_2O$ [13.9 mM], 2 g $FeCl_2$ · $6H_2O$ [7.40 mM], 0.4 g molybdic acid [2.47 mM], 1 g KI [6.02 mM], 0.4 g $CuSO_4$ ·$5H_2O$ [1.60 mM], and 10 g citric acid [47.6 mM]. Dissolve in deionized water and filter sterilize.

Selection media recipe

Media	FOA	Leucine	Uracil	Thiamine	G418
YES + G418	-	-	-	-	500 μg/mL
PMG + U + L + B1 + FOA	1 mg/mL	250 μg/mL	50 μg/mL	10 μM	-
PMG + U –L + B1 + G418	-	-	250 μg/mL	10 μM	500 μg/mL
PMG + U – L – B1 + G418	-	-	250 μg/mL	-	500 μg/mL
PMG + FOA	1 mg/mL	250 μg/mL	50 μg/mL	10 μM	-
PMG + FOA + G418	1 mg/mL	250 μg/mL	50 μg/mL	10 μM	500 μg/mL

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
5-Fluoroorotic acid (FOA)	USBiological	207291-81-4
Thiabendazole	Sigma	T8904
EDTA-free protease inhibitor cocktail	Roche	11836170001
G418	Sigma	A1720
ssDNA (Herring sperm DNA)	(Rai et al., 2018a, 2018b)	D3159
LiOAc	Sigma	517992
PEG4000	Sigma	N/A
Zymolyase 100T	Seikagaku Kogyo	N/A
Sorbitol	Sigma	S1876
Citric acid monohydrate	Sigma	C1909
Na_2HPO_4*$7H_20$	Sigma	431478
Tris	Sigma	10708976001
EDTA	Sigma	E9884
NaOH	Sigma	S8045
KOAc	Sigma	P1190
SDS	Sigma	L3771
NaCl	Sigma	S7653
Proteinase K	Thermo Fisher	EO0491
RNase A	Thermo Fisher	EN0531
T4 ligase	NEB	M0202M
10x Clontech buffer	Clontech	S1795
Titanium Taq polymerase	Clontech	639210
Critical commercial assays		
Agencourt AMPure XP beads	Beckman Coulter	A63880
PCR purification kit	Qiagen	N/A
KAPA library quantification kit	KAPA	28106
Experimental models: Organisms/strains		
YHL10155 (*mat1Msmt0; Leu1-32; ade6-M210; His2; Ura4 DS/E;* cen1 *otr1R(Sph1)::ura4+)*	(Chalamcharla et al., 2015)	N/A
YHL912 (*h- ura4-294 leu1-32*)	(Levin, 1995)	N/A
Oligonucleotides		
HL1870 (GTAATACGACTCACTATAGGGCTCCGCTTA AGGGAC)	(Lee et al., 2020)	N/A
HL1871 (5′Phos/TAGTCCCTTAAGCGGAG/3′AmMO/)	(Lee et al., 2020)	N/A
HL3508 (AATGATACGGCGACCACCGAGATCTACACT CTTTCCCTACACGACGCTCTTCCGATCTNNNNNCTATGT GGCTTACGTTTGCCTG)	(Lee et al., 2020)	N/A
HL3509 (CAAGCAGAAGACGGCATACGAGATCGGTCT CGGCATTCCTGCTGAACCGCTCTTCCGATCTGTAATAC GACTCACTATAGGGC)	(Lee et al., 2020)	N/A
Recombinant DNA		
pHL2577 (contains kanMX6 flanked by *Hermes* terminal inverted repeat (TIR) left, right)	(Evertts et al., 2007)	N/A
pHL2578 (expresses *Hermes* transposase)	(Evertts et al., 2007)	N/A
pHL1768 (Rep81X, empty vector lacking *Hermes* transposase, serves as control.	(Evertts et al., 2007)	N/A
Software and algorithms		
HTtools release v1.1.1: https://github.com/NICHD-BSPC/httools/releases/tag/v1.1.1	https://github.com/NICHD-BSPC/httools_py/	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Other		
Cooled micro centrifuge for 1.5–2.0 mL tubes	Eppendorf	N/A
Cooled tabletop centrifuge for 50 mL tubes	Beckman	N/A
Vortex	N/A	N/A
Rotating wheel at 32°C	N/A	N/A
Thermocycler	Bio-Rad	N/A

STEP-BY-STEP METHOD DETAILS

Step 1: Introduce the *Hermes* transposon and transposase plasmids sequentially into strains characterized by TIS

Timing: 10 days

Note: To identify genes important for heterochromatin formation we use two strains that differ in that one (YHL10155) has *ura4* inserted into the centromere of chromosome 1 (cen 1 *otr1R::ura4*) where it is silenced by heterochromatin. The other strain (YHL912) does not have *ura4* in the centromere (cen1 WT) (Figure 1). *Hermes* transposition is induced to generate high-density profiles of transposon integration in both strains. Integration of the *Hermes* transposon that disrupts genes required for heterochromatin silencing results in the expression of *ura4* in the centromere. These cells are removed from the culture by FOA. Genes with a potential role in forming heterochromatin accumulate significantly fewer insertions in the strain with *ura4* in centromere 1 that the strain with the wild type centromere. In this initial Step of the protocol we introduce the plasmid containing the *Hermes* transposon with *kanMX6* (pHL2577) and the plasmid that expresses the transposase (pHL2578) into the strains to be assayed by TIS (Figure 2A). The transposase excises the *Hermes* transposon and integrates it into the host genome (Figure 2B).

Prepare competent cells

1. Scrape a matchhead sized amount of YHL10155 and YHL912 cells from the YES plates and resuspend the cells thoroughly in 1 mL of YES media. Measure the OD_{600} of the resuspended cultures using dilutions with YES media to obtain a reading in the linear range of your spectrophotometer, usually between OD_{600} 0.1 and OD_{600} 0.6. Use these measurements to start a 50 mL culture of YHL10155 and YHL912 in YES media at OD_{600} = 0.05. Shake cultures at 32°C for 16 h.
2. When the cultures reach a density of OD_{600} = 0.5, centrifuge at 1600 *g*, 5 min at 25°C.
3. Resuspend cells with 50 mL sterile ddH_2O and centrifuge as above.
4. Resuspend cells in 25 mL TE + 0.1 M LiOAc and centrifuge as above.
5. Resuspend the cell pellet in 0.5 mL TE + 0.1 M LiOAc.
6. Put each strain in a 1.5 mL micro-centrifuge tube and roll at 32°C for 60 min.
7. Make 0.1 mL aliquots for immediate use.

First transformation to introduce plasmid containing Hermes transposon (pHL2577)

Transformation solution	
Premix	Volume (μL)
Competent cells	100
ssDNA (10 mg/mL)	5
Plasmid (~500 ng/μL)	5
PEG solution (40% PEG 4000 in 1xTE + 0.1 M LiOAc)	700
Total	810

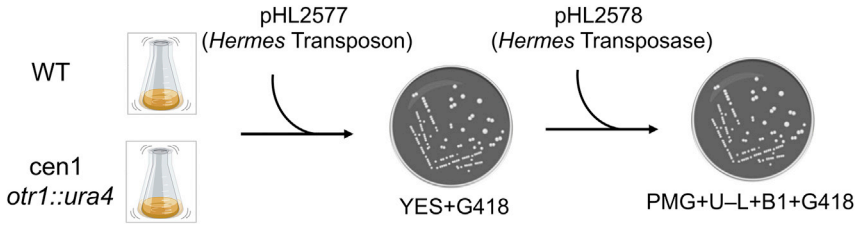

Figure 1. Step 1: Introduce *Hermes* transposon and *Hermes* transposase expression plasmids into wild type (WT) and reporter strains (otr1:: ura4)

The plasmid pHL2577 contains the *Hermes* transposon with kanMX6, the marker that encodes resistance to G418 and is used to select for the plasmid. The *Hermes* transposase is expressed from the plasmid pHL2578 which contains LEU2, the selection marker used to retain the plasmid.

Note: The PEG solution can be stored at 25°C for 6 month.

8. Add 5 μL of pre-boiled ssDNA (10 mg/mL), which is to enhance the uptake of plasmid.
9. Add 5 μL plasmid DNA to each aliquot except one which will serve as a transformation control to test whether colonies isolated in steps 14 and 15 are derived from the *S. pombe* being studied and not contamination of bacteria or mold.
10. Incubate the mixture on roller at 32°C for 30 min.
11. Add 0.7 mL PEG solution.
12. Incubate 60 min. at 32°C.
13. Heat shock 5 min. at 42°C.
14. Pellet cells with a brief spin in a microfuge, resuspend in 0.2 mL sterile ddH_2O and spread onto two YES plates equally. After 24 h, cell mass should form. Then replica-print the plates onto YES + G418 plates. After 3 days, cells containing the plasmid should form colonies.
    a. Pick 4 average-sized clones with a sterile toothpick.
    b. Streak each on YES + G418 plates to form single colonies.
    c. Use these purified colonies to inoculate patches on YES + G418. When cells are grown, place into vials with 1.5 mL YES containing 15% glycerol for storage at −80°C.

Pause point: The strains can be stored at −80°C indefinitely until ready to proceed to next step. To continue the protocol use the frozen vials to inoculate plates containing YES + G418.

Second transformation to add the plasmid with the Hermes *transposase (pHL2578)*

15. Repeat steps (1–14) for the 2nd transformation to introduce the transposase expression plasmid (pHL2578) or empty vector (pHL1768) into the strains containing the *Hermes* transposon plasmid (pHL2577) obtained in step 14 with the following modifications: In step 1 inoculate replicate strains containing pHL2577 and start the culture in YES + G418 for 16 h, which is to keep selection pressure for the *Hermes* transposon plasmid. In step 14, instead of YES, spread cells directly onto PMG + U – L + B1 + G418 plates to select for pHL2577 with G418, and pHL2578 with –Leucine. After 3 days, pick 4 single clones and streak on PMG + U – L + B1 + G418 plates. Use the purified colonies to inoculate patches on PMG + U – L + B1 + G418. Once grown at 32°C for 16 h these cells will be used immediately in step 16.

Note: We found maintaining cells with both plasmids causes genome instability because the *Hermes* transposon is very active. So every effort should be made to limit the number of cell divisions between transformation of the transposase plasmid and start of the induction cultures.

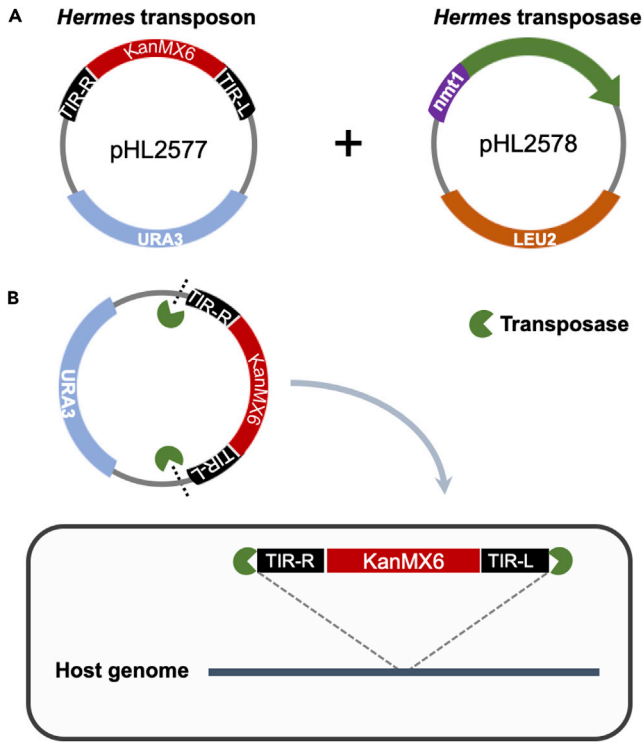

Figure 2. Induction of *Hermes* transposition *in S. pombe*

(A) Two plasmids were generated to induce *Hermes* transposition. pHL2577 provides the source of transposon DNA flanked by TIR-R and TIR-L. The kanMX6 cassette was inserted between the TIRs, giving cells with transpositions resistance to the drug G418. pHL2578 uses the *nmt1* promoter to express the *Hermes* transposase. TIR: terminal inverted repeats.

(B) The TIRs are bound by the transposase that excises the *Hermes* transposon and integrates the transposon DNA into a new genomic location.

Step 2: Generating library of Hermes insertions

Timing: 15 days

Note: This Step is to induce *Hermes* transposition and generate pools of cells with single integration events (Figure 3). The expression of *Hermes* transposase is induced in the absence of vitamin B1, which represses the *nmt* promoter. The *Hermes* transposase is expressed from the plasmid pHL2578 which also contains *LEU2*, the selection marker used to retain the plasmid. The plasmid pHL2577 contains the *Hermes* transposon with *kanMX6*, the marker that encodes resistance to G418 and is used to select for the plasmid. The expression cultures are passaged until approximately 10% of the cells have an integration as monitored by the quantitative transposition assay (step 22). Typically, 12 sequential passages (approximately 80 generations of cell division) are required. Additional growth risks introducing unneeded selection bias in the insertion strains and could generate too many cells with more than one insertion. Following transposition, pHL2577 is removed so that G418 can be used to identify cells with *de novo* insertions of *Hermes*. For this step *URA3* is used with FOA to evict pHL2577. FOA not only selects against the plasmid with *Hermes-kanMX6* but it also provides the selection that removes cells with defects of the centromeric heterochromatin that silences *ura4*.

Strains for integration profiling	
Strains used	Description
YHL10155 + pHL2577 + pHL2578	cen1 *otr1::ura4* with *Hermes* transposon and transposase
YHL10155 + pHL2577 + pHL1768	cen1 *otr1::ura4* with *Hermes* transposon, but without transposase
YHL912 + pHL2577 + pHL2578	cen1 WT with *Hermes* transposon and transposase
YHL912 + pHL2577 + pHL1768	cen1 WT with *Hermes* transposon, but without transposase

⚠ CRITICAL: To ensure robust statistical measures of integration density in each gene we recommend that four biologically independent cultures of each strain be processed as described below. Our independent cultures were also independent transformants of the plasmids.

16. For each strain, use the patches of step 15 to inoculate a matchhead amount of cells into one 5 mL PMG + U – L + B1 + G418 culture. Let the cells grow for 16 h at 32°C shaking at 230 rpm.
17. Next morning, briefly centrifuge and resuspend the cells to wash the starter culture four times with 5 mL of PMG + U – L – **B1** + G418 media.

⚠ CRITICAL: This step removes residual B1 which will inhibit the *nmt1* promoter that induces expression of transposase.

18. With these cells, inoculate 50 mL PMG + U – L – B1 + G418 cultures for each strain at a starting OD_{600} = 0.05.
19. Grow cultures in 32° C shaking incubator over night to achieve OD_{600} >2.0.
20. The next day measure OD_{600}, and use these cells to inoculate new 50 mL cultures of PMG + U – L – **B1** + G418 at OD_{600} = 0.05.
21. Repeat steps 19–21 until 10% of cells contain an integration event as monitored by the method described below (step 22). During the multiday procedure that measures integration frequency, continue the daily passages of the transposition cultures (steps 19–21).
22. Measure the transposition frequency every two or three days of passaged cultures.
    a. Plate 100 μL of OD_{600} = 0.05 and 100 μL of OD_{600} = 0.005 on PMG + FOA. Plate 100 μL of OD_{600} = 0.5 and 100 μL of OD_{600} = 0.05 on PMG + FOA + G418.
    b. After 3 days of incubation at 32° C, count the number of colonies. The transposition frequency is calculated as the number of colonies on PMG + FOA + G418 divided by the number of colonies on PMG + FOA taking into account dilution factors required to count colonies.
    c. Repeat until the transposition frequency > 10%.
23. Once the transposition frequency exceeds 10%, use the cells from the PMG + U – L –B1 + G418 culture of step 21 to inoculate a 500 mL culture of PMG + U + L + B1 + FOA at starting OD_{600} =0.5. This selects against the plasmid containing the *Hermes* transposon.
24. After 24 h of growth at 32°C, use these cells to inoculate a 500 mL culture of PMG + FOA + G418 at starting OD_{600} = 0.5. Grow this culture around 12∼16 h until it reaches saturation, approximately OD_{600} =6. This culture selects for cells with *Hermes* integration that also lack the plasmid containing the *Hermes* transposon.

Note: FOA is used to select against cells expressing *URA3* in the *Hermes* transposon plasmid. FOA is toxic to the yeast cells because *URA3* converts the FOA into the toxic substance 5-fluorouracil.

25. We reserve and freeze these cells in 10 mL aliquots at OD_{600} = 5.0. To make 25 10 mL aliquots determine the final OD of the PMG + FOA + G418 culture and calculate the volume of culture containing 1,250 OD units of cells.
26. Centrifuge this volume of culture at 1,700 *g* for 5 min to pellet the cells.

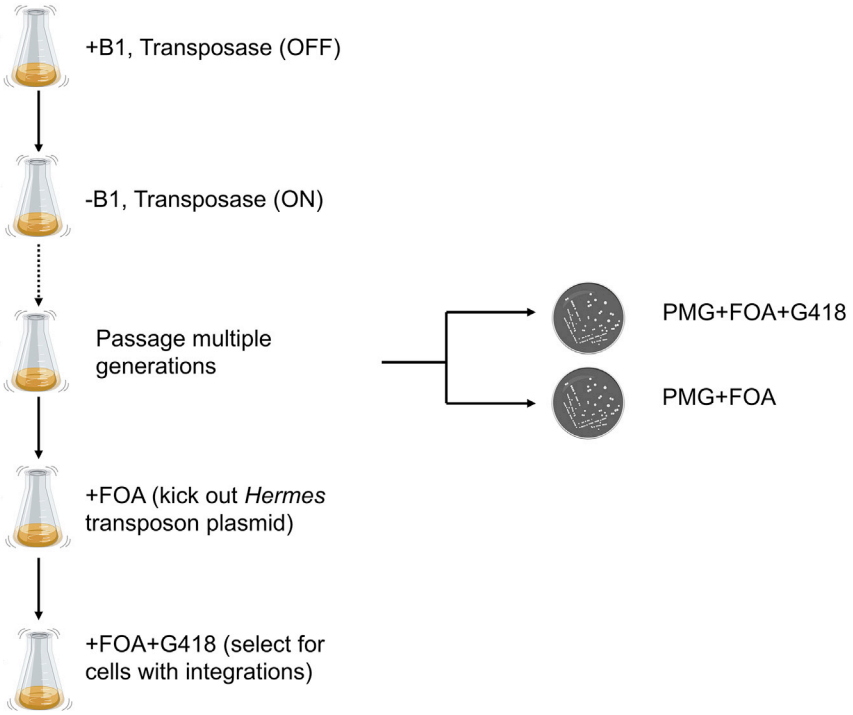

Figure 3. Step 2: Generation of *Hermes* integrations

Insertions of the transposable element *Hermes* were produced in cells with WT cen1 and cen1 *otr1R::ura4*. The transposition process began with expression of *Hermes* transposase. The cells were passaged for multiple generations until ~10% of cells had *Hermes* integrations which were monitored by comparing colony numbers on PMG + FOA plates with and without G418. Growth in liquid medium with FOA removed the expression plasmid. Subsequent growth in liquid medium containing FOA + G418, selected for cells that lacked the expression plasmid and had insertions.

27. Decant the supernatant and re-suspend in 250 mL of sterile 15% glycerol with YES media.
28. Distribute to twenty-five 15 mL snap-cap Falcon tubes, in 10 mL/aliquots and store at −80°C.

Pause point: The aliquots can be stored at −80°C until ready to proceed to next step.

Step 3: TIS to identify genes important for forming heterochromatin

Timing: 15 days

Note: The cells from the previous procedure have been grown for approximately five generations in the presence of FOA. This selection accomplishes two goals; it selects against the *Hermes* transposon containing donor plasmid and it begins the selection against *Hermes* insertions that disrupt genes important for forming heterochromatin. (Figure 4). Such mutations that impair heterochromatin in the centromere activate expression of *ura4* and as a result inhibit growth. The following procedure passages the cultures for 80 additional generations in FOA which allows the identification of many more genes that contribute to heterochromatin. Following growth in FOA the cells are used to perform high throughput sequencing to map insertion sites.

29. For each strain and its replicates, thaw 1 −80°C frozen aliquot (each aliquot is 10 mL of OD_{600} = 5.0) on ice.

30. Centrifuge cells at 1,700 *g* for 5 min to pellet.
31. Pour off glycerol, wash twice by resuspending in PMG + U + L + B1 liquid media.
32. Pour off media, resuspend in 10 mL of PMG + U + L + B1 + FOA.
33. Use 2 mL to inoculate a 100 mL culture of PMG + U + L + B1 + FOA.
34. Measure OD_{600}, should be ~ 0.1, which is a total of 2.0×10^8 cells.
35. Grow cultures for 16 h shaking in 32°C incubator.
36. The next day, the OD_{600} should be approximately 6.0. Use these cells to inoculate new 100 mL cultures of PMG + U + L + B1 + FOA at starting OD_{600} = 0.05.

Note: With the remaining cells of the culture, approximately 200 OD units, pellet and freeze the cells at −80°C. These will be used to isolate genomic DNA for deep sequencing insertions sites at this initial point of propagation in FOA.

37. Repeat inoculating fresh 100 mL PMG + U + L + B1 + FOA cultures at OD_{600} = 0.05 every day until the number of cumulative generations reaches 80. Calculate generations using equation:

$$\text{Generations} = \ln(OD_{final}/OD_{initial})/0.693$$

38. When number of generations reaches 80:
    a. Spin down a few 10 aliquots of culture and resuspend in 2 mL 15% glycerol to make perms and freeze at −80°C (in case you decide to grow for additional generations).
    b. Spin down remaining cultures at 1,700 *g* for 5 min and make 200 OD unit pellets to be stored at −80°C. These will be used for deep sequencing the insertion library at 80 generations of growth.

Step 4: Library preparation for deep sequencing of integration profiles

Timing: 6 days

Note: The goal of this Step is to prepare libraries for deep sequencing and identify *Hermes* integrations throughout the genome by bioinformatic analysis. Separate libraries are made for each replicate of each strain. The process is a specialized form of targeted sequencing using ligation mediated PCR (Guo et al., 2013). We incorporate Illumina's P5 and P7 binding sequences into primers that recognize the TIR-L of the *Hermes* transposon. We describe the extraction of genomic DNA, the digestion of the DNA with MseI, the ligation of linkers to the MseI fragments, the PCR amplification of insertion sites, the gel purification of the PCR products, and Illumina sequencing of the amplified DNA (Figure 5).

Extracting genomic DNA

39. To identify genes with strong contributions to heterochromatin we isolate DNA and sequence insertions from cells obtained early in the propagation in FOA (step 36). Many more candidate genes are identified from genomic DNA of cells grown for 80 additional generations in FOA (step 37). From steps 36 and 38b re-suspend cell pellets of 200 OD units in 5 mL of **SP1 buffer** with 30 mg zymolyase previously dissolved.

SP1 buffer (once sterilized, store at 25°C for 6 months)	
Reagent	Final concentration
Sorbitol	1.2 M
Citric Acid mohohydrate	50 mM
$Na_2PO_4.7H_2O$	50 mM
EDTA	40 mM
Adjust pH 5.6 with 1 M NaOH	

Note: Combine 50 mg of dry zymolyase with 5 mL of SP1 buffer and mix gently. The zymolyase may not dissolve completely, but do not heat for the solubilization. Use a short spin in a micro-fuge to remove the precipitate. Use the supernatant and store this solution in aliquots at −20°C.

40. Incubate at 37°C for 2 h or until cells are dark under a phase contrast microscope.
41. Pellet spheroplasted cells by spinning at 1600 *g* for 5 min.
42. Re-suspend in 15 mL 5X TE (50 mM Tris, 5 mM EDTA, pH 7.9). Add 1.5 mL 10% SDS and incubate at 25°C for 60 min. Then incubate at 65°C for 5 min.
43. Transfer to 50 mL Falcon screw-cap tube. Add 5 mL 5 M KOAc and incubate at 25°C for 30 min.
44. Centrifuge at 1600 *g* for 15 min to pellet debris. Pipette supernatant into high-resistance, thick-plastic tube (next step requires high-speed centrifugation).
45. Add 20 mL ice-cold isopropanol to supernatant, incubate on ice for 5 min.
46. Centrifuge at 8000 *g* for 10 min to pellet the nucleic acids. Decant and let pellet air-dry until liquid evaporates.
47. Re-suspend pellet in 6 mL 5X TE. Add RNase A to a final concentration of 100 μg/mL.
48. Incubate at 37°C for 1 h.
49. Add 0.2 mL 10% SDS and Proteinase K to final concentration of 50 μg/mL. Incubate for 2 h to 16 h at 50°C.
50. Add equal volume of equilibrated Phenol and mix vigorously. Centrifuge at 8000 *g* for 5 min. Extract the top aqueous layer to a fresh tube.
51. Repeat step 50 twice, or until interface between aqueous and organic layer is clear.
52. Add equal volume of Phenol/$CHCl_3$/IAA, mix, centrifuge at 8000 *g* for 5 min. Extract the top aqueous layer to a fresh tube.
53. Repeat step 52 twice, or until interface between aqueous and organic layer is clear. For last extraction, leave 20% of aqueous volume behind.
54. EtOH precipitate DNA by adding 1/10 volume 5 M NaCl and 2.5 volumes 100% ethanol.

Optional: keep in −20°C freezer for 2 h

55. Aliquot solution to micro-centrifuge tubes. Centrifuge at 8000 *g* for 20 min on table-top centri-fuge.
56. Wash pellet with 70% ethanol. Air dry the pellet.
57. As an optional step to improve purification re-suspend DNA in 200 μL TE and perform an addi-tional ethanol precipitation by adding 20 μL 5 M NaCl and 2.5 volumes 100% ethanol. Wash pel-let with 70% ethanol. Air dry the pellet.
58. Re-suspend DNA in 200 μL TE. Run small aliquot (5 μL) of DNA on 1% agarose gel with standards to gauge the quality of purified gDNA. Two examples of genomic DNA (gDNA) are shown below and we estimate the size of the gDNA is between 10 kb and 23 kb (Figure 6). Here you can use Qubit or Picogreen to quantify the gDNA concentration and normalize the concentration of each sample at 50 ng/μL. The total yield of gDNA extraction from 200 OD unit cells is around 10∼20 μg.

Pause point: The genomic DNA can be stored at −80°C for several months.

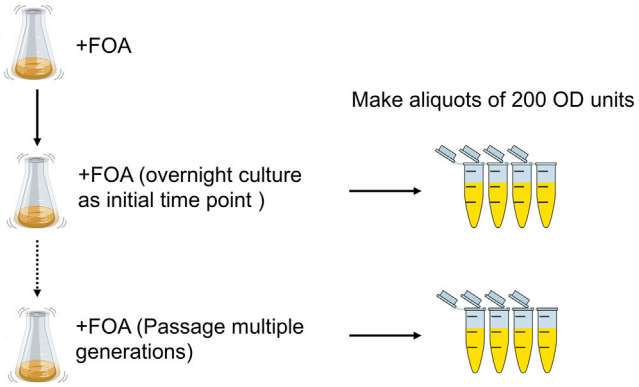

Figure 4. Step 3: TIS selection to identify genes important for forming heterochromatin

The cultures obtained from Step 2 were passaged for additional generations in the presence of FOA. The cells from the second FOA cultures represented the initial time point. Subsequent passages in FOA occurred until a total of 80 generations were achieved.

Cut genomic DNA with Mse I

Note: MseI, which recognizes TTAA cuts extensively the AT-rich genome of *S. pombe*. The digestion generates fragments containing *Hermes* transposon TIR-L and genomic flanking DNA (Figure 5).

59. Make a pool of common reagents (enough for 7 digests) for MseI digestion. The instructions and proportions below are used to make a high coverage library of a single culture replicate.

Mse I digestion reaction		
Component	X1	X8
10X NEB Buffer #4	10 µL	80 µL
100X NEB BSA	1 µL	8 µL
10 U/µL NEB MseI	3 µL	24 µL
dH_2O	66 µL	500 µL

60. To each of 7 micro-centrifuge tubes, add 80 µL of pool mix, 20 µL of gDNA (concentration ~ 50 ng/µL). Put in 37°C incubator O/N.

Purification of MseI-digest with magnetic beads

61. Pool together digests. Distribute to aliquots of 160 µL.
62. Add to each aliquot 1.8 × volume AMPure XP solution (Beckman). Pipette-mix 10 times. A short "touch" spin can bring down the solution stuck to wall.
63. Let sit at 25°C for 5 min.
64. Place tubes on magnetic stand, let sit for 2 min.
65. Pipette out solution.
66. Add 500 µL 70% ethanol, let sit for 30 sec. Pipette out Ethanol.
67. Repeat step 66, for 2 total washes.
68. Remove residual ethanol. Let air-dry for at least 5 min until there is no residual ethanol at the bottom of the tube.

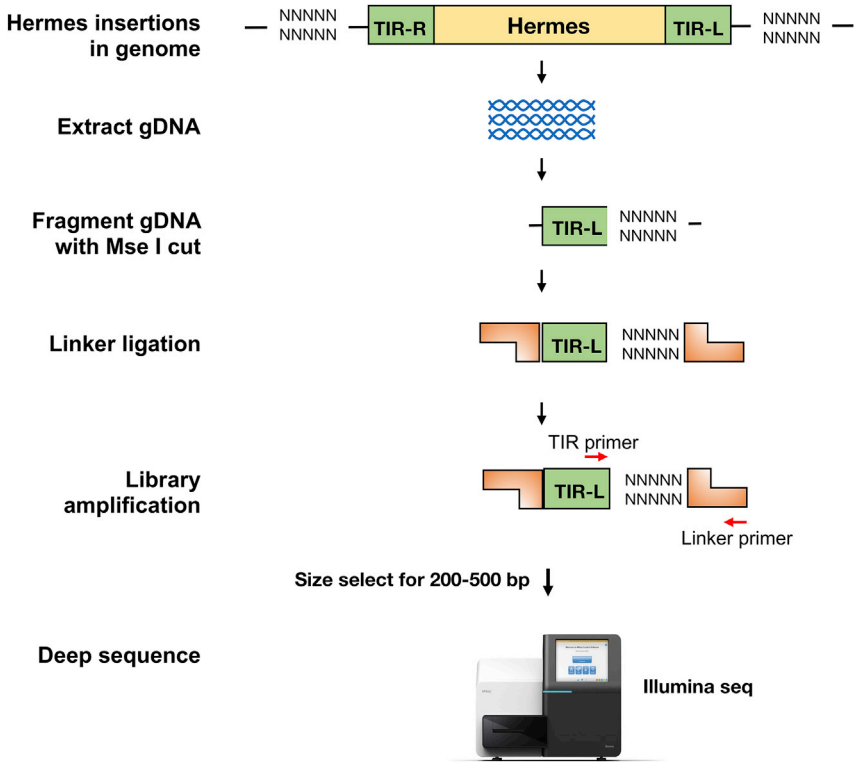

Figure 5. Step 4: Scheme of library preparation and deep sequencing

High-throughput sequencing of Hermes integrations depends on amplifying insertions with ligation-mediated PCR.

69. Elute with 50 μL Buffer EB. Pipette-mix thoroughly. A short "touch" spin can bring down the solution stuck to wall.
70. In order to separate the eluted DNA from the magnetic beads, place tubes back on magnetic stand, wait 3 min until the eluate is clear.
71. Collect eluate in new tube and try not to disturb the beads in the bottom.
72. Run a 1.0% agarose gel with standards to check yield after AMPure XP purification (Figure 7. lane 2–5).

Linker preparation

Note: It is critical that HL1871 have a 3′ blocked nucleotide. Otherwise the 3′ end of HL1871 in linker ligated to both ends of any fragment of genomic DNA would be extended and result in amplification during PCR (described below). With the 3′ block PCR specifically amplifies *Hermes* TIR-L and the adjacent genomic DNA. The linker annealing can be done in advance of the linker ligation to the genomic DNA fragments. The annealed linker can be stored at −20° C for several months.

73. Mix the following reagents:

Reagent	Volume
100 μM HL1870	5 μL
100 μM HL1871	5 μL
10X Clontech PCR Buffer	5 μL
dH_2O	35 μL

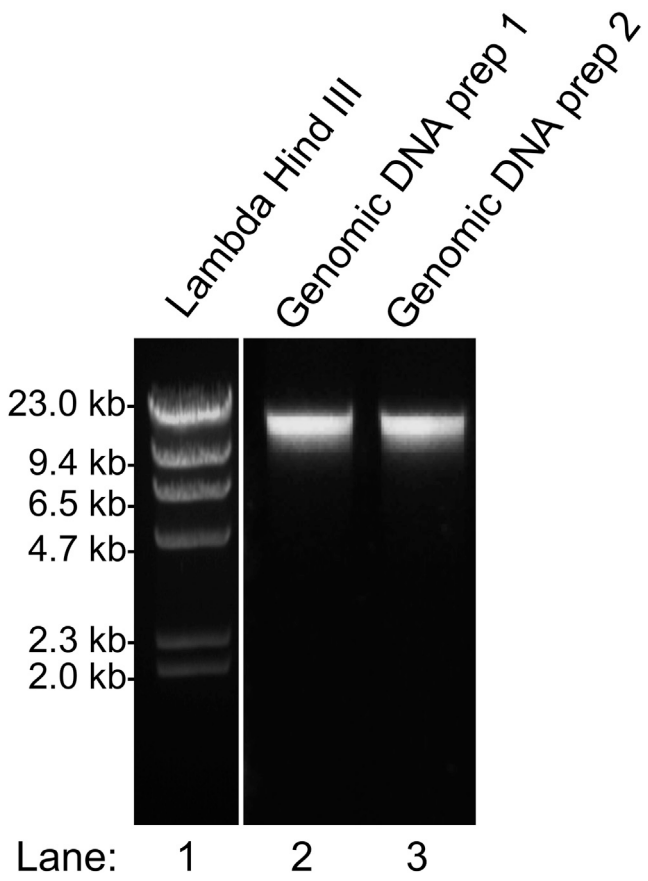

Figure 6. Gel of extracted genomic DNA

Lane 1: lambda phage DNA (250 ng) digested with HindIII; Lane 2 and 3: two genomic DNA samples in a 1% agarose gel run at 80 V for 1.5 h

Note : 10x Clontech PCR buffer: 100 mM Tris-HCl(pH 8.9), 500 mM KCl, 15 mM $MgCl_2$

74. Aliquot to small PCR tubes.
75. Run the following (pre-set) program in PCR machine (Bio-Rad Thermal Cycler T100):

PCR cycling conditions

Steps	Temperature	Time	Cycles
Initial denaturation	94°C	1:45 min	1
Annealing	80°C	10 min	1
Annealing	70°C	10 min	1
Annealing	60°C	10 min	1
Annealing	50°C	10 min	1
Annealing	40°C	10 min	1
Annealing	30°C	10 min	1
Annealing	20°C	10 min	1
Hold	4°C	Forever	

Ligate linker to MseI-digested fragments of gDNA

76. Set up following pool (× 12) of common reagents:

Reagent	1 ×	12 ×
10 × Ligation buffer (see below)	2 μL	24 μL
Purified MseI digest (~35 ng/μL)	12 μL	144 μL
10 mM ATP	2 μL	24 μL
Annealed linker	3.5 μL	42 μL
NEB T4 DNA Ligase	0.5 μL	6 μL

⚠ CRITICAL: Here, you need to set up a no-linker control to test for efficient ligation. You will see an upward shift due to ligation of larger fragments (Figure 7, lane 6–9). The upward shift is an indication of robust ligation. Low ligation efficiency results in poor library content.

77. Pipette 16.5 μL of mix to a tube (negative control, which has no linker). Add 3.5 μL of dH_2O.
78. For each of the replicate samples distribute 16.5 μL of mix and 3.5 μL 10 μM linker to 11 PCR tubes.
79. Run in PCR machine, set to program: 16°C for 16 h, 65°C for 10 min, 4°C until sample retrieved.
80. Run a 2% agarose gel with size standards to check the ligation efficiency (Figure 7, lane 6–9).

Composition of 10X Ligation buffer	
Reagent	Final concentration
Tris Buffer, pH 7.4	0.5 M
$MgCl_2$	0.1 M
Dithiothreitol	0.1 M
BSA (250 ug/mL)	1 mg/mL

Note: Filter-sterilize and make 25 μL aliquots. Store at −20°C

PCR amplification of insertions

Note: This set of steps is to use the linker-ligated genomic DNA fragments as templates for PCR amplification of genomic sequences flanking *Hermes* integration sites. TIR primer specifically binds to the end of *Hermes* TIR-L and extends across the linker filling in the 3′ end allowing the linker primer to anneal and initiate amplification (Figure 5).

⚠ CRITICAL: To produce libraries with the highest numbers of insertions we use up to 95 independent PCR reactions for each sample.

81. Set up following pool of common reagents:

Library PCR amplification reaction		
Reagent	1×	100×
10× Clontech buffer	2 μL	200 μL
50× dNTP mix (10 mM)	0.4 μL	40 μL
10 μM TIR primer HL3508	0.4 μL	40 μL
10 μM Linker primer HL3509	0.4 μL	40 μL
Clontech Titanium Taq	0.4 μL	40 μL
dH_2O	14.8 μL	1480 μL

82. To one PCR well in plate, add 18.4 μL of mix and 1.6 μL dH_2O (no DNA negative control). Each replicate sample requires its own 96 well plate and its own version of HL3508 with unique sequence in place of Ns (See step 94).
83. To rest of mix, add 158.4 μL of linker ligated DNA template (conc. ~ 20 ng/μL).
84. Distribute 20 μL of mix to 95 wells of PCR plate (or PCR tubes).
85. Run in PCR machine (Bio-Rad Thermal Cycler T100), set to program:

PCR cycling conditions			
Steps	Temperature	Time	Cycles
Initial denaturation	94°C	4 min	1
Denaturation	94°C	15 s	6
Annealing	65°C	30 s	
Extension	72°C	45 s	
Denaturation	94°C	15 s	24
Annealing	60°C	30 s	
Extension	72°C	45 s	
Extension	68°C	10 min	1
Hold	4°C	Forever	

86. Run a small aliquot (5~10 μL) on a 1.7% agar gel to visualize PCR products (Figure 8).

Size selection of PCR products on preparative gel

Note: Each pool of PCR products of a replica is size selected to optimize the number of reads produced by Illumina sequencing.

87. Collect the PCR reactions from each library in 15 mL tube and add 7.5 mL buffer PC from Qiagen PCR purification kit. Add 100 μL 3 M NaAc (pH5.2) to adjust the pH. Distribute equally on 6 Qiagen PCR purification columns (following kit instructions; yield is improved when DNA-buffer PI mixture is loaded on column twice; yield is improved when Buffer EB eluate is loaded on column twice). Elute with 50 μL Buffer EB on each column (total 300 μL).
88. Prepare a 2% preparative agarose gel with a large capacity well (by taping together 8 teeth in comb).
89. Add 10X loading dye to DNA sample.
90. Run preparative gel at 70 V for ~ 2 h. Excise the gel with single use razor between 200 bp and 500 bp (Figure 8).
91. Follow procedure in Qiagen Gel Purification Kit (yield is improved with above modifications, as well as loading DNA-Buffer QG mixture to column twice). Elute each column with 30 μL Buffer EB.
92. Concentrate the gel-extract product on one Qiagen PCR purification column (following kit instructions, with above modifications). Elute with 100 μL 1X TE. Store at −20°C.

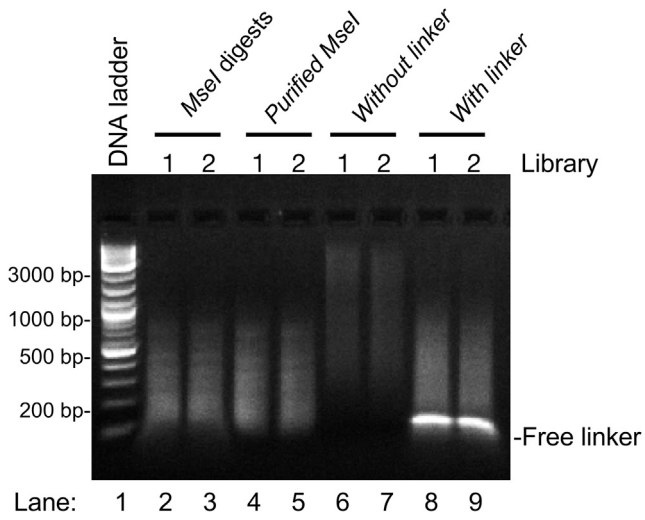

Figure 7. Ligation of linkers to MseI-cut genomic DNA fragments

Lane 1: 1 kb ladder; Lane 2 and 3: gDNA fragments with MseI digestion before purification; Lanes 4 and 5: purified gDNA fragments with MseI digestion; Lane 6 and 7: ligation without linkers; Lane 8 and 9: ligation with linkers. The two bright bands in lanes 8 and 9 are the free linker. Two independent library preps are shown.

93. Quantify DNA concentration following KAPA Library Quantification Kit (Roche). Typical concentrations are above 100 nM.
94. Library is now ready for sequencing. This DNA is sequence ready for Illumina machines. We do not add sequencing adapters because we have included the Illumina P5 and P7 sequences in our PCR primers. However, to achieve registration of clusters during the first four cycles of sequencing you need to combine and multiplex different samples for simultaneous sequencing using additional versions of primer HL3508 with different sequences at positions indicated with an N in the key resources table. Design the N sequences so that when the amplicons are combined all four nucleotides are detected at each N position.

EXPECTED OUTCOMES

This protocol is designed to identify genes important for heterochromatin by comparing *Hermes* integration densities in cells with and without cen1 *otr1R::ura4*. We found the total integration in each culture passaged in FOA averaged one insertion for every 29 bp of the genome. Of the 5,059 annotated genes of *S. pombe*, only 109 were free of insertions. The vast majority of these such as the retrotransposons were derived from duplicated sequence which prevented us from assigning unique insertions.

Genes that are already reported to be required for heterochromatin assembly, such as *mit1* and *sir2*, had substantially fewer insertions in the cells with cen1 *otr1R::ura4* compared with the cen1 WT strain (Figure 9).

To generate a list of candidate genes, we choose ORFs that have integration densities 2-fold higher in WT than the strain with the centromere reporter. The majority of ORFs have no significant change of insertion density, indicating they have no role in heterochromatin formation. We identified 199 genes with less *Hermes* integrations in cells with cen1 *otr1R::ura4*. Surprisingly, 65 candidate genes were previously reported to be essential for viability. We discovered that in WT cells integrations in many essential genes were enriched in 3′ regions where they did not impair viability. However, these 3′ insertions were reduced in cen1 *otr1R::ura4* cells because they impaired heterochromatin function.

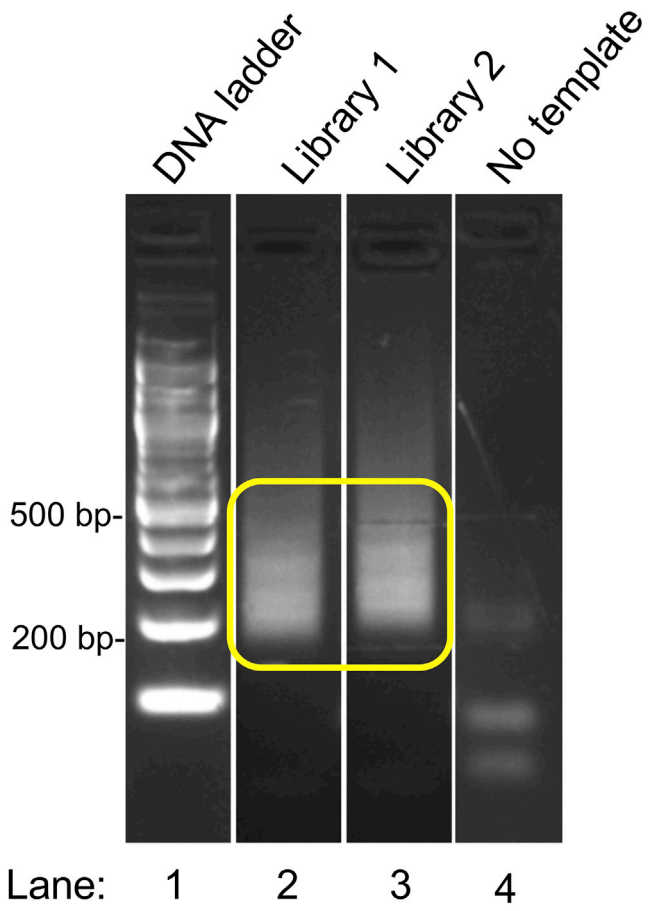

Figure 8. DNA library amplification

Lane 1: DNA ladder; Lane 2 and 3: PCR amplification fragments from two libraries; Lane 4: PCR reaction without template. The region in the yellow box is excised to purify DNA fragments with sizes of 200 bp to 500 bp. The lanes shown derive from the same gel, but some lanes were excluded from the figure as indicated by the white vertical lines.

QUANTIFICATION AND STATISTICAL ANALYSIS

Note: Resulting sequence reads were processed through HTtools to screen for those containing the *Hermes* left TIR end, map to unique positions in the *S. pombe* genome (ASM294v2.21) and quantify mapped *Hermes* insertions. HTtools is a Snakemake-based pipeline (Koster and Rahmann, 2012). The source code is available as a Github repository (https://github.com/nichd-bspc/httools). Documentation is available at https://nichd-bspc.github.io/httools/; as updates to the tool are made this documentation will be similarly updated.

HTtools filters the fastq files for sequences containing the barcode sequence and the reverse complement of the first 38-bp of the left *Hermes* TIR (GTGGCTTACGTT-TGCCTGTGGCTTGTTGAAG TTCTCTG) with up to 2 mismatches. The *Hermes* TIR-L is trimmed off, and remaining sequences are screened against sequences that would indicate amplification of the flanking sequence in the donor plasmid (CTCGAGGGGGGGCCCGGTACCAGCTTTTGTTC) and possible ligation chimeras and/or incomplete MseI digestion immediately after the end of *Hermes* transposon sequence (TTAA); see Advanced parameters in the documentation for more details. Trimmed reads matching either of these non-integration-specific fragments, or are shorter than 14 bp are discarded. HTtools maps the resulting reads to the reference genome using the blastx function of NCBI BLAST suite,

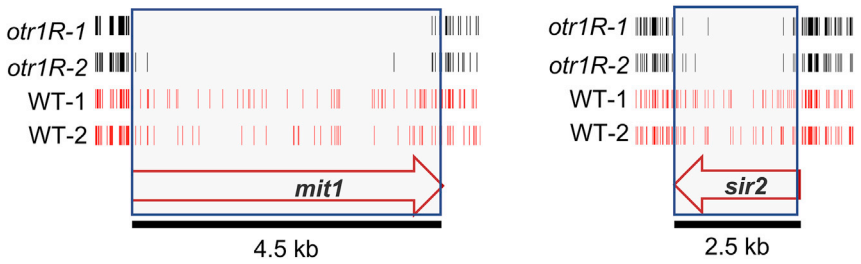

Figure 9. Two examples of *Hermes* integration density in genes involved in forming centromere heterochromatin

mit1 and *sir2* had fewer inserts in cells with the cen1 *otr1R::ura4* (black, duplicate libraries) relative to cells with WT cen1 (red, duplicate libraries). This figure is reprinted with permission from (Lee et al., 2020).

with an e-value threshold of 0.05. Passing alignments are sorted according to uniquely mapped and mapping to multiple positions. A ratio of 10^{-4} is required between e-values of the best match and second-best match for a read to be considered uniquely mapped. For metagene analysis uniquely mapped reads are also positioned relative to the closest ORF.

Since HTtools is based on Snakemake, the entire workflow can be executed on a single machine, submitted to a cluster, or run on cloud platforms (see the Snakemake documentation for details on these execution methods). Execution on a single machine is described below.

Hardware requirements: HTtools can be run on a laptop, but depending on library size, number of insertions, and other experimental details it could take hours due to the computational complexity. We recommend running HTtools on a high-performance cluster, if possible. The pipeline has been implemented and tested in Linux single machine, high-performance computing (HPC) cluster and MacOS environments.

Software requirements: All commands are run in a terminal. It is assumed git (https://git-scm.com/) and conda (https://docs.conda.io/en/latest/) are installed on the system. It is recommended to use an environment manager such as conda to install the dependencies required. This does not require any administrative privileges on the machine, and keeps all software dependencies isolated from the rest of the system. For long-term reproducibility, we recommend creating such an environment in each project directory as described below. This allows the versions of all installed packages to be maintained independently of any other projects or software on the system.

Installing the repository

1. Clone the repository with git by navigating to the location where you want to clone HTtools and:

git clonegit@github.com:NICHD-BSPC/httools.git

2. Navigate to your HTtools directory:

```
cd httools
```

All subsequent steps assume being in the HTtools directory.

Installing and activating the environment

3. Create the environment:

```
conda env create -p env/ -file requirements-linux.yaml (if on Linux)
```

```
or

conda env create -p env/ -file requirements-macos.yaml (if on MacOS)
```

4. Before using, the conda environment must be activated. This prepends the environment's executables directory to the users PATH environment variable, allowing use of all of the installed packages. Activate the environment:

```
conda activate env/
```

All subsequent steps assume the environment to be activated.

Running the tests

5. [optional] Run the tests:

```
bash test_snakemake.sh
```

6. Upon success, a message stating the tests passed successfully will be displayed. This ensures reproducibility by verifying that the current tools and packages versions are giving the expected results. This needs to be done only once after installation.

Configuring the workflow

7. The required input files are the sequence reads fastq file(s), optionally gzip compressed, and a copy of the HTtools repository. A YAML-format configuration file must be provided for each run. It contains the pipeline parameters, the path to the fastq(s) files, as well as the sample-specific parameters. A template configuration file with *Hermes*-specific parameters is included in the Github repository (see config-Hermes.yaml). Adjust the path to your fastq files, relative to the HTtools directory, and the individual samples parameters.

Sample block in the config.yaml file

Parameter	Description
Name	sample name. Must be unique within the file
barcode_start	position 1-based in the sequence reads of the barcode start. Indicate 'none' in absence of barcode
barcode_length	length of the barcode. Indicate 'none' in absence of barcode
Sequence	expected sequence, from the barcode (included, if applicable) to the end of the LTR. ***Note:*** if a Serial Number is included, it must be indicated with Xs
Integrase	whether the integrase was native (wt) or frameshift (fs)
lib_design	whether the sequence reads originate from the U5 (downstream) or the U3 (upstream) end of the retrotransposon
SN_position	(optional) start position of the Serial Number, indicate 'none' if no SN was used
SN_length	(optional) length of the Serial Number, indicate 'none' if no SN was used

Additional details about how the above parameters are used within the workflow can be found in the HTtools online documentation.

The data shown as example here has been processed under "legacy_mode", set in the configuration file and matching the parameters used in Li et al. (Lee et al., 2020). Subsequent improvements to the processing pipeline can be used by disabling this legacy mode.

8. Save the YAML-format configuration file in the HTtools directory under the name "config.yaml".

Running the workflow

9. Run the workflow:

```
snakemake –config fn=config.yaml –cores=1
```

The default –cores = 1 will work on any system. Increase the number of cores to what is available on your system to increase performance.

10. *Optional*) Alternatively, if parameters were modified in config.yaml, it is possible to only trigger the re-run of rules affected by those changes. This can save substantial computational time by not re-generating files that are unaffected by the respective parameter changes. Run the following (note that the command uses backticks (’), not single quote (’)):

```
snakemake –config fn=config.yaml -R `snakemake –config fn=config.yaml – list-par-
ams-changes` –cores=1
```

The command snakemake –config fn=config.yaml –list-params-changes lists the files affected by any parameter changes done in the config.yaml file since the last snakemake execution. -R triggers the rules that produce those files, effectively re-processing and updating any result file dependent of the changed parameters.

Expected results

11. Upon success, the pipeline creates a directory labeled "data/{name}" where name is the experiment name provided in the config.yaml file. It contains an HTML summary file with interactive histograms of integration genomic distribution and distribution relative to ORFs, heatmaps of the most targeted regions and links to the output TSV files.

The output files "data/{name}/location/ORF_{sample}.txt" list for individual sample the ORFs and the corresponding number of integrations; those files were used in subsequent analysis to generate the list of candidate genes. The online HTtools documentation has more details on the output files.

LIMITATIONS

The number of potential candidates can vary depending on the number of generations passaged in FOA media. The more cells are passaged, the greater the list of candidate genes. In our case, 28 were identified in cultures grown five generations in FOA, and 184 resulted from cultures grown for 80 generations in FOA. 13 genes were identified in both 5 and 80 generations. The most significant limitation of TIS is that it produces only candidates. Additional experiments with independent approaches must be conducted to validate candidates. Gene networks and biochemical pathways contribute significantly to regulating growth when cells are exposed to different environments. As a result TIS experiments can identify groups and clusters of factors that contribute to growth in the condition of study. If harsh or prolonged conditions of selection are used it is likely that expanded lists of candidates will have a ripple effect where the function of genes contribute indirectly to growth. Before embarking on a TIS study it is best to review previously published studies in order to identify appropriate conditions for your cultures. A recent publication provides an

extensive review of TIS experiments conducted in bacteria and yeast (van Opijnen and Levin, 2020). A highlight of this paper is a list of key issues to consider when designing a TIS study.

TROUBLESHOOTING

Problem 1 (Step 1)

Colonies on transformation plates, G418 or PMG, do not appear to be *S. pombe*.

Potential solution

It is possible that solutions used in transforming yeast get contaminated with bacteria or mold. To be sure that colonies are the *S. pombe* strains studied leave out the plasmid DNA from one of the aliquots of cells being transformed (step 9).

Problem 2 (Step 1)

Alterations in plasmid structures during transformation.

Potential solution

Do not transform both plasmids simultaneously. This commonly leads to plasmid-plasmid recombination due to large sections of homology.

Problem 3 (Step 1)

Transformation of pHL2577 does not lead to colonies on the G418 plates.

Potential solution

Reduce amount of cells spread onto YES plates by 2 to 4-fold. This can prevent G418 resistant cells from being overgrown by non-transformed cells.

Potential solution

Increase amount of pHL2577 DNA added up to 2 micrograms but do not increase the volume beyond 5 microliters since this will reduce transformation efficiency.

Problem 4 (Step 1)

Transformation of pHL2578 does not produce colonies on PMG + U –L + B1 + G418 plates.

Potential solution

Increase amount of DNA added up to 2 micrograms but do not increase the volume beyond 5 microliters.

Problem 5 (Step 2)

Transposition frequency may be too low to reach frequencies of 10%.

Potential solution

Remove any sources of residual vitamin B1 in the expression cultures to prevent shutting off the *nmt1* promoter.

Problem 6 (Step 2)

Independent transformants produce significantly different transposition frequencies.

Potential solution

It is common with yeast that during transformation plasmids acquire mutations. This is why we typically use 4 independent transformants of each strain. Should one transformant behave differently from the other three it is likely due to an altered plasmid.

Problem 7 (Step 2)

When measuring transposition frequency too many cells grow on the PMG FOA plates.

Potential solution

FOA takes up to 15 min to dissolve in PMG liquid and it is heat sensitive. When adding FOA you can warm up the liquid PMG to 40°C to dissolve it, but don't use a higher temperature, it will inactive the drug.

Problem 8 (Step 4)

Poor yield of genomic DNA from spheroplast method.

Potential solution

There are many steps in this protocol that are sensitive to small errors in volume. Consider an alternative DNA isolation method that relies on the MasterPure Yeast DNA purification kit from epicenter.

Problem 9 (Step 4)

MseI does not efficiently cut the genomic DNA

Potential solution

Residual phenol from the DNA purification can inhibit MseI digestion. An additional ethanol precipitation and wash with 70% ethanol will solve this problem.

Problem 10 (Step 4)

Low ligation efficiency at step 4.

Potential solution

This is a critical step where we have had trouble. That is why we monitor ligation efficiency using the no linker control. When ligation is efficient the lack of linker allows the fragmented genomic DNA to ligate into high weight species. If you don't see this you must troubleshoot the ligation. Here are some suggestions. During magnetic beads purification, try to avoid any residual ethanol when resuspending the beads with 1xTE. At the ligation step, ensure the annealed linkers are freshly made and the NEB T4 ligase is 2,000,000 units/ml. Use fresh 10 mM ATP stored at −80°C freezer (make aliquots of stock to minimize freeze-thawing). Alternatively, you may wish to try other commercial sources of ligase.

Problem 11 (Step 4)

PCR amplification of linker ligated insertions is dominated by jackpot products.

Potential solution

Be sure the concentration of DNA template is at least 20 ng/μL. The concentration can be measured with the PiCo-Green kit (Invitrogen) and a fluorimeter or with Qubit Fluorometric Quantification (Thermo Fisher).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Henry Levin (henry_levin@nih.gov)

Materials availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Henry Levin (henry_levin@nih.gov). All strains and plasmid constructs are available for sharing.

Data and code availability

The accession number for sequencing results is SRA#PRJNA517661 (*Hermes* integration profiles). The code used in this study is available in the Github (https://github.com/NICHD-BSPC/httools/releases/tag/v1.1.1).

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AUTHOR CONTRIBUTIONS

F.L. contributed to experimental methods and to the writing of the manuscript. S.H. contributed to experimental design and conducted the experiments. C.E. wrote the updated version of HTtools that maps and analyzes integration reads. H.L.L. contributed to the experimental design, supervised the project, and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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