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End-Labeling and Analysis of Spo11-Oligonucleotide Complexes in *Saccharomyces cerevisiae*

Matthew J. Neale and Scott Keeney*

Summary

During meiosis Spo11 catalyses the formation of DNA double-strand breaks, becoming covalently attached to the 5' ends on both sides of the break during this process. Spo11 is removed from the DSB by single-stranded endonucleolytic cleavage flanking the DSB, liberating a short-lived species consisting of Spo11 protein covalently linked to a short oligonucleotide. The method presented here details how to detect these Spo11-oligo complexes in extracts made from meiotic yeast cells.

Keywords

Spo11; meiosis; DNA double-strand break; end-labeling; terminal transferase

1. Introduction

Meiotic recombination is initiated by DNA double-strand breaks (DSBs) created by the evolutionarily conserved protein, Spo11 (*1*). Spo11 shares sequence similarity to subunit A of the type-II topoisomerase from archaea, Topo VI, and is thought to perform DSB catalysis in a similar way. Specifically, Spo11 dimerization creates two intermolecular DNA cleavage sites each consisting of a Mg^{2+} coordination site (Toprim domain) and the 5Y-CAP domain where the catalytic tyrosine residue resides. Concerted DNA strand breakage creates a DSB with a two-nucleotide 5' overhang with the 5' ends covalently linked to the catalytic tyrosine present in the active site of each monomer (residue 135 in yeast). Mutation of this residue, or indeed others within the active site, abolishes or reduces DSB formation (*2, 3*).

Topo VI is an A_2B_2 heterotetramer, which functions in isolation. Spo11, on the other hand, requires at least nine other proteins of unknown stoichiometry to support DSB catalysis (*1*). Most of these proteins have a poorly understood role in the catalytic process, and show poor evolutionary conservation (e.g., *4*). Nevertheless, a pattern of essential interactions between them is slowly emerging (*4-6*). Given that Spo11 shares homology with only the A subunit of Topo VI, it is possible that one or more of these proteins functions as an equivalent or substitute of the B subunit of Topo VI, which contains an essential ATPase domain.

In contrast to the transient lesions created by type II topoisomerases, Spo11-DSBs are not resealed by a reversal of the DNA cleavage reaction. Instead, Spo11-DSBs are channeled into a DNA repair pathway that involves 5'→3' single-stranded exonucleolytic resection, DNA strand invasion and extension of the exposed single stranded ends, and finally

*corresponding author.

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Holliday junction resolution to yield intact, and recombinant, DNA molecules (7). The first step in this processing pathway is the removal of the covalently bound Spo11 protein from the DSB end, a process that requires the Mre11 complex and the accessory protein, Sae2 (1).

In cell lysates from both yeast and mouse, a fraction of Spo11 protein is detected covalently attached to short oligos that end with free 3' OH termini (8). Detection of these Spo11-oligo complexes is dependent on meiotic DSB formation and on the activity of the Mre11 complex and Sae2, mutation in either of which prevents release of Spo11 from DSB ends. In both organisms, two forms of the Spo11-oligo complexes are detected, differing in the length of the attached DNA. In yeast, the shorter form has oligos of less than 12 nt, whereas the longer form has oligos between 21 and 37 nt in length. In mouse, the length distribution of the shorter oligos is more heterogeneous (12-26 nt), whereas the larger form is more discrete (28-34 nt) (8). In yeast, the two forms of Spo11-oligo complex are equally abundant, with kinetics of appearance and disappearance closely matching those of resected DSB molecules. Together these observations provide compelling evidence to support a model in which Spo11 is released from DSB ends via single-strand scissions flanking the DSB, a reaction that may be catalyzed by the Mre11 nuclease in combination with Sae2 (8).

The relationship between the long and short forms of Spo11-oligo complex is not known. However, one idea, inspired by the observation that the two forms are equally abundant, is that the nicks are positioned asymmetrically flanking the DSB, thus yielding one longer and one shorter Spo11-oligo complex at every DSB (Fig. 1). Moreover, if the DSB end is duplex at the time when the releasing nicks are created, differential stability of the Spo11-oligo complexes on either side of the DSB (due to the extent of DNA base pairing) could create one free DSB end and one DSB end blocked by the Spo11-oligo complexes. The lifespan of the Spo11-oligo complexes might then be expected to match that of unrepaired DSBs, as is observed. Direct physical evidence to support such a model is being sought.

Here, we present a method for detection of Spo11-oligo complexes from yeast (*see Fig. 2*). The methodology involves purifying Spo11 complexes from cell lysates by immunoprecipitation, followed by end-labeling any copurifying free 3' OH DNA ends with radiolabeled nucleotide by using the template-independent polymerase, terminal deoxynucleotidyl transferase (TdT). End-labeled Spo11-oligo complexes are separated from any end-labeled DNA contaminants by fractionation on SDS-PAGE, and are detected by autoradiography. We prepare yeast lysates by breaking cells in the presence of the strong denaturant, trichloroacetic acid (TCA), and dissolving the resulting precipitate in Tris-buffered SDS. This method minimizes proteolysis, which is otherwise a significant problem when preparing meiotic protein extracts. However, care should be taken to keep temperatures low at all times, since the strongly acidic conditions can lead to reduction in Spo11-oligo labeling yield most likely due to acid-induced DNA depurination. The relative abundance of the Spo11-oligos appears to be a good measure of the relative frequency of DSB formation within the cell. In principle, we believe that with suitable modification such methods could also be used to detect Spo11-oligo complexes in other organisms, and would thus provide a long-sought biochemical assay to measure DSB frequency.

2. Materials

2.1. Preparation of denatured yeast cell lysate

1. General yeast lab equipment, including: shaking incubator, temperature controlled floor standing centrifuge and rotors, swinging bucket bench top centrifuge, vacuum aspirator, dry heat block, tube mixing platform, microcentrifuge, water bath

2. Yeast growth media: YPD: 1% Yeast extract, 2% Peptone, 2% D-glucose. YPA: 1% Yeast extract, 2% Peptone, 2% potassium acetate, 0.001% antifoam (Sigma). SPM: 2% potassium acetate, 0.2% supplements, 0.001% antifoam.
3. 100× supplements: 1 mg/mL adenine, 1 mg/mL histidine, 3 mg/mL leucine, 1 mg/mL tryptophan, 1 mg/mL uracil. Prepare each separately and filter sterilise.
4. Bio-Spec mini bead beater 8 with 5-place, 7-mL tube holder, 7 mL polypropylene screw cap lysis tubes (Biospec Products Inc), and 0.5 mm zirconium/silica beads (BioSpec) (*see Note 1*).
5. 10% TCA. Dilute as needed from 100% stock.
6. Saturated bromophenol blue: approximately 0.5% bromophenol blue, dissolved and filtered.
7. β -mercaptoethanol (BME).
8. SDS extraction buffer: 2% SDS, 0.5 M Tris-HCl pH 8.1, 10 mM EDTA. Make up 1% of the volume with filtered saturated bromophenol blue (~0.005% final).

2.2. End-labeling of Spo11-oligo complexes

1. 2× IP buffer: 2% Triton X100, 30 mM Tris-HCl pH 8.1, 300 mM NaCl, 2 mM EDTA, 0.02% SDS. Prepare 1× IP buffer by diluting with ddH₂O.
2. Protein-G-agarose beads (Roche).
3. Mouse monoclonal anti-HA antibody (clone F7, Santa Cruz) and mouse monoclonal anti-HA-HRP conjugated antibody (clone F7, SantaCruz).
4. 10× TdT labeling buffer (500 mM potassium acetate, 200 mM Tris acetate, 100 mM magnesium acetate, 10 mM dithiothreitol, pH 7.9 at 25°C. Dilute to 1× as required with MilliQ water. This 10× buffer formulation is identical to 10× NEB4 buffer (New England Biolabs).
5. dCTP (α -³²P, 6000 Ci/mmol).
6. FPLC-pure terminal deoxynucleotidyl transferase (GE Healthcare).
7. 2.5 mM CoCl₂, dissolved in MilliQ water. Store at -20°C.
8. 2× LB: 4% SDS, 100 mM Tris-HCl pH 6.8, 20% glycerol, 1 mM EDTA. Make up 10% of the volume with saturated bromophenol blue (~0.05% final).
9. Prestained protein molecular weight marker.
10. Standard SDS-PAGE running/transfer/gel drying apparatus and reagents (e.g. BioRad Mini-Protean).
11. Phosphorimaging system and screens (e.g. Fuji FLA7000), or film.
12. Whatman 3MM paper.
13. DE81 paper (Schleicher and Schuell).
14. 1× CAPS transfer buffer: 10 mM CAPS-NaOH pH 11, 10% methanol.
15. 1× TBST: 25 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20.

¹If you do not have access to a dedicated bead beating apparatus, cell lysis can also be effected by vortexing vigorously in glass-walled tubes with zirconium beads. Perform multiple rounds of vortexing, 30 s at a time, with chilling on ice for > 1 min in between. To assess cell lysis efficiency, *see Note 5*. Continue until >95 % of cells appear lysed.

16. Immobilon PVDF membrane (Millipore).
17. Powdered non fat dry milk.
18. ECL+ reagent (GE Healthcare)

3. Methods

This protocol is optimised for sensitive detection of Spo11-oligo complexes from the synchronously sporulating *S. cerevisiae* strain, SK1. We use Spo11 that is fused at its C-terminus to the HA3-His6 epitope tag. In principle, we see no reason why it should not be possible to successfully detect Spo11-oligo complexes from other strain backgrounds or where Spo11 is fused to an alternative epitope tag, however, the efficiency of immunoprecipitation may require optimization. An outline of the various steps of the experiment is shown in **Fig. 2**.

3.1 Preparation of denatured yeast cell lysate

3.1.1 Preparing cells for synchronous yeast meiosis

1. From a -80°C glycerol stock, streak out yeast cells onto a YPD plate. Incubate 2 d at 30°C .
2. In a glass culture tube, inoculate 4 mL YPD liquid with a single colony taken from the YPD plate. Incubate 24 h at 30°C with shaking (250 rpm).
3. In a 2 L flask, inoculate 200 mL YPA to a cell density (OD_{600}) of 0.2 (this should require approx. 1:100 dilution of the YPD overnight culture). Incubate 14 h at 30°C with shaking (250 rpm; *see Note 2*).
4. Collect cells by centrifugation (3 min at 5000g, 4°C) and drain supernatant. Wash with 200 mL water by sealing bottle and shaking vigorously. Pellet cells again, drain, and resuspend with 200 mL SPM prewarmed to 30°C . Return to a 2 L flask and incubate at 30°C with shaking (250 rpm).

3.1.2 Cell lysis in TCA

1. Collect cells at 4 h in meiosis by centrifugation (3 min at 5000g, 4°C) (*see Note 3*).
2. Resuspend pellet with 8 mL ice-cold water, and transfer equal amounts into two 7-mL lysis tubes.
3. Spin down cells (3 min at 5000g) and aspirate supernatant. Cell pellets should be approximately 1 mL volume. Place tubes on ice.
4. Without disturbing the cell pellets, add an equal vol (1 mL) ice-cold 10% TCA. Pour chilled zirconia/silica beads into the tubes until the top of the beads is level with the final level of the TCA meniscus after it rises (approximately 2 mL of beads per tube). Tubes should be no more than half full.
5. Tightly secure tube caps, place into bead-beater and secure lid. Lyse for no longer than 30 s at full power. Quickly place tubes into an ice water bath and chill for 2 min, inverting a few times to speed the chilling process (*see Note 4*). Repeat this step twice more.

²After 14 h of growth, cell density should be $\sim 5 \times 10^7$ cells/mL (OD_{600} of a 10-fold dilution should be 0.3-0.6), with short chains of 5-10 enlarged, round cells with few (<10%) small buds. If cell density is significantly different, scale all subsequent volumes accordingly.

³Four hours normally represents the peak of both DSB formation and Spo11-oligo abundance. The protocol can be appropriately scaled to sample a culture at multiple time points.

6. Invert lysis tubes. Flame a 25-gauge needle and pierce the bottom of one of the tubes 2–3 times. Push the pierced 7-mL lysis tube into the top of a 14-mL conical centrifuge tube and collect lysate by centrifuging for 1 min at 1400g in a swinging bucket rotor. Return tube to ice.
7. Collect lysate from the second 7-mL tube in the same 14-mL conical tube by repeating step 6 (*see Note 5*).
8. Spin down precipitated protein 10 min, 16,000g, 4°C. Completely aspirate supernatant, and place tubes on ice. Proceed to the next section.

3.1.3. Protein extraction from denatured yeast lysate

1. Add 1 mL of SDS extraction buffer to the TCA precipitate. Break up the dense protein precipitate using a clean glass rod. Once a rough white paste is created, hold the glass rod loosely in your fingers and vortex the tube so that the rod spins inside the tube. A smooth paste should be created without significant foaming (*see Note 6*).
2. Add 1 mL of SDS extraction buffer and mix further with glass rod/vortex.
3. Add 4 mL of SDS extraction buffer, remove rod, and mix by gentle vortexing. Avoid foaming. The lysate should appear pale blue, homogeneous, and quite opaque.
4. Add 120 μ L BME and mix.
5. Cap tube loosely, and heat for 5 min on a dry heat block at 95–100°C. Cool lysate on ice for 5 min.
6. Spin down insoluble cell debris 10 min, 16,000g, 4°C.
7. Pour supernatant into a 15-mL conical tube (approx. 6 mL total). Mix and place on ice (*see Note 7*).

3.2. End-labeling of Spo11-oligo complexes

3.2.1. Immunoprecipitation of Spo11-oligo complexes

1. On ice, dilute 6 mL of cleared cell lysate with 6 mL of 2 $\times$ IP buffer and mix on a nutator for 10 min at 4°C (*see Note 8*).
2. Add 50 μ L mouse monoclonal anti-HA antibody and 100 μ L of protein-G-agarose bead suspension. Incubate overnight at 4°C on a nutator.

⁴It is essential not to allow the tubes to heat up significantly during the cell lysis step as high temperature reduces the observed signal, a process we believe to be due to acid-catalyzed depurination of the Spo11-oligo chain (*see Fig. 4*).

⁵Cell lysis efficiency can be checked by mixing 1 μ L of TCA lysate with 10 μ L of SDS extraction buffer on a microscope slide. Lysed cells appear as translucent ghosts and cell wall fragments. Intact cells will be phase dark. Lysis efficiency is usually >95%.

⁶The addition of too much buffer too soon can lead to partially solubilized protein aggregates that are subsequently difficult to completely resuspend, reducing yield.

⁷Cleared lysate can be stored at -20°C but should be reheated briefly and, if necessary, re-clarified by centrifugation prior to continuing with the immunoprecipitation.

⁸It is possible to detect Spo11-oligo complexes from as little as 60 μ L of cell lysate (equivalent to just 2 mL of meiotic culture; *see Fig. 3*). However, when performing this assay for the first time, or when assaying strains expected to contain fewer Spo11-oligo complexes, we suggest using 3–6 mL of lysate per assay as described here. When performing the assay for the first time, it may be useful to use half of the lysate for a mock (no antibody) control. If using less than 6 mL of lysate, scale reagent volumes proportionally in **Subheading 3.2.1**, steps 1 and 2. In the original protocol (8), the cell lysate was diluted 10-fold to final concentrations of 0.2% SDS, 1% Triton. However, for immunoprecipitation of Spo11-HA3His6 with anti-HA, we find that just 2-fold dilution (to 1% SDS, 1% Triton) is sufficient to achieve the same or higher yield, most likely due to the increased concentration of antibody and protein-G beads. Optimization of the immunoprecipitation (particularly the extent of dilution) will be necessary if attempting to use alternative antibody/affinity tag combinations.

3. Collect immune complexes by gentle centrifugation (1 min at 500g). Aspirate supernatant.
4. Suspend beads with 1 mL of 1× IP buffer and transfer to 1.5 mL Eppendorf tube. Gently pellet beads (1 min at 500g) and aspirate supernatant.
5. Wash beads with 1 mL of 1× IP buffer, re-spin, and aspirate supernatant. Repeat.

3.2.2. End-labeling of Spo11-oligo complexes using TdT

1. Wash IP beads twice with 0.5 mL 1× TdT labeling buffer, spin, and carefully remove supernatant (*see Note 9*).
2. Prepare TdT reaction buffer on ice as follows:

Component	Per reaction	Final concentration
10× TdT labeling buffer	5 µL	1×
2.5 mM CoCl ₂	5 µL	0.25 mM
MilliQ water	38 µL	
TdT	1 µL	
20 µCi dCTP (α - ³² P, 6000 Ci/mmol)	1 µL	

3. Add 50 µL TdT reaction buffer to beads and mix. Incubate at 37°C for 60 min. Mix reaction periodically (*see Note 10*).
4. Pellet beads 10 s at 500g, and carefully remove reaction supernatant using a pipetman. This and subsequent wash supernatants will contain a lot of unincorporated radioactivity: discard as radioactive liquid waste (*see Note 11*).
5. Wash beads with 1 mL 1× IP buffer. Pellet beads and remove supernatant with pipetman. Discard with radioactive liquid waste. Alternatively, a vacuum aspirator dedicated to radioactive work may be used. Repeat this step twice.
6. Elute complexes by adding 50 µL 2× LB and boiling for 5 min. Radiolabeled Spo11-oligo complexes can be stored at −20°C.

3.2.3. Detection of end-labelled Spo11-oligo complexes

1. Pour a standard 1-mm-thick 7.5% SDS-PAGE gel, with 4% stacking gel. We use a premixed 30% acrylamide solution (29:1 acrylamide:bisacrylamide; BioRad).
2. Boil samples for 5 min, pulse spin, mix, re-spin, and load up to 20 µL of supernatant per lane. For best results, load into the bottoms of wells using gel loading tips, and do not overload lanes. Load prestained marker to monitor band migration.

⁹When in 1× TdT labeling buffer (1× NEB4), the beads are easy to disturb during aspiration. We recommend using an aspirator tipped with an ultrafine sequencing gel loading tip. A second wash may not be necessary if: a) the volume of protein-G-beads used is low (due to scaling down the immunoprecipitation), and b) care is taken to remove all supernatant after the first wash.

¹⁰We generally mix the reactions about every 15 min by holding tube in a pair of long tweezers and agitating gently for a few seconds. Signal can be increased by incubation for longer than 60 min (e.g., 2–4 h). For accurate sizing of oligo complexes, use a chain terminating nucleotide such as cordycepin triphosphate.

¹¹Placing the reaction tube in a small hand-held acrylic block during this and the subsequent wash steps minimizes exposure to the radioactive buffer. A six-place micro-centrifuge dedicated to radioactive work is also useful.

3. Run gel at 150 V for 60–70 min (approximately 5–10 min after dye front has migrated out of the gel, or until the 37 kDa marker is at the bottom of the gel).
4. Dismantle gel apparatus and discard the running buffer as radioactive liquid waste. Rinse gel with water and process for drying (step 5) or transfer (step 6).
5. Alternative 1: For drying, immerse gel in water, capture from beneath with a piece of Whatman 3MM paper, and dry under vacuum over a sheet of DE81 paper at 80°C for 30 min.
6. Alternative 2: For transfer, soak gel 10 min in 1× CAPS transfer buffer, and transfer onto PVDF membrane in 1× CAPS transfer buffer. Transfer at 100 V for 60 min (approximately 300–500 mA) with buffer circulation and cooling. Following transfer, allow PVDF membrane to completely dry (about 15 min at room temperature).
7. Mark the position of protein standards with a radioactive pen. Wrap the dried gel or PVDF blot with plastic wrap and expose to a phosphor screen or to film (*see Note 12*). A sample labeling experiment is shown in **Fig. 3**.

3.2.4. Western detection of Spo11 (optional)—Spo11 can be detected on the PVDF membrane (**Subheading 3.2.3, step 6**) using mouse monoclonal anti-HA-HRP conjugated antibody.

1. Rewet the membrane in methanol, rinse with 1× TBST, and block for 15 min in 1× TBST containing 5% non-fat dry milk.
2. Incubate the membrane for 30 min with mouse monoclonal anti-HA-HRP conjugated antibody diluted 1:10,000 in 1× TBST containing 1% non-fat dry milk (*see Note 13*).
3. Wash the membrane three times with 1× TBST for 10–15 min each.
4. Incubate the membrane with ECL+ reagent and expose to film (5–120 s).

3.2.5. Sampling multiple time points—Although the methods presented above can be used to sample multiple time points by starting with a large (e.g., 1 L) culture, it is more convenient to sample smaller aliquots of cells from a 200 mL culture. The procedure works well (although with reduced sensitivity) starting with 20 mL aliquots of meiotic cell culture, and as such, permits performing cell lysis and the entire immunoprecipitation/labeling protocol in 1.5-mL tubes. All lysis and immunoprecipitation reagent volumes should be scaled accordingly, and extra care should be taken to keep things cold during cell lysis. Reagent volumes used for the radiolabeling steps should remain unchanged. Expect sensitivity to be reduced ~10-fold.

3.3 Potential problems

Failure to keep the cell lysate cool during TCA lysis can dramatically affect the detection of Spo11-oligo species. **Fig. 4** illustrates this point (compare lanes 1 and 2 with lanes 3 and 4). Based on western blot analysis of the recovery of Spo11 protein (data not shown), the reduced signal in lanes 3 and 4 does not appear to be a consequence of increased proteolysis.

¹²Specific labeling should be apparent when monitoring the gel or blot with a hand-held detector, and give readily detectable bands with 1–2 h exposure to a phosphor screen. For accurate quantification, vary the length of exposure to achieve an image with the best dynamic range without saturation.

¹³Optimal antibody concentration will depend on the scale of the immunoprecipitation. Use of an HRP-conjugated antibody prevents cross-reaction with the heavy chain of the antibody used for immunoprecipitation, which migrates at the same position as Spo11-HA3His6.

We surmise that acid-induced depurination, which would be greater at elevated temperature, renders the Spo11-associated oligo refractory to extension by TdT. Thus, it is important to use short bursts in the bead beater (<30 s) and to chill on ice in between.

Different TdT preparations contain different protein contaminants that can be labeled with TdT and α -³²P-labeled nucleotide. This point is also illustrated in **Fig. 4**, where parallel labeling was carried out with TdT from different commercial sources (compare lanes 1 and 3 with lanes 2 and 4). The source of these labeling artifacts is not known, although we speculate that the contaminant in the New England Biolabs preparation is a covalent complex of *E. coli* topoisomerase I and a short oligo (the TdT enzyme was expressed in recombinant form and purified from *E. coli*). These results show that care should be exercised in choosing a source of TdT.

References

1. Keeney S. Mechanism and control of meiotic recombination initiation. *Curr. Top. Dev. Biol.* 2001; 52:1–53. [PubMed: 11529427]
2. Bergerat A, de Massy B, Gadelle D, Varoutas PC, Nicolas A, Forterre P. An atypical topoisomerase II from Archaea with implications for meiotic recombination. *Nature.* 1997; 386:414–417. [PubMed: 9121560]
3. Diaz RL, Alcid AD, Berger JM, Keeney S. Identification of residues in yeast Spo11p critical for meiotic DNA double-strand break formation. *Mol. Cell. Biol.* 2002; 22:1106–1115. [PubMed: 11809802]
4. Maleki S, Neale MJ, Arora C, Henderson KA, Keeney S. Interactions between Mei4, Rec114, and other proteins required for meiotic DNA double-strand break formation in *Saccharomyces cerevisiae*. *Chromosoma.* 2007; 116:471–486. [PubMed: 17558514]
5. Arora C, Kee K, Maleki S, Keeney S. Antiviral protein Ski8 is a direct partner of Spo11 in meiotic DNA break formation, independent of its cytoplasmic role in RNA metabolism. *Mol. Cell.* 2004; 13:549–559. [PubMed: 14992724]
6. Li J, Hooker GW, Roeder GS. *Saccharomyces cerevisiae* Mer2, Mei4 and Rec114 form a complex required for meiotic double-strand break formation. *Genetics.* 2006; 173:1969–1981. [PubMed: 16783010]
7. Neale MJ, Keeney S. Clarifying the mechanics of DNA strand exchange in meiotic recombination. *Nature.* 2006; 442:153–158. [PubMed: 16838012]
8. Neale MJ, Pan J, Keeney S. Endonucleolytic processing of covalent protein-linked DNA double-strand breaks. *Nature.* 2005; 436:1053–1057. [PubMed: 16107854]

Fig 1.

Endonucleolytic release of Spo11 from DSB ends. Spo11 catalyses DSB formation, becoming covalently attached to the 5' ends at the break site. Single-stranded endonucleolytic cleavage flanking the Spo11-DSB permits release of Spo11 that is still covalently attached to a short oligonucleotide. Two equally abundant populations of Spo11-oligo complexes are detected, which differ in the length of the attached oligo, suggesting that the nicks could be positioned asymmetrically around each DSB. Adapted from Ref. 8.

Fig 2.

Scheme for detecting Spo11-oligo complexes from meiotic yeast cells. A) Meiotic cells are harvested and lysed in 10% TCA with zirconia beads. Precipitated protein in the extract is dissolved with 2% SDS and soluble protein diluted with Triton X100. Spo11-HA3His6 is immunoprecipitated with anti-HA antibody and protein-G-agarose beads. Immune complexes are washed, end-labeled using TdT and radioactive dCTP, then washed and eluted in gel loading buffer. Spo11-oligo complexes are detected by SDS-PAGE and autoradiography (autoradiograph adapted from Ref. 8). B) Schematic of the TdT end-labeling reaction.

Fig 3.

Sample experimental result demonstrating sensitivity of the labeling assay. Lane 1 shows the signal obtained from a cell extract prepared from 200 mL of wild-type culture. Lane 2 shows the signal obtained from a mixture of extract from 2 mL of wild-type culture mixed with extract prepared from 198 mL of culture of a *spo11(Y135F)* mutant, which produces no DSBs and contains no Spo11-oligo species. The assay is sensitive enough to quantitatively detect 1% or less of the wild-type level of Spo11-oligo species. Two exposures are shown. In the longer (20 h) exposure, the signal in lane 1 is heavily saturated. Arrowheads mark Spo11-oligo species.

Fig 4.

Potential problems. Identical cell pellets were lysed with 4 bursts of 30 s each with chilling on ice (≥ 2 min) between bursts (lanes 1, 2), or were lysed continuously for 2 min in the bead beater at 4°C, but with no other cooling (lanes 3, 4). Subsequent immunoprecipitation and labeling steps were performed in parallel as described in this protocol. Spo11-oligo species are marked with arrowheads. Overall signal was reduced >10 -fold in lanes 3 and 4, with complete loss of the upper Spo11-oligo band. In lanes 1 and 3, end-labeling with [α - 32 P]dCTP was carried out using FPLC-pure TdT from GE Healthcare, whereas labeling in lanes 2 and 4 was carried out with recombinant TdT from New England Biolabs. The asterisk marks an end-labeled contaminant from the latter preparation. See text (**Subheading 3.3**) for further discussion.