



MSM – TETRAD DISSECTION – QUICK START GUIDE

INTRODUCTION – CEREVISIAE



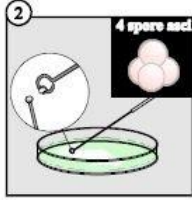


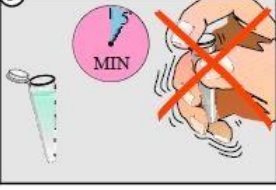


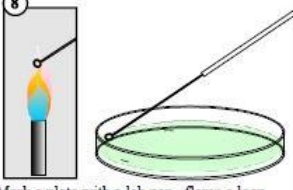
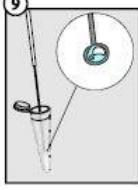
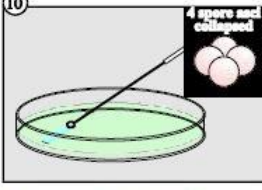

The following guide provides a series of steps which act as a reminder to those who have experienced the difficulties of successful tetrad dissection

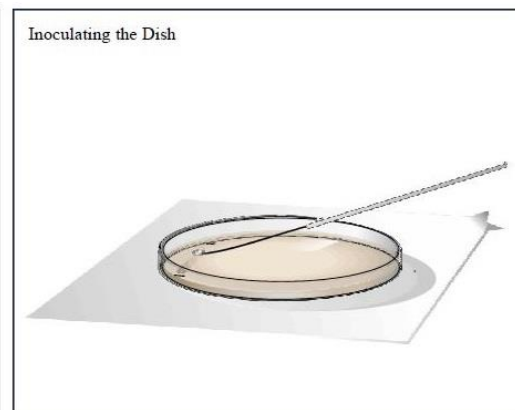
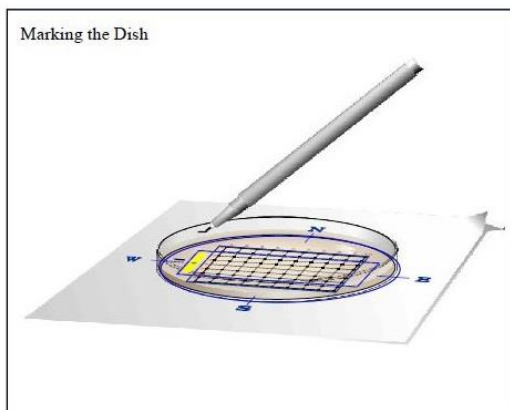
It may also serve as a teaching aid to those who have never dissected before.

NOTE : If you are using Pombe then digestion is not necessary as spontaneous lysing occurs although similar sporulation checks and volume (pin head) is used in the suspension.

(Information originally produced by Jan Saunders-Singer – 5th March 2010)

PROCEDURE

<p>Ten steps to perfect digestion</p> <p>Inspect the sporulated culture microscopically to ensure the presence of four spore asci and to assess the sporulation rate</p> 	<p>1</p>  <p>Using a flamed loop or sterile toothpick...</p>	<p>2</p> <p>4 spore asci</p>  <p>...take pinhead quantity of sporulated culture...</p>	<p>3</p> <p>50-100µl prepared enzyme solution</p>  <p>Suspend in 50-100µl of prepared enzyme solution incubate for 10-20 minutes depending on strain</p>
<p>4</p> <p>STOP Iced</p>  <p>Fill Eppendorf with ice cold water (1ml+).</p>	<p>5</p> <p>MIN</p>  <p>Leave to settle for 5 minutes (do not agitate)</p>	<p>6</p>  <p>Aspirate back to the original volume</p>	<p>Inoculating a Petri Dish</p> <p>7</p>  <p>Lightly re-suspend cells by hand</p>
<p>8</p>  <p>Mark a plate with a lab pen... flame a loop then dab it on an unused area of your plate to cool it before putting it into suspension.</p>	<p>9</p>  <p>Insert a 3mm flamed loop into your suspension</p>	<p>10</p> <p>4 spore asci collapsed</p>  <p>Mark the bottom of the dish with a lab pen and with a single (light) stroke inoculate the dish</p>	<p>X300</p>  <p>This would be an ideal inoculum. Not too dense.</p>





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1 On switching on the MSM System 400 the display will present you with a Splash Screen that lets you know that the program is loading.

Singer Program and Protocols

Note: Although you can use the joystick buttons to OK selection or to select the Menu it would be better to use the touch-screen only up to the point of dissection. The choice is yours.



OR



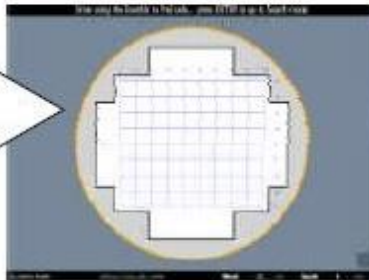


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6 On selecting OK you will be presented with the Matrix. Drive to your inoculum position ready to search.

Move North/South

Singer Program and Protocols



Note: for the purposes of visual clarity the camera is shown off. The default is on but the matrix shown left would be in overlay mode until changed. You may leave it in overlay mode if preferred and continue without selecting the below menu bar.

7 When you have found the inoculum accept position by clicking the black button.



Non Operational Menu Bar

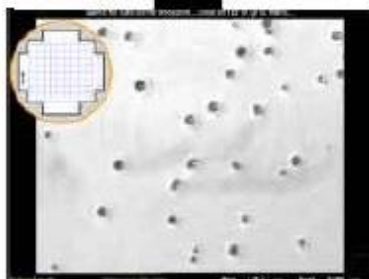


8 Touch Screen Control only to change screen layout



9 You can step in any direction within the inoculum.

Move North/South



Seeing the formation of the meniscus around the needle tip confirms that it is on the surface

Needle in focus not touching Needle not in focus but touching Needle in focus and touching

10 Use the fine focus knob to adjust focus

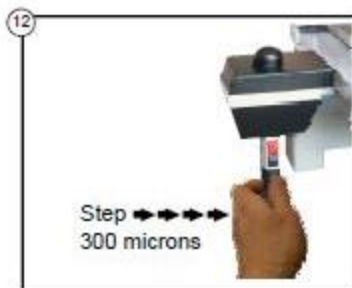
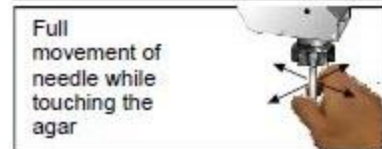
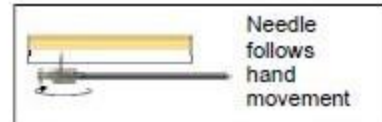
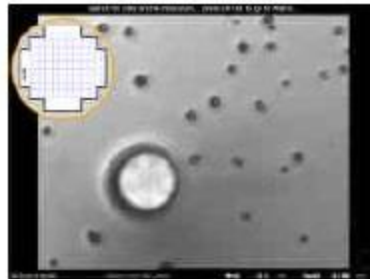


Needle to the surface of the agar

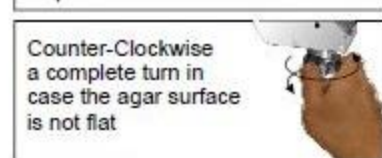
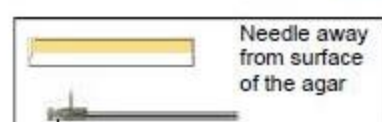
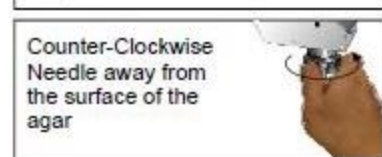
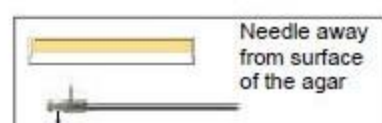
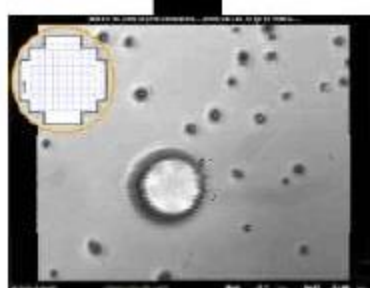
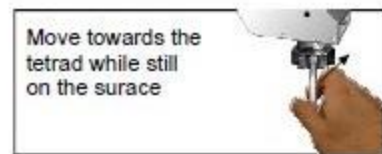
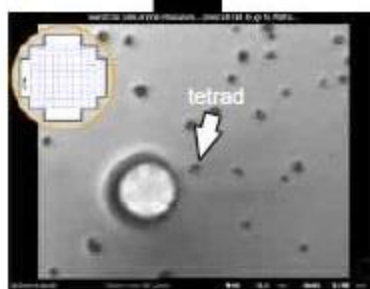
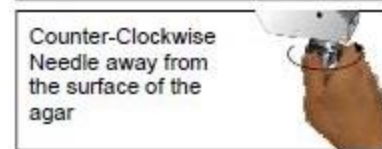
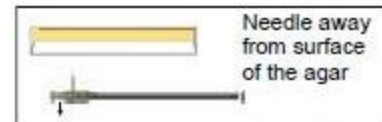
Clockwise Needle to the surface of the agar



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It is good practise to move (step) to the side of the inoculum bring the needle up to the surface and plunge the needle tip into the sterile agar and move the tip rigorously in to remove contaminates. Lower the needle and return to the original position and search for a tetrad.







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Remember watch for the meniscus first then...

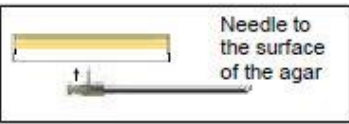
16 Use the fine focus knob to adjust focus




...focus




Needle to the surface of the agar




17







Clockwise Needle to the surface of the agar




18 Use the fine focus knob to adjust focus




ZAP




After dropping tetrad move towards it to separate the spores




19







Zapping the tetrad can separate one from three or in pairs or even single spores. Whatever the case try and leave one here and take the others to the next position A2




20 After moving the needle away from the surface of the agar pull joystick towards you once to move to position A2

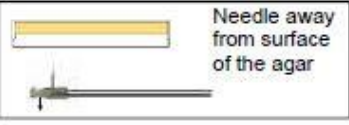





On separating the tetrad move needle to pick up three spores and leave one at A1



Needle away from surface of the agar



Counter-Clockwise a complete turn in case the agar surface is not flat





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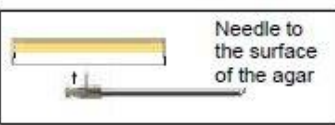

Use the fine focus knob to adjust focus

...focus

Remember watch for the meniscus first then...

Needle to the surface of the agar

Clockwise Needle to the surface of the agar



Separating the three spores is much the same as separating the four spore asci. You may use the MicroZapper, separate them pick up two and move to position A3, separate and pick up one spore move to A4 and place it there. The technique is virtually the same and very quick.

It is also good practise to move back up the column, refocussing at each grid point, to check for a spore. This ensures that you haven't mistaken a bubble in the agar as a spore. It also serves to put you back to A1. At this position you will mark the agar so that you may find this datum position after plate removal and incubation or a break in tetrad dissection. This is done by first moving the spore to an area away from the centre, plunging the needle into the agar and jiggling it a bit creating a hole. Don't worry that you will break the needle, you won't.

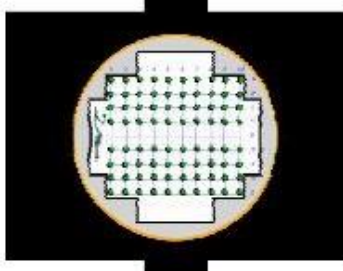
22

Return to the inoculum by pressing the black button on the joystick control

The stage will return to the last position always. You can continue the search for tetrads using the joystick control as before.

The authogonal array at 6mm centres will allow you to dissect 20 tetrads. There is no need to measure distances as the MSM System 400 automatically uses this grid setup when in the Matrix. On moving to the inoculum it reverts to 300 micron steps and 24 micron nudge movements automatically. The appropriate automation allows users to concentrate on selecting good asci



Being able to return to the the same position in the inoculum serves two useful purposes; one is from a point you have already searched and two if you have over digested then you may return to pick up a dropped spore without ruining the experiment.

You may also use the nudge facility in the Matrix if a spore moves outside the ellipse of the dissecting needle.

Step, Nudge and Matrix parameters can be changed from the menu bar.

The position of the Matrix display can be changed from the menu bar.

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Pressing the red button will bring up the Main Menu which allows you to dissect another dish or quit







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CONCLUSION

We hope that this explanation will assist you in performing the above procedure. If you need further assistance do not hesitate to contact the Technical Support Team at Singer Instruments.

CONTACT INFORMATION

Name(s): *Ian Morgan*

Phone : *+44 (0) 1984 640226*

Email: *technicalsupport@singerinstruments.com*

