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Cultivation Conditions After Monoclonal Selection Using VELSSON PLATES

INTRODUCTION

VELSSON's series of consumables, developed in-house, boasts exceptional technical performance and quality. Our product quality rivals that of leading international companies, while our pricing retains a competitive advantage. These products are compatible with Velsson's smart solutions and can be flexibly customized to meet diverse customer needs. Velsson's plate consumables include cell culture plates, shallow well plates, PCR plates, and agar plates. The culture plates are available in 24-well and 96-well formats, made entirely of polypropylene (PP), ensuring stable chemical properties that comply with USP Class VI standards. Designed to ANSI specifications, they are suitable for multi-channel pipetting and are compatible with Velsson's automated operating units. They are sterile, free from DNase and RNase, and lack pyrogens, having been sterilized via gamma radiation for enhanced safety. They can also withstand high-temperature and high-pressure sterilization at 121°C for 15 minutes, endure low temperatures down to -80°C, and tolerate centrifugal forces of up to 3500xg, thereby broadening their applicability. The plates allow for complete liquid aspiration (no residual liquid), minimal wall adhesion, and low residual volumes, particularly when used with deep well plate lids, which reduce evaporation.

EXPERIMENTAL PROCEDURE

1. Experiment Title

Acceptance Testing for Escherichia coli Selection.

2. Experiment Objective

To use monoclonal equipment for the selection of Escherichia coli colonies and evaluate the culture results post-selection.

3. Experimental Plan

Select three agar plates with Escherichia coli colonies and inoculate them into 24/96 well culture plates. Incubate overnight on a shaking incubator at 37°C. Compare the number of selected colonies with the turbidity of the inoculated cultures, calculate the success rate of Escherichia coli selection, and summarize the cultivation results.

4. Experimental Procedure

4.1 Medium Preparation and Distribution

Preparation of LB Liquid Medium:
Combine 10 g peptone, 5 g yeast extract, and 10 g sodium chloride in 1000ml of distilled water. Stir until homogeneous, sterilize at 121°C, and cool to 50-60°C before adding AMP antibiotic to a final concentration of 100 ng/µl. Store in the refrigerator.

Medium Distribution:
Dispense the prepared LB medium into 24/96 deep well plates, 1ml per well, filling ten 24/96 deep well plates, and store them at 4°C.

4.2 Experimental Process

UV Sterilization:
Sterilize the monoclonal equipment with UV light for 30 minutes.

Preparation for Selection:
Retrieve three overnight cultured 12-well plates with E. coli colonies and place them alongside three 24/96 deep well plates at the selection position on the MONOCLONE sliding platform.

Selection Process:
Initiate the selection process. After completion, dip the selected picking tool into each well of the 24/96 deep well plates containing 1ml LB. Follow up with a cleaning process after selection.

Repetition and Documentation:
Repeat the selection for three agar plates, recording the number of selected colonies for each.
Document any issues encountered during the selection process.

Incubation:

After selection, place the 24/96 deep well plates in a shaking incubator at 37°C and 250 rpm overnight, observing and recording the cultivation results.

Calculation of Success Rates:

Calculate the selection success rate and inoculation success rate:

Selection Success Rate = Actual Identified Count / Required Selection Count

Inoculation Success Rate = Turbid Wells Count / Selected Colonies Count

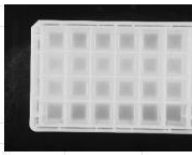
A success rate greater than 99% is considered acceptable.

RESULTS

The cultivation conditions for the 24-well plate selection are as follows:

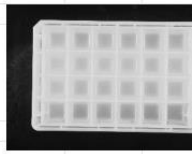
2023.10.19 Selection of monoclonal clones of Escherichia coli followed by OD600 measurement after cultivation (cultivation conditions: 37°C, 200 rpm for 15 hours)."

24/24	1	2	3	4	5	6	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.25	4.37	4.54	4.38	4.4	4.34	100.00%	4.38875	0.117299783	2.67%
B	4.53	4.28	4.5	4.33	4.54	4.5				
C	4.45	4.26	4.39	4.29	4.56	4.28				
D	4.53	4.55	4.31	4.28	4.29	4.18				



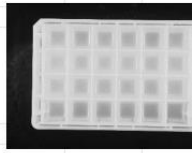
2023.10.26Selection of monoclonal clones of Escherichia coli followed by OD600 measurement after cultivation (cultivation conditions: 37°C, 200 rpm for 15 hours)."

24/24	1	2	3	4	5	6	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.32	4.55	4.54	4.76	4.78	4.7	100.00%	4.691666667	0.145830952	3.11%
B	4.35	4.66	4.62	4.8	4.8	4.69				
C	4.76	4.68	4.67	4.88	4.75	4.8				
D	4.89	4.78	4.63	4.58	4.76	4.85				



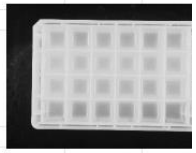
2023.11.15Selection of monoclonal clones of Escherichia coli followed by OD600 measurement after cultivation (cultivation conditions: 37°C, 200 rpm for 15 hours)."

24/24	1	2	3	4	5	6	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.2	4.38	4.28	4.33	4.3	4.32	100.00%	4.259166667	0.071985304	1.69%
B	4.27	4.35	4.21	4.35	4.28	4.16				
C	4.21	4.33	4.19	4.13	4.18	4.25				
D	4.35	4.31	4.22	4.16	4.2	4.26				



2023.11.23Selection of monoclonal clones of Escherichia coli followed by OD600 measurement after cultivation (cultivation conditions: 37°C, 200 rpm for 15 hours)."

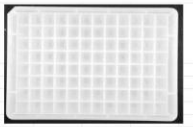
24/24	1	2	3	4	5	6	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.35	4.16	4.28	4.19	4.26	4.15	100.00%	4.20875	0.072430626	1.72%
B	4.17	4.28	4.25	4.2	4.23	4.08				
C	4.2	4.15	4.14	4.21	4.16	4.2				
D	4.27	4.35	4.1	4.11	4.27	4.25				



The cultivation conditions for the 96-well plate selection are as follows:

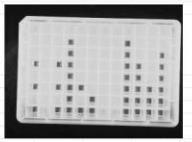
2023.10.18 The selection of monoclonal clones of Escherichia coli was conducted, followed by measurement of optical density at 600 nm (OD600) after cultivation under the following conditions: 37°C, 700 rpm for 15 hours.

96/96	1	2	3	4	5	6	7	8	9	10	11	12	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	3.79	4.01	4.25	4.25	4.17	4.23	3.95	4.03	4.04	4.16	4.01	3.97				
B	4.1	4.18	4.2	4.08	4.26	4.06	4	4.13	4.06	4.12	4.02	3.99				
C	3.77	4.16	4.23	3.98	4.14	3.78	4.15	4.1	4.11	4.05	4.05	4				
D	4.02	4.15	4.07	3.94	3.99	3.8	4.15	4.1	4.2	4.17	4.1	4.12				
E	3.88	3.99	4.04	4.06	3.87	3.9	4.06	4.15	4.17	4.22	4.17	4	100.00%	4.060833333	0.119795147	2.95%
F	3.94	4.21	4	4.13	3.98	4.22	3.88	4.28	3.84	3.9	4.14	4.06				
G	4.12	4.04	4.22	3.87	4.18	4.12	4.02	4.2	3.99	4.15	4.08	3.99				
H	4.07	3.98	4.12	3.95	4.11	3.89	3.91	4.18	4.06	3.87	4.03	4.21				



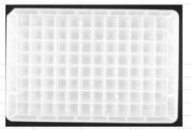
2023.10.25 The selection of monoclonal clones of Escherichia coli was conducted, followed by measurement of optical density at 600 nm (OD600) after cultivation under the following conditions: 37°C, 700 rpm for 15 hours.

56/56	1	2	3	4	5	6	7	8	9	10	11	12	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.28	4.33	4.2	4.11	4.08	4.4	4.31	4.2	4.31	4.36	4.45	4.34				
B	4.56	4.24	4.05		4.25	4.54	4.26	4.19		4.4	4.43	4.3				
C	4.39	4.31	4.16		4.37	4.38	4.51	4.37		4.36	4.5					
D		4.45			4.32	4.27	4.5	4.3			4.37		100.00%	4.298448276	0.131693447	3.06%
E	4.24	4.4	4.17		4.26	4.2	4.41	4.12			4.32					
F		4.28				4.33	4.39	4.21								
G	3.87	4.17	4.16		4.45		4.37	4.22								
H		4.11					4.3	4.18								



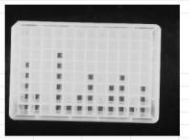
2023.11.13 The selection of monoclonal clones of Escherichia coli was conducted, followed by measurement of optical density at 600 nm (OD600) after cultivation under the following conditions: 37°C, 700 rpm for 15 hours.

96/96	1	2	3	4	5	6	7	8	9	10	11	12	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.22	4.16	4.44	4.35	4.27	4.2	4.18	4.35	4.32	4.26	4.25	4.27				
B	4.34	4.13	4.34	4.52	4.18	4.22	4.19	4.37	4.31	4.37	4.27	4.29				
C	4.11	4.12	4.28	4.11	4.16	4.37	4.11	4.32	4.33	4.33	4.26	4.27				
D	4.42	4.33	4.2	4.17	4.33	4.32	4.17	4.18	4.19	4.3	4.16	4.13				
E	4.33	4.46	4.31	4.24	4.41	4.39	4.32	4.16	4.28	4.08	4.18	4.43	100.00%	4.261875	0.100935753	2.37%
F	4.19	4.09	4.11	4.39	4.24	4.38	4.27	4.36	4.22	4.22	4.16	4.21				
G	4.12	4.22	4.29	4.21	4.18	4.35	4.25	4.29	4.29	4.38	4.15	4.2				
H	4.38	4.29	4.28	4.38	4.1	4.41	4.21	4.12	4.19	4.45	4.11	4.39				



2023.11.21 The selection of monoclonal clones of Escherichia coli was conducted, followed by measurement of optical density at 600 nm (OD600) after cultivation under the following conditions: 37°C, 700 rpm for 15 hours.

64/64	1	2	3	4	5	6	7	8	9	10	11	12	Success Rate	Average OD Value	Standard Deviation	Coefficient of Variation
A	4.42	4.16	4.36	4.22	4.37	4.36	4.2	4.54	4.38	4.28	4.33	4.25				
B	4.11	4.15	4.33	4.28	4.3	4.13	4.35	4.32	4.57	4.21	4.25	4.16				
C	4.06	4.23	4.25		4.18	4.16	4.44	4.42	4.55	4.37	4.37	4.36				
D		4.27	4.11		4.01	4.26	4.4	4.32	4.42	4.35	4.35	4.39	100.00%	4.28453125	0.118065487	2.76%
E		4.4	4.05		4.37	4.28		4.26	4.31		4.25	4.4				
F		4.38	4.09		4.28	4.19		4.29			4.18					
G			4.19		4.22			4.3			4.2					
H			4.27													



CONCLUSIONS

1、Single clones selected from the screening process were cultured using 96-well and 24-well plates. After incubation at 37°C for 15 hours, the culturing success rate for both the 96-well and 24-well plates was 100%. The results corresponded with the inoculated cell wells, indicating that the cell culture performance of the plates was satisfactory and that there was no contamination.

2、The optical density (OD) values of the bacterial suspensions in each well were measured. The coefficient of variation for the OD values in each plate after cultivation was within 5%, indicating good uniformity in the culture across the wells.