Genotype Confirmation Testing of S. typhimurium Ames Strains

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Genotype	Test Media	Assay Setup – Day 0	Assay Performance – Day 1	Expected Results – Day 2
his⁻	MGA[1] w/ 0.75 µg/mL biotin	Inoculate cultures[2] (15 – 18 h prior to assay performance)	Grow cultures to 1 – 2 x 10^9 cfu/ml[2]; Using a 10 µl inoculating loop[3] or a swab[3,4], streak culture(s) across MGA	No growth
bio⁻[5]	MGA[1] w/ 50 µg/mL histidine	Prepare phenotype plates (if applicable)	Incubate *overnight*, 37°C	No growth except for TA102 which is biotin independent
his⁻, *bio*⁻	MGA[1] w/ 0.75 µg/mL biotin & 50 µg/mL histidine			Growth

[1] Minimal Glucose Agar. Reference Mortelmans and Zeiger (2000), The Ames *Salmonella*/microsome mutagenicity assay, *Mut Res.* 455, pp 29-70.
[2] Reference MOLTOX® Technical Bulletin *Lyophilized Culture Discs.*
[3] Sterile
[4] Press swab against the side of the growth vessel to remove excess broth prior to inoculating MGA plate. This helps prevent nutrient carryover to the MGA plate which can cause erroneous results.
[5] If testing TA102, streak both it and a biotin dependent strain (i.e., any other Ames *S. typhimurium* strain) on the same plate for growth comparison after incubation.

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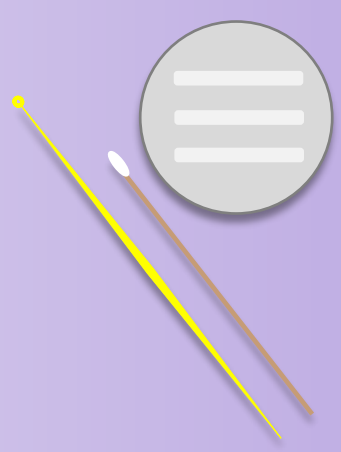
Genotype	Test Media	Assay Setup – Day 0	Assay Performance – Day 1	Expected Results – Day 2
rfa[6]	MGA[1] w/ 0.75 µg/mL biotin & 50 µg/mL histidine[7]	Inoculate cultures[2] (15 – 18 h prior to assay performance)	Grow cultures to 1 – 2 x 10^9 cfu/ml[2]; Using a 10 µl inoculating loop[3] or a swab[3, 4], streak culture(s) across MGA; Incubate *overnight*, 37°C	Growth; zone of inhibition around crystal violet disc[6]
uvrB	N/A[8]			N/A[8]
pKM101[9]	MGA[1] w/ 0.75 µg/mL biotin, 50 µg/mL histidine & 24 µg/mL ampicillin	Prepare phenotype plates (if applicable)		Growth of pKM101-containing strains only
pAQ1[10]	MGA[1] w/ 0.75 µg/mL biotin, 50 µg/mL histidine & 2 µg/mL tetracycline			Growth of pAQ1-containing strain (i.e., TA102) only



[6] After streaking, place a filter paper disc containing 10 µg of crystal violet in the center of the streak.
[7] Nutrient Agar prepared with Oxoid Broth No.2 can also be used.
[8] The deletion mutation is across the *bio-uvrB* chromosomal region; it cannot be reverted to wild-type. If the strains are biotin dependent one can infer they are *uvrB d*efective. Reference Mortelmans and Zeiger (2000), The Ames *Salmonella*/microsome mutagenicity assay, *Mut Res.* 455, pp 29-70.
[9] Streak a pKM101-containing and a pKM101-free strain on the same plate for growth comparison after incubation. Reference MOLTOX® Technical Bulletin *Lyophilized Culture Discs.*
[10] Streak a *pAQ1*-containing (i.e., TA102) and a pAQ1-free strain on the same plate for growth comparison after incubation. Reference MOLTOX® Technical Bulletin *Lyophilized Culture Discs.*