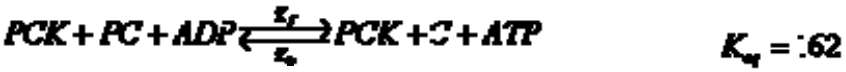
ATP regeneration system:

This set of reactions regenerates ATP from ADP thus keeping the ATP concentration high enough for the ATP dependent reactions of the RecA.

Aberrations:

ATP – Adenosine Tri Phosphate
ADP – Adenosine Di Phosphate
CPK - Creatine Phospho Kinase
PC – PhosphoCreatine
C - Creatine

This is an equilibrium reaction:



The PCK enzyme has a random sequential mechanism – the order of addition of substrates and release of products is random.

1) Avram lysates' system:

Cell extract + 20x Creatine kinase + 10x energy mix + H_2O = 10ul / reaction
30^oC, 0 – 3 hours

20x Creatine kinase =**1mg/ml** (stock = 5mg/ml)

10x energy mix = **75mM Phosphocreatine**
10mM ATP
10mM $MgCl_2$

2) For add-back use in Valary's Bomb lysis buffer:

Noc lysate + 10x Regeration mix + beads + H_2O = 50ul/ reaction (30^oC, 30min)

10x Regeration mix:	**1M Tris.HCl pH7.2**	**20ul (final conc. 0.2M)**
	1M $MgCl_2$	**20ul (final conc. 0.2M)**
	1M Phosphocreatine (CP)	**20ul (final conc. 0.2M)**
	10mg/ml Creatine Kinase (CPK)	**10ul (final conc. 1mg/ml)**
	0.1M ATP	**10ul (final conc. 10mM)**
	0.2M βME	**5ul (final conc. 10mM)**
	H_2O	**=➔ 100ul (total volume)**

NOTE: Modified Bomb Lysis Buffer - (βME instead of DTT, omit EDTA)

For 500ml:

0.5 M Hepes pH7.4	**40ml (final conc. 40mM)**
14.3 M βME (original bottle)	**70ul (final conc. 2mM)**
β-gp (glycerolphosphate)	**6g (final 60mM)**

Other inhibitors (1000x) added upon use.